

**CIMAR – LA**  
**EVALUATION REPORT 2003-2007**

October 2008



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## **1. MAIN PAGE**

### **1.1 Original research contract**

CIMAR Research Contract (See [Annex 1](#))

### **1.2 Director**

João José Oliveira Dias Coimbra

### **1.3 Denomination**

Centro Interdisciplinar de Investigação Marinha e Ambiental - CIIMAR

### **1.4 Units involved**

### **1.5 Reporting period**

2003-2007

### **1.6 Institution(s) on which it is based**

Centro Interdisciplinar de Investigação Marinha e Ambiental

### **1.7 Additional information**

CIMAR became an Associated Laboratory (CIMAR LA) in 2002 as a partnership between CIIMAR (University of Porto) and CCMAR (University of Algarve) having as main objectives “to understand natural processes in the ocean and the coastal zone, to study and implement sustainable exploitation of aquatic resources and the impact of human activities in the environment”. With these objective two main thematic areas were outlined in the research contract. The first, Conservation and Management of Aquatic Ecosystems, included in the original contract research the following research lines:

- a) Ecology and Management of Freshwater and Coastal Ecosystems;
- b) Biodiversity and Management of Threatened Ecosystems;
- c) Sustainable Exploitation of Aquatic Resources;
- d) Environmental Impact of Anthropogenic Activities and Ecological Risk;
- e) Development of Methodologies for Biomonitoring.

The second, Aquaculture, Marine Biotechnologies and Quality Control, included the following research lines: a) Marine Biotechnologies; b) Technology and Management in Aquaculture; c) Biology, Nutrition and Pathology of Industrially Cultivated Species; d) Potential New Species for Aquaculture and e) Quality Control and Food Safety.

## R&D PROGRAMME

During 2003-2007, the ten research lines set out in the original contract were condensed to four lines, which have particular approaches or share specific types of equipment. These lines of research are: 1) Biodiversity and Management of Aquatic Resources; 2) Environmental Chemistry and Toxicology; 3) Marine Biotechnology; 4) Aquaculture. In addition, support infrastructure and logistics for some of these lines is being provided by 5) Interface of Marine Genomics (IMG).

### 1) Biodiversity and Management of Aquatic Resources

Planning agencies, resource managers, local authorities, etc – rely on knowledge of coastal ecosystems and their models as management tools for sustainable use of aquatic resources and development of the coastal zone. Agreement and implementation of International Conventions and EU Directives also require a sound scientific basis. Integrating research results in environmental management and policy at the EU level is one of the objectives of the thematic network European Platform for Biodiversity, that integrates 28 partners from 28 European countries and that is coordinated by CIMAR - UP. CIMAR also integrates the European Marine Biodiversity Network of Excellence, Marbef, funded under the 6th Framework Program that aims at structuring the European research in Marine Biodiversity and Ecosystem Functioning and is being pivotal for the development of the research in this field.

#### 1.1 Dynamics of estuarine and coastal waters

Airborne remote sensing plays a key role in mapping applications. Remote sensing satellites provide information at a regional scale; aircraft based systems are appropriate for local scales.

With the miniaturization of navigation and sensing instruments, the use of small aircraft is becoming suitable for high resolution applications at small scales. The project aims to develop expertise in airborne systems for estuarine, coastal and oceanic applications. These include water colour studies; identification and monitoring of pollutants: beach dynamics and management; mapping and monitoring of shore belts; erosion and damage assessment in beaches, estuaries, flooded areas; current modeling. The research includes i) Sensor integration and adaptation of a data acquisition unit, ii) Flight tests for system validation together with ground measurements, iii) Establish an observation strategy for monitoring periodical events, iv) Seasonal anchor stations during consecutive tidal cycles and for periods 15 days v) Longitudinal gradient surveys in river estuaries and associated river plumes. The CIMAR - UP team has previous experience in the implementation of airborne systems for marine applications. Preliminary flights have already provided coastal maps for mapping algal communities. In the case of unexpected events such as accidental or natural pollution discharges a fast response can be provided.

#### 1.2 Coastal and estuarine ecosystem functioning

Macrophyte communities of estuaries and tidal lagoons, provide two crucial environmental services: 1) saltmarshes serve as buffers between the land and sea, intercepting a significant fraction of terrestrially derived nitrogen; 2) tidal marshes and seagrasses drive much of the secondary production within the coastal system. It is commonly accepted that the main route for organic matter to enter the food web is detritivore, but the microphytobenthos cannot be underestimated. We are applying two levels of investigation. The first characterizes the sources and patterns of primary production and makes a global assessment of organic matter transfers along the food web through multiple stable isotope analysis. The second, at the organism level

uses stable isotopes to reveal the physiological mechanisms of uptake using  $^{13}\text{C}$  labeled molecules. At the ecosystem level, the analysis of the natural abundance of stable isotopes is combined with experiments with controlled additions of stable isotope labeled compounds to test hypothesis about trophic linkages between primary producers and consumers. For the research on ecophysiology, molecular biology techniques are being used to address ecological and evolutionary questions of intertidal algae and seagrasses as main models. Events of importance in desiccation tolerance are being studied at the level of gene identification and expression to understand (a) similarities and differences in molecular responses of poikilohydric algae to those of lower and higher plant models, and (b) evolutionary aspects of stress responses (local adaptation at the population level, variation between recently-evolved conspecifics).

Investigation of plastid genome organization and the regulation of chloroplast gene expression in response to the extreme environmental fluctuations in intertidal habitats, including roles of circadian/circatidal rhythms, light, and inorganic carbon supply is ongoing and research on evolution of reproductive strategies is being done using closely related hermaphroditic and dioecious species that will be compared for their a) population genetic structure, and b) reproductive ecology, to understand the evolution of reproductive strategies in marine algae and seagrasses.

In the Douro and Lima rivers a monitoring program started in 1985 will continue, providing data for modelling and the basis for the design of scientifically sound restoration plans for the two watersheds. Molecular methods are being used to detect Eubacteria, Archaea and nitrifying bacteria, while gas chromatography is used to detect  $\text{N}_2\text{O}$  and epifluorescence to quantify sediment bacteria.

### 1.3 Resource management and conservation

The impact of fishing activity on species, populations, communities and the ecosystem is a research line involving researchers from a number of different groups. Several projects deal with by-catch, discards, gear selectivity, ghost fishing and ways of mitigating some of the negative impacts of fishing with certain gears. Other lines of research focus on the biology and ecology of commercially important species. For example, following a successful tagging program to study the migratory behaviour and habitat use of juvenile sea breams in the Ria Formosa, new projects are extending these studies to adult stages. This research is being carried out in order to evaluate the potential of restocking coastal waters and the usefulness of artificial reefs. External T-tagging and acoustic telemetry is monitoring daily movements, habitat preferences and use, and inshore/offshore migration. For long-term monitoring ultrasonic VR1 Receivers/Monitors is being used. Individual fish are being also tagged and tracked from a small boat for periods of at least 24 hours. Factors affecting the recruitment of bivalves, cephalopods and fish are also being studied.

Most commercially important species have planktonic life stages and upwelling events during the spawning season may drastically affect their survival. Meroplankton abundance is being studied by obtaining biological, atmospheric and oceanographic data during a seasonal cycle, especially during the major spawning season of selected species using in situ observations and satellite remote sensing to cover the period of upwelling events and outside upwelling events. Laboratory grazing rates and growth are being quantified for key meroplankton species. Lobster puerulus collectors for estimation of temporal and spatial patterns of settlement for the rock lobster (*Panulirus elephas*) are being deployed along the SW coast.

## 1.4 Biodiversity

Global climate change together with human activity is affecting species and ecosystems. Portuguese coastal waters, influenced by the Mediterranean, the North Atlantic and the African coastal zone are especially important zones in terms of biodiversity, being the southern or northern limit for the distributions of many species. This is an important area of research for the next years, involving many CIMAR researchers. A new project will continue to describe the long-term changes in the benthic marine flora of the continental coast of Portugal by extending south ongoing studies to compare the actual situation with the only available description of the Portuguese marine flora, which was done in the 1960's. Range shifts of species, variations in their abundance or new introductions is being analyzed to test the hypotheses that the richness and abundance of warm-water species has increased, as opposed to cold-water species. This work is providing an updated database on the benthic marine communities of the Portuguese coast that will continue to be integrated in global biodiversity databases as ALGAEBASE, Species 2000 and ERMS (European Register of Marine Organisms) as part of EU projects that CIMAR integrates. A project to study the populations from seaweeds that have their Southern distribution limits in the Northern coast of Portugal was proposed to FCT. It integrates researcher from CIMAR-UP and CCMAR to study growth, reproduction, demography, genetic structure and resistance to disturbance of these populations to find if they are more adapted to these conditions than others in the centre of distribution and predict what will happen if there is an increase in seawater temperature. This study is being done also with other Marbef partners.

Recent concerns of biodiversity loss worldwide and in Europe in particular, have raised the importance of this type of work. The need for the integration of the available data has led to the development of integrated networks on marine biodiversity for conservation purposes, but the available data on the Portuguese algal communities is still scarce.

Other studies are examining changes in diversity of other organisms, such as fish in the Ria Formosa lagoon and the spatial variability of intertidal rocky shore assemblages in the northwest coast of Portugal as well as that of macroalgal communities in littoral rock pools in this region. Experiments in the rocky shore are studying the effects of the introduced species *Grateloupia turuturu* on native communities. Special habitats such as temporary ponds and the ecology of species living in them are also continuing to be studied. A sample of the previously studied ponds are being observed for understanding year-to-year variation.

## 2) Environmental Chemistry and Toxicology – (From analytical chemistry to physiological and molecular approaches)

### 2.1 Assessment of contamination in aquatic ecosystems

Assessment of contaminated ecosystems using chemical analyses and speciation (including thermodynamic and kinetic aspects) of pollutants is a fundamental tool in aquatic ecotoxicology to understand the route of entrance, distribution and fate of xenobiotics in the environment and the chemicals transformations and interactions between the abiotic and biotic compartments.

Heavy metals and other persistent toxic substances (PTS) like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and pesticides are the main contributors to environmental contamination and are being quantified in sediments, water and organisms. The identification and qualification of those PTS provides support to the research described below and the data are being correlated with the biological alterations of aquatic organisms.



## 2.2 Biomarkers of environmental contamination

Available biomarkers cannot predict accurately adverse effects at the population level, limiting their value for risk assessment. The challenge is to modulate the cascade of events to predict population effects using models based on sub-individual parameters. Resistance to environmental contaminants are being studied in representative species of different trophic levels in marine, estuarine and freshwater ecosystems, including algae, molluscs, crustaceans and fish.

In the next years, we will continue to develop methods for biomonitoring and risk assessment in both freshwater and saltwater environments, including biomarkers (e.g. cholinesterases, lactate dehydrogenase, glutathione s-transferases, P450), in situ assays, and laboratory toxicity tests, particularly with autochthonous species. The effects of environment contaminants (including metals, pesticides, petroleum derived products and pharmaceuticals for human and veterinary use), their degradation products and metabolites on both freshwater and saltwater species are being studied in the sequence of previous studies, using parameters at different biological organization levels, from molecular to population levels. Several parameters are being investigated, including biomarkers (of neurotoxicity, oxidative stress, energetic mechanisms, detoxification, stress proteins, genetic damage and organ disruption), mortality, growth, reproduction, population < intrinsic growth rate. Particular attention is being given to the mechanisms of metal interaction with the acetylcholinesterase molecule of *Palaemon serratus*. This subject is being investigated using techniques of molecular genetics, enzymology methods and ecotoxicology approaches.

Another relevant line of investigation is the study of biochemical mechanisms contributing to genetic resistance to metals and pesticides in non-target species (cladocerans), evolution due to pollution in copepods the impact of pollution in the contribution of specific populations to the European stock renewal of *Anguilla anguilla*. We will also continue the work in course on tropical ecotoxicology in the scope of which we have been developing and validating toxicity assays and other ecotoxicological methods for use in the tropics, using tropical and sub-tropical species (e.g. *Chironomus xanthus*, *Poecilia reticulata*, *Gambusia yucatana*, *Dendrocyga autumnalis*) and studying the effects of chemicals with relevant impact on local aquatic systems on local species. Finally, we are studying the impact of pesticides on rural human populations.

Chronic sublethal contamination of estuaries and coastal areas from agriculture, industry and urban sewage sources can lead to alterations on reproduction, osmoregulation, liver metabolism and DNA in fish. Physiological responses in selected fish species are used as biomarkers of environmental contamination; these include the determination of plasmatic vitellogenin levels, gonad feminization in males after estrogenic compounds exposure (estradiol, ethinylestradiol, bisphenol A, nonylphenol, octylphenol), DNA damage (micronucleous assay and comet assay), oxidative stress, lipid peroxidation, oxidated proteins. The induction of hepatic cytochrome P450 (CYP1A) activity has been used as biomarker of exposure to persistent PCBs and organochlorine pesticides (OCPs) which accumulate in liver, muscle, eggs and fish larvae. PCBs also decrease circulating thyroid hormone levels due to competitive binding to thyroxine binding protein (TTR).

The presence of organochlorine pesticides (OCPs) in the environment induces alterations in Plasma cortisol levels and Na/K ATPase in gills and kidney which indicates disruption of the ionic and osmotic regulation. As a complement of field studies, laboratory experiments are being conducted in order to explain the mechanism underlying endocrine disruption in fish and marine invertebrates.

## 2.3 Histopathology

The rate of toxicopathic injuries in wild fish is steadily increasing, in correlation with the pollution degree; namely the preneoplastic, neoplastic, and necrotic liver lesions, and also ovotestis. If there is universal agreement that fish toxicopathic lesions do represent environmental problems, paradoxically, there is a complete information gap about the long-term meaning of the injuries for the fish health and consequent ecological impact. It is clearly relevant to know the functional translation of those lesions in fish. So, we are using protocols of experimental induction of neoplasia and necrosis, later making experiences in which the injured fish are challenged. We are using as models: brown trout, zebrafish, and tilapia. Hepatic function is being evaluated by a plethora of standardized tests, including of detoxifying capacity. Injuries are being diagnosed by histopathology, additionally using morphometry and immunocytochemistry. Neoplasia progression are being better known, being related for the first time with growth rates, survival and reproduction. We expect this approach leads to a breakthrough in understanding the negative consequences and health impact of the toxicopathic injuries in fish.

## 2.4 Natural toxicants from marine and freshwater cyanobacteria

The isolation of cyanobacteria and their culture is followed by chemical and biological assays so as to understand the role of the toxins and to identify them. We have been using HPLC with diode array detection and also with fluorescent detection for neuro and hepatotoxin analysis. The diversity of these toxins with more than hundred variants found until now and the lack of commercial standards for most make it necessary to use HPLC with MS detection. This is also essential to identify unknown compounds that we have been detecting by bioassays. Some compounds inhibit phytoplankton, others kill zooplankton and others that may be important in terms of human health as anticancer compounds or antiviral. The search and characterization of these new compounds is one of our goals for the near future.

## 2.5 Chemical speciation of culture and natural media of cyanobacteria from both fresh and sea waters

In addition to be able to produce intra-and extracellular toxins, cyanobacteria are highly effective biological metal sorbents, representing an important sink for metals in aquatic environment and playing an important role in affecting metal speciation and bioavailability. Understanding the fundamental physicochemical mechanisms of trace metal bio-uptake by aquatic microorganisms in natural systems involves research focused on identifying under what conditions uptake fluxes are limited by physical as opposed to biological processes. Further, the complexation of the trace metals in the aquatic medium has to be examined from both thermodynamic (stability) and kinetic (lability) perspective in order to elucidate its effect on the overall uptake flux. Investigation on this topic was recently launched to be developed in the next years.

With the final goal of identifying the major factors that condition the appearance of cyanobacteria blooms in the natural waters, particularly drinking water, a PhD project is being carried out using as case study the “Torrão” reservoir. This reservoir has potential to supply drinking water to the Great Porto area. The primary objective of this project is the thorough characterization of the trace chemical composition (in addition to major components and physical parameters) of water and sediments of “Torrão” reservoir in different seasons, to gain a better knowledge of the parameters that may be relevant in cyanobacteria blooms’ occurrence. Secondly, in vitro cultures of dominant cyanotoxins-producing species, in the studied reservoir, are being carried out to investigate the influence of some relevant chemical parameters on the biological response of

these microorganisms. The project deals with a novel topic of water chemistry and toxicology, with high relevance in public health, since potable drinking water is a scarce natural resource.

## 2.6 Rhizosphere biogeochemistry and phytoremediation at estuarine environment.

Estuarine sediments can be strongly influenced by plants activity. The biogeochemistry at the rhizosphere of plants is so intense and diversified that the rhizosphere can be considered as an independent system inside the sediment or soil where the plants grow. Even though it is known plants can play important roles in biotransformation of toxicants, bioaccumulation and phytoremediation, studies using salt marsh plants have been limited. A project was launched in order to attain a better understanding of the biogeochemistry of the rhizosphere of vascular plants that can grow at the Lima and Sado estuaries, for future application in toxicity end points and phytoremediation. It is intended to: (a) characterize the composition of the rhizosphere of some marsh plants (to be selected), in terms of biomass, physico-chemical parameters, total organic matter, inorganic macro and micro constituents (Including heavy metals) and organic contaminants (eg., PAHs, PCBs, butyltins, BTs, pesticides) and compare it with that of the surrounding sediments of similar grain size distributions but without vegetation; (b) study, in vitro (simple models), the effects of some contaminants on the biological response of the plants (eg., exudates release, ratio of subterranean/aerial biomass and chlorophyll; (c) estimate mechanisms used by plants to immobilize heavy metals; and (d) study in vitro and in field (two bio-geographic environments with different sedimentary dynamics, vegetal population, mean temperature, etc) the role that plants can play in some remediation process.

## 3) Basis for Marine Biotechnology

Basis for marine biotechnology has an organismic and/or cellular approach and focuses mainly on species which are cultivated, can be used as laboratory models for cultivated species, or have potential to be used for biotechnological applications. However, the objectives are not necessarily applied and some of the research is of a fundamental nature.

### 3.1 Skeletal development and calcium homeostasis

Osteocalcin (bone gla protein or BGP), a bone-specific protein, and Matrix Gla protein (MGP) are involved in the calcification process in lower vertebrates. We have purified and cloned BGP/MGP, and determined their primary protein structure, gene organization and tissue distribution, developed specific antibodies and the required fish/amphibian bone-derived cell lines capable of mineralizing in vitro. We propose to identify nuclear factors that regulate MGP/BGP gene expression and the signal transduction cascade involved in their regulation by extracellular signals such as increased calcium concentration; to characterize the response to mineralization of our in vitro cell systems by analysis of 2D protein profiles; and perform structural studies to contribute to elucidate BGP/MGP mode of action at the molecular level, still unclear even in mammals.

Although parathyroid hormone (PTH), the hypercalcemic hormone in tetrapodes, has not been identified in fish we have shown that a related factor PTHrP has hypercalcemic functions in fish through its N-terminal region. The gene for PTHrP has been isolated and its expression determined by immunocytochemistry and in situ hybridization. We are now investigating its gene regulation and other possible functions of PTHrP, which is multifunctional in mammals, such as regulation of normal chondrogenesis, regulation of vitellogenesis, and bone development and immune response, using a range of techniques including specific radioimmunoassay, microarrays, Ussing chambers, etc. Other fish hormones (prolactin, somatolactin) are being investigated to

establish the mechanisms regulating calcium homeostasis in fish. A comparative study of ion transport proteins is being carried using immunofluorescence techniques aiming at defining a suitable freshwater model for the branchial epithelial cells complementary or in alternative to cell culture.

### 3.2 Fish reproduction and sex differentiation

We have established that teleosts produce a large diversity of steroids which led to the development of unique radioimmunoassays used worldwide, investigate the relationship between sex hormones and behaviour and the function of steroid receptor (estrogen, androgen) isoforms, estrogen responsive genes and steroidogenic enzymes. We are identifying sex differentiation (SD) pathways in *Dicentrarchus labrax*, which in farms can differentiate to 100% males. Several putative SD genes have been cloned (e.g. SF-1, DAX-1, DMRT-1) and are being analysed for their pattern of expression using microarrays, PCR, etc. Subtractive hybridisation methods are used to search for unknown SD genes.

### 3.3 Estrogenic regulation of fish peroxisomes

We have shown that estradiol cause a reduction of peroxisomal size and repress peroxisomal metabolism in trout liver. Since Peroxisome Proliferator-Activated Receptors (PPARs) are the only known regulators of peroxisome function at DNA level, to elucidate the mechanism of estrogenic regulation of fish peroxisomes, we are investigating the expression of PPARs in different trout tissues and its variations during the annual reproductive cycle. Additionally, the concentrations of estrogens and xenoestrogens required for modifying fish peroxisomes in cell culture and the kinetics of these events will be determined. The expression of peroxisomal matrix enzyme genes is being quantified in fish exposed to estrogens.

### 3.4 Fish chemoreception

*S. pavo* males produce a putative pheromone that promotes female attraction to nesting sites. *S. aurata* and *O. mossambicus* males show strong olfactory responses to females holding water and to their urine and bile and intestinal fluid. We want to isolate the putative pheromones using a combination of HPLC with extracellular electrophysiological recording. Isolates are being identified by GC-MS and/or HPLC-MS, according to their nature. Function are being addressed in the response of *S. aurata* and *C. auratus* to alterations in environmental  $[Ca^{2+}]$ , possibly mediated by an extracellular  $Ca^{2+}$ -sensing receptor. Olfactory neurons that carry this information and pathways to the brain are being identified, followed by the olfactory transduction pathways using whole-cell patch-clamp techniques and the excised olfactory system in vivo from *S. aurata*, and later cell lines transfected with olfactory receptors.

### 3.5 Migratory fishes

The migratory phases of the life histories of many fishes are characterized by significant changes in environmental conditions that require physiological adaptations. One area of focus is the elucidation of the mechanisms involved in salinity adaptation in migratory as well as estuarine fishes. Working is being conducted on economically important (*A. anguilla*) as well as model species (*Tetraodon nigroviridis*).

Research in this area also covers the role of diurnal pressure changes (vertical migration) on gonadal maturation during the spawning migration of the European flounder (*P.flesus*), and the

study of the life history of the anguillids *C. conger*, and *A. anguilla* through otolith and morphometric analysis.

### 3.6 Chemical structure and biological activity of natural compounds

The structure of compounds isolated from marine sponges collected from the Gulf of Thailand and Moroccan Coast is being elucidated by spectroscopic methods. Antitumor and antimicrobial activity of these compounds is being evaluated. We also plan to study the apoptotic activity of plant compounds and bioactive secondary metabolites from marine sponges.

### 3.7 Parasitology

For the study of the bivalve parasite *Perkinsus atlanticus* the development of pure parasite cultures to allow in vitro analysis of host cells infection processes and contribute to understand the effect of environmental factors such as temperature, salinity and water pollution on parasite growth and development. An epidemiological study of the *Perkinsus* infection along the Portuguese coast over a three-year period is on going and a pure culture of *P. atlanticus* suitable for in vitro studies of infection and identification of the effects of environmental factors on parasite growth is being currently developed. Other protozoan and digenean parasites are also being studied using microscopy and molecular biology techniques. These parasites will be searched in fish and shellfish collected in Portugal, Spain and Brazil.

## 4) Aquaculture

Research covers various aspects of aquaculture including optimisation of farming output, mitigation of stress conditions for the farmed fish, pathology and control of environmental impacts.

### 4.1 Fish immunology and vaccinology

The objective is to investigate the immunological mechanisms, cellular changes and cytokine/chemokine profiles, related to the intraperitoneal immune granuloma seen in vaccinated farmed fish after use of oil adjuvanted vaccines. The following topics are under investigations: a) the quantitative reaction of sea bass (*Dicentrarchus labrax*) to the inoculation of different vaccine formulations, using cytological and cytochemical methods including TEM and SEM, b) the relationship of eosinophilic granular cells (EGC) to the inflammatory response triggered by vaccination. It involves FACS analysis and cell sorting for better characterisation.

4.2 Nutritional requirements and optimisation of feeding regimes of fish species which are candidates for aquaculture Amino acid (AA) and fatty acid requirements are studied mainly in *Solea senegalensis* and *Pagellus bogaraveo*. <sup>15</sup>N-labelled food and GC-IRMS are being used to study simultaneously the bioavailability of several individual AA or fatty acids. Micro-injection are being used to estimate metabolic budgets of individual amino acids and fatty acids and classical methodologies to study protein and energy requirements. A self-feeding system is being used to monitor feeding activity and zootechnical performance without human disturbance within the fish biologic feeding rhythms and enable the optimisation of feeding regimes and avoid feed waste.

4.3 Interaction between stressful conditions and nutritional requirements of cultured fish diets is being supplemented with key AA to test whether effects of stressful fish husbandry conditions can be mitigated. The effect of stress is being assessed by measurement of growth, survival,

protein turnover, plasma levels of free AA, cortisol, glucose and lactate. <sup>15</sup>N given as tracer tends to increase when there is AA imbalance and is being used to monitor o protein turnover.

#### 4.4 Nutrient fluxes in fish and estimation of waste output in fish farming.

Fish grown in ponds is being fed diets containing <sup>15</sup>N and <sup>13</sup>C as tracers. They are allowing to following the energy and nitrogen fluxes through the trophic chain in the ponds, compare different diets and determine the relative importance of artificial (feeds) and natural food for fish growth. This should allow the implementation of better management in pond culture systems and reduce waste output.

#### 4.5 Development of indicators physiological condition, nutritional status, and growth potential in fish larvae

The activity of the several digestive enzymes (e.g., trypsin, alkaline phosphatase and aminopeptidase) is being assayed in vitro. Assimilation efficiency of the main nutrients is being determined through in vivo tube-feeding of a known amount of radio-labelled proteins and triglycerides. Protein/lipid ratio, DNA/RNA ratio and Protein/RNA ratio will be tested as larval condition factors.

#### 4.6 Fish pigmentation

Farmed P. bogaraveo do not develop normal skin pigmentation. The red colour of salmonids flesh comes from dietary keto-carotenoids, such as astaxanthin and canthaxanthine. Analysis of blood carotenoids showed that dietary astaxanthin can be bio-absorbed by seabream, but is not deposited in the skin. The emphasis is to incorporate the desired levels of pigmentation in the skin rather than in the muscle tissues.

### 5) Marine Genomics Interface

The marine environment and in particular the organisms that inhabit this vast habitat still remain poorly studied despite the importance of this resource. However, only the genomes of a few marine organisms, mainly microorganisms and a vertebrate (*Fugu rubripes*), have been sequenced so far. At present in CCMAR genomics is being used to study issues related to biodiversity, stock assessment, ecology and aquaculture and CIMAR aims to extend genomics to ecotoxicology. In addition to the various projects using genomics approaches indicated above in the area of aquaculture, investigation into comparative genomics and gene mapping has been initiated and strong links have been created with European Platforms in the context of Marine Genomics Europe. CIMAR is involved as a partner in genome mapping of sea bass and seabream as well as the sea bass BAC/genome sequencing consortium led by the Max Plank Institute of Molecular Genetics (Berlin, Germany). The Marine Sciences are a priority for Portugal and inclusion of Marine Genomics is key to new scientific developments and even economic benefits. We have initiated steps towards integrating various genomics efforts in Portugal leading to the creation of a network and to try pool resources in a Marine Genomics Interface (MGI) for the benefit of the existing community. We are developing a common infrastructure which functions as a Marine Resource Centre and is sited at CCMAR. It includes in the first phase of its development, generation of Marine genomic resources such as low cost sequencing, generation of gridded libraries, gene isolation and small scale proteomics facilities. This is being complemented by development of database services and bioinformatics.



In summary, the CIMAR - Associated Laboratory research programme reflects the multidisciplinary nature of Marine Sciences ranging from cellular to ecosystem approaches and the monitoring of relevant environmental parameters. It includes the recent developments of Molecular Biology and strategically, it aims at making these tools available throughout the programme. Another target is the development of appropriate Analytical Chemistry tools to support the biological activities. The recent addition of two groups of chemistry researchers reinforces this objective.

The development of these strategic areas in CIMAR – a resource centre of Marine Genomics in Algarve and an Environmental Chemistry and Toxicology Unit and associated services in Porto – will guarantee the needed support for the entire programme and correspond to the natural distributions of competences existing in the two centres. Our task for the next years is to reinforce them and make possible the spreading of these competences among the groups, taking advantage of their integration in CIMAR. The new equipment granted through the Re-equipment Programme of FCT and the hiring of post-doctoral researchers guarantee the reinforcement of the Associate Laboratory.

The Universities, University of Porto and University of Algarve, are the mother-institutions of CIIMAR and CCMAR, respectively, provide a significant part of the salaries (those from university staff) and the infrastructure for research. Both include in their strategic plans a strong support for research activities of excellent quality, namely in the Marine and Environmental areas.

As part of the strategic plan, CIMAR LA has also established a formal institutional agreement with the Instituto Geológico e Mineiro (IGM, now the Marine geology department of INETI), especially in the areas of marine geology covering aspects related to climate change. This Collaborative Agreement, signed in 2003, involves postdoctoral researchers contracted by CIIMAR working in INETI's Marine Geology group, by the Principal Investigator Fátima Abrantes. This PI is an invited member of the Board of Directors, but since she is a member of INETI staff, a State Laboratory, she can not be formally included as an integrated member of the LA and PI of the research group. To overcome this situation, João Coimbra appears in the present report as the PI of the Geology and Paleo-Oceanography Group.

This relationship will continue and it is expected to become more formally integrated as we develop a common strategy and complementarily of human resources for investigating the marine environment. More recently, and as result of an already significant level of collaborative work in research projects and post graduate student supervision in the areas of behavioural endocrinology, evolutionary biology and Conservation CIMAR LA has established formal links with the Research Unit on Eco-Ethology (UIEE) at the Higher Institute of Applied Ethology with the aim of UIEE becoming part of CIMAR during 2008. An agreement has also been established since 2004 with CESAM – Associated Laboratory (Centre for Environmental and Marine Studies), with the aim of reinforcing the common lines of research and particularly to create a Doctoral School in collaboration with the Universities of Porto, Aveiro and Algarve (1<sup>st</sup> edition in 2007/2008).

## POST-DOCTORAL RESEARCH CONTRACTS

An integral part of the CIMAR-LA contract with FCT, to be renewed in 2008, included the hiring of 28 post-doctoral researchers; so far, 23 were hired in the last five years.

These have been deployed to strengthen existing research groups. In 2007, an initiative to reinforce the human resources of the research centers has been launched by FCT (Ciência 2007) and CIMAR submitted a proposal to hire 12 researchers on two scientific areas of specialized competence but with a high potential to create synergies with the ongoing research lines, while at the same time significantly reinforcing national capacity in these areas, which are lacking. The two proposed areas were Marine Genomics (including proteomics and post-genomics) and Physical Oceanography. The first is an emerging discipline which has, for example, brought to light worlds which were until recently inaccessible such as the non-cultivable microbes. The second corresponds to a gap identified by the reviewers in the last evaluation of the Associated Laboratory. In addition it is a strategic area considering, among others, the ongoing changes in the oceans and in the global system as a result of anthropogenic interventions and its relationship to resource management. It is also an area in which Portugal clearly lacks critical mass and requires investing in human resources.

### Marine Genomics

The utilization of high throughput technologies to access genomes and their products enables new approaches and to obtain answers inaccessible a few years ago, both of a scientific or a practical interest. Examples for aquatic or polluted environments are biomineralization processes, community structure, and environmental adaptation - including extreme environments, or genetic and epigenetic effects of pollutants and other factors. It should be also emphasized the role of genomics approaches in the finding of new molecules for biotechnology and pharmaceuticals. The development of Marine Genomics approaches (including proteomics) has as main objective to establish a group with a minimum dimension to initiate a coherent research programme aiming at a high level of scientific excellence.

### Physical Oceanography

With the proposal to hire Physical Oceanographers the objective is to acquire capacities that enable CIMAR LA to intervene in a more effective way in the response to the challenges associated with global change, in which the oceans have a fundamental role, and as support to research related to resource dynamics and distribution. The adoption of an European Strategy for the Protection and Conservation of the Marine Environment, such as proposed in the Directive for the Marine Environment, will imply not only knowledge about the various ecosystems in our EEZ, but those same regions of the Biosphere are likely to undergo climatic and anthropogenic changes.

The oceanographic processes at the scale of the sub-basin influence the dynamics of the climate system as a whole at different temporal scales. Monitoring and experimentation may provide important information about high frequency processes, but for lower frequencies, i.e. climate and oceanic variability at longer time scales, it is necessary to consider paleoceanographic / paleoclimatic studies. The Portuguese margin is, because of its geographic location, of high importance for the diversity of oceanographic processes and extension of the EEZ. It is an area of seasonal upwelling where high productivity at different trophic levels is elevated making it an important region for fisheries. Nevertheless, with global warming its potential as a CO<sub>2</sub> sink makes it necessary to understand the physical and biogeochemical processes both those ongoing and those associated with passed climatic variations of the geological passed, so that future conditions can be estimated / mitigated.

Another area which has become relevant in oceanic observation is satellite altimetry because of its capacity to generate information day and night, in any climatic conditions. Continuous data



collection, now spanning 15 years, has revolutionized oceanic studies such as global sea level variations, determination of surface oceanic currents and monitoring large impact climatic phenomena such as El Niño, among others. The characteristics of the instrumentation aboard altimetric satellites and the geophysical corrections which are necessary to apply to obtain centimetric precision, has limited their use in the coastal zone. The development of methodologies adequate for the processing of altimetric measures and to the development of models for local corrections which will enable the extension of altimetry to coastal regions it is a research area of high interest, which requires the contribution of several complementary areas by a team of adequate dimension. In particular, the capacity to generate high precision altimetric products in the coastal zone for operational purposes is an aim that no other international group has yet attained.

These interrelated topics, each one with large implications for human society, require collaborations within and between disciplines for substantial progress to be achieved. But progress will be dependent essentially on many observations, appropriate processing of data, and numerical modelling which assimilates the data from observation and enables the establishment, for example, of a reference state (without human intervention), but also forecast with a certain degree of confidence.

From the 12 post-doctoral researchers proposed to Ciência 2007, 11 were hired during 2008. This initiative also allowed the submission of individual proposals directly to FCT and four of those proposals were oriented to CIMAR, including one in the area of science management, two in the area of Chemistry (Environmental Chemistry and Chemistry of Natural Products) and one in the area of Paleo-geology (to be hired by the Marine geology department of INETI). The Ciência 2008 initiative will allow the hiring of 5 researchers to work on Marine Biodiversity and Ecosystem Functioning and up to 3 researchers on Physiological and Nutritional Genomics, in Porto, and up to 7 researchers on Metagenomics, Environmental Genomics, Marine Biofuels, Chemical Ecology, Gelatinous zooplankton, Algal bloom, and Marine Bioeconomics, in Algarve.

## HORIZONTAL PROGRAMMES

Concerning its engagements towards society, the CIMAR-Associate Laboratory has become a renown formation centre for young researchers, welcoming about three hundred research grant holders, BSc, Masters and PhD students, and Post-Docs from different nationalities. Between 2003 and 2007, 142 MSc students and 87 PhD students have completed their degree under the (co)-supervision of CIMAR members. In 2007, can also be stressed the agreement with the Ministry of Science, Technology and Education for the creation of a Doctoral School in Marine and Environmental Sciences, in which the University of Porto and of Aveiro are CIMAR partners, and CESAM with whom CIMAR has a collaboration agreement. Simultaneously, CIMAR participates actively in various thematic networks, particularly as member of the Executive Committee of two Networks of Excellence (Marbef – Marine Biodiversity and Ecosystem Functioning, and Marine Genomics Europe). It is also essential to emphasize that CIMAR is becoming more and more active in supporting business and industry, being founding member of the Institute for the Development of the Knowledge and the Economy of the Sea (Instituto para o Desenvolvimento e Conhecimento da Economia do Mar – IDCEM), which acts as a promotion agency of scientific knowledge, research valorization, technology service agreements and promotion of entrepreneurship, which started its activities this year.

Regarding the outreach/science and society activities, the CIMAR has coordinated a number of high impact programmes, for example the educational programme Latitude 60, as part of the International Polar Year, and the Sea Itinerant University aboard the vessel Creoula from the

Portuguese Navy. The CIMAR also coordinates the scientific part of a network of Environmental Monitoring and Interpretation Centres, notably the CMIA in Vila do Conde and Matosinhos in 2007, and maintains close collaboration with the Ciência Viva Centre in Faro, the Aquamuseu from Minho River and the Littoral Station of Aguda.

Finally, it is also very important to stress the role of the Associate Laboratory as support of public policies, at the regional, national and European level. In 2007, and in collaboration with the North Regional Coordination and Development Commission, the CIMAR played an active role in the elaboration of the Ocean Agenda for the North Region and in the definition of the cooperation between North Portugal and Galicia. The contribution of CIMAR in the definition of the Ocean National Strategy and the European Maritime Policy, and as active partner within the European Science Foundation – Marine Board (Marine and Maritime) is also of particular interest. In 2007, the CIMAR organized two international conferences as part of the Portuguese Presidency of the European Union (The Role of Marine Sciences in Ocean Sustainability and Global Change, 8 October, Lisbon; Life on the Blue Planet: Biodiversity Research and the New EU Marine Policies, 7-9 November, Porto), and participated in the organization of other high relevance scientific conferences like the SETAC Europe (Society for Environmental Toxicology and Chemistry) Annual Meeting, in collaboration with the CESAM. The Centre also maintains its partnership as Third Party within two ERA-Nets: AMPERA and BIODIVERSA.

#### CIMAR IN NUMBERS

Maybe not the most significant but certainly of interest are the numbers that express the development of the CIMAR: from 113 to 154 PhD holders, and an increase in the number of articles published in international journals from 120 to 233 (with a maximum of 270 in 2006) corresponding to a total of 1002 articles in the last five years. An increase number in R&D projects (112 national and 29 European) and in contracts with industry, a considerable augmentation in scientific equipment investment with the attribution of 2MEuros through the Re-equipment Programme of FCT, the enlargement and improvement of the installations especially in Porto, can also be highlighted. The CIMAR also saw a remarkable increase in the number of research grant holders and post-graduation students, as well as an increase in the number of non-teaching researchers: 35 after the recent appointments through the programme Ciência 2007. At the same time the number of research groups dropped to 24, from the 38 existing in 2003. Moreover, more than 60 institutional agreements were signed during the last five years.

The internationalization of CIMAR is reflected in the number of scientific articles published in collaboration with foreign institutions, about 2/3 of the total number in the last five years, mainly from Spain, UK, USA, Brazil, France, Norway, Netherlands, Greece, Thailand, Germany, Italy, Belgium, Singapore, Canada and Mexico. The attractiveness of CIMAR at the international level is also reflected in the number of non-national researchers, about 15% of the total research team, from 23 nationalities. A gender balance was achieved with a steadily increase in the number of woman researchers working at CIMAR during the last years (ca. 60% of woman in 2007). The average age of researchers decreased with a more balanced number of junior and senior members.

#### MAJOR CONSTRAINS

The major constrains to the development of CIMAR during the last five years were the financial instability and the strong administrative effort required to manage the research projects. For example, during 2005 and 2006, only 50-60% of the total funding contracted with FCT (Programmatic and Pluriannual) was delivered to the LA, representing a deficit of ca. 2 MEUR. The complexity and time-consuming administrative work needed for the reimbursement of

expenses from the running projects also introduced a delay in the financial stability of the centre with 1.5 MEUR in debt from national projects at the end of 2007. The main reason for these constraints may be attributed to the existing obstacles faced by FCT in obtaining EU funding. A similar situation was faced with the European projects managed by CIMAR, namely those financed by the Interreg programme, with ca. 0.6 MEUR in debt at the end of 2007.

These financial constraints render very difficult the mobility of researchers between Porto and Faro and the implementation of common projects, contributing to a delay on the effective integration of the two centres.

Although we were allowed to hire 10 technicians in the last years (the contract with FCT only foresees the hiring of 8 technicians) the lack of technicians needs also to be pointed out since the present number is clearly insufficient for the objectives of CIMAR-LA. The lack of space facilities in Faro was an additional constraint to the development of CCMAR. New facilities are expected in Porto for 2011.

## 2. RESEARCH TEAM

### 2.1 Researchers w/ PhD.

- 1. Abraham Pérez Pastor - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 2. Adelino Vicente Mendonca Canario - (Agregação / Professor Catedrático / 50%)
- 3. Adriano Agostinho Donas Bôto Bordalo e Sá - (Agregação / Professor Associado / 40%)
- 4. Agostinho Antunes Pereira - (Doutoramento / Investigador Auxiliar / 100%)
- 5. Aires Manuel Pereira Oliva Teles - (Agregação / Professor Catedrático / 50%)
- 6. Alberto Teodorico Rodrigues Moura Correia - (Doutoramento / Professor Auxiliar / 50%)
- 7. Alexandra Paula Mimoso Henriques Cunha - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 8. Alexandre Manuel Silva Lobo Cunha - (Agregação / Professor Associado / 60%)
- 9. Alfredo Nuno Damasceno Pinto de Oliveira - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 10. Ana Maria de Melo Sampaio de Freitas - (Doutoramento / Professor-Adjunto / 25%)
- 11. Ana Maria Ferreira Bio - (Doutoramento / Investigador Auxiliar / 100%)
- 12. Ana Maria Monteiro Paiva Coimbra - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 13. Ana Paula de Campos Mucha - (Doutoramento / Investigador Auxiliar / 100%)
- 14. Anake Kijjoa - (Agregação / Professor Catedrático / 60%)
- 15. António de Vilhena Andrade Ferreira Sykes - (Agregação / Não aplicável (bolseiro) / 100%)
- 16. António José Rogeiro Gouveia - (Agregação / Professor Associado / 50%)
- 17. António Manuel Santos Afonso - (Doutoramento / Professor Associado / 70%)
- 18. António Paulo Alves Ferreira de Carvalho - (Doutoramento / Professor Auxiliar / 50%)
- 19. Aschwin Hillebrand Engelen - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 20. Aurélia Maria de Pinho Marques Saraiva - (Agregação / Professor Auxiliar / 50%)
- 21. Carlos Alexandre Sarabando Gravato - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 22. Carlos Gil Ribeiro Martins - (Doutoramento / Outra / 100%)
- 23. Carlos José Correia de Azevedo - (Agregação / Professor Catedrático / 75%)
- 24. Catarina Maria Pinto Mora Pinto de Magalhães - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 25. Channarayapattana Narasimhamurthy Prabhu - (Doutoramento / Investigador Auxiliar / 100%)
- 26. Christophe Haond - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 27. Cláudia Raquel Cêa de Aragão Teixeira - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 28. Cristina Isabel Coelho Dias Lopes - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 29. Cristina Maria Bravo de Faria Cruz - (Doutoramento / Professor Auxiliar / 50%)
- 30. Cristina Marisa Ribeiro de Almeida - (Doutoramento / Investigador Auxiliar / 100%)
- 31. Deborah Mary Power - (Agregação / Professor Catedrático / 50%)
- 32. Dina Cristina Fernandes Rodrigues da Costa Simes - (Doutoramento / Professor Auxiliar / 50%)
- 33. Dr Antje Helga Luise Voelker - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 34. Eduardo Bruno Oliveira Esteves - (Doutoramento / Professor-Adjunto / 50%)
- 35. Eduardo Jorge Sousa Rocha - (Agregação / Professor Associado / 50%)
- 36. Eduardo Nuno Picoto Lopes Barata - (Doutoramento / Professor Auxiliar / 50%)
- 37. Elsa Alexandra Martins Silva Cabrita - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 38. Emidio Ferreira Santos Gomes - (Agregação / Professor Catedrático / 65%)
- 39. Erik-jan Malta - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 40. Fernanda Russell Pinto - (Doutoramento / Professor Associado / 60%)
- 41. Filipe Alexandre Oliveira dos Santos Alberto - (Doutoramento / Investigador Auxiliar / 100%)
- 42. Florbela Maria Benjamim Soares - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 43. Francisco Arenas Parra - (Doutoramento / Investigador Auxiliar / 100%)

- 44. Gareth Anthony Pearson - (Doutoramento / Investigador Auxiliar / 100%)
- 45. Gerhard Michael Weber - (Doutoramento / Professor Auxiliar / 30%)
- 46. Isabel Cristina Guimarães Nogueira - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 47. Isabel Cristina Oliveira Gonzalez Cunha - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 48. Isabel Gonçalves de Barbosa Araújo - (Doutoramento / Ciência 2007 / 100%)
- 49. Isabel Maria Trigueiros Sousa Pinto Machado - (Doutoramento / Professor Auxiliar / 50%)
- 50. Isabel Martins Gil - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 51. Joana Fernandes da Fonseca da Costa Martins Osswald - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 52. Joana Ferreira Marques Ferreira Cardoso - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 53. João Carlos dos Reis Cardoso - (Doutoramento / Investigador Auxiliar / 100%)
- 54. João Carlos Serafim Varela - (Doutoramento / Professor Auxiliar / 50%)
- 55. João José Oliveira Dias Coimbra - (Agregação / Professor Catedrático / 75%)
- 56. João Miguel Sousa da Silva - (Doutoramento / Investigador Auxiliar / 100%)
- 57. João Paulo de Sousa Cabral - (Doutoramento / Professor Associado / 35%)
- 58. Jonathan Mark Wilson - (Doutoramento / Investigador Auxiliar / 100%)
- 59. Jorge Afonso Martins da Palma - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 60. Jorge Guimaraes da Costa Eiras - (Agregação / Professor Catedrático / 50%)
- 61. Jorge Manuel dos Santos Goncalves - (Doutoramento / Investigador Auxiliar / 100%)
- 62. Jorge Pereira Machado - (Doutoramento / Professor Associado / 60%)
- 63. Jorge Proença Dias - (Doutoramento / Investigador Auxiliar / 100%)
- 64. José Carlos Caetano Xavier - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 65. Jose Carlos Fernandes Antunes - (Doutoramento / Professor Auxiliar / 50%)
- 66. José Fernando Magalhães Gonçalves - (Doutoramento / Professor Auxiliar / 60%)
- 67. José Pedro de Andrade e Silva Andrade - (Agregação / Professor Catedrático / 50%)
- 68. Juan Fuentes Diaz - (Doutoramento / Investigador Auxiliar / 100%)
- 69. Justyna Kopecka-Pilarczyk - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 70. Karim Erzini - (Agregação / Professor Associado / 50%)
- 71. Laura Maria Simões Coutinho Guimarães - (Doutoramento / Investigador Auxiliar / 100%)
- 72. Laurence Alexandrine Mathilde Deloffre - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 73. Lúcia de Abreu - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 74. Lúcia Maria das Candeias Guilhermino - (Agregação / Professor Catedrático / 50%)
- 75. Luis Eugenio Castanheira Da Conceicao - (Doutoramento / Investigador Auxiliar / 100%)
- 76. Luís Filipe Costa Castro - (Doutoramento / Investigador Auxiliar / 100%)
- 77. Luis Filipe Pereira de Oliva Teles - (Doutoramento / Professor Auxiliar / 50%)
- 78. Luis Manuel Zambujal Chicharo - (Agregação / Professor Associado / 45%)
- 79. Luísa Maria Pinheiro Valente - (Doutoramento / Professor Associado / 75%)
- 80. Machiel Simon Bos - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 81. Manuel Aureliano Pereira Martins Alves - (Doutoramento / Professor Associado / 50%)
- 82. Margarida de Lurdes de Jesus Bastos Cristo - (Doutoramento / Professor Auxiliar / 50%)
- 83. Margarita Guennadievna Evtyugina - (Doutoramento / Outra / 100%)
- 84. Maria Alexandra Anica Teodosio Chicharo - (Agregação / Professor Associado / 50%)
- 85. Maria Antonia Santos Mendes Salgado - (Doutoramento / Professor Auxiliar / 50%)
- 86. Maria Armanda Reis Henriques - (Agregação / Professor Catedrático / 50%)
- 87. María Asunción Lago Lestón - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 88. Maria Begona Redruello Trelles - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 89. Maria Clara Semedo da Silva Costa - (Doutoramento / Professor Auxiliar / 50%)
- 90. Maria del Mar Huertas Pau - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 91. Maria do Rosário Fidalgo Martins - (Doutoramento / Professor-Adjunto / 40%)
- 92. Maria Dulce da Mota Antunes de Oliveira Estêvão - (Doutoramento / Professor-Adjunto / 25%)



- 93. Maria Elena Varela Alvarez - (Doutoramento / Não aplicável (bolseiro) / 95%)
- 94. Maria Emília Carvalho Salgueiro - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 95. Maria Ester Tavares Alvares Serrao - (Agregação / Professor Auxiliar / 50%)
- 96. Maria Helena Tabuaço Rêgo Martins Peres - (Doutoramento / Investigador Auxiliar / 100%)
- 97. Maria João Faria Leite Dias dos Santos - (Doutoramento / Professor Auxiliar / 50%)
- 98. Maria Joao Tome Costa Sousa Rocha - (Doutoramento / Professor Associado / 40%)
- 99. Maria Laura Braga Ribeiro - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 100. Maria Leonor Fidalgo - (Agregação / Professor Associado / 50%)
- 101. Maria Leonor Nunes Ribeiro Cruzeiro - (Doutoramento / Professor Associado / 50%)
- 102. Maria Leonor Quintais Cancela Fonseca - (Agregação / Professor Catedrático / 50%)
- 103. Maria Luisa Machado Cerqueira Bastos - (Doutoramento / Investigador Principal / 50%)
- 104. Maria Lurdes Santos Cristiano - (Agregação / Professor Associado / 50%)
- 105. Maria Margarida M Castro - (Agregação / Professor Associado / 50%)
- 106. Maria Natividade Ribeiro Vieira - (Agregação / Professor Auxiliar / 50%)
- 107. Maria Teresa Calvinho Cerveira Borges - (Doutoramento / Professor Auxiliar / 50%)
- 108. Maria Teresa Dinis - (Agregação / Professor Catedrático / 50%)
- 109. Maria Teresa Martins Borges - (Doutoramento / Professor Auxiliar / 40%)
- 110. Maria Teresa Sa Dias de Vasconcelos - (Agregação / Professor Catedrático / 50%)
- 111. Marta Sofia Sa Ferreira - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 112. Miguel Alberto Fernandes Machado e Santos - (Doutoramento / Investigador Auxiliar / 100%)
- 113. Nadège Richard - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 114. Natércia Maria da Silva Conceição - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 115. Nuna Cláudia Peixoto de Araújo - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 116. Nuno Miguel Pinto de Sousa Monteiro - (Doutoramento / Professor Auxiliar / 30%)
- 117. Onno Everhardus Diekmann - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 118. Patrícia Isabel Silvestre Pinto - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 119. Paulo Jorge Travessa Gavaia - (Doutoramento / Investigador Auxiliar / 100%)
- 120. Paulo José de Azevedo Pinto Rema - (Doutoramento / Professor Auxiliar / 50%)
- 121. Paulo Jose Talhadas Santos - (Doutoramento / Professor Auxiliar / 50%)
- 122. Paulo Manuel Rodrigues Martins da Costa - (Doutoramento / Professor Auxiliar / 50%)
- 123. Paulo Manuel Rodrigues Vaz-Pires - (Doutoramento / Professor Associado / 50%)
- 124. Paulo Renato Enes Baganha Baptista - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 125. Pedro Miguel Guerreiro Costa Guerreiro - (Doutoramento / Investigador Auxiliar / 100%)
- 126. Pedro Miguel Leal Rodrigues - (Doutoramento / Professor Auxiliar / 50%)
- 127. Pedro Miguel Lopes Gonçalves - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 128. Peter Colin Hubbard - (Doutoramento / Investigador Auxiliar / 100%)
- 129. Radhouane Ben Hamadou - (Doutoramento / Investigador Auxiliar / 100%)
- 130. Rawiwan Watanadilok - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 131. Rita Alexandra Duarte Borges - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 132. Rita Castilho - (Doutoramento / Professor Auxiliar / 50%)
- 133. Rodrigo Ozorio - (Doutoramento / Investigador Auxiliar / 100%)
- 134. Rogério Alves Ferreira Monteiro - (Agregação / Professor Catedrático / 40%)
- 135. Rui Manuel do Amaral Branco de Oliveira Quartau - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 136. Rui Miguel Andrade Caldeira - (Doutoramento / Ciência 2007 / 100%)
- 137. Rui Miguel Fonseca Neves de Sá - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 138. Rui Orlando Pimenta Santos - (Agregação / Professor Associado / 50%)
- 139. Rui Pedro Goncalves Pereira - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 140. Rute Andreia Rodrigues da Fonseca - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 141. Sandra Cristina da Costa e Silva Ramos - (Doutoramento / Não aplicável (bolseiro) / 100%)

- 142. Sara Maria Mira da Silva - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 143. Sergio Reis Cunha - (Doutoramento / Professor Auxiliar / 30%)
- 144. Sofia Jacinto Morais - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 145. Stefano Vaselli - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 146. Susana Maria Pereira da Costa Moreira - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 147. Susana Rodrigues Pereira - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 148. Teodor Ludmilov Stoichev - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 149. Teresa Isabel Mendonca Modesto - (Doutoramento / Professor Auxiliar / 50%)
- 150. Teresa Paula Martins Tiago - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 151. Vanessa Schein - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 152. Vincent Laizé - (Doutoramento / Investigador Auxiliar / 100%)
- 153. Vitor Hugo da Silva Magalhães - (Doutoramento / Não aplicável (bolseiro) / 100%)
- 154. Vitor Manuel Oliveira Vasconcelos - (Agregação / Professor Associado / 70%)

## 2.2 Researchers w/ PhD. (not eligible)

- 1. Abdelaziz Fassouane - (Doutoramento / Não aplicável (bolseiro) / n/r%)
- 2. Ana Dulce de Ascensao Almeida Correia - (Doutoramento / Não aplicável (bolseiro) / n/r%)
- 3. Ana Maria Pimentel Rodrigues - (Doutoramento / Professor Auxiliar / n/r%)
- 4. Ana Paula Pereira Paiva - (Doutoramento / Outra / n/r%)
- 5. Antonio Jose Estevas Grande Candeias - (Doutoramento / Outra / n/r%)
- 6. Cecile, Marie-Alexane Perrin - (Doutoramento / Investigador Auxiliar / n/r%)
- 7. Christopher Anthony Cooper - (Doutoramento / Investigador Auxiliar / n/r%)
- 8. Corália Maria Fortuba de Brito Vicente - (Agregação / Professor Catedrático / n/r%)
- 9. Cristina Isabel Ramires Rigaud de Abreu - (Doutoramento / Assistente / n/r%)
- 10. Dr. Roderic William Wilson - (Doutoramento / Professor Associado / n/r%)
- 11. Fatima Filomena Guedes Abrantes - (Doutoramento / Investigador Principal / n/r%)
- 12. Helena Maria Leite Pato Granja - (Agregação / Professor Catedrático / n/r%)
- 13. Idílio Jorge Matias Pereira Pinto - (Doutoramento / Ciência 2007 / n/r%)
- 14. Ivar Ronnestad - (Doutoramento / Professor Associado / n/r%)
- 15. Jaime Rendón von Osten - (Doutoramento / Professor Auxiliar / n/r%)
- 16. José Américo Pereira de Sousa - (Doutoramento / Professor Auxiliar / n/r%)
- 17. José Manuel Cardoso Duarte - (Doutoramento / Outra / n/r%)
- 18. José Manuel Lage Campelo Calheiros - (Agregação / Professor Catedrático / n/r%)
- 19. Josep Rotllant - (Doutoramento / Investigador Auxiliar / n/r%)
- 20. Juan Bosco Ortiz Delgado - (Doutoramento / Não aplicável (bolseiro) / n/r%)
- 21. Laurence Myriam Elandalloussi - (Doutoramento / Não aplicável (bolseiro) / n/r%)
- 22. Manuela Maria Teixeira Basto de Faria Frasco - (Doutoramento / Não aplicável (bolseiro) / n/r%)
- 23. Maria Clara Ramalho Monteiro Pires Basto - (Doutoramento / Professor Auxiliar / n/r%)
- 24. Maria da Conceicao Constantino Fernandes - (Mestrado / Outra / n/r%)
- 25. Maria Leonor Faleiro - (Doutoramento / Outra / n/r%)
- 26. Maria Margarida da Fonseca e Castro Cardoso - (Doutoramento / Professor Auxiliar / n/r%)
- 27. Maria Teresa Garrett Silveirinha Sottomayor Neuparth - (Doutoramento / Outra / n/r%)
- 28. Martin Leslie Saker - (Doutoramento / Investigador Auxiliar / n/r%)
- 29. Mathilakath M. Vijayan - (Agregação / Professor Catedrático / n/r%)
- 30. Olga Maria Oliveira da Silva Lage - (Doutoramento / Professor Auxiliar / n/r%)
- 31. Pedro Alexandre Garcia Range - (Doutoramento / Investigador Auxiliar / n/r%)
- 32. Pedro Manuel Silva Duarte - (Doutoramento / Professor Associado / n/r%)
- 33. Pedro Miguel Alfaia Barcia Re - (Doutoramento / Professor Associado / n/r%)

- 34. Pedro Miguel Coutinho Victorino Borges Moraes - (Doutoramento / Investigador Auxiliar / n/r%)
- 35. Raquel Beatriz Ribeiro de Mesquita - (Doutoramento / Não aplicável (bolseiro) / n/r%)
- 36. Raquel Carmona Fernandez - (Doutoramento / Outra / n/r%)
- 37. Raul Jose Jorge de Barros - (Doutoramento / Outra / n/r%)
- 38. Ricardo Jorge Guerra Calado - (Doutoramento / Não aplicável (bolseiro) / n/r%)
- 39. Rui Manuel Vitor Cortes - (Agregação / Professor Catedrático / n/r%)
- 40. Rute Andreia Rodrigues da Fonseca - (Doutoramento / Não aplicável (bolseiro) / n/r%)
- 41. Sandra Maria Pinto Marques - (Doutoramento / Outra / n/r%)
- 42. Sophie Arnaud-Haond - (Doutoramento / Investigador Auxiliar / n/r%)
- 43. Susana Isabel dos Santos Cabaço - (Doutoramento / Investigador Auxiliar / n/r%)
- 44. Vanesa Robles Rodríguez - (Doutoramento / Investigador Auxiliar / n/r%)
- 45. Vijayakumar Parameswaran - (Doutoramento / Não aplicável (bolseiro) / n/r%)
- 46. William Lawrence Silvert - (Doutoramento / Outra / n/r%)

### 2.3 Researchers w/o PhD.

- 1. Agnès Marhadour - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 2. Albina Dolores Cardoso da Silva Castro Resende - (Mestrado / Assistente / n/r%)
- 3. Alexandra Ernestina Fernandes Barbosa - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 4. Amélia Cláudia Figueiredo Silva - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 5. Ana Alexandra Pedrosa Ramos - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 6. Ana Cláudia Moreira Dias - (Licenciatura / Outra / n/r%)
- 7. Ana Cristina da Silva Gomes - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 8. Ana Cristina Lemos de Matos - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 9. Ana Cristina Silva Rocha - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 10. Ana Filipa dos Santos Gonçalves - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 11. Ana Isabel Delfim dos Santos Alexandre - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 12. Ana Isabel Gaspar Meirinho - (Licenciatura / Outra / n/r%)
- 13. Ana Isabel Santos Couto - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 14. Ana Mafalda Rocha Tavares - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 15. Ana Mafalda Saraiva Baptista - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 16. Ana Margarida Araújo Amaral - (Mestrado / Assistente de Investigação / n/r%)
- 17. Ana Margarida da Silva Faria - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 18. Ana Margarida Pereira - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 19. Ana Margarida Pinto Henrique Machado - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 20. Ana Maria dos Santos Rocha - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 21. Ana Paula Magalhaes Lima - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 22. Ana Rita de Araújo Ribeiro - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 23. Ana Rita Neves Sousa - (Bacharelato / Outra / n/r%)
- 24. Ana Rita Pimenta Falcao Marques - (Licenciatura / Assistente de Investigação / n/r%)
- 25. Ana Sousa Ramos Ramalho Ribeiro - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 26. Anabela Ben-Simon Brito - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 27. André Gonçalo Antunes dos Santos - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 28. André Gonçalo Antunes dos Santos - (Bacharelato / Outra / n/r%)
- 29. André Miguel Moura da Costa e Sousa - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 30. Andreas Guntram Jurgen Schunhoff - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 31. Andreia Cristina Domingues Bringela - (Ensino Secundário / Estagiário de Investigação / n/r%)
- 32. Andreia Filipa Domingues Braga Henriques - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 33. Andreia Monteiro da Cruz Ribeiro Gouveia - (Licenciatura / Não aplicável (bolseiro) / n/r%)



- 34. Andreia Seia Rebotim - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 35. Ângela Alexandra Martinho Ramos - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 36. Bárbara Bilreiro de Mendóça Frazão - (Licenciatura / Outra / n/r%)
- 37. Begona Fernandez Duran - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 38. Begona Fernandez Duran - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 39. Benjamín Costas Refojos - (Mestrado / Assistente de Investigação / n/r%)
- 40. Brigitte Sandra Nunes Simões - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 41. Bruno Emanuel Pereira Louro - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 42. Bruno Graziano da Silva Turini - (Licenciatura / Outra / n/r%)
- 43. Carina Santos da Silva - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 44. Carla Alexandra da Silva Monteiro - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 45. Carla Alexandra Martins Peixe - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 46. Carla Alexandra São Bento Viegas - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 47. Carla Batista de Carvalho Batista Pinto - (PAPCC / Assistente / n/r%)
- 48. Carla Helena Dias Ventura - (Licenciatura / Outra / n/r%)
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- 53. Catarina Fernanda de Carvalho Pinheiro Teixeira - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 54. Catarina Figueiredo da Mota - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 55. Cátia Andreia Lourenço Marques - (Licenciatura / Não aplicável (bolseiro) / n/r%)
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- 58. Célia Teresa Neto dos Santos - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 59. Céline Rodrigues Madeira - (Licenciatura / Não aplicável (bolseiro) / n/r%)
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- 62. Claire Juliana Francisco - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 63. Claudia de Fátima Escórcio Pereira - (Licenciatura / Outra / n/r%)
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- 75. David Maria Aguiar Abecasis - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 76. David Veríssimo Piló - (Licenciatura / Não aplicável (bolseiro) / n/r%)
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- 86. Emmanuelle Billard - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 87. Estibaliz Berecibar - (Mestrado / Não aplicável (bolseiro) / n/r%)
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- 91. Filipa Margarida Barroso Ferreira - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 92. Francisca Isabel Merino Nunes Cabral Cavaleiro - (Licenciatura / Outra / n/r%)
- 93. François Noël Hubert - (Licenciatura / Não aplicável (bolseiro) / n/r%)
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- 100. Helena Rita de Carvalho Ferraz Pedrosa Teodósio - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 101. Helena Sofia Fernandes Teixeira - (Ensino Secundário / Não aplicável (bolseiro) / n/r%)
- 102. Hugo Manuel Silva Ribeiro - (Licenciatura / Não aplicável (bolseiro) / n/r%)
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- 105. Inês Paixão Nunes Figueiredo - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 106. Isa Cristina Teixeira Santos - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 107. Isabel Maria Sena Morgado - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 108. Joana Alexandra Teixeira Rosa - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 109. Joana Catarina Rocha Moreira da Silva - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 110. Joana Costa Vilhena de Bessa Campos - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 111. Joana do Passo Carneiro Azevedo - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 112. Joana Filipa de Sousa Micael Pereira - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 113. Joana Helena Goncalves da Costa Padrao - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 114. Joana Isabel Correia Bondoso - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 115. Joana Isabel Ferreira Marques - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 116. Joana Margarida Guimarães e Silva - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 117. Joana Reis de Almeida - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 118. Joana Ruela Boavida - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 119. Joana Savva Bordalo e Sa - (Licenciatura / Outra / n/r%)
- 120. Joana Silveira Soares - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 121. Joana Soares de Oliveira Martins - (Licenciatura / Outra / n/r%)
- 122. Joana Sousa Rodrigues - (Licenciatura / Outra / n/r%)
- 123. João da Mota e Silva Rodrigues de Carvalho - (Licenciatura / Outra / n/r%)
- 124. João Filipe Pereira Vilares Neves - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 125. João Miranda Neiva - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 126. João Paulo Ramalho Marreiros - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 127. João Paulo Rodrigues Machado - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 128. João Paulo Soares - (Licenciatura / Outra / n/r%)
- 129. João Pedro Gonçalves Cardeira da Silva - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 130. João Soares Carrola - (Mestrado / Assistente / n/r%)
- 131. Joaquim Miguel Neves Ribeiro - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 132. José Carlos de Moraes Martins - (Mestrado / Não aplicável (bolseiro) / n/r%)

- 133. José Miguel Pereira Gomes - (Licenciatura / Outra / n/r%)
- 134. Katia Pinto Pedrosa - (Licenciatura / Outra / n/r%)
- 135. Leonardo Filipe Rodrigues da Mata - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 136. Leonor Isabel Moreira de Araújo - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 137. Licínia Cristina Freitas Gouveia - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 138. Lília Isabel Lameirinhas Cabral - (Ensino Secundário / Estagiário de Investigação / n/r%)
- 139. Liliana Isabel Queirós de Almeida Carvalho - (Mestrado / Outra / n/r%)
- 140. Liliana Isabel Tome Dos Anjos Guerreiro - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 141. Luis Filipe da Cunha Melo Silva Rangel - (Licenciatura / Outra / n/r%)
- 142. Luis Filipe Rodrigues Batista - (Licenciatura / Outra / n/r%)
- 143. Luis Manuel Correia Ferreira Bentes - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 144. Luis Miguel dos Santos Russo Vieira - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 145. Luís Miguel Teodoro Frija - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 146. Mafalda Rangel Malheiro Dias de Oliveira - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 147. Mahaut Diane Marie Stephanie de Labroue de Vareilles Sommieres - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 148. Manuela Fernanda Gomes Moreira da Silva - (Licenciatura / Outra / n/r%)
- 149. Marco António do Nascimento Sequeira de Jesus Campinho - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 150. Marcos Rubal García - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 151. Margarida Dulce da Conceição Aragão Hermida - (Licenciatura / Outra / n/r%)
- 152. Maria Alexandra Santos Martins - (Licenciatura / Outra / n/r%)
- 153. Maria da Nazaré Parada Figueiredo de Sousa Couto - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 154. Maria Esmeralda de Sá Leite Correia da Costa da Fonseca - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 155. Maria Fernanda Ferreira Marques - (Licenciatura / Outra / n/r%)
- 156. Maria Helena Trindade de Abreu - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 157. Maria Inês de Almeida Páscoa - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 158. Maria Isabel da Silva Costa Azevedo - (Mestrado / Outra / n/r%)
- 159. Maria Joao Rodrigues Pereira - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 160. Maria Manuel Valente Correia da Silva Moreira - (Licenciatura / Outra / n/r%)
- 161. Maria Margarida Alves da Silva de Almeida Saavedra - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 162. Maria Margarida Oliveira Maló Machado - (Licenciatura / Assistente de Investigação / n/r%)
- 163. Maria Raquel Assuncao Goncalves Machas - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 164. Maria Regina Oliveira Lopes da Cunha - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 165. Mariana Dias Almeida - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 166. Mariana Fonseca Hinzmann - (Ensino Secundário / Outra / n/r%)
- 167. Marisa Sárria Pereira de Passos - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 168. Marlene Cristina da Silva Antunes - (Licenciatura / Outra / n/r%)
- 169. Marta Catarina Veiga de Faria Rodrigues - (Licenciatura / Assistente de Investigação / n/r%)
- 170. Marta Isabel da Silva Rafael - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 171. Marta Sofia Alves Gomes - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 172. Marta Sofia Mendes Valente Bernardo - (Licenciatura / Assistente de Investigação / n/r%)
- 173. Marta Tibaldo - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 174. Michael Nogueira Viegas - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 175. Miguel Alexandre Medeiros Vidigal Caldeira Pais - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 176. Mirjam Susanne van de Vliet - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 177. Monica Sofia Furtado Martins - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 178. Monya Mendes Costa - (Licenciatura / Não aplicável (bolseiro) / n/r%)

- 179. Nádia Margarida Rosário Silva - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 180. Natalia Paola Moncaut - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 181. Nelson Alexandre Castilho Coelho - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 182. Nikolay Kolmakov - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 183. Nuno Filipe Alexandre Henriques - (Bacharelato / Outra / n/r%)
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- 185. Odete Marinho Gonçalves - (Licenciatura / Não aplicável (bolseiro) / n/r%)
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- 187. Pablo Manent Sintes - (Licenciatura / Investigador Auxiliar / n/r%)
- 188. Patricia Alexandra Cavaleiro Diogo - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 189. Patrick Neil Bowskill Reis dos Santos - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 190. Paula Cristina Paulo Videira da Silva - (Mestrado / Assistente Convitado / n/r%)
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- 192. Paulo Alexandre dos Santos Silva - (Mestrado / Assistente de Investigação / n/r%)
- 193. Paulo César Nunes Pereira do Rêgo - (Licenciatura / Não aplicável (bolseiro) / n/r%)
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- 196. Pedro Alexandre Coelho Borges - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 197. Pedro Alexandrino Silva Ferreira Martins Monteiro - (Mestrado / Não aplicável (bolseiro) / n/r%)
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- 199. Pedro Fernandes Seixas - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 200. Pedro Filipe Duarte Alves da Veiga - (Licenciatura / Não aplicável (bolseiro) / n/r%)
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- 202. Pedro Luís Martins de Castro Pinheiro - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 203. Pedro Miguel Coutinho Victorino Borges Morais - (Doutoramento / Não aplicável (bolseiro) / n/r%)
- 204. Pedro Neves de Carvalho - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 205. Pedro Nuno da Costa Leão - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 206. Pedro Nuno Ribeiro Rodrigues - (Licenciatura / Outra / n/r%)
- 207. Rafael Domingues Evangelista - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 208. Raquel Andreia Pinheiro Vieira - (Licenciatura / Outra / n/r%)
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- 210. Ricardo Bruno de Araújo Severino - (Licenciatura / Outra / n/r%)
- 211. Ricardo do Amaral Ribeiro - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 212. Ricardo Estêvão Oliveira Almeida - (Bacharelato / Outra / n/r%)
- 213. Ricardo Jorge Pereira Córdova Marcos - (PAPCC / Assistente / n/r%)
- 214. Ricardo Manuel Campinho Capela - (Ensino Secundário / Outra / n/r%)
- 215. Ricardo Manuel Rafael Afonso - (Licenciatura / Assistente de Investigação / n/r%)
- 216. Ricardo Mario Bastos Leite - (Mestrado / Assistente de Investigação / n/r%)
- 217. Richard Deurloo - (Mestrado / Não aplicável (bolseiro) / n/r%)
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- 219. Rita Isabel Pontes Barbosa Colen - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 220. Rita Margarida Teixeira Ascenso - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 221. Rita Micaela dos Santos Fernandes de Araujo - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 222. Ronaldo Gomes de Sousa - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 223. Rosa Maria Faustino Brissos - (Ensino Secundário / Outra / n/r%)
- 224. Rui Filipe de Ascensao Almeida - (Licenciatura / Assistente de Investigação / n/r%)
- 225. Rui Jorge Fernandes Miranda Rocha - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 226. Rui Pedro Andrade Coelho - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 227. Rui Pedro Peres dos Santos - (Licenciatura / Não aplicável (bolseiro) / n/r%)



- 228. Rui Seabra Alves Martinho - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 229. Rute Isabel Pinto de Brito - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 230. Rute Sofia Tavares Martins Brazona - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 231. Sacha Nicole Coesel - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 232. Sandra Maria Sengo Mesquita - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 233. Sandra Sofia Ganchas Soares - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 234. Sara Isabel da Silva Pires Marques Barrento - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 235. Sara Judite Lopes Teixeira - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 236. S rgia Catarina de Amorim Costa Dias - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 237. Sofia Alexandra Dias Engrola - (Licenciatura / Assistente de Investiga  o / n/r%)
- 238. Sofia Gabriel Garcia Santos - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 239. Sofia Isabel Franco Cavaco - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 240. Sonia Alexandra da Rocha Dias Gomes - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 241. S nia Isabel Rodrigues Aldeia Sanches Massa - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 242. Sonia Maria De Sousa Olim - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 243. Susana Maria Rocha Pina - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 244. Susana Micaela Machado Ferreira do Vale - (Mestrado / Outra / n/r%)
- 245. T nia Angelina de Sousa Leit o - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 246. T nia Marisa Ramos Pipa - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 247. T nia Raquel Santos Aires - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 248. T nia Vieira Madureira - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 249. Tom  Pereira de Azevedo Santos Silva - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 250. Tom  Pereira de Azevedo Santos Silva - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 251. V nia Cristina Palma Roberto - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 252. Vasco Manuel Nobre de Carvalho da Silva Vieira - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 253. Vera Alexandra Garcia da Fonseca Batista - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 254. Vera L cia Fernandes Rodrigues - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 255. Vitor Manuel Capela Ramos - (Mestrado / Não aplicável (bolseiro) / n/r%)
- 256. Viviana Raquel Vieira da Silva Lopes - (Licenciatura / Não aplicável (bolseiro) / n/r%)
- 257. Wilson Gabriel Poseiro Coutinho Pinto - (Licenciatura / Não aplicável (bolseiro) / n/r%)

### **3. MANAGEMENT & FUNDING**

#### **3.1 Management structure of the LA (2000 ca.)**

CIMAR performs mainly two types of activities: basic (oriented) research and programs involving socio-economic issues. Both require creativity and innovative attitude. To maintain the right environment for the scientific productivity and the high level of the horizontal programs, the managerial structure of CIMAR has to be light and decentralized while other aspects, namely internal/external services, require a more rigid hierarchical organisation (e.g administrative services, international cooperation, chemistry laboratory, IT services, seawater system, maintenance).

Management bodies described in the contract with FCT include:

- the General Assembly (GA) composed by the GAs of both Centres;
- a Board of Directors composed by both Directors (SC) and 2 other elements from the two centres nominated by the SCs. The Direction of the AL is alternate, on a 3-y basis between the two SCs;
- a Scientific Committee composed by the PhD holders;
- an External Advisory Board;
- a Financial Council.

During these first 6 years of CIMAR activity, a number of management initiatives and small modifications were introduced as adaptations to the singular situation of the AL, the two Centres being separated by 600 Km and subject to long periods of financial difficulties due to obstacles on the access of FCT to the EU funds.

In 2006, it was decided to replace the Executive Board created in 2003 by an enlarged Board of Directors, from 3 to 5 elements both in CIIMAR and CCMAR, and to give to their members the responsibility of the horizontal programs.

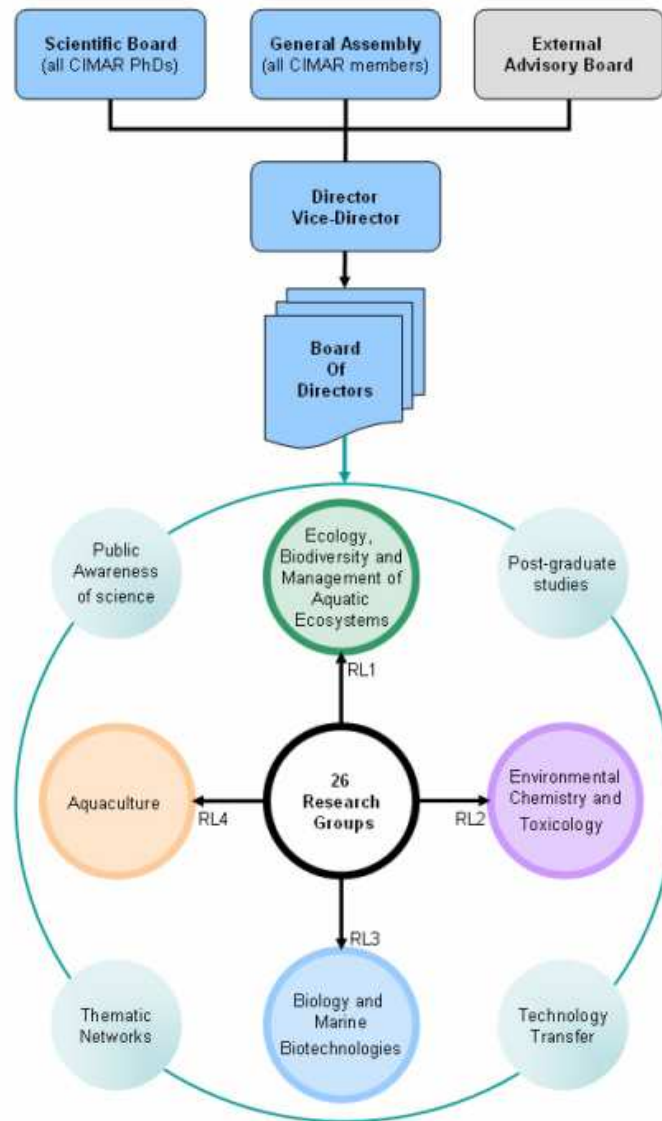
To increase the efficiency of the BD, the representatives of both Centres started to be the members of each BD.

It was also decided to include a representative of IGM/INETI in the BD following the 2003 agreement.

The Annual Meeting is the most important event in the AL life, when members of the SC, PhD and MSc students, guests and members of the Advisory Board meet. This is also the occasion for the BD to discuss with our advisors, formal members of the AL BD or advisors of the individual Centres.

Due to the distance, the BD now meets through teleconference. From the operational point of view, however, the most important are the daily phone calls between the Directors of the two Centres.

### 3.2 Management struture (diagram)



### 3.3 Funding

Origin	2003	2004	2005	2006	2007	Total
LA FCT *	580041	1586158	1536008	1092380	2913911	7708498.00
Units FCT **	0	0	0	0	0	0.00
Project FCT	274687	453275	552605	570952	827775	2679294.00
Other National	132068	159133	108507	197458	240018	837184.00
Other International	370942	681716	704412	659879	1082379	3499328.00
National Industry	77626	233887	110233	140634	258989	821369.00
International Industry	0	0	0	0	0	0.00
<b>Total</b>	1435364	3114169	3011765	2661303	5323072	15545673.00

(\*) Base + Programmatic; (\*\*) Base + Programmatic of the units before the creation of the LA.



## 4. GENERAL INDICATORS

### 4.1 Composition and training

	2003	2004	2005	2006	2007	Total
No. of researchers proposed	11	6	6	5	0	28
No. of researchers hired	8	0	9	4	2	23
<b>Balance</b>	-3	-6	3	-1	2	-5
No. of researchers (FTE) (*)	8	0	9	4	2	
Training Masters (Master thesis completed)	27	24	25	17	49	142
Training PhDs (PhD thesis completed)	10	8	8	23	38	87

(\*) Base + Programmatic; (\*\*) Base + Programmatic of the units before the creation of the LA.

#### 4.2 Researchers hired under LA contract

Name	Start date	End date
Juan Fuentes Diaz	02-01-2003	01-01-2009
Radhouane Ben Hamadou	01-02-2006	31-01-2009
Jorge Proença Dias	01-05-2006	30-04-2009
Gareth Anthony Pearson	01-09-2003	31-08-2009
Jorge Manuel dos Santos Goncalves	01-09-2005	01-09-2008
Vincent Laizé	01-10-2005	01-10-2008
Pedro Miguel Guerreiro Costa Guerreiro	01-07-2007	30-06-2010
Paulo Jorge Travessa Gavaia	01-08-2007	31-07-2010
Miguel Alberto Fernandes Machado e Santos	01-04-2003	03-04-2009
Rodrigo Ozorio	02-06-2003	02-06-2009
Jonathan Mark Wilson	02-01-2003	02-01-2009
Maria Helena Tabuaço Rêgo Martins Peres	01-04-2003	03-04-2009
Ana Maria Ferreira Bio	01-09-2005	01-09-2008
Cristina Marisa Ribeiro de Almeida	01-09-2005	01-09-2008
Martin Leslie Saker	21-06-2005	21-06-2008
Francisco Arenas Parra	21-06-2005	21-06-2008
Luís Filipe Costa Castro	02-12-2005	02-12-2008
Ana Paula de Campos Mucha	02-12-2005	02-12-2008
Laura Maria Simões Coutinho Guimarães	28-12-2005	28-12-2008
Channarayapattana Narasimhamurthy Prabhu	14-07-2006	14-07-2009
Agostinho Antunes Pereira	03-07-2007	02-07-2010
Luis Eugenio Castanheira da Conceicao	01-08-2003	31-07-2009
Peter Colin Hubbard	02-01-2003	01-01-2009
João Miguel Sousa da Silva	01-06-2006	31-05-2009

### 4.3 Technical personal hired under LA contract

Name	Start date	End date
Hugo Gabriel Maia da Silva Santos	01-04-2003	03-04-2009
Pedro Alexandre Pereira dos Reis	20-03-2003	20-03-2009
Pedro Romeu Baixinho Rodrigues	01-09-2003	01-09-2009
Anabela Outeiro Martins	01-04-2003	31-03-2006
Carlos Gil Ribeiro Martins	21-12-2005	21-12-2008
Margarita Guennadievna Evtyugina	02-05-2007	01-05-2010
Andreia Sofia Pires Pinto	01-04-2006	31-03-2009
Beatriz Maria Alvega Cardoso	01-03-2004	28-02-2010
Ana Margarida Araújo Amaral	19-04-2004	18-04-2010
Carla Alexandra São Bento Viegas	01-04-2003	01-04-2005
Marta Sofia Mendes Valente Bernardo	01-10-2005	01-10-2008

### 4.4 Additional comments

#### Technical personal

Although we were allowed to hire 10 technicians in the last years (the contract with FCT only foresees the hiring of 8 technicians) the present number is clearly insufficient for the objectives of CIMAR-LA. Considering the present number of members, the total estimated number of technicians needed to support CIMAR activities and services is 20, not only for laboratory but also for the use of scientific equipments of vessels.

#### Researchers

The prevision for new researchers indicated in section 9 of this report assumes that the individual proposals submitted directly to FCT under Ciência 2007 initiative (4 proposals) will be hired during 2008.

## **5. RESEARCH LINES (THEMATIC AREAS)**

### **RL1**

#### **5a. General description**

##### **1. Designation**

**Ecology, Biodiversity and Management of Aquatic Ecosystems**

##### **2. Principal investigator**

**Karim Erzini**

##### **3. Research area**

Marine Sciences

##### **4. General objectives (1000 ca.)**

The general objectives of this Line are to study selected aspects of marine geology, oceanography, marine biodiversity and ecosystems, integrating research to provide a sound scientific basis for assessment, management and policy related with the marine ecosystems. Within this framework, the objectives in the evaluation period covered the following:

- To promote the geological research of the oceans and coastal regions, the paleoceanography, and the processes related to the occurrence of marine mineral resources.
- To determine the composition, abundance and distribution of the national marine biodiversity;
- To monitor and study coastal and estuarine ecosystem functioning, and long-term changes in marine communities and habitats to determine the impacts of climate change and local human action;
- To study the genetics, population biology, ecology and recruitment of ecologically and commercially important species;
- To determine the impacts of fishing at different levels, from genetics to ecosystems;
- To investigate the physiological adaptations of migratory fishes, to different salinity and hydrostatic pressure;
- To participate in International networks on marine biodiversity;
- To train young scientists and to disseminate scientific knowledge and link with managers and policy makers to contribute to evidence based management and sustainability.

## 5. Major achievements (2000 ca.)

- Significant advances in biogeography and marine reproductive ecology, including identification of population structure and barriers to gene flow in several fish, algae and seagrass species, the discovery of independent colonization events in endemic gastropods, and the development of novel molecular and statistical methods for clonal population genetics.
- Important contributions to the knowledge of the marine biodiversity and its distribution and abundance , producing 4 new checklists of species from different areas along the coast of Portugal, and including many new records. The information collected allowed analysis of changes during the last 40 years and detection of invasive species.
- Development of ecological and ecohydrologic models that allow the evaluation of human impacts on ecosystems, in particular estuarine and coastal.
- Scientific contributions for the assessment and management of human impacts on protected coastal systems such as the North and southwest coast of Portugal and the Ria Formosa lagoon, and for management of commercially exploited species.
- Monitoring of the Minho, Lima and Douro estuaries, the Ria Formosa lagoon and the Litoral Norte site.
- Initiation of a new and innovative approach aimed at understanding the effects of endocrine disrupting chemicals under complex mixtures.
- Development of a multiparametric monitoring system for the quality and control assessment of oil pollution in estuarine and marine areas.
- Development of a new laboratory (Multispecies Marine Biomonitor) for recording the behavioural responses of fish and mussel to oil.
- Construction of a reference frame of the main dynamic aspects of the northwest Iberian region.
- Development of a new method to analyze temporal correlated time-series, such as GPS data, that is one order of magnitude faster than those which were available before.
- Launch of the National network for Marine and Coastal biodiversity to integrate data and research on marine biodiversity at National level.
- Participation in EU networks including NoE Marbef and Marine Genomics and ENCORA: coastal network, and the international Program Census of Marine Life. Participation in the Erasmus Mundus MSc in Marine Biodiversity and Conservation. Contribution to the Editorial Board of the new Journal of Marine Biodiversity.

## 5b. Research groups

Principle Investigator	Name of the Research Groups
Maria Ester Tavares Alvares Serrao	Biogeographical Ecology and Evolution (BEE)
Luis Manuel Zambujal Chicharo	Ecology and Restoration of Estuarine and Coastal Habitats (ECOREACH)
Rui Orlando Pimenta Santos	Marine Plant Ecology Research Group (ALGAE)
José Pedro de Andrade e Silva Andrade	Fisheries Biology and Hydroecology Research Group (FBHRG)
Karim Erzini	Fisheries, Biodiversity and Conservation
Adriano Agostinho Donas Bôto Bordalo e Sá	Hydrobiology
João José Oliveira Dias Coimbra	Ecophysiology
Maria Armada Reis Henriques	Environmental Toxicology
Lúcia Maria das Candeias Guilhermino	Ecotoxicology (ECOTOX)
Isabel Maria Trigueiros Sousa Pinto Machado	Biodiversity of Aquatic Ecosystems
Maria Luisa Machado Cerqueira Bastos	Oceanic and Coastal Dynamics
João José Oliveira Dias Coimbra	Geology and Paleo-oceanography

## 5c. Research lines output

- 1. Collaborative publications in peer review journals** (2000 ca.) (Include only if more than one Research Groups of the LA is involved up to max of 10. Give title and full citation in original language)

1. Abrantes, F, Gil, I, Lopes, C, Castro, M. 2005. Quantitative diatom analyses - A faster cleaning procedure. Deep-Sea Res. Part I – Oceanographic Research Papers 52: 189-198. IF=1.795, n°C=5

2. Alexandre, A, Cabaço, S, Santos, R, Serrão, EA. 2006. Timing and success of reproductive stages in the seagrass *Zostera noltii*. *Aquatic Botany* 85: 219-223. IF=1.497, n°C=0
3. Alexandre, A, Santos, R, Serrão, E. 2005. Effects of clam harvesting on sexual reproduction of the seagrass *Zostera noltii*. *Marine Ecology Progress Series* 298: 115-122. IF=2.546, n°C=4
4. Castro, M, Santos, MM, Monteiro, NM, Vieira, N. 2004. Measuring lysosomal stability as an effective tool for marine coastal environmental monitoring. *Marine Environmental Research* 58: 741-745. IF=2.055, n°C=10.
5. Gómez-Ariza, JL, Santos, MM, Morales, E, Giráldez, I, Sánchez-Rodas, D, Vieira, N, Kemp, J.F., (...), Ten-Hallers-Tjabbes, CC. 2006. Organotin contamination in the Atlantic Ocean off the Iberian Peninsula in relation to shipping. *Chemosphere* 64: 1100-1108. IF=2.442, n°C=8
6. Santos, MM, Castro, LFC, Vieira, MN, Micael, J, Morabito, R, Massanisso, P, Reis-Henriques, MA. 2005. New insights into the mechanism of imposex induction in the dogwhelk *Nucella lapillus*. *Comparative Biochemistry and Physiology.C - Toxicol. and Pharmacol.* 141: 101-109. IF=1.456, n°C=19.
7. Santos, MM, Vieira, N, Reis-Henriques, MA., et al. 2004. Imposex and butyltin contamination off the Oporto Coast (NW Portugal): A possible effect of the discharge of dredged material . *Environmental International* 30: 793-798. IF=2.335, n°C=10
8. Sousa, R, Antunes, C, Guilhermino, L. 2007. Species composition and monthly variation of the Molluscan fauna in the freshwater subtidal area of the River Minho estuary. *Estuarine, Coastal & Shelf Science* 75: 90-100. IF=1.799, n°C=2
9. Sousa, R., Freire, R, Rufino, M, Mendez, J, Gaspar, M, Antunes, C, Guilhermino, L. 2007. Genetic and shell morphological variability of the invasive bivalve *Corbicula fluminea* (Müller, 1774) in two Portuguese estuaries. *Estuarine, Coastal & Shelf Science* 74: 166-174. IF=1.799, n°C=3.
10. Valente, LMP, Gouveia, A, Rema, P, Matos, J, Gomes, EF, Pinto, IS. 2006. Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture* 252: 85-91. IF 2006=2.081, n°C=3

**2. Collaborative other publications** (2000 ca.) (Include only if more than one group is involved and only include here Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**3. Master and PhD thesis completed** (3000 ca.) (Co-supervision or clearly multidisciplinary projects are allowed here)

## MSc THESIS

Alexandre, A. 2004. Impacto da actividade de marisqueio na floração e esforço reprodutivo de *Zostera noltii* Hornemann na Ria Formosa. Mestrado em Estudos Marinhos e Costeiros (EMAC). Co-supervision: E Serrão (BEE), R Santos (ALGAE).

Lopo, M. 2004. Avaliação do impacto da extracção de inertes em populações naturais de peixes. MSc on Sea Sciences and Marine Resources of ICBAS. Supervisor: L Guilhermino (Ecotoxicology Lab). Co-Supervisor: C Antunes (Ecophysiology Lab).

## **PhD THESIS**

Araújo, R . Population dynamics, geographical distribution and genetic diversity of macroalgal species at their southern limits of distribution. Univ. Algarve. Co-supervision: E Serrão (CCMAR), I Sousa-Pinto (CIIMAR).

Sousa, R. 2008. Factors contributing to the invasive success of *Corbicula fluminea* (Müller, 1774) PhD in Aquatic Sciences, ICBAS, University of Porto. Supervisor: L Guilhermino (Ecotoxicology Lab), Co supervisor: C Antunes (Ecophysiology Lab).

## **5d. Future research**

- 1. Other information** (4000 ca.) (Include only if more than one group is involved. Patents/prototypes, organization of conferences, industry contract research)

In 2007 members of the CCMAR (Fisheries, biodiversity and conservation group) and CIIMAR participated in an environmental impact assessment of dredging along the Portuguese continental coast entirely financed by the industry (Amb & Veritas/Marinertes). The objective of the project was to evaluate the impacts of dredging on the marine life, namely the benthic organisms.

In 2007 Cimar LA signed a memorandum of understanding with CESAM-LA to launch the “Rede de Biodiversidade Marinha e Costeira” that integrates mainly the groups from the research line: Ecology, Biodiversity and Management of Aquatic Ecosystems, together with the groups that will integrate CIMAR from the ISPA. This network is now being extended to all major marine biology groups in Portugal and had until now five plenary meetings and an intense electronic exchanges to discuss objectives and action plans, and to plan research and monitoring at a National level. Research grants were already presented for funding and the first two monitoring groups did already a first monitoring exercise that will be followed by workshops to agree on fine details of methodology. An exchange of researchers between the groups and smaller meetings with more focused objectives have been happening and are foreseen in order to ensure a tighter collaboration between the different groups and some uniformity of field research methods for monitoring and joint experiments.

The main objectives of the network are the development of joint research and a research agenda for marine biodiversity, the formation of a strong group to enter European and International projects, and the development of a database and a long-term data collection at national level to be able to examine patterns and dynamics of biodiversity and ecosystem change over wider temporal and spatial scales. We aim also at working with the managers and policy makers from local and regional to the National level, targeting more the Environmental and Fisheries Ministries, but also to provide data and knowledge on how to develop sustainable use of other under/misused marine resources, including algae. We aim also at doing research that will support policy and the private initiative for the exploitation of marine genetic resources. We believe that these objectives will be attained better at a national level and that our groups can have a bigger impact if we join forces. More information on <http://www.cimar.org/divmar/>.



In 2007, a protocol was signed with the Moroccan National Fisheries Institute (Institut National de Recherche Halieutique – INRH). The protocol envisages collaboration in research projects, training of scientists and organization of workshops. An example of collaboration in progress is the participation of INRH and CIMAR in the Ocean Tracking Network (<http://oceantrackingnetwork.org/>). The objective of this global project, involving institutions from around the world, is to deploy arrays of acoustic receivers in key locations around the world, that will be used to track marine organisms implanted with acoustic tags (“pingers”). CIMAR and INRH are involved in the deployment of the Strait of Gibraltar array. This array, will provide important information on the movements between the Atlantic and the Mediterranean of fish (especially large pelagic), marine mammals and turtles.

## **2. Future plans (2000 ca.)**

Continue integration of the groups, and with the groups from ISPA, continuing the steady work that has already produced joint publications and the Network described above. We will also expect to integrate 16 new researchers that already joined Cimar in 2008 or will be hired at the beginning of 2009. These will reinforce existing lines of research, e.g. phylogenetics, ecosystem functioning, ecological modeling and will open new research lines as biodiversity valuation and oceanography that will greatly improve our collective research capacity and the response to societal needs. So within the continuation of the general objectives defined above we will:

- increase our research on phylogeography, edge population genetics, genetics of metapopulation systems, coastal and deep sea connectivity, invasive species, and genome-wide patterns of stress-responsive gene expression
- use otolith finger printing as biological tracers of fish migration and for stock: discrimination, and of transcriptomics to study physiological adaptation in fishes
- enhance research on sea horses and pipefish, including larval production, status of wild populations, variation in population abundance, and use of re-stocking for conservation
- monitor erosion phenomena, calibrate coastline evolution models, improve geoid description in coastal areas, produce detailed sea surface, topography maps, and of sea level variations, and monitor coastal biodiversity and habitats to study the effects of global environmental changes and local anthropogenic effects, and to assess and monitor the Ecological Quality of coastal and marine ecosystems within the Water Framework Directive
- develop and implement hydrodynamic and biogeochemical models, and decision support systems (DSS), to integrate knowledge about coastal ecosystems and to be used as management tools
- continue to work on restoration of biodiversity and habitats and evaluation of the benefits of a marine protected areas, including valuation of biodiversity and ecosystem based management
- assessment of toxicity effects of wastewaters using different test organisms and development and application of eco-technology techniques for improvement of water quality
- continue to isolate compounds extracted from marine organisms and assess in vitro anticancer and antifungal activities

- to build an international reference group in the marine geology and paleo-oceanography areas, with the paleo team following up the study of the five most important coastal upwelling regions.

## **RL2**

### **5a. General description**

#### **1. Designation**

#### **Environmental Chemistry and Toxicology**

#### **2. Principal investigator**

**Maria Teresa Sa Dias de Vasconcelos**

#### **3. Research area**

Environment

#### **4. General objectives (1000 ca.)**

The aims were to foster cooperation and chase high quality research, whereas developing innovative solutions and applications, in three strategic areas:

Chemical speciation of xenobiotics and natural species, in order to get answers for the following questions: (1) how does the chemical composition of a medium influence the responses of an organism in contact with it; and (2) how do organisms affect the chemical composition of the surrounding medium?

Evolution and functioning of ecosystems under anthropogenic and natural stress, in order to better understand the effects of pollutants (e.g. endocrine disrupting compounds, pharmaceuticals, persistent organic and organo-metallic pollutants, PAHs and heavy metals) on aquatic organisms and ecosystems. For this, we aimed (and managed) to use integrated strategies, including methods and tools from ecotoxicology, ecology, genetics and chemistry. We also aimed to develop novel holistic approaches, namely those based on the use of batteries of sensitive biological tests for diagnosis, monitoring, modelling and remediation of pollution.

Ecotoxicology of cyanobacteria and of other potentially toxic organisms (in marine, brackish and freshwaters), and characterization of toxins, using molecular and computational genomics / proteomics.

#### **5. Major achievements (2000 ca.)**

#### **TECHNOLOGICAL**

- Validation of biomarkers from 21 native species to use in biomonitoring studies in temperate and tropical ecosystems.
- Development of multiparametric monitoring systems for assessment of pollution and its effects on estuarine and marine ecosystems.
- Implementation of new analytical methods for identification/determination of chemical and biological molecules (toxicants or produced in response to toxicity).

- Optimization of biological remediation solutions to solve problems of pollution in confined areas.

## SCIENTIFIC

The achievements were materialized through publication of more than 200 papers in peer reviewed international and national journals. From these, we emphasize work done on:

- Identification of mechanisms of action of several environmental contaminants on aquatic organisms and those involved in genetic resistance to chemical contamination, including molecular mechanisms underlying imposex in gastropods, xenoestrogens in vitellogenin induction in fish, biochemical and populational mechanisms of genetic resistance in cladocerans and marine copepods, mechanisms of cholinesterase ChE inhibition by metals, among others.
- Portuguese marine Cyanobacteria that produce apoptotic activity in neuroblastoma cells and that are good candidates for pharmaceutical research. The use of ELISA and of multiplex PCR proved that many dietary supplements are contaminated with the hepatotoxic microcystin. Genomic studies across organisms contributed to understand the functional changes of various gene/proteins involved in the metabolism of drugs and toxins.
- Contamination levels in NW Portuguese coast in Minho, Lima, Cávado and Douro Rivers estuaries and Aveiro lagoon, using integrated approaches including water quality variables, chemical analysis (metals, PAHs, pesticides), natural toxins, bioaccumulation factors, biomarkers and condition indexes.

## SUPPORT FOR ADVANCED TRAINING

Know-how and technical facilities have been used to support a large number of undergraduate, Master and PhD students during the preparation of their theses.

## INTERNATIONAL RECOGNITION BY PUBLISHING HOUSES AND EDITORS

Several members of ETC Line are members of Editorial Board of International Journals, and one of them is Editor-in-chief. Line members have been regular reviewers of manuscripts submitted to ISI indexed journals.

## 5b. Research groups

Principle Investigator	Name of the Research Groups
Manuel Aureliano Pereira Martins Alves	BioVanadium Research Group
Adriano Agostinho Donas Bôto Bordalo e Sá	Hydrobiology
Maria Armanda Reis Henriques	Environmental Toxicology
Maria Teresa Sa Dias de Vasconcelos	Chemical Speciation and Bioavailability (CS&B)
Lúcia Maria das Candeias Guilhermino	Ecotoxicology (ECOTOX)
Vitor Manuel Oliveira Vasconcelos	Ecotoxicology, Genomics and Evolution (LEGE)
Isabel Maria Trigueiros Sousa Pinto Machado	Biodiversity of Aquatic Ecosystems
Eduardo Jorge Sousa Rocha	Cellular and Molecular Studies (LECEM)

## 5c. Research lines output

These sections allow you to provide a description of the Research line, as well as its productivity, collaborative results and future plans.

### 1. Collaborative publications in peer review journals (2000 ca.) (Include only if more than one Research Groups of the LA is involved up to max of 10. Give title and full citation in original language)

1. Castro, LFC, Lima, D, Machado, A, Melo, C, Hiromori, Y, Nishikawa, J, Nakanishi, T, Reis-Henriques, MA, Santos, MM. 2007. Imposex induction is mediated through the Retinoid X Receptor signaling pathway in the neogastropod *Nucella lapillus*. *Aquatic Toxicology* 85: 57-66. IF 2007=2.975, n°C=6.
2. Nogueira, ICG, Lobo-da-Cunha, A, Vasconcelos, VM. 2006. Effects of cyanobacteria ingestion on *Daphnia magna* midgut and associated diverticula epithelium. *Aquatic Toxicology* 80: 194-203. IF 2006/07=2.964/2.975, n°C=0.

3. Almeida, CMR, Mucha, AP, Bordalo, AA, Vasconcelos, MTSD. 2004. Influence of the sea rush *Juncus maritimus* on metal concentration and speciation in estuarine sediment colonized by the plant. *Environmental Science and Technology* 38: 3112-3118. IF 2004/07= 3.557/4.363, n°C=14.
4. Mucha, AP, Bordalo, AA, Vasconcelos, MTSD. 2003. Macrobenthic community in the Douro Estuary: Relations with trace metals and natural sediment characteristics, *Environmental Pollution* 121:169-180. IF 2003/07=2.002/3.135, n°C=38.
5. Correia, AD, Freitas, S, Scholze, M, Gonçalves, JF, Booij, P, Lamoree, MH, Mañanós E, Reis-Henriques, MA. 2007. Mixtures of estrogenic chemicals enhance vitellogenic response in sea bass. *Environmental Health Perspectives* 115: 115-121. IF 2007=5.636, n°C=0.
6. Cunha, I, Garcia, LM, Guilhermino, L. 2005. Sea-urchin (*Paracentrotus lividus*) glutathione S-transferases and cholinesterase activities as biomarkers of environmental contamination. *Journal of Environmental Monitoring* 7: 288-294. IF 2005/07=1.578/1.833, n°C=10.
7. Santos, MM, Vieira, N, Reis-Henriques, MA, Santos, AM, Gómez-Ariza, JL, Giraldez I, Ten Hatters-Tjabbes, CC. 2004. Imposed and butyltin contamination off the Oporto coast: a possible effect of dredged material. *Environment International* 30: 793-798. IF 2004/07=2.335/2.797, n°C=10.
8. Sousa, R, Freire, R, Rufino, M, Méndez, J, Gaspar, M, Antunes, C, Guilhermino, L. 2007. Genetic and shell morphological variability of the invasive bivalve *Corbicula fluminea* (Müller, 1774) in two Portuguese estuaries. *Estuarine, Coastal and Shelf Science* 74: 166-174. IF 2007=1.799, n°C=1.
9. Castro, E, Santos, MM, Monteiro, N, Vieira, N. 2004. Measuring lysosomal stability as an effective tool for marine coastal environmental monitoring. *Marine Environmental Research* 58: 741-745. IF 2004/07=2.055/1.930, n°C=10.

**2. Collaborative other publications** (2000 ca.) (Include only if more than one group is involved and only include here Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Sousa, R, Antunes, C, Guilhermino, L. 2007. Bivalves de água doce presentes no troço internacional do rio Minho. *Actas do III Simpósio Ibérico sobre a bacia hidrográfica do rio Minho*, V. N. de Cerveira, Junho de 2006.
2. Sousa, R, Dias, S, Antunes, C, Guilhermino, L. 2007. Fauna exótica presente no estuário do rio Minho: distribuição e impactos potenciais. *Actas do III Simpósio Ibérico sobre a bacia hidrográfica do rio Minho*, V. N. de Cerveira, Junho de 2006.

**3. Master and PhD thesis completed** (3000 ca.) (Co-supervision or clearly multidisciplinary projects are allowed here)

In addition to Master and PhD Theses completed with collaboration with groups external to CIMAR LA, including international cooperation (see details provided by each group / lab), collaborative Theses existed both involving groups within the Research Line and involving other groups of the LA, ie, primarily belonging to other Research Lines. We list below the Theses from collaborations “within Line” and “between Lines”. Moreover,

there are currently ongoing collaborative Theses projects (see details provided by each group / lab).

## **MSc THESIS**

### **WITHIN THE RESEARCH LINECS&B and LEGE**

Leão, PNC. 2006. Interactions between marine cyanobacteria and trace metals in seawater. MSc in Hidrobiologia by Faculdade de Ciências da Universidade do Porto, Porto, Portugal. Supervisor: MTSD Vasconcelos (CS&B Lab); Co-supervisor: V Vasconcelos (LEGE Lab).

### **WITHIN THE LA**

#### **CS&B and Ecophysiology**

Lyra, FJPCF. 2007. Caracterização da comunidade de macroinvertebrados bentónicos do estuário do Rio Minho – sua relação com a distribuição de poluentes no sedimento. MSc in Ecologia Aplicada by Faculdade de Ciências da Universidade do Porto, Porto, Portugal. Supervisor: C Antunes (Ecophysiology Lab), Co-supervisor: MCP Basto (CS&B Lab).

Reis, PA. 2007. Estudo Geoquímico de Metais em Sedimentos do Sapal dos Rios Minho e Coura. MSc in Ciências do Mar e Recursos Marinhos by Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Portugal. Supervisor: CMR Almeida (CS&B), Co-supervisor: C Antunes (Ecophysiology).

#### **Ecotoxicology and Ecophysiology**

Lopo, M. 2004. Avaliação do impacto da extracção de inertes em populações naturais de peixes. MSc on Sea Sciences and Marine Resources of ICBAS. Supervisor: L Guilhermino (Ecotoxicology Lab). Co-Supervisor: C Antunes (Ecophysiology Lab).

## **PhD THESIS**

### **WITHIN THE LA**

#### **Environmental Toxicology and Ecophysiology**

Damasceno-Oliveira, A. 2007. Regulação hormonal da reprodução na solha (*Platyichthys flesus* L.); relação com o ciclo migratório. PhD, ICBAS, University of Porto. Supervisor: J Coimbra (Ecophysiology); Co-supervisor: MA Reis-Henriques (Environmental Toxicology).

Coimbra, AM. 2006. Efeito de poluentes organoclorados no comportamento reprodutivo e no desenvolvimento larvar da tilápia nilótica, *Oreochromis niloticus*. PhD, ICBAS, University of Porto. Supervisor: MA Reis-Henriques (Environmental Toxicology); Co-supervisor: J Coimbra (Ecophysiology).



## 5d. Future research

1. **Other information** (4000 ca.) (Include only if more than one group is involved. Patents/prototypes, organization of conferences, industry contract research)

As to industry contract research, the aim is to continue to provide advanced services to industry, not only via the current established contracts (eg, detection of natural toxins in consuming water – see more information in the details provided by the Groups / Labs) but also promoting new ones. As to the Organization of Conferences, in the area of the Line we can affirm that the pole holds all the know-how, networking, and other bases to attract and house internationally established meetings and even launching new important series of conferences; one is already programmed:

1st Iberian Conference on Cyanotoxins. Porto, FCUP, 6 and 7 of July 2009. Organized by CIMAR (Group LEGE, with collaboration of LE. Chairman of the conference: V. Vasconcelos).

2. **Future plans** (2000 ca.)

### RESEARCH TARGETS

Current projects and pending proposals assure funding to sustain high quality research.

Integrating methods at different levels of biological organization, i.e., from changes in gene expression up to alterations on population dynamics and ecosystem functioning will be one of the main goals. The effects of chemical pollution and natural stressors on the cited functioning will be investigated using both in situ and laboratory experiments. We want to implement risk assessment, namely targeting both natural toxins and man made xenobiotics (e.g. oil and chemical spills, PAH, pharmaceuticals), using multidisciplinary approaches in marine, brackish water and freshwater environments. Risk assessment will also integrate the potential impact of global changes on the effects of pollution in temperate and tropical ecosystems.

The variation of the levels of contamination will continue to be quantified with the collaboration of groups from other research lines within CIMAR, articulating such information with the development of early warning methods for the detection and quantification of cyanotoxins and of chemical contaminants, using molecular and ecotoxicological approaches.

Efforts at cell level will continue, targeting effects and mechanisms. The effects of vanadium compounds with insulin mimetic properties in calcium regulation and also the mechanisms of cell death induced by vanadate oligomers will be studied. In the field of endocrine disruption we will continue the research on chemical mixtures with additive and antagonistic effects. Evolutionary genomics/proteomics studies of protein-coding genes operating in detoxification, development, immune system and genetic disease onset will expand previous approaches. Also, we aim to better clarify the roles of “phases I, II and III” of detoxification, namely the transport proteins involved in the elimination of xenobiotics and natural toxins.

### COOPERATIVE NETWORKING

Members of this research line will continue to participate in several networks and in the organization of some national and international conferences. Cooperation with national and local authorities e.g. the National Environmental Agency, Water Institute and Health Authorities will support policy developments and hazard assessment of chemicals. The international cooperation

with countries from Central and South America, North Africa and Asian will assess the effects of the contamination, climate changes, biodiversity conservation and sustainable development.

## **RL3**

### **5a. General description**

#### **1. Designation**

#### **Biology and Marine Biotechnologies**

#### **2. Principal investigator**

**Deborah Mary Power**

#### **3. Research area**

Biological Sciences

#### **4. General objectives (1000 ca.)**

Extend fundamental knowledge about marine organisms at a molecular to whole organism level and across the evolutionary scale. Establish how natural and man made environmental challenges impact organismal function. Promote the identification of potential “spin-offs” arising from basic biology and stimulate pilot studies and links with industry. Develop “near market” solutions, such as, genotyping for aquaculture species, molecular bar codes, diagnostic tests in pathology and biomarkers to monitor environmental pollution and as performance indicators. Promote the identification and exploration of marine organisms of direct commercial interest or which produce biologically active compounds or biomaterials, with the long term aim of patenting and exploitation. Actively encourage and educate stake holders (scientists, policy makers, industry, teachers and the public) about the diversity, complexity and importance of marine ecosystem. Promote partnership within CIMAR and work towards internationalization. Help increasing public awareness about marine science.

#### **5. Major achievements (2000 ca.)**

A diversity of subjects is encompassed within the research line in which scientific and technical achievements have been made (see group reports). Herein, only major achievements, those perceived as having a higher impact are indicated.

- Promotion of and participation in sequencing and characterization of the genome of important Southern European aquaculture species sea bream and sea bass. Participation in phase one trials of phenotype selection based on molecular genetics.
- Scientific and technical contribution to understanding shell, skeletal and liver development, structure and function, and factors contributing to development of malformations in aquaculture.
- Characterization and monitoring programs for pathogens, principally parasites, of marine vertebrates (fish) and invertebrates (clams) in European waters.
- Gain of mechanistic insights of metabolic pathways via experimentation, including toxicological assays, whereas contributing to monitoring programs with biomarkers targeting marine invertebrates and vertebrates.

- Development and application of low/non invasive techniques for monitoring fish physiology and chemoreception and identification of novel food attractants with potential application in fisheries, conservation biology and aquaculture.
- Advances in the understanding of reproductive endocrinology for important commercial species.
- Establishment of a range of new technologies and capacities by groups in the research line to enhance R&D capabilities.
- Partner in Marine Genomics Europe, funded under the FP6 and coordinated in CIMAR by AVM Canario, promoting a European Network of Excellence in the field, with strong international partnership, and facilitating Group cooperation at CIMAR.
- Over 25 PhDs and 30 MScs have developed capacities in the research line, many of whom have integrated in other research groups in Portugal and Europe. Additional training and technology transfer activities include specialist workshops and training and technology transfer to developing countries.
- Interaction with the general public and schools has been actively pursued through open lectures, open days, short-study visits by students and resource development.
- Development of a hyperbaric system for the long-term study and conservation of intermediate- and deep-depth aquatic organisms. CIMAR/INEGI. Portuguese invention patent n°103774 and International patent pending. Seafloor: Portuguese trade mark n° 423824.

## 5b. Research groups

Principle Investigator	Name of the Research Groups
Adelino Vicente Mendonca Canario	Comparative Molecular Endocrinology (CME)
João Carlos Serafim Varela	Biotechnology and Molecular Biology of Microalgae (BMBM)
Maria Leonor Nunes Ribeiro Cruzeiro	Biophysics
Maria Leonor Quintais Cancela Fonseca	Molecular Biology of Marine Organisms (MBMO)
João José Oliveira Dias Coimbra	Ecophysiology
Eduardo Jorge Sousa Rocha	Cellular and Molecular Studies (LECEM)

## 5c. Research lines output

### 1. Collaborative publications in peer review journals (2000 ca.) (Include only if more than one Research Groups of the LA is involved up to max of 10. Give title and full citation in original language)

1. Gonçalves, AF, Castro, LF, Pereira, CM, Coimbra, J, Wilson, JM. 2007. Is there a compromise between nutrient uptake and gas exchange in the gut of *Misgurnus anguillicaudatus*, an intestinal air breathing fish? *Comparative Biochemistry and Physiology D* 2: 345-355. IF 2007=1.391, n°C=0.
2. Castro, LFC, Lima, D, Machado, A, Melo, C, Hiromori, Y, Nishikawa, J, Nakanishi, T, Reis-Henriques, MA, Santos, MM. 2007. Imposex induction is mediated through the Retinoid X Receptor signalling pathway in the neogastropod *Nucella lapillus*. *Aquatic Toxicology* 85: 57-66. IF 2007=2.975, n°C=6.
3. Damasceno-Oliveira, A, Fernandez-Duran, B, Goncalves J, Serrao P, Soares-da-Silva P, Reis-Henriques MA, Coimbra, J. 2007. Effects of cyclic hydrostatic pressure on the brain biogenic amines concentrations in the flounder, *Platichthys flesus*. *General and Comparative Endocrinology* 153: 385–389. IF=2.562, n°C=0.
4. Sousa, R, Antunes, C, Guilhermino, L. 2007 . Species composition and monthly variation of the Molluscan fauna in the freshwater subtidal area of the River Minho estuary. *Estuarine, Coastal and Shelf Science* 75: 90-100 IF 2007 1.799, n°C= 2.
5. Correia, AD, Freitas, S, Scholze, M, Gonçalves, JF, Booj, P, Lamoree, MH, Mañanos, E, Reis-Henriques, MA. 2007. Mixtures of estrogenic chemicals enhance vitellogenic response in sea bass. *Environmental Health Perspectives* 115: 115-121. IF 2007=5.636, n°C=0.
6. Russell-Pinto, F, Gonçalves, JF, Bowers, E. 2006. Digenean Larvae Parasitizing *Cerastoderma edule* (Bivalvia) and *Nassarius reticulatus* (Gastropoda) From Ria de Aveiro, Portugal. *Journal of Parasitology* 92: 319-332. IF 2006/2007=1.300/1.129, n°C=2.
7. Figueiredo-Fernandes, AM, Fontáinhas-Fernandes, A, Monteiro, RAF, Reis-Henriques, MA, Rocha, E. 2006. Temperature and gender influences on the hepatic stroma (and associated pancreatic acini) of Nile tilapia, *Oreochromis niloticus* (Teleostei, Cichlidae): A stereological analysis by light microscopy. *Journal of Morphology* 267: 221-230. IF 2006/2007=1.553/1.621, n°C=3.
8. Castro, LFC, Santos, MM, Reis-Henriques, MA. 2005. The genomic environment around the Aromatase gene: evolutionary insights. *BMC Evolutionary Biology* 5: 43. IF 2005/2007=4.447/4.091, n°C=4.
9. Santos, MM, Castro, LFC, Vieira, MN, Micael, J, Morabito, R, Massanisso, P, Reis-Henriques, MA. 2005. New insights into the mechanism of imposex induction in the dogwhelk *Nucella lapillus*. *Comparative Biochemistry and Physiology C* 141: 101-109. IF 2005/2007=1.456/2.345, n°C=20.

- 2. Collaborative other publications** (2000 ca.) (Include only if more than one group is involved and only include here Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Pittman, K, Rønnestad, I, Gavaia, PJ, Cancela, ML, Guerreiro, P, Ribeiro, L, Aragão, C, Hamre, K, Moren, M, Yúfera, M, Conceição, L. 2007. FISH LARVAL RESEARCH: A tool for sustainable food production and understanding environmental impacts on developing organisms. *Aquaculture Europe* 32(4): 5-10 (Outcomes of the LARVAR 06 - Workshop in Fish Larval Research held at the CCMAR in 2006).

- 3. Master and PhD thesis completed** (3000 ca.) (Co-supervision or clearly multidisciplinary projects are allowed here)

## **MSc THESIS**

Lopo, MP. 2004. Avaliação do impacto da extracção de inertes em populações naturais de peixes. MSc. CIIMAR, Marine Science and Resources. Supervisor: L Guilhermino (Ecotoxicology Lab), Co supervisor: C Antunes (Ecophysiology Lab)

## **PhD THESIS**

Damasceno-Oliveira, A. 2007. Regulação hormonal da reprodução na solha (*Platyichthys flesus* L.); relação com o ciclo migratório. PhD in Aquatic Sciences, ICBAS, University of Porto. Supervisor: J Coimbra (Ecophysiology Lab), Co supervisor: MA Reis-Henriques (Env. Toxicology Lab).

Coimbra, AM. 2006. Efeito de poluentes organoclorados no comportamento reprodutivo e no desenvolvimento larvar da tilápia nilótica, *Oreochromis niloticus*. PhD in Aquatic Sciences, ICBAS, University of Porto. Supervisor: MA Reis-Henriques (Env. Toxicology Lab), Co supervisor: JCoimbra (Ecophysiology Lab).

Moncaut, N. 2006. Isolation and functional characterization of gonadotrophin releasing hormone receptors. ICBAS, University of Porto. Supervisor: A Canário (CME Lab), Co supervisor: J Coimbra (Ecophysiology Lab).

## **5d. Future research**

- 1. Other information** (4000 ca.) (Include only if more that one group is involved. Patents/prototypes, organization of conferences, industry contract research)

### **PATENTS/PROTOTYPES:**

In the wake of the patented Hyperbaric system for the long-term study and conservation of intermediate- and deep-depth aquatic organisms (Sistema hiperbárico para o estudo e conservação por longos períodos de organismos aquáticos de média/grande profundidade). CIMAR/INEGI. Portuguese invention patent nº103774 and International patent pending. Seafloor: Portuguese trade mark nº 423824. Other developments are expected for the near future.

## **2. Future plans (2000 ca.)**

### **CONSOLIDATION & IMPACT**

All the research groups involved in the present research line will naturally aim to consolidate / increase their impact on their existing areas of expertise and capacities by seeking funding and when appropriate establishing complimentary collaboration. Capacities and resources developed in the preceding 5 years will be exploited in order to increase impact in the respective research areas. Current and pending funding from projects support the aims.

### **STRATEGIC GOALS**

Build / integrate a task force and develop a strategy to meet the challenge of determining the impact of environmental change and anthropocentric factors on marine organisms.

Implement and gain National recognition of monitoring programs in the area of toxicology and disease of Marine organisms and strengthen links with European collaborators / networks in this area.

Take steps to structure the research line and identify common and specific research goals and priorities and develop a cohesive plan to stimulate excellence in basic and applied research.

Identify emerging research challenges and set-up mechanisms to ensure rapid response to such challenges and also facilitate the adoption of new research lines through the integration of research expertise in the area.

Stimulate discussion about the new paradigm in Education and develop joint resources for training excellence in the area of marine biology and biotechnology.

Strengthen existing National and International collaboration and seek to develop new collaborations in key research initiatives to permit transfer of new expertise and heighten the profile of the research line.

Aim to strengthen the link to regional and national needs by stimulating exchange of information and increase the visibility of Marine Biology and Biotechnology by representation of its importance to Industry and society.



## **RL4**

### **5a. General description**

#### **1. Designation**

#### **Aquaculture**

#### **2. Principal investigator**

**Maria Teresa Dinis**

#### **3. Research area**

Marine Sciences

#### **4. General objectives (1000 ca.)**

The objective of the Aquaculture Research Line is to promote the generation of high quality scientific knowledge, through basic and applied research, to tackle some of the sustainability challenges facing the aquaculture industry.

Several strategic research areas were defined and have been clearly consolidated within the reporting period:

1. Biologic assessment of the Cultivation Potential of New Aquaculture Species, for the: Establishment of sustainable culture practices to facilitate the industrialization process in areas of reproduction, farming and husbandry practices of selected new species.
2. Fish Nutrition, which fostered: a) a better understanding of the nutritional modulation of intermediary metabolism, stress resistance and skeletal abnormalities in fish; b) estimation of nutritional requirements and optimal formulations for enhanced performance, improved quality of fish and lower environmental impact.
3. Research on Fish Immunology & Health dealt with: a) a better understanding of host/pathogens interaction mechanisms; b) the establishment of immunostimulation strategies to enhance stress resistance and welfare of farmed fish.

#### **5. Major achievements (2000 ca.)**

#### **INSTITUTIONAL**

The collaboration links between the several research groups of the Aquaculture Research Line has grown in terms of the rational implementation of experimental/analytical capacities and scientific and technical expertise, resulting in a progressive rise on shared publications. In 2003-2007, 5 collaborative projects (4 National and 1 European) were established between the groups, generating funds of about 1.13 Million Euros. Additionally, 3 joint PhD projects are currently underway. The distinctiveness and complementary expertise of each group has been capitalized.

#### **SOCIETAL**

The major achievement of the Aquaculture Research Line is its contribution through basic knowledge and practical collaboration with the industry to the establishment of intensive and semi-intensive farming systems for new species in the Mediterranean area (namely Senegalese sole, blackspot seabream and white bream).

## SCIENTIFIC

### Cultivation Potential of New Aquaculture Species:

- The reproduction under captivity of Senegalese sole and Dusky grouper has been successfully established.
- Weaning protocols have been optimized for sole larvae.
- The potential of polyculture of sole with gilthead seabream has been demonstrated.

### Fish Nutrition:

- Nutritional requirements and optimal practical formulations were established for Senegalese sole, blackspot seabream and white bream.
- Several pathways on the nutritional modulation of intermediary metabolism in marine fish have been elucidated.
- Tracer studies techniques were developed for direct estimation of feed intake, and protein and lipid metabolism in fish larvae.
- Some mechanisms underlying the dietary modulation of bone protein expression in marine fish larvae have been elucidated.
- The establishment of fish as a functional food, through a dietary supplementation of conjugated linoleic acid, has been assessed in European seabass and rainbow trout.
- Progress was achieved in the development of plant protein-rich diets as a tool to promote the sustainable growth of aquaculture.

### Fish Immunology & Health:

- Immunostimulation and vaccination strategies have been successfully established for European seabass, sole and turbot.

## 5b. Research groups

Principle Investigator	Name of the Research Groups
Maria Teresa Dinis	Aquaculture Research Group (Aquagroup)
Luísa Maria Pinheiro Valente	Nutrition, Growth and Quality of Fish (LANUCE)
Aires Manuel Pereira Oliveira Teles	Fish Nutrition

## 5c. Research lines output

These sections allow you to provide a description of the Research line, as well as its productivity, collaborative results and future plans.

- 1. Collaborative publications in peer review journals** (2000 ca.) (Include only if more than one Research Groups of the LA is involved up to max of 10. Give title and full citation in original language)

With groups associated to the Aquaculture Research Line:

1. Aragão, C, Conceição, LEC, Martins, D, Rønnestad, I, Gomes, E, Dinis, MT. 2004. A balanced dietary amino acid profile improves amino acid retention in post-larval Senegalese sole (*Solea senegalensis*). *Aquaculture* 233: 293-304.
2. Imsland, AK, Foss, A, Conceição, LEC, Dinis, MT, Delbare, D, Schram, E, Kamstra, A, Rema, P, White, P. 2003. A review of the culture potential of *Solea solea* and *S. senegalensis*. *Reviews in Fish Biology and Fisheries* 13: 379-407.
3. Dias, J, Rueda-Jasso, R, Panserat, S, Conceição, LEC, Gomes, E, Dinis, MT. 2004. Effect of dietary carbohydrate to lipid ratios on growth, lipid deposition and metabolic hepatic enzymes in juvenile Senegal sole (*Solea senegalensis*). *Aquaculture Research* 35: 1122-1130.
4. Figueiredo-Silva, A, Rocha, E, Dias, J, Silva, P, Rema, P, Gomes, E, Valente, LMP. 2005. Partial replacement of fish oil by soybean oil on lipid distribution and liver histology in European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) juveniles. *Aquaculture Nutrition* 11: 147-155.
5. Ozório, ROA, Valente, LMP, Pousão-Ferreira, P, Oliva-Teles, A. 2006. Growth performance and body composition of white seabream (*Diplodus sargus*) juveniles fed diets with different protein and lipid levels. *Aquaculture Research* 37: 255-263.

6. Alves Martins, D, Gomes, E, Rema, P, Dias, J, Ozório, ROA, Valente, LMP. 2006. Growth, digestibility and nutrient utilization of rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax*) juveniles fed different dietary soybean oil levels. *Aquaculture International* 14: 285-295.

With other groups of CIMAR LA:

1. Sarmiento, A, Guilhermino, L, Afonso, A. 2005. Mercury chloride effects on the function and cellular integrity of sea bass (*Dicentrarchus labrax*) head kidney macrophages. *Fish and Shellfish Immunology*. 17: 489-498.

2. Engrola, S, Conceição, LEC, Gavaia, PJ, Cancela, ML, Dinis, MT. 2005. Effects of pre-weaning feeding frequency on growth, survival, and deformation of Senegalese sole, *Solea senegalensis* (Kaup, 1858). *The Israeli Journal of Aquaculture - Bamidgeh* 57: 10-18.

3. Silva, P, Andrade, CAP, Timóteo, VMFA, Rocha, E, Valente, LMP, 2006. Dietary protein, growth, nutrient utilization and body composition of juvenile blackspot seabream, *Pagellus bogaraveo* (Brunnich). *Aquaculture Research* 38: 1007-1014.

**2. Collaborative other publications (2000 ca.)** (Include only if more than one group is involved and only include here Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

Shared publications of the groups associated to the Aquaculture Research Line of CIMAR LA:

1. Conceição, LEC, Pinto, W, Costas, B, Bruwier, S, Aragão, C. 2006. Stressful husbandry conditions and amino acids requirements in Senegalese sole. COST 867 Workshop on Welfare of Fish in European Aquaculture, 9-11 October, Arcachon, France.

2. Silva, JMG, Conceição, LEC, Espe, M, Valente, LMP. 2006. Lysine requirements in diets for juveniles Senegalese sole (*Solea senegalensis*). Book of abstracts of the XII ISFNF, 28 May-1 June, Biarritz, France.

3. Aragão, C, Martins, D, Rønnestad, I, Yúfera, M, Gomes, E, Dinis, MT, Conceição, LEC. 2004. Diets balanced in amino acids improve nitrogen utilisation in fish larvae. Book of abstracts of the XI ISFNF, 2-7 May, Phuket, Thailand.

4. Ozório, R, Gouveia, N, Andrade, CAP, Conceição, LEC, Peleteiro, T, Gomes, EFS, Valente, LMP. 2004. Performance of blackspot seabream (*Pagellus bogaraveo*, Brunnich 1768) fed on different protein/lipid ratios. Book of abstracts of the XI ISFNF, 2-7 May, Phuket, Thailand.

5. Ozório, ROA, Conceição, LEC, Andrade, C, Timóteo, VMFA, Valente, LMP. 2006. Restricting feeding reduces body fat in Blackspot seabream (*Pagellus bogaraveo*). Book of abstracts of the XII ISFNF, 28 May-1 June, Biarritz, France.

6. Silva, JMG, Conceição, LEC, Costas, B, Dias, J, Espe, M, Valente, LMP. 2007. Evaluation of a plant protein diet to be used in dose-response trials for Senegalese sole juveniles (*Solea senegalensis*). Book of abstracts of the EAS Aquaculture Europe 07, 24-27 October, Istanbul, Turkey.

7. Ozório, ROA, Andrade, C, Conceição, LEC, Timóteo, VMFA, Valente, LMP. 2007. Growth response, body composition and energy expenditure in Blackspot seabream *Pagellus bogaraveo* growing from 65 to 175 grams body weight. Book of abstracts of the EAS Aquaculture Europe 07, 24-27 October, Istanbul, Turkey.

Shared publications with other groups of CIMAR LA:

1. Rodrigues, PML, Cordeiro, O, Silva, TS, Conceição, LEC. 2007. Skeletal development analysis by two-dimensional electrophoresis (2-DE) in white bream (*Diplodus sargus*), fed with different diets. *Journal of Bone and Mineral Research* 22 (1), T210- S282.

### **3. Master and PhD thesis completed (3000 ca.) (Co-supervision or clearly multidisciplinary projects are allowed here)**

The groups associated to the Aquaculture Research Line of CIMAR LA have supervised and co-supervised several collaborative master and PhD Thesis.

#### **MSc THESIS**

Moreira, S. 2004. Variability of myostatin genes in rainbow trout strains exhibiting distinct hyperplastic growth. Master in Animal Production, University of Trás-os-Montes and Alto Douro. Supervisor: LMP Valente (U. Porto, CIMAR), Co-supervisor: Pierre-Ives Rescan (SCRIBE-INRA, Rennes, France).

Silva, AC. 2005. Effects of dietary conjugated linoleic acid in rainbow trout juveniles. Master in Aquatic Sciences – Marine Resources, Instituto de Ciências Biomédicas Abel Salazar, University of Porto. Supervisor: LMP Valente (U. Porto, CIMAR), Co-supervisor: P Rema (University of Trás-os-Montes and Alto Douro).

Ramos, MA. 2007. Time course deposition of conjugated linoleic acid in market size rainbow trout (*Oncorhynchus mykiss*) muscle. Master in Animal Production, Faculty of Veterinary Science of the University of Lisboa. Supervisor: LMP Valente (U. Porto, CIMAR), Co-supervisor: P Rema (University of Trás-os-Montes and Alto Douro).

Oliveira, AP. 2007. Validation of the food safety system of Diversumos. Master in Food Technology & Quality, Faculty of Sciences of University Nova de Lisboa, Co-supervisor: JF Santos Oliveira and P Vaz-Pires (CIMAR).

#### **PhD THESIS**

Rueda-Jasso, R. 2003. Interaction between nutrition, oxidative status and condition of *Solea* spp. post-larvae and juveniles. University of Ghent. Supervisors: P Sorgeloos (University of Ghent, Belgium), Supervisor CCMAR: L Conceição.

Morais, S. 2006. Digestive physiology and food intake in marine fish larvae with respect to dietary neutral lipids. University of Algarve. Supervisors CCMAR: MT Dinis and L Conceição, External supervisor: I Rønnestad (University of Bergen, Norway).

Currently, shared co-supervision within the research line groups involves 3 PhD projects.

## 5d. Future research

1. **Other information** (4000 ca.) (Include only if more than one group is involved. Patents/prototypes, organization of conferences, industry contract research)

The strategy of reinforcing the partnership between various research groups of the Aquaculture Research Line, to gain competitiveness in research consortia, continues to prove beneficial. The joint application of the LANUCE and CIIMAR colleagues of the AQUAGROUP was recently financed in a National SME collaborative project “Research and development on the production of sole juveniles: enhanced resistance to pathologies and growth”. Funding by QREN I&DT Co-Promoção (2008-2011).

One additional joint PhD project has just been approved by FCT and will start in October 2008.

### 2. Future plans (2000 ca.)

The aquaculture industry in Portugal is suffering profound transformations. The massive increase in production with off-shore cage producing systems clashes with the necessity to maintain competitiveness and profitability of semi-intensive aquaculture production in earth ponds. Simultaneously, various new species will enter into commercial phase as a strategy to diversify the offer. Consumer awareness on the quality and safety of seafood and the sustainability issues of aquaculture as a food production system is also growing.

These evolving challenges have been identified as research opportunities for CIMAR LA. A consolidated view on the best organizational structure was discussed within CIMAR LA – Aquaculture. It was clear that throughout the consolidation of the various groups, the Fish Nutrition area had gained a central role in their research interests and activities. Such discussions fostered the idea that there were clear benefits, both for individual groups and for CIMAR LA to federate future research efforts on this area under a common umbrella, the designated CIMAR LA – Fish Nutrition Unit. In general terms, its mission is to promote and consolidate the rationale and strategic development of the CIMAR LA expertise and research activities in the area of Fish Nutrition.

From 2008 on, the groups hosted by the CIMAR LA – Fish Nutrition Unit are the current:

- Fish Nutrition - CIIMAR.
- Fish Nutrition, Growth and Quality - CIIMAR.
- Aquaculture Research Group – CCMAR + CIIMAR.

The major competences foreseen for the future CIMAR LA – Fish Nutrition Unit are:

- Assume a clear responsibility on the establishment of CIMAR LA research strategy for aquaculture, and become an indispensable contributor in the elaboration of regional priorities for the sustainable development of marine research.
- Reinforce the joint application of Unit members to research funds, gaining a stronger competitiveness and visibility in both national and international research consortiums.

Given the growing importance of Fish Welfare as a societal issue, this research area will be further consolidated within the Aquaculture Research Line. Focus will be given to the generation of basic knowledge regarding the modulation of stress in fish, aggressive behavior and the evaluation of bioactive compounds for enhanced health status.



## 6. RESEARCH GROUPS

### 1. Research group

Group no.	Principal Investigator	Name of the Research Groups
RG-Norte-Porto-750015-3189	Adelino Vicente Mendonca Canario	Comparative Molecular Endocrinology (CME)
RG-Norte-Porto-750015-3233	Maria Teresa Dinis	Aquaculture Research Group (Aquagroup)
RG-Norte-Porto-750015-3234	João Carlos Serafim Varela	Biotechnology and Molecular Biology of Microalgae (BMBM)
RG-Norte-Porto-750015-3235	Maria Ester Tavares Alvares Serrao	Biogeographical Ecology and Evolution (BEE)
RG-Norte-Porto-750015-3236	Luis Manuel Zambujal Chicharo	Ecology and Restoration of Estuarine and Coastal Habitats (ECOREACH)
RG-Norte-Porto-750015-3237	Rui Orlando Pimenta Santos	Marine Plant Ecology Research Group (ALGAE)
RG-Norte-Porto-750015-3238	Maria Clara Semedo da Silva Costa	Environmental Technologies
RG-Norte-Porto-750015-3239	José Pedro de Andrade e Silva Andrade	Fisheries Biology and Hydroecology Research Group (FBHRG)
RG-Norte-Porto-750015-3240	Maria Leonor Nunes Ribeiro Cruzeiro	Biophysics
RG-Norte-Porto-750015-3241	Maria Lurdes Santos Cristiano	Group of Synthesis and Organic Reactivity
RG-Norte-Porto-750015-3242	Manuel Aureliano Pereira Martins Alves	BioVanadium Research Group
RG-Norte-Porto-750015-3243	Maria Leonor Quintais Cancela Fonseca	Molecular Biology of Marine Organisms (MBMO), now consolidated as group EDGE (Evolution, Development and Gene Expression)
RG-Norte-Porto-750015-3301	Karim Erzini	Fisheries, Biodiversity and Conservation
RG-Norte-Porto-750015-3329	Adriano Agostinho Donas Bôto Bordalo e Sá	Hydrobiology
RG-Norte-Porto-750015-3331	João José Oliveira Dias Coimbra	Ecophysiology

RG-Norte-Porto-750015-3332	Maria Armanda Reis Henriques	Environmental Toxicology
RG-Norte-Porto-750015-3334	Maria Teresa Sá Dias de Vasconcelos	Chemical Speciation and Bioavailability (CS&B)
RG-Norte-Porto-750015-3335	Lúcia Maria das Candeias Guilhermino	Ecotoxicology (ECOTOX)
RG-Norte-Porto-750015-3336	Vitor Manuel Oliveira Vasconcelos	Ecotoxicology, Genomics and Evolution (LEGE)
RG-Norte-Porto-750015-3339	Jorge Guimaraes da Costa Eiras	Pathology
RG-Norte-Porto-750015-3340	Luísa Maria Pinheiro Valente	Nutrition, Growth and Quality of Fish (LANUCE)
RG-Norte-Porto-750015-3341	Aires Manuel Pereira Oliva Teles	Fish Nutrition
RG-Norte-Porto-750015-3342	Isabel Maria Trigueiros Sousa Pinto Machado	Biodiversity of Aquatic Ecosystems
RG-Norte-Porto-750015-3343	Maria Luisa Machado Cerqueira Bastos	Oceanic and Coastal Dynamics
RG-Norte-Porto-750015-3500	João José Oliveira Dias Coimbra	Geology and Paleo-oceanography
RG-Norte-Porto-750015-3512	Eduardo Jorge Sousa Rocha	Cellular and Molecular Studies (LECEM)

## **Comparative Molecular Endocrinology (CME)**

### **6a. Group description**

#### **1. Group name / denomination**

**Comparative Molecular Endocrinology (CME)**

#### **2. Principal investigator**

**Adelino Vicente Mendonca Canario**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Homeostasis, Development, Behaviour, Evolution

#### **5. Funding, source, dates (1000 ca.)**

Average annual CME budget approximately 400.000,00 EUR (50% European).

Source: European Commission

239.832 EUR; Q5RS-CT-2000-31365; 2001-2003

244.286 EUR; Q5RS-CT-2001-01465; 2001-2005

211.906 EUR; Q5RS-CT-2001-0179; 2001-2005

217.536 EUR; Q5RS-CT-2002-01192; 2002-2005

63.493 EUR; FP6 n° 513692; 2004-2008

162.700 EUR; FP6 n° 505403; 2004-2008

28.516 EUR; FP6 n° 022685 SSA; 2006-2007

92.108 EUR; FP6 n° 044481 SSA; 2007-2008

160.400 EUR; FP6 n° 012451; 2005-2009

Source: Fundação para a Ciência e Tecnologia (FCT)

130.000 EUR; Plurianual; 2003-2007

219.470 EUR; PDCTM/P/MAR/15270/1999; 2001-2003

83.000 EUR; POCTI/BSE/38815/2001; 2002-2005

100.000 EUR; POCTI/CVT/38831/2001; 2003-2006

59.706 EUR; POCTI/BSE/45843/2002; 2004-2006

50.000 EUR; POCTI/CVT/48946/2002; 2005-2007

82.500 EUR; POCI/BIA-BDE/55463/2004; 2005-2008

45.000 EUR; POCI/BIA-BCM/55467/2004; 2005-2008

15.300 EUR; POCTI/MAR/58244/2004; 2005-2008

50.000 EUR; POCI/BIA-BCM/60554/200; 2005-2008

74.000 EUR; POCTI/CVT/55683/2004; 2005-2008

83.598 EUR; POCI/CVT/61052/2004; 2005-2008

50.000 EUR; POCTI/CVT/47124/2002; 2005-2008

83.963 EUR; POCI/MAR/61091/2004; 2006-2008

185.091 EUR; PTDC/CVT/66735/2006; 2007-2010

Source: Others

85.000 Euro; several sources and services; 2003-2007

## **6b. Group team**

### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Adelino Vicente Mendonca Canario (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

002. Deborah Mary Power (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

003. Eduardo Nuno Picoto Lopes Barata (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

004. Teresa Isabel Mendonca Modesto (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

005. Maria Dulce da Mota Antunes de Oliveira Estêvão (Cat.: Professor-Adjunto, Gr. Acad.: Doutoramento)

006. João Carlos dos Reis Cardoso (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

007. Peter Colin Hubbard (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

008. Christophe Haond (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

009. Laurence Alexandrine Mathilde Deloffre (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

010. Maria Begona Redruello Trelles (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

011. Maria del Mar Huertas Pau (Cat.: Não aplicável (bolseiro) Gr. Acad.: Doutoramento )

012. Patrícia Isabel Silvestre Pinto (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

013. Vanessa Schein (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

## **2. Other researchers in the group (Include here collaborators with PhD. only)**

001. Josep Rotllant (Cat.: Investigador Auxiliar Gr. Acad.: Doutoramento)

## **3. Other researchers in the group (non PhD.)**

001. Ana Cristina da Silva Gomes (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

002. Ângela Alexandra Martinho Ramos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

003. Bruno Emanuel Pereira Louro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

004. Cristina Maria Simões de Jesus Rocha (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

005. Elsa Maria (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

006. Helena Rita de Carvalho Ferraz Pedrosa Teodósio (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

007. Isabel Maria Sena Morgado (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

008. Liliana Isabel Tome Dos Anjos Guerreiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

009. Marco António do Nascimento Sequeira de Jesus Campinho (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

010. Nádia Margarida Rosário Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

011. Natalia Paola Moncaut (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

012. Nikolay Kolmakov (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

013. Olinda Gomes de Almeida (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

014. Pedro Luís Martins de Castro Pinheiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

015. Rita Alves Costa (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

016. Rute Sofia Tavares Martins Brazona (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

## 6c. Objectives & achievements

### 1. Objectives (2000 ca.)

The main aim of the group is study the role of hormones and their metabolites on physiology and the evolution of the endocrine system, using fish as the principal model together with other key invertebrate and vertebrate models. The research follows four main interrelated themes which have developed from earlier work: 1) Hormonal control of Reproduction and Development; 2) Calcitropic hormones, mineral metabolism and environmental adaptation, 3) Hormones, pheromones and behavior, 4) The impact of Genotype on phenotype. Specific objectives are:

#### Hormonal control of Reproduction and Development

- a) To establish the identity, function and mechanisms of action of key regulatory factors in relation to sex differentiation, puberty and gonad maturation.
- b) To understand the process of metamorphosis and skeletal development in fishes, and the role of thyroid and metabolic hormones.

#### Calcitropic hormones, mineral metabolism and environmental adaptation

- c) To understand the evolution of mineral homeostasis and the role of endocrine regulating factors, their site of action and the molecular mechanisms responsible for their biological actions.
- d) To understand the process of ossification and regeneration in fishes and the role of the extracellular matrix, minerals and endocrine factors.

#### Hormones, pheromones and behavior

- e) To understand the underlying mechanisms regulating the interaction between hormones and behaviour in fishes.
- f) To understand how fish detect biologically important environmental chemical cues - pheromones, food-related odorants and inorganic cations ( $\text{Ca}^{2+}$  and  $\text{Na}^{+}$ ) and how this olfactory input is centrally processed to evoke the appropriate physiological and behavioural responses.

#### The impact of Genotype on phenotype

- g) To apply an integrative and systems approach, from the genome to individuals and populations, to the study of the evolution of endocrine hormones/systems and their impact on phenotype.

### 2. Main achievements (2000 ca.)

#### Scientific discoveries:

- 1) Established that in fishes the parathyroid family is composed of 5 peptides originating from different genes, three of which are present in all vertebrates. The target organs and calcitropic activity of the members of this family has been characterised.

- 2) Identified parathyroid hormone-related protein responsive genes and that this peptide mediates hypercalcemia during vitellogenesis and influences intestinal calcium fluxes, cortisol production in the interrenal and scale metabolism.
- 3) Proposed evolutionary model of family 2 G protein coupled receptors and established that 5 genes encode gonadotrophin releasing hormone receptors in fish.
- 4) Identified novel estrogen signaling molecules in fish testis and their transcriptional response to estrogen receptor agonists and antagonists.
- 5) Discovered a new aquaporin which controls egg hydration and floatability.
- 6) Discovered that chemical signalling of dominance status occurs via controlled urination in tilapia and other freshwater fish
- 7) Established in tilapia that unlike in dyadic interactions, fighting against its own image in a mirror does not modify endogenous androgens.
- 8) Established that androgens control the development of an external gland (anal gland) in male blennies, which produces a species-specific pheromone which attracts spawning females and enhance male reproductive success.
- 9) Established olfactory sensitivity to  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  as a real biological phenomenon in fish possibly related to rapid environmental adjustments.

#### Technology development /transfer:

- 10) Contributed to European aquaculture genetics and breeding through consortia which generated genomics resources for sea bass and sea bream: cDNA and BAC libraries, EST and genome sequences, microarrays, linkage and radiation hybrid maps.
- 11) Produced a dozen fish specific recombinant proteins as well as antisera and immunoassays for newly discovered peptides proteins, including parathyroid hormones and transthyretin.
- 12) Introduced transcriptome analysis technologies: SuperSAGE (I. Matsumura, Iwate Biotechnology Research Center, Japan); cell transfection, RNAi and transgenics (J Rotllant, Baltimore Biotechnology Centre).

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

Between 2003-2006 the CME group published 96 SCI articles, of which 4 reviews: 24 in journals of  $\text{IF} > 3$ ;

1. Barata, EN, Hubbard, PC, Almeida, OG, Miranda, A, Canario, AVM. 2007. Male urine signals social rank in the Mozambique tilapia (*Oreochromis mossambicus*). *Bmc Biology* 5: 54. IF 2007=5.059, n°C=1.



2. Oliveira, RF, Carneiro, LA, Canario, AVM. 2005. No hormonal response in tied fights. *Nature* 437: 207-208. IF 2007=28.751, n°C=12.
3. Redruello, B, Estevao, MD, Rotllant, J, Guerreiro, PM, Anjos, LI, Canario, AVM, Power, DM. 2005. Isolation and characterization of piscine osteonectin and downregulation of its expression by PTH-related protein. *Journal of Bone and Mineral Research* 20: 682-692. IF 2007=6.004, n°C=9.
4. Rotllant, J, Guerreiro, PM, Anjos, L, Redruello, B, Canario, AVM, Power, DM. 2005. Stimulation of cortisol release by the N terminus of teleost parathyroid hormone-related protein in interrenal cells in vitro. *Endocrinology* 146: 71-76. IF 2007=5.045, n°C=14.
5. Fuentes, J, Figueiredo, J, Power, DM, Canario, AVM. 2006. Parathyroid hormone-related protein regulates intestinal calcium transport in sea bream (*Sparus auratus*). *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 291: R1499-R1506. IF 2007=3.661, n°C=6.
6. Canario, AVM, Rotllant, J, Fuentes, J, Guerreiro, PM, Teodosio, HR, Power, DM, Clark, MS. 2006. Novel bioactive parathyroid hormone and related peptides in teleost fish. *Febs Letters* 580: 291-299. IF 2007=3.263, n°C=7.
7. Santos, CRA, Estevao, MD, Fuentes, J, Cardoso, JCR, Fabra, A, Passos, AL, Detmers, FJ, Deen, PMT, Cerda, J, Power, DM. 2004. Isolation of a novel aquaglyceroporin from a marine teleost (*Sparus auratus*): function and tissue distribution. *Journal of Experimental Biology* 207: 1217-1227. IF 2007=2.972, n°C=12.
8. Fabra, M, Raldua, D, Power, DM, Deen, PMT, Cerda, J. 2005. Marine fish egg hydration is aquaporin-mediated. *Science* 307: 545-545. IF 2007=26.372, n°C=15.
9. Cardoso, JCR, Pinto, VC, Vieira, FA, Clark, MS, Power, DM. 2006. Evolution of secretin family GPCR members in the metazoa. *Bmc Evolutionary Biology* 6: 108. IF 2007=4.091, n°C=7.
10. Cavaco, JEB, Santos, CRA, Ingleton, PM, Canario, AVM, Power, DM. 2003. Quantification of prolactin (PRL) and PRL receptor messenger RNA in gilthead Seabream (*Sparus aurata*) after treatment with Estradiol-17 beta. *Biology of Reproduction* 68: 588-594. IF 2007=3.670, n°C=14.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Louro, BEP, Passos, ALS, Power, DM. 2006. Transcriptome Analysis of the gilthead sea bream (*Sparus auratus*) Pituitary gland: type I markers for molecular genetics. *Revista Portuguesa Zootecnia*, XII (II): 91-104.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

Between 2003-2006 the CME group published 19 book chapters/proceedings internationally:

1. Ingleton, PM, Power, DM. 2004. Evolution of Prolactin. In: Luciano, M (ed) Encyclopedia of Endocrine Diseases. New York: Elsevier, pp. 104-110. ISBN 978-0-12-475570-3.
2. Canário, AVM, Fuentes, J, Guerreiro, PM, Power, DM. 2007. Parathyroid Hormone and Related Peptides in Fish: From Sequence to Function. In: Novel Aspects of PTHrP Physiopathology. Luparello, C (Ed.), pp. 27-40. Nova Publishers, ISBN:1-60021-857-1.
- Guerreiro, PM, Fuentes, J. 2007. Control of calcium balance in fish. In: Fish Osmoregulation. B Baldisserotto, JM Mancera, BG Kapoor (Eds.), pp. 427-495, Science Publisher, Inc., USA, ISBN 978-1-57808-447-0/ 2007.
3. Mancera, JM, Fuentes, J. 2006. Osmoregulatory action of hypophyseal hormones in Teleosts. In: Fish Endocrinology. BG Kapoor, G Zaccone, M Reinecke (Eds), pp. 393-417. Science Publishers, Inc. Enfield (NH). USA; Plymouth, U.K. & Oxford & IBH Publishing Co. Pvt. Ltd., ISBN 1-57808-415-6.
4. Redruello, B, Rotllant, J, Guerreiro, PM, Estêvão, D, Anjos, L, Power, DM, Canário, AVM. 2003. Osteonectin from sea bream: phylogeny, ontogeny and hormonal regulation. In: Avances en Endocrinología Comparada, vol. II. JP Castaño, MM Malagon, S Garcia-Navarro (Eds), pp. 125-128. Universidad de Córdoba: Córdoba, España.
5. Rotllant, J, Fernandes, H, Anjos, L, Guerreiro, PM, Redruello, B, Fuentes, J, Canário, AVM, Power, DM. 2003. Studies on truncated forms of the (1-34) N-terminal peptide of parathyroid hormone-related protein defines regions important for activity. In: Avances en Endocrinología Comparada, vol. II. JP Castaño, MM Malagon, S Garcia-Navarro (Eds), pp. 107-112. Uni. Córdoba: Córdoba, España.
6. Guerreiro, PM, Canário, AVM, Fuentes, J, Rotllant, J, Redruello, B, Power, DM, Renfro, JL. 2003. Possible actions of PTHrP on calcium and phosphate transport by winter flounder (*Pseudopleuronectes americanus*) renal proximal tubule cells. . En “Avances en Endocrinología Comparada” vol. II. JP Castaño, MM Malagon, S Garcia-Navarro (eds). pp. 113-117. Universidad de Córdoba: Córdoba, España.
7. Campinho, MA, Silva, N, Sweeney, GE, Power, DM. 2007. Molecular, histological and morphological changes in skin of halibut during metamorphosis. In: Avanços em Endocrinologia Comparativa, Vol III. Canário AVM and Power DM. (Eds), pp. 155-158. Univ. Algarve, Faro.
8. Silva, N, Baptista, VM, Power, DM. 2007. Expression patterns of the main MLC isoforms during halibut (*Hipoglossus hipoglossus* L.) metamorphosis. In: Avanços em Endocrinologia Comparativa. Vol III, Canário AVM and Power DM (Eds), pp. 165-168. Univ. Algarve, Faro.
9. Louro, B, Power, DM. 2007. Identification of polymorphisms in endocrine related transcripts in the sea bream (*Sparus auratus*). In: Avanços em Endocrinologia Comparativa, Vol III. Canário AVM and Power DM (Eds), pp. 187-190. Univ. Algarve, Faro.
10. Cardoso, J, de Vet, E, Elgar, G, Power, DM. 2007. Functional Characterization of PACAP receptors from sea bream, *Sparus auratus*. In: Avanços em Endocrinologia Comparativa, Vol III. Canário AVM and Power DM (Eds), pp. 79-82. Univ. Algarve, Faro.

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Serrano, RM. 2008. Pheromones in the reproduction of *Salaria pavo* and *S. fluviatilis* (Pisces: Blenniidae): a comparative study. University of Évora. Co-supervisors: EN Barata, PC Hubbard.

Brinca, LF. 2007. Characterization of prolactin from *Sparus aurata* L. and the mechanisms controlling its synthesis and secretion. Univ. Algarve, 2007. Supervisor: DM Power.

Campinho, M. 2007. The molecular and endocrine basis of finfish embryo development and metamorphosis. University of Wales Cardiff, UK. Co-supervisors: DM Power and Glen Sweeney (Univ Cardiff).

Cruz, M. 2007. Ciclo Hormonal Reprodutor, Clonagem e Expressão da Enzima P450 Aromatase na Tilápia, *Oreochromis mossambicus* (Teleostei: Cichlidae). University of Algarve. Supervisor: AVM Canário.

Estêvão, MD. 2007. Comparative study of hormonal regulation of calcium in vertebrates. University of Algarve. Supervisor: DM Power.

Ramos, P. 2007. Contribution to the study of thyroid hyperplasia in pleuronectids. Technical Univ. of Lisbon. Co-supervisors: DM Power and Maria da Conceição Peleteiro (Tech. Univ Lisbon).

Morgado, I. 2007. The role of transthyretin in thyroid hormone transport in fishes and the effect of endocrine deregulators in this process. University of Algarve. Supervisor: DM Power.

Moncaut, N. 2006. Isolation and functional characterization of gonadotrophin releasing hormone receptors. ICBAS, University of Porto. Supervisor: AVM Canário.

Pinto, P. 2006. Diversity, expression and mechanism of action of estrogen receptors in seabream, *Sparus aurata*. University of Algarve. Supervisors: AVM Canário and Glen Sweeney (Cardiff University, UK).

Modesto, T. 2003. Hormonal control of the reproductive cycle of the Lusitanian Toadfish, *Halobatrachus didactylus*. University of Algarve. Supervisor: AVM Canário.

##### **MSc THESIS (out of >10)**

Diogo, ML. 2003. Role of the interrenal in temperature-induced sex differentiation in tilapia, *Oreochromis mossambicus*. MSc Aquaculture, University of Algarve. Supervisor: AVM Canário.

Martins, R. 2003. Does the interrenal influence sex differentiation in sea bass, *Dicentrarchus labrax*? MSc Aquaculture, University of Algarve. Supervisor: AVM Canário.

Oléjua, MAP. 2007. Effects of the goitrogenic compounds methimazole, thiourea and propylthiouracil on the fish thyroid axis, in vivo: the sea bream (*Sparus auratus*) model. MSc Marine Biology, University of Algarve. Supervisors: AVM Canário, P Pinto.

## **5. Patents/propotypes (2000 ca.)**

Patent in process of submission in Portugal "Food additives for aquaculture and the aquarium". Based on the identification of a compound released to the water by a prey which is detected by the olfactory system and promotes feeding activity in the predator.

Patent in process of submission in Denmark (collaboration with Dr Adrian Harrison) "Motion detector and ECG for fish monitoring". Based on the development of equipment to test response of fish to compounds added to water.

## **6. Organization of conferences (2000 ca.)**

V National Ethological Meeting. 18th – 19th September 2003, Faro, Portugal. Portuguese Society of Ethology.

Theoretical-practical seminar "A field Guide to GenBank and NCBI Molecular biology resources". In collaboration with the National Centre for Biotechnology Information (USA). Faro (Portugal). April 2004. 35 participants.

"5th Congress of the Iberian Association of Comparative Endocrinology". Faro, 7th-9th September, 2005. 100 Participants. Book of proceedings edited by A.V.M. Canario and D.M. Power.

Co-organizers of 3 Symposia part of the 23rd Conference of European Comparative Endocrinologists, Manchester, UK, 29th August - 3rd September, 2006: "Pheromone and other chemical communication"; "Endocrinology of calcium homeostasis"; "Comparative Thyroid Endocrinology". Corresponding Editorial Material in special issue of vol 52 (2007) of General Comparative Endocrinology (Elsevier).

Workshop of the European seabass genome sequencing consortium, Faro, 21-22 June 2007. The objective was to analyse progress and the next steps of the project.

SAGE (Serial Analysis of Gene Expression) Workshop, directed by Dr. Hideo Matsumura, Iwate Biotechnology Research Centre, Japan, 22nd and 23th June 2007. The objective was to explain and discuss the applications of this technique of global transcriptome analysis.

Workshop "Bioactive Water Borne Chemicals: Pheromones and Welfare Indicators in Fish". Faro, Portugal, 17th - 19th September, 2006. 45 scientists from 10 countries. Special Issue of volume 45 (2008) of journal Behaviour (Brill).

Workshop "Integrated knowledge on functional genomics in sustainable aquaculture", Universidade do Algarve, Faro, Portugal, 27-28 February 2006. 40 scientists from 10 countries. Co-organised with Prof K Sundell (University of Gothenburg, Sweden). Special issue of Reviews in Fisheries Science (Nov 2008)

## **7. Industry contract research (2000 ca.)**

**8. Government/organization contract research** (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

The CME has a strong international position, as indicated by 1) approximately 132 international co-authors from 66 institutions and 23 countries, 2) the number of European projects and networks excellence, they either coordinator or participate in (see funding).

Member of Steering Committee and Coordinator of Fish and Shellfish node of NoE Marine Genomics Europe (MGE). Leading role in post-MGE solution, including approved FP7 I3-infrastructures “Assemble” project (10 Meuro – 8 institutes). FP7 project “Lifecycle” approved (5 Meuro – 14 partners). Active member of seabass (*D. labrax*) and sea bream (*S. aurata*) genome sequencing consortium led by Richard Reinhardt at the Mac Plank Institute-Molecular Genetics (sea bass completed 2x genome coverage; sea bream BAC ends sequenced). Members of COST Actions 867 (Welfare of fish in European aquaculture) and 925 (The importance of prenatal events for postnatal muscle growth in relation to the quality of muscled based foods). Leading role in European Specific Support Actions Aquafunc and Aquagenome. National representative at Scientific Committee for Antarctic Research. Multiple International roles: advising (e.g., EuropoleMer, Genoma Espanã), evaluators (e.g., European Commission, NSF, BBSRC, INRA, Genoscope, Ministry Science Argentina, etc.), journal editors (*Acta Ethologica*, *General Comparative Endocrinology*, *Journal of Pre-Clinical and Clinical Research*) and referees (more than 20 journals). Several bilateral exchange funded agreements with groups in Spain, Argentina, Brazil, Russia and UK (FCT, GRICES and NATO funded). Plenary/state-of-the-art/keynote lectures at 4 international conferences.

Internationalization of training activities through hosting MSc & PhD student exchange (~12) and undergraduate ERASMUS/SOCRATES scheme. Host for several visiting researchers in the context of sabbaticals or short term training visits (Norway, Sweden, Spain, India, USA, UK).

## 6e. Future research

### 1. Objectives (3000 ca.)

One aim for the next 5 years is to exploit new sequencing technologies, analytical capacities, imaging systems and transgenic models to potentiate the current approach by integrating them with physiological, endocrinological, transcriptomics and biochemical studies. Specific objectives are:

- 1) What genes determine sex in fish? The focus is on the identification of early genes of the cascade of sex differentiation using transcriptomic and transgene approaches, with a view of understanding how environmental factors such as temperature can modify sex ratios.
- 2) What signals direct fish to grow or to mature? There are metabolic, behavioural and neuroendocrine components which need to be dissected using global transcriptomics approaches

to help us understand the mechanisms underlying the development of alternative mating tactics in fish and other vertebrates as well as precocious puberty in farmed fish.

3) Develop a model system for characterisation of TH on organogenesis and functional modifications during fish metamorphosis in order to study how asymmetry is generated. Establish the role of TH receptors, deiodinases and TH binding proteins and cell transporter proteins in regulating TH tissue responsiveness during fish metamorphosis.

4) To establish a model for the mechanism of action and physiological roles of the parathyroid (PTH) family of peptides in fish. Identification of signaling pathways, downstream responsive genes in key ion regulatory tissue (bone, intestine and kidney) and upstream biological responses. Physiological techniques, transcriptome and proteome analysis, RNA interference and transgenic approaches will be used.

5) Deorphanisation and functional characterisation of potential calcitonin/calcitonin related peptide and PTH-like proteins and their receptors in invertebrates and vertebrates. The approach includes physiological studies, RNA interference, gene expression and ligand peptide screening.

6) Establish how pheromones regulate reproductive and social interactions in fish by determining the chemical nature of pheromones, site of production and regulation of synthesis and their mode of action. Electrophysiology, behaviour, chemistry and genomics approaches will be used to study this question. Identification of ligands for olfactory receptors, the signaling pathways and mapping of olfactory neurones to olfactory bulb centers are some of the expected outcomes.

7) Identification of odorants present in natural diets of economically important fish, which may be used as attractants in additives to inert-feed for improvement of feed ingestion, growth and feed conversion rates in aquaculture. This work is particularly directed at *Solea senegalensis*, a new species for aquaculture for which feeding and the production of attractive dry diets is a bottleneck.

Existing collaboration will be continued and new collaborations sought to build new expertise and bring new approaches and capacities to the resolution of the objectives identified.

## **2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

Source: European Commission

92.108 EUR; FP6 n° 044481 SSA; 2007-2008

160.400 EUR; FP6 n° 012451; 2005-2009

366.304 EUR; FP7 project n° 222719; 2009-2012

Source: Fundação para a Ciência e Tecnologia (FCT)

20.000 EUR/year; Plurianual

45.000 EUR; POCI/BIA-BCM/55467/2004; 2005-2008

15.300 EUR; POCTI/MAR/58244/2004; 2005-2008



50.000 EUR; POCI/BIA-BCM/60554/200; 2005-2008

74.000 EUR; POCTI/CVT/55683/2004; 2005-2008

83.598 EUR; POCI/CVT/61052/2004; 2005-2008

50.000 EUR; POCTI/CVT/47124/2002; 2005-2008

83.963 EUR; POCI/MAR/61091/2004; 2006-2008

185.091 EUR; PTDC/CVT/66735/2006; 2007-2010

7.200 EUR; PTDC/MAR/72117/2006; 2008-2011

69.592 EUR; PTDC/MAR/69749/2006; 2008-2011

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Fuentes, J, Guerreiro, PM., Modesto, T, Rotllant, J, Canario, AVM, Power, DM. 2007. A PTH/PTHrP receptor antagonist blocks the hypercalcemic response to estradiol-17 beta. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 293, R956-R960.

2. Pinto PIS, Teodosio HR, Galay-Burgos M, Power DM, Sweeney GE, Canario, AVM. 2006. Identification of estrogen-responsive genes in the testis of sea bream (*Sparus auratus*) using suppression subtractive hybridization. Molecular Reproduction and Development 73: 318-329. IF=2.538, n°C=8.

3. Morgado, I, Hamers, T, Van der Ven, L, Power, DM. 2007. Disruption of thyroid hormone binding to sea bream recombinant transthyretin by ioxinyl and polybrominated diphenyl ethers. Chemosphere 69: 155-163. IF=2.739, n°C=0.

4. Velez, Z, Hubbard, PC, Barata, EN, Canario, AVM. 2007. Differential detection of conspecific-derived odorants by the two olfactory epithelia of the Senegalese sole (*Solea senegalensis*). General and Comparative Endocrinology 153: 418-425. IF=2.7562; n°C=2.

5. Kolmakov, NN, Kube, M, Reinhardt, R, Canario, AVM. 2008. Analysis of the goldfish *Carassius auratus* olfactory epithelium transcriptome reveals the presence of numerous non-olfactory GPCR and putative receptors for progestin pheromones. BMC Genomics 9: 429. IF=4.18, n°C=0.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

To consolidate and develop new capacities and to fully exploit discoveries and take advantage of the “omics” revolution needs have been identified:

Personnel



Two full-time technicians: a) histology/immunohistochemistry/in situ hybridization and immunoassays, radioligand and western blotting and b) production and maintenance of transgenic fish lines/storage

#### Enabling technologies

Imaging – a) confocal laser scanning microscope for live cells and fixed tissue (cell culture, immuno and in situ hybridization) b) upgrade of imaging system (to strengthen proteomics, transgenic fish body scan, cell based assays)

#### Biomolecule analysis

a) Workstation for preparative and analytical chemistry/biochemistry to carry out chromatographic analysis (standard pressure, HPLC analysis or FPLC and other types of chromatographic fractionation/purification coupled to detection system, UV, fluorescence, chemiluminescence);

b) Enhance recombinant protein production suite -upgrade incubators, AKTA for protein purification and computer for logging, -80oC for cell line storage;

c) Protein analysis: CD spectrometer and spectrofluorometer

d) Fast LC system (UltraPerformance LC – UPLC, equipped with a 3D diode array and/or mass spectrometer detector) for fast high-resolution separation of compounds in water and body fluids enabling a direct link to electrophysiological recordings (EOG and EEG) in fish olfactory system - increases analysis throughput.

#### Lab management

Electronic lab book (e.g Agilent Kalabie) for storing and sharing of information and quality control.

## **Aquaculture Research Group (Aquagroup)**

### **6a. Group description**

#### **1. Group name / denomination**

**Aquaculture research group (Aquagroup)**

#### **2. Principal investigator**

**Maria Teresa Dinis**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Aquaculture, Larval stages, New species, Nutrition

#### **5. Funding, source, dates (1000 ca.)**

The Aquagroup was involved in 25 research projects; both at National (19) and European level (6). The Aquagroup assumed the role of coordinator in 13 of these projects, a clear sign that the group gathers the managerial competences and scientific expertise to successfully pursue its objectives. Total funds attained 2.44 Million EUR. The large majority of such funds were obtained through research projects (93.7%), while “Plurianual FCT funds” represented 4.8% and contract-research activities 1.5% of the total funds.

Funds attracted through research projects accounted for 2.28 Million EUR. The financing agencies have been diverse, sign of a constant and active search of funding opportunities. Funding on a National level, through competitive calls from FCT (10: 654.734 EUR), Agência da Inovação (2: 23.8297 EUR), DGP-MARE (2: 268.380 EUR) and INTERREG Program (3: 380.000 EUR) accounted for 65% of total project funds. EU funding schemes, FP5 (3: 215.423 EUR), FP6 (1: 312.088 EUR) and CRAFT (2: 215.480 EUR) accounted for 33%. About 20% of the project funds were associated to collaborative research with SME's (EU-CRAFT and Agência da Inovação). In short, the Aquagroup is currently identified as a highly valuable partner in research consortiums at both National and European level.

### **6b. Group Team**

#### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Maria Teresa Dinis (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

002. António Manuel Santos Afonso (Cat.: Professor Associado, Gr. Acad.: Doutoramento)

003. Jorge Proença Dias (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

004. Luis Eugenio Castanheira Da Conceicao (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

005. Cláudia Raquel Cêa de Aragão Teixeira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
006. Elsa Alexandra Martins Silva Cabrita (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
007. Florbela Maria Benjamim Soares (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
008. Maria Laura Braga Ribeiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
009. Nadège Richard (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
010. Sofia Jacinto Morais (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

## **2. Other researchers in the group (Include here collaborators with PhD. only)**

001. Ricardo Jorge Guerra Calado (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

## **3. Other researchers in the group (non PhD.)**

001. Benjamín Costas Refojos (Cat.: Assistente de Investigação, Gr. Acad.: Mestrado)
002. Sofia Alexandra Dias Engrola (Cat.: Assistente de Investigação, Gr. Acad.: Licenciatura)
003. Ana Rita de Araújo Ribeiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
004. Ana Sousa Ramos Ramalho Ribeiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
005. André Gonçalo Antunes dos Santos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
006. Dorinda Marques da Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
007. Elisabete Alexandra Dias de Matos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
008. François Noël Hubert (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
009. Helena Sofia Fernandes Teixeira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Ensino Secundário)
010. Leonor Isabel Moreira de Araújo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
011. Mahaut Diane Marie Stephanie de Labroue de Vareilles Sommieres (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
012. Maria Margarida Alves da Silva de Almeida Saavedra (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
013. Odete Dmingues Cordeiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
014. Patricia Alexandra Cavaleiro Diogo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
015. Rita Isabel Pontes Barbosa Colen (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
016. Rui Jorge Fernandes Miranda Rocha (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

017. Sonia Alexandra da Rocha Dias Gomes (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

018. Tomé Pereira de Azevedo Santos Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

019. Vera Lúcia Fernandes Rodrigues (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

020. Wilson Gabriel Poseiro Coutinho Pinto (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

021. Helena Maria Lopes Damásio Luis (Cat.: Outra, Gr. Acad.: Ensino Secundário)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

- Establishment of successful weaning protocols for marine fish larvae, mainly sole
- Ontogeny of larval digestive functionality and control in relation to feeding
- Estimation of nutritional requirements, in particular of amino acids, for enhanced performance of fish larvae and juveniles

#### **FISH IMMUNOLOGY & HEALTH:**

- Better understanding of host/pathogens interaction mechanisms
- Establishment of immunostimulation strategies to enhance stress resistance and welfare of farmed fish.

### **2. Main achievements (2000 ca.)**

#### **SOCIETAL**

A major achievement of the Aquagroup has been its contribution to the optimization of Senegalese sole farming in intensive and semi-intensive systems. Knowledge generated contributed decisively for today's progressive establishment of large scale commercial farming of sole in Portugal and Spain.

#### **SCIENTIFIC**

##### **1. Reproduction**

- Sole sperm is produced in very low quantities, but is produced all year around.
- Male to female ratio in sole should be higher than 1.
- Free amino acid content in sole eggs can be used as indicator of egg quality.
- A protocol for Dusky grouper artificial reproduction has been successfully established.

##### **2. Farming**

- Bacteria from microalgae improve seabream larvae rearing.
- Green water technique increases ingestion rate in fish larvae.
- Optimized sole weaning protocols leading to enhanced fry quality have been transferred to several commercial fish farms.
- Polyculture of sole with gilthead seabream in earth ponds has been demonstrated.
- Rearing protocols for ornamental decapods crustaceans have been established and transferred to commercial companies.

### 3. Nutrition

- Live preys used in commercial hatcheries have amino acid imbalances, that causes lower survival rates and higher incidence of skeletal deformities in sparids.
- Tracer studies techniques were developed for direct estimation of feed intake, and protein and lipid metabolism in fish larvae; these techniques overcome major limitation in the use of traditional methodologies in nutritional studies with very small fish.
- Alkaline phosphatase activity is a good indicator of fish larvae nutritional condition during weaning.
- Nutrition modulates bone protein expression in marine fish larvae.
- A practical diet for Senegalese sole juveniles has been optimized.
- Stressful husbandry conditions affect amino acid metabolism in sole juveniles.

### CAREER BUILDING

Four post-doc researchers at the Aquagroup (2003-2007) found stable careers elsewhere:

- Neil Ruane - Research Manager at the Marine Institute (Ireland).
- Pavlos Makridis - Researcher at the Hellenic Centre for Marine Research (Greece).
- Ricardo Calado - Researcher at the CESAM Associated laboratory (Portugal).
- Elsa Cabrita - Researcher at the ICMAN-CSIC (Spain).

### 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Afonso, A, Gomes S., Silva, J, Marques, F, Henrique, M. 2005. Side-effects in sea bass (*Dicentrarchus labrax* L.) due to intraperitoneal vaccination against vibriosis and pasteurellosis. *Fish & Shellfish Immunology* 19: 1-16. IF 2007=3.160, n°C=5.
2. Aragão, C, Conceição, LEC, Dinis, MT, Fyhn, HJ. 2004. Amino acid pools of rotifers and *Artemia* under different conditions: nutritional implications for fish larvae. *Aquaculture* 234: 429-445. IF 2007=1.735, n°C = 18.
3. Aragão, C, Conceição, LEC, Martins, D, Rønnestad, I, Gomes, E, Dinis, MT. 2004. A balanced dietary amino acid profile improves amino acid retention in post-larval Senegalese sole (*Solea senegalensis*). *Aquaculture* 233: 293-304. IF 2007=1.735, n°C=16.
4. Cabrita, E, Robles, V, L, Rebordinos, C, Sarasquete, MP, Herráez (2005). Evaluation of DNA damage in rainbow trout (*Oncorhynchus mykiss*) and gilthead sea bream (*Sparus aurata*) cryopreserved sperm. *Cryobiology* 50: 144-153. IF 2007=1.936, n°C=4.
5. Calado, R, Vitorino, A, Dionísio, G, Dinis, MT. 2007. A recirculated maturation system for marine ornamental decapods. *Aquaculture* 263: 68-74. IF 2007=1.735, n°C=2.
6. Conceição, LEC, Grasdalen, H, Rønnestad, I. 2003. Amino acid requirements of fish larvae and post-larvae: new tools and recent findings. *Aquaculture* 227: 221-232. IF 2007=1.735, n°C=18.
7. Conceição, LEC, Grasdalen, H, Dinis, MT. 2003. A new method to estimate the relative bioavailability of individual amino acids in fish larvae using <sup>13</sup>C -NMR spectroscopy. *Comparative Biochemistry and Physiology* 134B: 103-109. IF 2007=1.651, n°C=11.
8. Imsland, AK, Foss, A, Conceição, LEC, Dinis, MT, Delbare, D, Schram, E, Kamstra, A, Rema, P, White, P. 2003. A review of the culture potential of *Solea solea* and *S. senegalensis*. *Reviews in Fish Biology and Fisheries* 13: 379-407. IF 2007=2.531, n°C=32.
9. Morais, S, Cahu, C, Zambonino-Infante, JL, Robin, J, Rønnestad, I, Dinis, MT, Conceição, LEC. 2004. Dietary triacylglycerol source and level affects performance and lipase expression in larval seabass (*Dicentrarchus labrax*). *Lipids* 39: 449-458. IF 2007=1.419, n°C=8.
10. Morais, S, Koven, W, Rønnestad, I, Dinis, MT, Conceição, LEC. 2005. Dietary protein/lipid ratio and lipid nature affects fatty acid absorption and metabolism in a teleost larva. *British Journal of Nutrition* 93: 813-820. IF 2007=2.339, n°C=6.

The Aquagroup has published a total of 57 ISI-listed peer-reviewed articles in the period 2003-2007. Most of these publications (72%) resulted from international collaborations. The mean Impact factor (based on ISI JCR 2007) for those 57 publications has been 1.597, a value just within the top 10 threshold value for the “Fisheries” subject category (ISI JCR 2007). From the 57 articles in the period, 58% were published in this last subject category. From the total published articles, 44 and 60% were published in top 10 and top 20, respectively, journals of a subject category of ISI JCR 2007. During the period 2003-2007, the Aquagroup has published an average of 1.7 publications (ISI) per year per Aquagroup member with a PhD degree.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Rønnestad, I, Conceição, LEC. 2005. Aspects of protein and amino acid digestion and utilization by marine fish larvae. In *Physiological and Ecological Adaptations to Feeding in Vertebrates* (J. Matthias Starck and Tobias Wang. eds.). Science Publishers: Enfield. New Hampshire, USA. 389-416 pp.

2. Soares, F, Conceição, L. 2007. Maneio de reprodutores de linguado (*Solea senegalensis*). In *Manual de Cultivo de Linguado e de outros Peixes Planos*. (Junta de Andalucia, Consejaria de Agricultura y Pesca ed.). Viceconsejaria. Servicio de Publicaciones y Divulgacion. 15-24 pp.

3. Engrola, S. 2007. Adaptação do linguado a alimento inerte. In *Manual de Cultivo de Linguado e de outros Peixes Planos*. (Junta de Andalucia, Consejaria de Agricultura y Pesca ed.). Viceconsejaria. Servicio de Publicaciones y Divulgacion. 55-65 pp.

**4. Master and Ph.D. thesis completed** (3000 ca.)

## **PhD THESIS**

Carvalho, S. 2007. Papel das Comunidades de Macrofauna Bentónica na Gestão de Tanques para Piscicultura. University of Algarve. Supervisor CCMAR: MT Dinis.

Morais, S. 2006. Digestive physiology and food intake in marine fish larvae with respect to dietary neutral lipids. University of Algarve. Supervisors CCMAR: MT Dinis and L Conceição. External supervisor: I Rønnestad (University of Bergen, Norway).

Aragão, C. 2004. Towards the assessment of amino acid requirements in seabream and sole larvae. University of Algarve. Supervisors CCMAR: MT Dinis and L Conceição.

Rueda-Jasso, R. 2003. Interaction between nutrition, oxidative status and condition of *Solea* spp. post-larvae and juveniles. University of Ghent. Supervisors: P Sorgeloos (University of Ghent, Belgium). Supervisor CCMAR: L Conceição.

Ribeiro, ML. 2003. Ontogenic development of *Solea senegalensis*: digestive system and nutritional aspects. University of Algarve. Supervisor CCMAR: MT Dinis.

## **MSc THESIS**

Baptista, I. 2007. Outdoor mass production of marine diatoms for bivalve hatcheries using saline ground water – A case study in Yerseke (Netherlands) and the improvement of the culture systems in Bouin (France). MSc of Marine Biology, University of Algarve. Supervisors CCMAR: MT Dinis.



Mota, E. 2007. Biochemical markers for the quality of *Solea senegalensis* eggs. MSc of Marine Biology, University of Algarve. Supervisors CCMAR: MT. Dinis, E Cabrita and F Soares.

Bruwiere, S. 2006. The effects of amino acid supplementation on stress response and performance of juvenile sole (*Solea senegalensis*). European MSc of Aquaculture and Fisheries, Ghent University. Supervisors CCMAR: L Conceição and C Aragão.

Canoza, B. 2006. Effects of copper in plasma, gills, liver and muscle of seabream (*Sparus aurata*, Linnaeus 1758) juveniles. European MSc of Aquaculture and Fisheries, University of Algarve. Supervisors CCMAR: F Soares and R Cabral e Silva.

Costas, B. 2006. Effect of high densities on growth, stress response and plasma amino acid levels in Senegalese sole (*Solea senegalensis*, Kaup 1858). European MSc of Aquaculture and Fisheries, University of Algarve. Supervisors CCMAR: L Conceição and C Aragão.

Weber, R.A. 2005. Determinação do efeito de condição de stress agudo e crónico no cultivo de pós-larvas de *Solea senegalensis*. Diploma de Estudos Avançados, Institute of Aquaculture, University of Santiago de Compostela. Supervisors CCMAR: C Aragão and MT Dinis.

Anastassiades, G. 2003. Early weaning of sole, *Solea senegalensis*, onto microencapsulated diets, with different levels of protein hydrolysates. European MSc of Aquaculture and Fisheries, Ghent University. Supervisors CCMAR: MT Dinis, L Conceição and L Ribeiro.

Pita, C. 2003. O perfil socio-económico de uma comunidade costeira portuguesa: a Fuseta. MSc in Marine and Coastal studies, University of Algarve. Supervisors CCMAR: MT Dinis and K Erzini.

Violante, A. 2003. Estudo da doença de inverno em dourada (*Sparus aurata* L.) de cultivo, a Sul de Portugal. MSc in Marine and Coastal Studies, University of Algarve. Supervisors CCMAR: MT Dinis and F Soares.

## **5. Patents/propotypes (2000 ca.)**

## **6. Organization of conferences (2000 ca.)**

- “Seminário de Aquacultura e Novas Espécies”, 12-15th September 2004, Funchal, Portugal. Maria Teresa Dinis and Luis Conceição were members of the organizing and scientific committees. The main results from research projects on aquaculture of new species were presented and discussed. A total of 60 persons, from Portugal and Spain, participated, including researchers, University students, and fish farmers.

- Larvi'05 – 4th international Fish & Shellfish Larviculture Symposium, 5-8th September 2005, Gent University, Belgium. Sept 2005. Maria Teresa Dinis was member of the Scientific Committee. Recent results in the fields of larval research and rearing were presented in the major international scientific meeting in the field. Over of 400 persons from all around the world participated.

- LARVAR 06 - Workshop in Fish Larval Research, 9th January 2006, Centre of Marine Sciences (CCMAR), Faro, Portugal (Organizers: Maria Teresa Dinis, Luís Conceição and Karin Pittman,

from University of Bergen, Norway). The main issues and research perspectives for basic and applied research on fish larvae were discussed by 20 researchers, from 6 institutions in Portugal, Norway, Israel and Spain.

- 3rd Workshop on the Cultivation of Soles. 22 – 23rd March 2006, CIFPA El Toruño, Cádiz, Spain. (Organizers: Bari Howell, Pedro Cañavate, Richard Prickett and Luis Conceição). The purpose of the meeting was to review the current status of commercial experience and corresponding research in key areas of the cultivation of soles and to provide information that would guide the future actions of both sectors. This Workshop was attended by 46 participants from both the research (59%) and commercial (41%) communities, representing 5 European countries (Spain, Greece, The Netherlands, Portugal and the UK).

- Jornadas de Aquacultura 2006, 5th December 2006, University of Algarve, Faro, Portugal (Organizers: Florbela Soares and Elsa Cabrita). The main objective was the dissemination of research results obtained by the Aquagroup to the fish farmers. In particular, results from the project REPROSOL, namely on the management of sole breeding, one of the major constraints for the cultivation of this species, were discussed. Results from other projects were also presented, and a round table was organized where farmers and researchers discussed the challenges and strategies for the development of the Portuguese aquaculture. A total of 90 persons participated, including researchers, students and 30 fish farmers.

#### **7. Industry contract research (2000 ca.)**

Over recent years, the scientific expertise and reliable experimental facilities associated to the Aquaculture Research Group have gained recognition by the aquaculture industry. The Aquagroup has conducted three contract-research trials with some of the most influential players in the aquafeed industry. Furthermore, the leading company in the area of feed additives for aquaculture (DSM Nutritional Products, former Roche Vitamins) relied on our scientific competences to provide a Training Seminar to the R&D personnel of their key customers in the Turkish market.

- INVE, 2004. Benchmarking trial with rotifer enrichments. Responsible: P. Makridis.
- EWOS, 2005. Benchmarking trial with larvae microdiets. Responsible: L. Conceição.
- DSM Nutritional Products, 2007. Customer Training Seminar in Nutrition and Feeding of Mediterranean farmed fish. Responsible: J. Dias.
- DSM Nutritional Products, 2007. Efficacy trial for the development of new feed additives. Responsible: J. Dias.

#### **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

- MT Dinis, 2003. Working Group of the Ministry of Agriculture and Fisheries for the Aquaculture Sector in Portugal. The objectives of this WG were to identify and propose measures to promote the aquaculture development in Portugal.

- MT Dinis, 2006. Coordinator of the CESO R&D Report for the Portuguese Directorate of Fisheries regarding the Evaluation of the impact of the European Community programs on Fisheries (2003-2005).

#### **9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

The Aquaculture Research Group has a long standing tradition of associating itself to international cooperation windows. Most of its researchers have carried part or the totality of their PhD work or post-doc activities under collaboration with other European research institutions. Four of the five PhD thesis completed within Aquagroup in 2003-2007 benefited from structural collaborations with labs elsewhere in Europe. This fact greatly contributed to the establishment of a strong and stable network of international cooperation's. This is reflected in the fact that 40 out of the 57 (72%) publications of the Aquagroup in 2003-2007 were written in collaboration with colleagues from institutions outside Portugal.

Major collaborations:

- Dept. Biology of Univ. Bergen, NIFES, Dept. Biotechnology & Biology of Norwegian Univ. Science and Technology (Norway).
- Institute for Zoology, Salzburg (Austria).
- ICRAM, Rome (Italy).
- ICMAN-CSIC, IFAPA, Univ. Leon (Spain).
- INRA, IFREMER (France).
- HCMR (Greece).
- Wageningen Univ. (The Netherlands).

Outside Europe, members of the Aquagroup have also been involved in scientific collaborations with:

- Vietnam – participation in two projects: 1) “Marine aquaculture in Vietnam”, funded by NUFU (Norway), in collaboration with the Nha Trang University (Vietnam) and NTNU (Trondheim, Norway), with participation in research activities related to reproduction and larval rearing, and in teaching of MSc disciplines; 2) “Improving Training and Research Capacity of the University of Fisheries, funded by NORAD SRV2701 (Norway), in collaboration with the Nha Trang University (Vietnam) and NTNU (Trondheim, Norway), with participation in teaching of MSc disciplines and training of PhD students.
- Brazil – Teaching of MSc disciplines at the UNESP (Jaboticabal, São Paulo, Brasil) and at the Federal University of Rio Grande (FURG, Rio Grande, Rio Grande do Sul, Brasil).

The Aquagroup has also hosted several foreign scientists and PhD students:

- Carlos Rojas-Garcia (Out 06). Professor at the Catholic University of Temuco, Chile.

- Pedro Pablo Ambrósio (Aug 04 – Feb 05). Associate Professor at the Polytechnic University of Catalonia, Spain.

- José Angel Ronson (Apr – Ago 04). PhD student at the University of Santiago de Compostela, Spain.

- Sagiv Kolkovski (Jun – Jul 03). Principal Research Scientist, Department of Fisheries WA, Australia.

- L.M. Diep (Oct- Nov 2005). PhD student at the University of Fisheries, Nha Trang, Vietnam.

The Aquagroup members have also been active participants in the following networking structures:

- COST Action 867 – Welfare of fish in European aquaculture. L. Conceição is one of the Portuguese representatives in the Management Committee.
- European Aquaculture Technology Platform (EATP).
- COST Action FA0801 “LARVANET - Critical success factors for fish larval production in European Aquaculture: a multidisciplinary network”. L. Conceição is the Portuguese representative in the Management Committee.

## **6e. Future research**

### **1. Objectives (3000 ca.)**

The mission of the Aquaculture Research Group will continue to be the generation of high quality scientific knowledge, through basic and applied research, to tackle some of the evolving sustainability challenges facing the aquaculture industry. Major strengths in terms of scientific skills gathered within of the Aquagroup rely heavily on our expertise on broodstock management, larvae rearing and nutrition in new species for Mediterranean aquaculture.

#### **CONSOLIDATION OF ESTABLISHED AREAS**

- The metabolic use of dietary amino acids in fish larvae, by means of tracer studies, is now a clear distinctive research topic of the Aquagroup. The area of Nitrogen Nutrition in Fish is to be further consolidated and reinforced, through integrative studies on the role of amino acids and peptide fractions in areas such as digestive efficiency, immune competence and skeletal deformities in marine fish. The access to functional genomic and proteome expression approaches is already under implementation, with one post-doc researcher and two new PhD students (2008) fully dedicated to this area.

- Research on Broodstock Management of new species (sole, grouper) will be continued, with a stronger focus on topics related to the broodstock nutrition and welfare. Future work on this area aims to develop a broodstock selection program for sole and grouper, based on egg and sperm quality, and subsequent larval and juvenile performance. One new post-doc researcher will be dedicated to this area.

- The area of Marine Ornamentals will pursue its objectives of developing farming protocols for new local marine ornamental species of high market potential, guaranteeing sustainable practices and maintenance of nature's biodiversity.

## NEW AREAS

- Activities on the modulation of Flesh Quality in Fish have been initiated as a research topic in the Aquagroup. Focus is given to the assessment of nutritional factors and farming practices as tools to enhance flesh quality and safety of farmed fish. The implementation of this topic is foreseen in close collaboration with the Laboratory of Nutrition, Growth and Quality of Fish (LANUCE), from CIIMAR. Two new PhD students (2008) are to be fully dedicated to this area.
- The aquaculture industry in Portugal faces profound changes in a near future. Increased production with the new off-shore cage farming systems, the entry into commercial phase of several new species and an obligation to maintain competitiveness and profitability of semi-intensive aquaculture production. In this context, the Aquagroup identified the Production Systems research area as a necessity and an opportunity to strengthen its support to industrial partners.
- Given its growing importance as a societal issue, the area of Welfare in Fish is to be consolidated within the Aquagroup. Research will focus on gaining basic knowledge regarding the stress resistance in fish, aggressive behavior, evaluation of bioactive compounds for enhanced health status. The implementation of this area will be progressive within CCMAR and in close collaboration with the Immunobiology Laboratory, from CIIMAR. One new PhD student (2008) will be fully dedicated to this area.

Areas of collaboration to be initiated or reinforced

- Members of the Aquagroup have recently created a feed technology based company, which will have a pilot-scale feed technology platform. Some potential research activities associated to a facilitated access to such structure have already been identified.

## **2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

Several areas previously identified as strategic priorities for consolidation and growth of the Aquagroup are already supported by recently granted research projects.

Projects Funding Agency Funds (EUR) Time frame

FISHWELL DGP-MARE 50.000 Jan 07 - Sep 08

SAARGO FCT 55.400 Nov 05 - Oct 08

SEACASE EU-FP6 312.088 Jan 07 - Dec 09

FATTYBONE FCT 108.002 Jun 08 - May 11

TEXBREAM FCT 155.128 Jun 08 - May 11

HYDRRA FCT 171.002 Jul 08 - Jun 11

CRYOSPERM FCT 189.785 Apr 08 - Mar 11

EFARFish FCT 95.122 Oct 08 - Sep 11

PROSPAWN EU-FP7 RB-SME 99.000 Jan 09 - Dec 10

ECOAQUA INTERREG (pending) 105.000 Jan 09 - Dec 10

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TOTAL PROJECTS 1340,531

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Aragão, C, Corte-Real, J, Costas, B, Dinis, MT, Conceição, LEC. 2008. Stress response and changes in amino acid requirements in Senegalese sole (*Solea senegalensis* Kaup 1858). *Amino Acids* 34: 143-148. IF=2.780, n°C=0.
2. Calado, R, Dionísio, G, Dinis, MT. 2007. Decapod crustaceans associated with anemone *Anemonia sulcata* – living there or just passing by? *Scientia Marina* 71: 287-292. IF= 0.945, n°C=0.
3. Conceição, LEC, Ribeiro L, Engrola, S, Aragão, C, Morais, S, Lacuisse, M, Soares, F, Dinis MT. 2007. Nutritional physiology during development of Senegalese sole (*Solea senegalensis*). *Aquaculture* 268: 64-81. IF=1.735, n°C=1.
4. Martinez-Pastor, F, Cabrita, E, Soares, F, Anel, L, Dinis, MT. 2008. Multivariate cluster analysis to study motility activation of *Solea senegalensis* spermatozoa: a model for marine teleosts. *Reproduction* 135: 449-459. IF=2.962, n°C=0.
5. Morais, S, Koven, W, Rønnestad, I, Dinis, MT, Conceição, LEC. 2005. Dietary protein/lipid ratio affects growth and amino acid and fatty acid absorption and metabolism in Senegalese sole (*Solea senegalensis* Kaup 1858) larvae. *Aquaculture* 246: 347-357. IF=1.735, n°C=10.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

At present, one of the major threats conditioning the activities of the Aquagroup is the poor incentive for stable and progressive research careers within CCMAR. Over the years, this incapacity of retaining high valuable personnel and expertise leads to sub-optimal internal organization, creates great difficulties to consolidate research axis and does not promote the transition to independence of post-doc researchers. In what concerns the FCT programs Ciência 2007 and Ciência 2008, the policy adopted by CCMAR in promoting the establishment of new research groups at the expenses of consolidating the existing ones has not strengthen the Aquagroup. A recent self-evaluation of the Aquagroup identified two research profiles which seem strategic for the development of the Aquagroup:

- Welfare in Fish and Sustainable aquaculture practices

The necessities in terms of equipment and facilities, for the implementation of future research activities within the Aquagroup rely essentially on the following issues:

- Dedicated facilities for tracer studies in fish
- Elemental Analyser (EA/LC-IRMS) for tracer studies using stable isotopes
- Lab space and equipment (essentially a flow cytometer) for immunology work
- Office space for PhD students and research assistants



## **Biotechnology and Molecular Biology of Microalgae (BMBM)**

### **6a. Group description**

#### **1. Group name / denomination**

**Biotechnology and Molecular Biology of Microalgae (BMBM)**

#### **2. Principal investigator**

**João Carlos Serafim Varela**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Carotenoid biosynthesis, Biodiesel, Biotechnology, Environmental

#### **5. Funding, source, dates (1000 ca.)**

Indicate funding (amount):

167.219,00 EUR.

### **6b. Group team**

#### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. João Carlos Serafim Varela (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

#### **2. Other researchers in the group (Include here collaborators with PhD. only)**

#### **3. Other researchers in the group (non PhD.)**

001. Ana Rita Pimenta Falcao Marques (Cat.: Assistente de Investigação, Gr. Acad.: Licenciatura)

002. Marta Catarina Veiga de Faria Rodrigues (Cat.: Assistente de Investigação, Gr. Acad.: Licenciatura)

003. Ana Alexandra Pedrosa Ramos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

004. Sacha Nicole Coesel (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

## 6c. Objectives & achievements

### 1. Objectives (2000 ca.)

The BMBM research group is focused on the following interrelated research lines:

- 1) molecular basis of carotenoid biosynthesis in microalgae;
- 2) isolation of carotenoid-hyperproducing microalgal strains;
- 3) optimization of carotenoid production at laboratory and pilot scale by means of photobioreactors (in collaboration with our entrepreneurial partner Necton, S.A.);
- 4) isolation of lipid-hyperproducing microalgal strains for biodiesel production;
- 5) optimization of microalgal lipid biosynthesis for biodiesel production.

### 2. Main achievements (2000 ca.)

Between 2003-2008, the BMBM research group has carried out several projects concerning all the research lines given above. Within the OVERCAROTEN project, for instance, we were able to isolate several carotenoid-hyperproducing strains of *Dunaliella salina* by means of chemical mutagenesis and Fluorescent-Activated Cell Sorting. In this project we were also able to determine that sample humidity is crucial for efficient carotenoid extraction by means of supercritical fluids and that carotenoid-overproducing strains often develop a cold-sensitive phenotype that limits their use under cold weather. This project proved not only that Nile red-coupled FACS is a useful high throughput methodology to isolate hyperproducing strains from chemically mutagenized pools, but also that seemingly minor procedural innovations can be critical for the successful isolation of hyperproducers. For example, instead of using solid media plates for isolation of clonal populations, we resorted to serial dilution and cultivation in 96-well plates containing liquid growth media suitable for microalgal growth. This allowed us to obtain microalgal populations coming from a single cell (verified microscopically) in a relatively short time, as we avoided one of the critical bottlenecks of microalgal isolation: growth in solid media followed by transfer / adaptation to liquid media, an extremely stressful condition for flagellated microalgae. This know-how was later employed in the BOTRYOFUEL project for the isolation of microalgal hyperproducers of lipids suitable for biodiesel production. Within the INTERREG SAL project, we were able to characterize *D. salina* strains at molecular level concerning the gene expression of enzymes involved in carotenoid biosynthesis, namely phytoene synthase (Psy) and phytoene desaturase (Pds). Using a combination of Northern and Quantitative PCR analyses we were able to conclude that regulation at the transcriptional level may be one of the most crucial factors determining carotenoid biosynthesis in *D. salina*. Recently, we have cloned a cDNA encoding a third enzyme of the pathway: lycopene beta-cyclase (Lcy). Our results indicate all these enzymes are co-ordinately expressed and highly induced by abiotic stress when nutrients are depleted. Conversely, high nutrient levels abolish this response.

## 6d. Productivity

- 1. Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Ramos, A, Varela, J. 2008. Biochemistry and molecular biology in Portugal: an overview of past and current contributions. IUBMB Life 60: 265-269. IF 2007=2.857, n°C=0.
2. Ramos, A, Coesel, S, Marques, A, Rodrigues, M, Baumgartner, A, Noronha, J, Rauter, A, Brenig, B, Varela, J. 2008. Isolation and characterization of a stress-inducible *Dunaliella salina* Lcy- $\beta$  gene encoding a functional lycopene  $\beta$ -cyclase. Applied Microbiology and Biotechnology 79: 819-828. IF 2007=2.475, n°C=0.
3. Mendoza, H, Freijanes, K, Carmona, L, Ramos, A, Duarte, V, Varela, J, de la Jara, A. 2008. Characterization of *Dunaliella salina* strains by flow cytometry: a new technique to select  $\beta$ -carotene hyperproducing strains. Electronic Journal of Biotechnology, in press. IF 2007=0.859, n°C=0.
4. Coesel, SN, Teles, LM, Baumgartner, AC, Henriques, NM, Cancela, L, Varela, JC. 2008. Nutrient limitation is the main regulatory factor for massive  $\beta$ -carotene accumulation and for Psy and Pds transcript levels in *Dunaliella salina* exposed to light and salt stress. Marine Biotechnology, in press. IF 2007=2.503, n°C=0.
5. Coesel, S, Oborník, M, Varela, J, Falcatore, A, Bowler, C. 2008. Evolutionary origins and functions of the carotenoid biosynthetic pathway in marine diatoms. PloS ONE, in press.

- 2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Ramos, AA, Rodrigues, MC, Marcelo, F, Lamela, T, Duarte, V, Baumgartner, AC, Coesel, SC, Morais, R, Gouveia, L, Mendes, R, Brenig, B, Navalho, J, Varela, J. 2005. Isolation of carotenoid-overproducing *Dunaliella salina* strains. 6th European Society of Microalgal Biotechnology European Workshop, Potsdam, Germany, 23-24 May.
2. Baumgartner, AC, Coesel, SN, Marques, AR, Ramos, AA, Rodrigues, MC, Brenig, B, Varela, JC. 2005. Optimising carotenoid production in *Dunaliella salina*: the effect of salinity and nutrient availability. 6th European Society of Microalgal Biotechnology European Workshop, Potsdam, Germany, 23-24 May.
3. Rodrigues, MC, Baumgartner, AC, Ramos, AA, Marques, AR, Henriques, NM, Varela, JC. 2005. Isolation and characterization of a *Dunaliella salina* eEF1A homologue and its possible role in the regulation of carotenoid biosynthesis. The 14th International Symposium on Carotenoids, Edinburgh, UK, 17-22 July.
4. Ramos, AA, Coesel, SN, Marques, AR, Rodrigues, MC, Brenig, B, Varela, JC. 2005. Isolation and expression patterns of a cDNA encoding *Dunaliella salina* Lycopene beta-cyclase. The 14th International Symposium on Carotenoids, Edinburgh, UK, 17-22 July.

5. Marques, AR, Baumgartner, AC, Ramos, AA, Rodrigues, MC, Henriques, NM, Brenig, B, Varela, JC. 2005. Isolation and characterization of a *Dunaliella salina* ClpC homologue. The 14th International Symposium on Carotenoids, Edimburgh, UK, 17-22 July.
6. Baumgartner, AC, Henriques, NM, Coesel, SN, Duarte, VS, Brenig, B, Varela, JC. 2005. The molecular regulation of abiotic stress-induced carotenogenesis in the microalga *Dunaliella salina*. The 14th International Symposium on Carotenoids, Edimburgh, UK, 17-22 July.
7. Ramos, A, Noronha, JP, Coesel, S, Baumgartner, A, Rauter, A, Varela, J. 2006. Functional characterization of *Dunaliella salina* Lcy-beta gene encoding lycopene beta-cyclase, an enzyme involved in the massive accumulation of carotenoids induced by abiotic stress. 15th National Congress of Biochemistry, Aveiro, Portugal, 8-10 December.
8. Varela, J, Ramos, A, Marques, AR, Coesel, S, Henriques, N, Brenig, B, Baumgartner, A. 2006. Functional characterization of *Dunaliella salina* Lytb / Hdr gene encoding a key enzyme involved in the biosynthesis of carotenoid precursors. 15th National Congress of Biochemistry, Aveiro, Portugal, 8-10 December.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**4. Master and Ph.D. thesis completed** (3000 ca.)

## PhD THESIS

Coesel, S. 2008. Isolation and characterization of regulatory and biosynthetic genes involved in carotenogenesis in the microalga *Dunaliella salina*. University of Algarve. Supervisors: J Varela and Chris Bowler (Stazione Zoologica, Italy).

## MSc THESIS

Ivanna, AP. 2006. Expression patterns of Hdr and Lcy, two enzymes involved in the carotenoid biosynthetic pathway of *Dunaliella salina* (Teod.). University of Algarve – University of Gent Joint MSc Thesis. Supervisors: J Varela and P Bossier (University of Gent).

Mestrinho, I. 2004. Isolation of antioxidant compounds from *Cecropia leucocoma*, Cecropiaceae, a plant from the Amazon's rain forest. Graduation Thesis. University of Algarve.

Silva, J. 2003. Comparison of three photobioreactors for the production of astaxanthin by *Haematococcus pluvialis* (Chlorophyceae). Graduation Thesis. University of Algarve.

**5. Patents/prototypes** (2000 ca.)

Due to a strategic option by our entrepreneurial partner, no patents could be filed. The high costs of worldwide patent maintenance and the risks of disclosure of critical information have prevented us from following this path concerning the protection of Intellectual Property.

## **6. Organization of conferences (2000 ca.)**

2004 - Congresso Nacional de Bioquímica. ML Cancela, MA Alves, J Martins, P Rodrigues, D Simes & J Varela (Organizers). Tivoli Marinotel, Vilamoura, Algarve, 2-4 December. This is the largest and most prestigious national meeting in the Biochemistry / Molecular Biology field.

## **7. Industry contract research (2000 ca.)**

NITEC-GENTEC contract research for the development of an R&D unit at CIMAR (CCMAR) and technology and know-how transfer from BMBM research group to Necton S.A., a Portuguese SME dedicated to biotechnological applications of microalgae. Currently, BMBM and Necton have the following common research interests: 1) isolation of microalgal lipid-hyperproducing strains for biodiesel production; 2) isolation of novel microalgal strains suitable for the cosmetics industry; and 3) isolation of carotenoid-hyperproducing strains for the aquaculture, pharmaceutical and pigment industries. This agreement has resulted in the transfer of know-how / procedures for monitoring genetic stability of microalgal strains grown in photobioreactors; transfer of carotenoid-hyperproducing strains; determination of critical factors for increased lipid production by microalgae; and identification of regulatory switches at the physiological and molecular level. A great part of this research is under a Non-Disclosure Agreement, which has had a negative impact on the number of publications by the BMBM research group. This explains why all publications of the research group are concentrated in the year of 2008. More publications will follow after getting the necessary clearances.

## **8. Government/organization contract research (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)**

In order to achieve the best performance and profit from complementary know-how and research staff, the BMBM has collaborations with Technological Institute of Canarias (Canarias Islands, Spain) for the development of FACS methodologies for high throughput isolation of hyperproducing strains; Chris Bowler's labs (Stazione Zoologica, Italy and École Supérieure de Paris, France) for joint training of students at the PhD level and joint publication efforts concerning carotenoid biosynthesis and development of know-how for microalgal DNA transformation; Francis Cunningham & Elizabeth Gantt's lab (U. Maryland, USA) for exchange of molecular tools for the isolation of carotenogenic enzymes as well as the development of in vivo functional assays; Terry Kinzy's lab (University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical School, New Jersey, USA) for collaborative work on the development of functional assays for the multifunctional Eukaryotic Elongation Factor 1A in yeast, as the D. salina homolog has been found in a subtractive cDNA screen for genes induced during carotenogenesis (M. Rodrigues, S. Gross, A. Baumgartner, T. Kinzy & J.Varela, manuscript in preparation). Concomitantly, the BMBM research group has joined the EU-funded ALGINET, whose main goal is the establishment of a thematic network of European researchers interested in microalgae as natural sources of high value-added biochemicals.

## **9. Internationalization (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)**

## 6e. Future research

### 1. Objectives (3000 ca.)

Recently, by means of a yeast two-hybrid screen, we have identified a gene product that seems to interact with lycopene beta-cyclase. This result is potentially of high significance because, so far, little is known how the carotenoid biosynthetic pathway in photosynthetic organisms is regulated. Interestingly, this interactor with unknown function is highly conserved throughout evolution in photosynthetic organisms but is virtually absent in organisms lacking carotenoids. Therefore, the elucidation of its role in the microalga *Dunaliella salina* and other photosynthetic organisms will be a top priority. In order to accomplish this, we intend to collaborate with Arabidopsis research groups to test whether this interaction takes place in terrestrial plants as well. Other heterologous gene expression systems will also be used to demonstrate the interaction in vitro and in vivo.

Another area of importance within the BMBM research group will be the development of microalgae-derived biofuels, which corresponds to a strategic area identified by CIMAR. With the rise in the oil prices, alternative fuels are urgently needed. Unlike terrestrial plants, microalgae are fast-growing photosynthetic organisms that do not compete for food crop land and thus these organisms have been selected as possible sources of biodiesel. However, for the successful development of this research line we will need to address the following: 1) the need of modelling the energetic balance of biofuels produced by microalgae according to the size of photobioreactors / open ponds; 2) improve the harvest of microalgae; 3) optimize growth conditions of lipid hyperproducing strains; and 4) optimize lipid extraction / biodiesel from microalgae. All these items are important to reduce production costs, a key factor for the successful production and usage of marine biofuels. In order to accomplish this, the BMBM group, along with our entrepreneurial partner (Necton S.A.) and other European partners is preparing an FP7 project on this very theme. Other projects in this area have also been submitted. This effort will be enhanced by the opening of two post-doctoral positions under the Ciência 2008 programme umbrella (see below).

### 2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)

2007-Present. POCI 2010 - IDEIA. BOTRYOFUEL 13-05-04-FDR-00041. Hydrocarbon hyperproducing strains. Agência de Inovação, S.A., 1 yr. Team member. 13428 EUR.

2007-Present. NITEC-GENTEC 161/26/06. Stimulating systems for the creation of research & technological development nuclei in entrepreneurial sector. Agência de Inovação, S.A., 3 yrs. Team member. 6.000 EUR.

2005-Present. SAL - Salinas do Atlântico. Recovery of the identity of Atlantic salt pans (salinas). Recovery and promotion of the biological, economical, and cultural potential of humid coastal areas. INTERREG IIIB - Atlantic, 159 SAL, 3 yrs., Team Member. 41.200 EUR.

### 3. Previous publications in the area (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Ramos, A, Coesel, S, Marques, A, Rodrigues, M, Baumgartner, A, Noronha, J, Rauter, A, Brenig, B, Varela, J. 2008. Isolation and characterization of a stress-inducible *Dunaliella salina*

Lcy- $\beta$  gene encoding a functional lycopene  $\beta$ -cyclase. *Applied Microbiology and Biotechnology* 79: 819-828. IF=2.475, n°C=0.

2. Mendoza, H, Freijanes, K, Carmona, L, Ramos, A, Duarte, V, Varela, J, de la Jara, A. 2008. Characterization of *Dunaliella salina* strains by flow cytometry: a new technique to select  $\beta$ -carotene hyperproducing strains. *Electronic Journal of Biotechnology*, in press. IF=0.859, n°C=0.

3. Coesel, SN, Teles, LM, Baumgartner, AC, Henriques, NM, Cancela, L, Varela, JC. 2008. Nutrient limitation is the main regulatory factor for massive  $\beta$ -carotene accumulation and for *Psy* and *Pds* transcript levels in *Dunaliella salina* exposed to light and salt stress. *Marine Biotechnology*, in press. IF=2.503, n°C=0.

4. Coesel, S, Oborník, M, Varela, J, Falciatore, A, Bowler, C. 2008. Evolutionary origins and functions of the carotenoid biosynthetic pathway in marine diatoms. *PloS ONE*, in press. IF= n°C=0.

**4. Special requirements (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)**

As part of the strategic development of the LA, two post-doctoral positions for researchers with a track record of publications in the broad field of marine biofuels have been opened under the umbrella of the Ciência 2008 programme. This will enable the formation of the needed critical core of researchers to develop two main lines of research: 1) photobioreactor engineering and harvest of microalgae; and 2) metabolic and solar energy bio-conversion engineering. Other necessary equipment to be acquired is a flow cytometer with cell sorting for Fluorescent-Activated Cell Sorting. Photobioreactors needed for modelling the energetic balance of biodiesel production by microalgae have already been purchased by our entrepreneurial partner.



## **Biogeographical Ecology and Evolution (BEE)**

### **6a. Group description**

#### **1. Group name / denomination**

**Biogeographical Ecology and Evolution**

#### **2. Principal investigator**

**Maria Ester Tavares Alvares Serrao**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Biogeography, Reproductive ecology, Population genetics, Dispersal connectivity

#### **5. Funding, source, dates (1000 ca.)**

Funds indicated are only those for the team BEE (total 2.324.003,00 EUR)

EU funds:

LIFE 06 NAT/P/000192. (BIOMARES) 2007-2010 (891.160 EUR)

NEST-2005-Path-COM/043251. (EDEN) 2007- 2009 (295.908 EUR)

EVK3-CT-2000-00044. (M&MS) 2001-2005 (330.000 EUR)

Plus various projects in MARBEF and MGE networks

FCT:

As coordinators:

POCI/MAR/61105/2004. (ADAPT) 2006-2009 (86.400 EUR)

POCI/MAR/60179/2004. DIVSTAB) 2006-2009 (82.800 EUR)

POCI/MAR/57499/2004. (MATING) 2006-2008 (89.100 EUR)

POCI/MAR/57342/2004. 2005-2007 (NETWORK) (45.000 EUR)

POCI/MAR/60044/2004. (DIVIDE) 2005-2008 (41.400 EUR)

POCTI/39431/BSE/2001. (GAMETE) 2002-2005 (45.000 EUR)

POCTI/BSE/48317/2002. (STRESSREG) 2004-2007 (105.491 EUR)

POCTI/38863/BSE/2001. (CHLORGEN) 2002-2005 (61.632 EUR)

POCTI/BSE/35045/99. (GENFUCUS) 2001-2004 (150.000 EUR)

PNAT/1999/BIA/15003/C. (SEAGRASSRIA) 2001-2004 (75.000 EUR)

PDCTM/P/MAR/5292/1999. (BIOPORT) 2001-2004 (225.000 EUR)

As partners:

POCI/MAR/58837/2004. 2005-2008 (32.500 EUR)

POCI/MAR/56149/2004. (LIMITS) 2006-2009 (25.020 EUR)

Subcontracts:

(ECOKELP), ANR, France, 2007-2009 (30.000 EUR)

ICCM, Spain 2006-2009 (8.500 EUR)

Other collaborating projects not listed (space limitations)

## **6b. Group team**

### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Maria Ester Tavares Alvares Serrao (Cat.: Professor Auxiliar, Gr. Acad.: Agregação)

002. Rita Castilho (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

003. Filipe Alexandre Oliveira dos Santos Alberto (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

004. Gareth Anthony Pearson (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

005. María Asunción Lago Lestón (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

006. Maria Elena Varela Alvarez (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

007. Onno Everhardus Diekmann (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

### **2. Other researchers in the group (Include here collaborators with PhD. only)**

001. Cecile, Marie-Alexane Perrin (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

002. Sophie Arnaud-Haond (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

### **3. Other researchers in the group (non PhD.)**

001. Pablo Manent Sintes (Cat.: Investigador Auxiliar, Gr. Acad.: Licenciatura)

- 002. Catarina Figueiredo da Mota (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 003. Cláudia Sofia Patrão Beliz Rosa e Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 004. Emmanuelle Billard (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 005. Gonçalo Jorge Franco Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 006. João Miranda Neiva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 007. Licínia Cristina Freitas Gouveia (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 008. Maria Regina Oliveira Lopes da Cunha (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 009. Mirjam Susanne van de Vliet (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 010. Nelson Alexandre Castilho Coelho (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 011. Rita Micaela dos Santos Fernandes de Araujo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 012. Sara Judite Lopes Teixeira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 013. Sónia Isabel Rodrigues Aldeia Sanches Massa (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 014. Tânia Raquel Santos Aires (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

We aim at understanding patterns and processes mediating population biology from ecological to evolutionary scales. Topics include phylogeography, population dispersal/connectivity, abiotic stress-driven evolution, reproductive ecology and roles of mating systems in population divergence and speciation. Biological models include marine plants, algae, animals, using a variety of approaches, from molecular to experimental ecology and phylogenetic reconstruction.

A large focus of current research is the central question in evolutionary ecology of the nature of environmental barriers that limit gene flow and induce population genetic divergence, a first step towards speciation. We identify gene flow barriers in marine populations, a subject particularly poorly understood for marine taxa given the apparent continuity of marine environments for dispersal, and we track genetic imprints of ocean-climate cycles (lower temperatures and sea levels) from Pleistocene ice ages, on several taxa of pelagic and benthic marine species across various oceans and seas, inferred from genetic structure and molecular phylogenies.

In biogeographical theory we also study evolutionary patterns of archipelago colonizations and we empirically test theoretical predictions from evolutionary ecology of species' ranges, whereby distributional edges and marginal habitats would be expected to have higher clonality and inbreeding, selected for reproductive assurance and local adaptation, and higher genetic differentiation and lower genetic diversity which trade-off with higher selective pressures for stress-driven local adaptation at range edges.

We address leading questions in marine ecology, such as maximizing fertilization success in exposed shores by timing broadcast spawning synchrony, comparing population mating systems in order to understand their roles in enabling species to respond to variable conditions and eventually driving reproductive isolation and speciation, maintaining species integrity in sister taxa that can hybridize and introgress.

These objectives have required significant effort into methodological objectives, including development of various types of molecular methods and molecular markers, novel population genetics analyses methods, and specific software.

## **2. Main achievements (2000 ca.)**

Significant advances in biogeography and marine reproductive ecology:

(45 papers 2003-2007, already +14 in 2008):

Population structure and barriers to gene flow were identified, with genetic imprints of Pleistocene glacial periods on several pelagic and benthic species, ranging from between oceans to within Atlantic and Mediterranean basins. Marks of displacements, vicariance, extinctions/recolonizations and contemporary gene flow restrictions were revealed in several fish, algae and seagrass species.

Independent colonizations events with remarkably recurring biogeographical patterns of diversification were discovered in endemic gastropods (*Conus*) in Cape Verde archipelago. Canary islands seagrasses show founder effects during archipelago colonization from a single source, decreasing genetic diversity with distance to mainland.

Population genetic structure at species biogeographical distributional limits revealed high inbreeding and clonality for seagrasses, mangrove trees and algae. Secondary evolution of asexual reproduction was discovered in marginal Baltic populations of algae.

Genetic entities identified in a species complex (*Fucus*) revealed hybridization/introgression but divergent mating systems contribute to species integrity, the self-compatible hermaphroditic mostly selfing, contrasting to the outcrossing species, despite biparental inbreeding from restricted gamete dispersal.

Restricted intra-population dispersal (sexual and clonal) was shown (spatial autocorrelation analyses, microsatellites) in several seagrass and algal species, associated with inbreeding and outbreeding depression, dependent on outcrossing distance.

Complex network analysis revealed the contribution of different reproductive and dispersal processes to population structure.

Coast/estuary habitat linked population differentiation and convergent adaptation to marginal habitats, including by hybridization and polyploidy, were revealed in *Fucus* algae across European and Pacific shores.

Synchronous spawning patterns and physiological signals were identified in furoid algae, restricting gamete release to periods promoting reproductive success.

We reviewed and developed novel molecular and statistical methods for clonal population genetics.

## 6d. Productivity

### 1. Publications in peer review journals (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Alberto, F, Gouveia, L, Arnaud-Haond, S, Pérez-Lloréns, JL, Duarte, CM, Serrão, EA. 2005. Within population genetic structure, neighbourhood size and clonal subrange in the seagrass *Cymodocea nodosa*. *Molecular Ecology* 14: 2669-2681. IF 2005/2007=4.30/5.169, n°C=20.
2. Arnaud-Haond, S, Duarte, CM, Alberto, F, Serrão, EA. 2007. Standardizing methods to address clonality in population studies. *Molecular Ecology* 16: 5115-5139. IF 2007=5.169, n°C=0.
3. Arnaud-Haond, S, Teixeira, S, Massa, S, Billot, C, Saenger, P, Coupland, G, Duarte, CM, Serrão, EA. 2006. Genetic structure at range-edge: low diversity and high inbreeding in SE Asia mangrove (*Avicennia marina*) populations. *Molecular Ecology* 15: 3515-3525. IF 2006/2007=4.83/5.169, n°C=8.
4. Cunha, RL, Castilho, R, Rüber, R, Zardoya, R. 2005. Patterns of Cladogenesis in the Venomous Marine Gastropod Genus *Conus* from the Cape Verde Islands. *Systematic Biology* 54(4): 634–650. IF 2005=10.33, n°C=4.
5. Engel, CR, Daguin, C, Serrão, EA. 2005. Genetic entities and mating system in hermaphroditic *Fucus spiralis* and its close dioecious relative *F. vesiculosus* (Fucaceae, Phaeophyceae). *Molecular Ecology* 14: 2033-2046. IF 2005/2007=4.30/5.169, n°C=19.
6. Olsen, JL, Stam, WT, Coyer, JA, Reusch, TBH, Billingham, M, Bostrom, C, Calvert, E, Christie, H, Granger, S, La Lumiere, R, Milchakova, N, Oudot-Le Secq, M-P, Procaccini, G, Sanjabi, B, Serrão, E, Veldsink, J, Widdecombe, S, Wyllie-Echeverria, S. 2004. North Atlantic phylogeography and large-scale populations differentiation of the seagrass *Zostera marina* L. *Molecular Ecology* 13: 1923-1941. IF 2004/2007=4.38/5.169, n°C=48.
7. Patarnello, T, Volckaert, F, Castilho, R. 2007. Pillars of Hercules: Is the Atlantic-Mediterranean transition a phylogeographic break? *Molecular Ecology* 16: 4426–4444. IF 2007=5.169, n° C=3.
8. Pearson, GA, Serrão, EA, Dring, M, Schmid, R. 2004. Blue and green-light signals for gamete release in the brown alga *Silvetia compressa*. *Oecologia* 138: 193-201. IF 2004/2007=2.90/2.973, n°C=3.
9. Tatarenkov, A, Bergström, L, Jönsson, R, Serrão, EA, Kautsky, L, Johannesson, K. 2005. Intriguing asexual life in the brown seaweed *Fucus vesiculosus*. *Molecular Ecology* 14: 647-651. IF 2005/2007=4.30/5.169, n°C=11.

10. Zardoya, R, Castilho, R, Grande, C, Favre-Krey, L, Caetano, S, Marcato, S, Krey, G, Patarnello, T. 2004. Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*) in the Mediterranean. *Molecular Ecology* 13: 1785-1798. IF 2004/2007=4.38/5.169, n°C=30.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Serrão, EA, Havenhand, J. Fertilization strategies. Chapter 3.1 in: Wahl M (ed.) *Hard bottom communities. Patterns, scales, dynamics, functions, shifts.* Ecological Studies, Springer Verlag. In press.

2. Hernández-García, E, Herrada, EA, Rozenfeld, AF, Tessone, CJ, Eguíluz, VM, Duarte, CM, Arnaud-Haond, S, Serrão, E. 2007. Evolutionary and Ecological Trees and Networks. In: *Nonequilibrium Statistical Mechanics and Nonlinear Physics.* Descalzi, O, Rosso, OA and Larrondo HA (Eds). AIP Conference Proceedings Volume 913, American Institute of Physics (New York, 2007), pp. 78-83.

3. Christensen, PB, Diaz-Almela, E, Diekmann, O. 2004. Can transplanting accelerate the recovery of seagrasses?. In: *European seagrasses: an introduction to ecology monitoring and management.* Borum, J, Duarte, CM, Krause-Jensen, D (Eds). Chapter 13, pp. 77-82, EU project EVK3-CT-2000-00044, ISBN 87-89143-21-3.

4. Kennedy, H, Papadimitriou, S, Marba, N, Duarte, C, Serrao, EA, Arnaud-Haond, S. 2004. How are seagrass processes, genetics and chemical composition monitored? In: *European seagrasses: an introduction to ecology monitoring and management.* Borum, J, Duarte, CM, Krause-Jensen, D (Eds). Chapter 9, pp. 54-62, EU project EVK3-CT-2000-00044, ISBN 87-89143-21-3.

**4. Master and Ph.D. thesis completed** (3000 ca.)

## **PhD THESIS**

Alberto, Filipe. 2005. Dispersal, sex and clonality in the marine environment: population genetic structure of the seagrass *Cymodocea nodosa* on Mediterranean and Atlantic coasts. University of Algarve. Co-supervisors: E Serrao, C Duarte (CSIC, Spain).

Billard, Emmanuelle. 2007. Evolution of reproductive strategies in closely related brown seaweeds. Co-tutored thesis, University of Paris VI (France) and University of Algarve. Co-supervisors: E Serrao, M Valero (CNRS, France).

Lago-Leston, Asuncion. 2007. The molecular basis for differential stress-tolerance in co-existing, ecologically similar algal species. University of Algarve. Co-supervisors: E Serrao, G Pearson (CCMAR).

## MSc THESIS

Caetano, Sofia. 2003. Diversidade molecular de *Salamandra salamandra* no Sul de Portugal. Mestrado em Conservação da Natureza, Universidade do Algarve. Supervisor: R Castilho.

Alexandre, Ana. 2004. Impacto da actividade de marisqueio na floração e esforço reprodutivo de *Zostera noltii* Hornemann na Ria Formosa. Mestrado em Estudos Marinhos e Costeiros (EMAC). Co-supervisors: E Serrao, R Santos, team ALGAE.

Frutuoso, Ana Luísa. 2006. Diversidade de macrofungos em sobreiral serrano do sul de Portugal: sua relação com a gestão florestal. Mestrado em Gestão e Conservação da Natureza, Universidade do Algarve. Co-supervisors: E. Serrao, M. Honrubia (Univ. Murcia), P. Beja (CIBIO, ERENA).

Fragoso, Bruno. 2007. Assessment of biofouling pressure in an oyster aquaculture longline. Mestrado em Biologia Marinha, Universidade do Algarve. Co-supervisors: E. Serrao, J. Icely (Sagremarisco).

Soares, Miguel. 2007. Análise da Variabilidade genética e demografia das espécies *Atherina presbyter* e *Atherina boyeri* (Família: Atherinidae). Mestrado em Biologia Marinha e Pescas, Universidade do Algarve. Supervisor: R. Castilho.

### **5. Patents/propotypes (2000 ca.)**

### **6. Organization of conferences (2000 ca.)**

### **7. Industry contract research (2000 ca.)**

### **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

### **9. Internationalization (2000 ca.)** (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

- USA-NSF Research Coordination Network - CORONA: Historical Ecology of the trans-Atlantic Marine Biota. 2002-2006. PI C.Cunningham. Scientific steering committee members: G.Pearson, E.Serrao.

Two team joint review papers published in Ecology (IF4.78).

EU Networks of Excellence:

- MARBEF (Marine Biodiversity and Ecosystem Function)



Projects:

- GBIRM-Genetic Biodiversity
- MARPACE-Marine Propagation Along the Coasts of Europe
- BIOFUSE-Effects of biodiversity on the functioning and stability of marine ecosystems
- MGE (Marine Genomics Europe)

Environmental genomics workpackage, Algal Node, coordinated by G.Pearson

Projects:

- Microsatellite development in algal species
- EST banks for studying adaptive evolution in *Fucus*
- Microarray development in *Fucus*

Bilateral exchange projects: 3 with CNRS, France (M.Valero) and 2 with CSIC, Spain (C.Duarte)

International graduate training:

Two PhD students under joint degree agreements Univ.Algarve-Univ.ParisVI (E.Billard, J.Neiva).

All our Portuguese PhD students have international co-supervisors.

Joint international publications:

Most of our projects (see funding above) include international collaborations with other international teams, such as for example:

Magoulas, A, Castilho, R, Caetano, S, Marcato, S, Patarnello, T. 2006. Mitochondrial DNA reveals a mosaic pattern of phylogeographical structure in Atlantic and Mediterranean populations of anchovy (*Engraulis encrasicolus*). *Molecular Phylogenetics and Evolution* 3: 734-746. IF=3.53, n°C=4.

Martinez, P, Gonzalez, EG, Castilho, R, Zardoya, R. 2006. Genetic diversity and historical demography of Atlantic bigeye tuna (*Thunnus obesus*). *Molecular Phylogenetics and Evolution* 2: 404-416. IF=3.53, n°C=6.

Rozenfeld, AF, Arnaud-Haond, S, Hernández-García, E, Eguíluz, VM, Serrão, EA, Duarte, CM. (accepted). Network analysis identifies weak and strong links in a metapopulation system. *Proceedings of the National Academy of Sciences USA*. IF=9.60, n°C=n.a.

Rozenfeld, AF, Arnaud-Haond, S, Hernández-García, E, Eguíluz, VM, Matías, MM, Serrão, EA, Duarte, CM. 2007. Spectrum of genetic diversity and networks of clonal organisms. *Journal of the Royal Society Interface* 4: 1093-1102. IF=3.09, n°C=0.

## 6e. Future research

### 1. Objectives (3000 ca.)

Besides continuation of past research objectives outlined above, we highlight here novel research objectives in newly funded projects:

Phylogeography, edge population genetics:

The level and influence of Pleistocene gene flow between North Africa and Europe (mainly Iberia and Southern Italy) will be determined in various organisms, including vertebrates and invertebrates with varying colonizing abilities.

Phylogeography and historical demography will be compared at retreating edges and leading edges of cold temperate and warm water organisms respectively, in coastal European waters. Main focus will be on key canopy-forming habitat structuring species and associated animal taxa.

Genetics of metapopulation systems:

Metapopulation genetics of forests of giant kelp *Macrocystis pyrifera*, that sustain one of the most diverse, productive, and dynamic ecosystems on the planet, will unravel gene-flow patterns at different spatial scales, from intra- to metapopulation scales in California, to global ocean biogeography, including various Pacific biogeographic boundaries and effects of the west wind drift that connects Pacific, Atlantic, and Indian ocean along the Southern Ocean.

Gene flow analyses within and between patches of Iberian kelp forests will infer reproductive ecology and assess population fragmentation. Kelp forest mapping is helped by volunteer participation (project FINDKELP).

Seagrass habitat fragmentation effects assessed on genetic structure and gene flow of associated seahorses and pipefishes that depend largely on seagrass habitat.

Deep sea connectivity:

Spatial distribution of intraspecific biodiversity in bony fish and sharks will be studied along continental slopes and Mid-Atlantic Ridge. Integrating genetic, phenotypic and oceanographic data will unravel processes shaping population connectivity patterns in the deep-sea, contributing to management of deep-sea fisheries and other anthropogenic pressures.

Phylogeny and phylogeography of deep-sea shrimp (Alvinocarididae) across Atlantic and Pacific deep-sea chemosynthetic ecosystems will trace back colonization pathways along ridges.

Invasive species genetics and bacterial interactions:

The evolution of invasive *Caulerpa* species and its associated bacterial flora will be compared in Australian (native range) and Mediterranean (invaded range) coasts, assessing also the possible flow of associated microorganisms with the native congeneric species of the Mediterranean.

Polar phytoplankton stress-responses:

In the Southern Ocean, an ocean disproportionately influencing global climate, genome-wide patterns of stress-responsive gene expression will be characterized in natural phytoplanktonic

assemblages with key roles in macronutrient fluxes and export of fixed carbon. In polar research cruises, experiments will be conducted with natural phytoplanktonic assemblages, in which major hypothesized bottom-up drivers of ecosystem function will be manipulated (Fe, Si, UVR, light).

Genomic responses near climate-induced tipping points for Arctic phytoplankton will be assessed as short-term responses of phytoplankton community transcriptome in situ during bloom-forming events, and for selected major bloom-forming diatoms (basis of Arctic food-webs). Deep transcriptome coverage in both polar projects will be achieved using improved Serial Analysis of Gene Expression (SuperSAGE).

**2. Funding, source, dates** (1500 ca.) (Indicate in full including amount of current and pending funding)

FP7-ENV-2008-1-226248. Arctic Tipping Points (ATP)–2009-2012, PI P.Wassmann, U.Tromsø, Nat. PI G. Pearson (at EU negotiation stage) (190.672 EUR).

MarinERA-189570. Marine phylogeographic structuring during climate change: the signature of leading and rear edge of range shifting populations (SHIFTING)-2008-2011, PI V. Almada, ISPA (78.000 EUR).

EuroCores 1 390. Unravelling population connectivity for sustainable fisheries in the Deep Sea (DEECON). 2007-2009, PI N.C. Stenseth (17.500 EUR).

PTDC/MAR/72630/2006. Environmental Genomics of Southern Ocean Phytoplankton Communities, Nov 2008-Nov 2011, PI G. Pearson (190.672 EUR).

PTDC/MAR/65461/2006. Molecular ecology of the giant kelp *Macrocystis pyrifera*, Oct 2008-Oct 2011, PI F. Alberto (191.441EUR).

PTDC/MAR/70921/2006. Population genetic structure, molecular phylogeny and historical biogeography in species of the genus *Caulerpa*... Oct 2008-Oct 2011, PI E. Varela (182.630 EUR).

PTDC/MAR/64749/2006. The success of invasive species: exploring the joint role of biotic interactions and of founder effect in *Caulerpa* species, Oct 2008-Oct 2011, PI S. Arnaud (187.140 EUR).

PTDC/BIA-BDE/74349/2006. Gene flow across the pillars of Hercules: the role of North Africa in the postglacial recolonization of Europe (HERCULES). 2008-2010, PI J. Harris (40.000 EUR).

PTDC/BIA-BDE/68730/2006. Spatial structure of amphibian (meta)populations in Mediterranean farmland. Jan 2008-Dec 2010. PI P. Beja, CIBIO (53.648 EUR).

Full genome sequencing of *Zostera marina*, PI J. Olsen, core partner E. Serrao. Joint Genome Institute (JGI)-Community Sequencing Program (CSP2009).

FINDKELP (3200 EUR). AWARE Foundation Europe.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Alberto, F, Massa, S, Manent, P, Diaz-Almela, E, Arnaud-Haond, S, Duarte, CM, Serrão, EA. 2008. Genetic differentiation and secondary contact zone in the seagrass *Cymodocea nodosa* across the Mediterranean-Atlantic transition region. *Journal of Biogeography* 35: 1279-1294. IF=3.54, n°C= 0.
2. Arnaud-Haond, S, Migliaccio, M, Diaz-Almela, E, Teixeira, S, van de Vliet, MS, Alberto, F, Procaccini, G, Duarte, CM, Serrão, EA. 2007. Vicariance patterns in the Mediterranean Sea: East-West cleavage and low dispersal in the endemic seagrass *Posidonia oceanica*. *Journal of Biogeography* 34: 963-976. IF=3.54, n°C=8.
3. Cunha, RL, Tenório, MJ, Afonso, C, Castilho, R, Zardoya, R. 2008. Replaying the tape: recurring biogeographical patterns in Cape Verde Conus after 12 million years. *Molecular Ecology* 17(3): 885-901. IF=5.17, n°C=0.
4. Maggs, CA, Castilho, R, Foltz, D, Henzler, C, Jolly, T, Olsen, J, Perez, KE, Stam, W, Vainola, R, Viard, F, Wares, J (accepted). Evaluating signatures of glacial refugia for North Atlantic Marine Organisms. *Ecology*. IF=4.78, n°C=n.a.
5. Schmidt, PS, Serrao, EA, Pearson, GA, Riginos, C, Rawson, PD, Hibish, TJ, Brawley, SH, Trussell, GC, Carrington, E, Wetthey, DS, Grahame, JW, Bonhomme, F, Rand, DM (accepted). Ecological genetics in the North Atlantic intertidal: environmental gradients, replicated clines, and adaptation at specific loci. *Ecology*. IF=4.78, n°C=n.a.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

CCMAR is fully equipped with the molecular, physiological, and ecological equipment and laboratory facilities to conduct our planned research when used together with our international collaborations, as in the examples below.

We will make use of international sequencing or genotyping facilities for fostering particular research lines, e.g., the Joint Genome Institute (JGI Community Sequencing Program, California) will provide full genome sequencing for *Zostera marina*; the Spanish National Genotyping Center (Santiago) will provide SNP genotyping of loci designed from our EST libraries from *Fucus* and *Z. noltii*, for which large-scale sequencing was conducted by the Max-Planck Institute for Molecular Genomics.

Our novel projects involve international collaborations that make it logistically possible to study various marine ecosystems throughout the world. Examples:

Collaboration with CSIC, Spain (Carlos Duarte, Susana Augusti) allow us access to polar research cruises and laboratory and shipboard experimental set-ups for our studies of phytoplankton transcriptomics stress-responses.

Collaborations with Univ. California (Pete Raimondi, Daniel Reed) provide us access to long term ecological research GIS data (LTER-SBC) on giant kelp *Macrocystis pyrifera*, to overlap with our gene flow data and describe landscape genetics of giant marine forests, whereby we will

contribute to design the first managed network of marine protected areas (MPAs) for the state of California.

Collaboration with IFREMER, Brest, project DeepOases (Daniel Desbruyères) provides us access to samples of shrimp from deep sea vents throughout the world's ridges, and characterization of their associated bacteria for co-evolution questions.

## **Ecology and Restoration of Estuarine and Coastal Habitats (ECOREACH)**

### **6a. Group description**

#### **1. Group name / denomination**

**Ecology and Restoration of Estuarine and Coastal Habitats (ECOREACH)**

#### **2. Principal investigator**

**Luis Manuel Zambujal Chicharo**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Marine Benthos and plankton ecology, Alien species, Ecological modelling, Coastal Ecohydrology and climate change

#### **5. Funding, source, dates (1000 ca.)**

6.000 EUR, Student guide for ecohydrology experiments, UNESCO, 2007.

10.000 EUR, Grant for research mobility under ecohydrology programme UNESCO, 2007.

42.620 EUR, project Nutritional condition of fish larvae in major protected marine areas of the South Portugal (Ria Formosa and Estuário do Guadiana) GUADIRIA FCT - POCI/BIA-BDE/59200/2004, 2005-2008.

10.000 EUR, Guadiana Demo site: EH solutions for the estuary and coastal areas. UNESCO-ICE-PAS, 2005–2007.

30.247 EUR, "Fishing impacts. Use of control areas", SAPIENS (POCTI/MGS/42319/2001), 2002-2006.

2.500 EUR, Contract UNESCO-ROSTE 875.842.5 – EH management models for the Guadiana estuary: Sub-model A: Using bivalves specific filtration rates to reduce algal blooms; Sub-model B: Using freshwater discharge pulses to eliminate toxic algal blooms; Sub-model C: Managing (dam) freshwater discharge to maintain/restore biodiversity and to enhance coastal fisheries, 2003.

5.000 EUR, Contract UNESCO-ROSTE 875.766.4 – EH Guadiana model (predictions of changes in biotic interactions as functions of different flow discharges), 2003.

## **6b. Group team**

### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Luis Manuel Zambujal Chicharo (Cat.: Professor Associado, Gr. Acad.: Agregação)

002. Maria Alexandra Anica Teodosio Chicharo (Cat.: Professor Associado, Gr. Acad.: Agregação)

003. Radhouane Ben Hamadou (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

004. Rita Alexandra Duarte Borges (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

### **2. Other researchers in the group (Include here collaborators with PhD. only)**

001. Pedro Alexandre Garcia Range (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

002. Pedro Miguel Coutinho Victorino Borges Morais (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

### **3. Other researchers in the group (non PhD.)**

001. Ana Margarida Araújo Amaral (Cat.: Assistente de Investigação, Gr. Acad.: Mestrado)

002. Ana Margarida da Silva Faria (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

003. Carmen Sofia Cardoso Mateus (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

004. David Veríssimo Piló (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

005. Eloise Abelha César de Sá (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

006. Rute Isabel Pinto de Brito (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

007. Tânia Angelina de Sousa Leitão (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

Our research is mainly focused on biological and physical-chemical processes in transitional waters, from freshwater catchments to coastal marine ecosystems. We are particularly interested in primary and secondary production, nutrient cycles, the characterisation of habitats, populations, trophic interactions and community dynamics. The purpose of our research is to understand the natural variability of aquatic ecosystems (e. g., tides, upwelling, winds) and the ecologic impacts by caused by human activities (fisheries, pollution, dams, alien species, etc). All this information is necessary to develop and test Ecohydrology ecosystem-based solutions for reverting degradation, sustaining functioning and promoting adaptation of aquatic environments to impacts, considering the current scenarios for climatic changes.



## 2. Main achievements (2000 ca.)

A deep understanding of how the quality, quantity and seasonality of freshwater discharges affect estuarine and coastal ecosystems was the main achievement of the group during 2003-2007. This information was integrated in ecological models that allow the evaluation of human impacts on ecosystems, by simulating different scenarios, in order to develop better guidelines and strategies for managing ecosystems. Particular emphasis was placed on the interactions between rivers and the coastal zone, through freshwater flow regimes. This knowledge was linked to human uses of these transitional waters, such as fisheries and tourism, and models were developed to mitigate impacts and improve the carrying capacity of these ecosystems. The issue of global changes and the predicted scenarios of intense retention of freshwater in dams were also studied using this new approach:

Model A. Ecohydrologic (EH) Guadiana model (predictions of changes in biotic interactions as functions of different flow discharges)

A 1- A ecohydrology model was proposed that integrates physical, chemical and biological processes in the Guadiana Estuary under conditions of reduced flow. It predicts “ecosystem health” as determined by the following variables: river discharge, nutrients, suspended particulate matter, phytoplankton, zooplankton, bivalves, zooplanktivorous fish and carnivorous/omnivorous fish... (+)

Model B. Ecohydrologic (EH) models to control phytoplankton succession

Two generic models to illustrate the major dynamic properties of phytoplankton succession under bottom-up and top-down control was developed using MATLAB 6.5 Software

The edition of a special issue in the journal *Estuarine and Coastal Shelf Science* about “Ecohydrology and Phytotechnologies approaches in Guadiana estuary” contributed to the dissemination of new management options for dams among the scientific community and governmental agencies.

In the field of ecology of fish larvae the major achievement of the group was the analysis of the effects of upwelling and river discharge on the condition of sardine larvae and the development of a light trap that allowed the capture of late clupeid larvae, which are usually not represented with common ichthyoplankton gear. This methodology, combined with RNA:DNA ratios to determine the eco-physiological condition of larvae, will contribute to advance our understanding about the mechanisms determining the variability of recruitment of small pelagic fish.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Garrido, S, Rosa, R, Ben-Hamadou, R, Cunha, ME, Chícharo, MA, van der Lingen, CD. 2007. Effect of maternal fat reserves on the fatty acid composition of sardine (*Sardina pilchardus*) oocytes. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 148(4): 398-409. IF 2007=1.651, n°C= 2.

2. Santos, AMP, Chícharo, MA, Dos Santos, A, Moita, T, Oliveira, P.B., Peliz, Á, Ré, P. 2007. Physical–biological interactions in the life history of small pelagic fish in the Western Iberia Upwelling Ecosystem. *Progress in Oceanography* 74(2-3): 192-209. IF 2007=2.102, n°C=3.
  3. Chícharo, L, Chícharo, MA, Ben-Hamadou, R. 2006. Use of a hydrotechnical infrastructure (Alqueva Dam) to regulate planktonic assemblages in the Guadiana estuary: basis for sustainable water and ecosystem services management. *Estuarine Coastal and Shelf Science* 70 (1-2): 3-18. IF 2007=1.799, n°C=2.
  4. Chícharo, MA, Chícharo, L, Morais, P. 2006. Inter-annual differences of ichthyofauna structure of the Guadiana estuary and adjacent coastal area (SE Portugal/SW Spain): before and after Alqueva dam construction. *Estuarine Coastal and Shelf Science* 70 (1-2): 39-51. IF 2007=1.799, n°C=4.
  5. Murphy, RJ, Underwood, AJ, Pinkerton, MH, Range, P. 2005. Field spectrometry: New methods to investigate epilithic micro-algae on rocky shores. *Journal of Experimental Marine Biology and Ecology* 325: 111-124. IF 2007=1.750, n°C=3.
  6. Ben Hamadou, R, Ibanez, F, Picheral, M, Gorsky, G. 2005. Identification of water columns boundaries. An application of the Walsh transform to Underwater Video Profiler-data from NE Atlantic. *Ecological Modelling* 182(1): 11-24. IF 2007=2.077, n°C=2.
  7. Wolanski, E, Boorman, LA, Chícharo, L, Langlois-Saliou, E, Lara, R, Plater, AJ, Uncles, RJ, Zalewski, M. 2004. Ecohydrology as a new tool for sustainable management of estuaries and coastal waters. *Wetlands Ecology and Management* 12(4): 235-276. IF 2007=, n°C=4.
  8. Chicharo, MA, Amaral, A, Condinho, S, Alves, F, Regala, J, Gaspar, M, Chicharo, L. 2003. Adenylic-derived indices and reburying time as indicators of the effects of dredging-induced stress on the clam *Spisula solida*. *Marine Biology* 142: 1113-1117. IF 2007=2.215, n°C=3.
  9. Chícharo, MA, Esteves, AE, Santos, MP, dos Santos, A, Peliz, A, Ré, P. 2003. Are sardine larvae caught during a winter upwelling event off northern Portugal starving? An approach using RNA/DNA ratios. *Marine Ecology Progress Series* 257: 303-309. IF 2007=2.32, n°C=10.
  10. Gaspar, MB, Leitaó, F., Santos, M. N., Chicharo, L., Dias, MD, Chicharo, A, Monteiro, C. 2003. A comparison of direct macrofaunal mortality using three types of clam dredges. *Ices Journal of Marine Science* 60: 733-742. IF 2007=1.934, n°C=3.
- Morais, P, Chicharo, MA, Barbosa, A. 2003. Phytoplankton dynamics in a coastal saline lake (SE-Portugal). *Acta Oecologica* 24: S87-S96. IF 2007=1.306, n°C=7.

**2. Other publications national (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)**

1. Sadat, M, Domingues, R, Chícharo, M, Chícharo, L. 2006. Roteiro Ecológico da Ria Formosa, Vol. V, Unicululares, Edição Universidade do Algarve/CIMA, 89 p.
2. Chicharo, MA. 2005. Desenvolvimento de novos métodos de amostragem de larvas de peixe no meio marinho. *Mudança Global Notícias*, volume de nº 2 Outubro.

3. Bejaoui, B, Ben-Charrada, R, Moussa, M, Ben-Hamadou, R, Harzalla, A, Chapelle, A. 2005. Caracterisation hivernale de la lagune de Bizerte. Bulletin de l'Institut National des Sciences et Technologies de la Mer (Tunisia) 33: 26-39.

**3. Other publications international (3000 ca.)** (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Wolanski, E, Chicharo, L, Chicharo, MA. 2007. Ecological Engineering: Estuarine Ecohydrology. Chapter for the "Encyclopaedia of Ecology" (Ed. S. Jorgensen), Elsevier.

2. Gaspar, M, Chicharo, L. 2007. Modifying dredges to reduce by-catch and impacts on the benthos. In: By-catch reductions in the World's Fisheries. Steve Kennelly (Ed), vol 7, pp. 95-140, Springer.

3. Chicharo, L, Chicharo, MA, Ben Hamadou, R, Morais, P. 2005. Ecohydrology in estuaries and coastal areas: the Guadiana estuary case. In: Water and Ecosystems: Water Resources Management in Diverse Ecosystems and Providing for Human Needs. UNU/INWEH publications. 13 pp.

4. Ben-Hamadou, R, Chicharo, L, Chicharo, M. 2005. Characterization of the zooplankton community on the Algarve coastal zone - Relation with the Mediterranean. Proceedings do workshop MEDZOO Impact du changement climatique sur le zooplankton mediterraneen, 26-30 October 2005, Villefranche Sur Mer, p. 3.

5. Chicharo, L. 2004. Estuarine & Coastal Areas: How To Prevent Degradation And Restore. In: Integrated Watershed Management - Ecohydrology & Phytotechnology – Manual. Zalewski, M, Wagner-Lotkowska, I. (Eds), Chapter 13, pp. 202-208, Japan, UNEP - UNESCO – IHP.

6. Chicharo, MA, Chicharo, L. 2004. Estuarine & coastal areas.: How & What to measure. In: Integrated Watershed Management - Ecohydrology & Phytotechnology – Manual. Zalewski, M, Wagner-Lotkowska, I. (Eds), Chapter 8, pp. 124-131, Japan, UNEP - UNESCO – IHP.

7. Wagner-Lotkowska, I, Magnuszewski, A, Kaczmarek, Z, Trojanowska, A, Krauze, K, Lapinska, M, Izydorczyk, K, Wojtal, A, Frankiewicz, P, Chicharo, L. 2004. Basic concepts and definitions. In: Integrated Watershed Management - Ecohydrology & Phytotechnology - Manual. Zalewski, M, Wagner-Lotkowska, I. (Eds), Chapter 3, pp. 30-40, Japan, UNEP - UNESCO – IHP.

**4. Master and Ph.D. thesis completed (3000 ca.)**

**PhD THESIS**

Borges, Rita. 2006. Composition, Temporal and Spatial Patterns of Very nearshore Larval Fish Assemblages at the Arrábida Marine Park. PhD thesis, University of Algarve. Supervisors: MA Chicharo, E Gonçalves (ISPA), P Ré (UL).

Morais, Pedro. 2007. Engraulis encrasicolus sensus lato population dynamics in the Guadiana estuary and adjacent coastal area. PhD thesis, University of Algarve. Supervisors: MA Alexandra Chicharo, L Chicharo.

Garrido, Susana. 2007. Trophic Ecology of the Atlanto-Iberian Sardine (*Sardina pilchardus*). PhD thesis, University of Algarve. Supervisors: MA Chícharo, E Cunha (IPIMAR), Carl Van der Lingen (Offshore Resources Marine and Coastal Management South Africa).

Moschino, Vanessa. 2007. Physiological impact of clams fishery in the Venice lagoon. PhD thesis, University of Algarve. Supervisors: L Chícharo, M Gabriella Marin (University of Padova).

## **MSc THESIS**

Xavier, Barbara. 2007. Indicadores de eutrofização em comunidades bentónicas na Ria Formosa. Master thesis, University of Algarve. Supervisors: L Chícharo, F Martins (CIMA).

Piló, David. 2007. Análise do impacto da ETAR de Faro Noroeste (Montenegro) nas comunidades bentónicas. Master thesis in Marine biology, University of Algarve. Supervisors: L Chícharo, MA Chícharo.

Pedro, Tania. 2007. Padrões de Crescimento em Otólitos de Larvas de *Pomatoschistus pictus* (Pisces, Gobiidae). Master Thesis in Marine Biology, University of Algarve. Supervisors: MA Chícharo, E Gonçalves (ISPA).

Monteiro, Joana. 2005. Descrição do desenvolvimento larvar de *Gobius xanthocephalus* (Pisces: Gobiidae). Master Thesis in Marine Biology, University of Algarve. Supervisors: MA Chícharo, E Gonçalves (ISPA).

Dias, Joana. 2004. Contribuição para o estudo da pescaria de *Solen marginatus* na Ria Formosa. Master thesis in Marine Biology, University of Algarve. Supervisors: L Chicharo, A Amaral.

Faria, Ana. 2004. Estudo do ictioplâncton do estuário do Guadiana e zona costeira adjacente. Master Thesis in Marine biology, University of Algarve. Supervisors: MA Chicharo, P Morais.

## **5. Patents/propotypes (2000 ca.)**

## **6. Organization of conferences (2000 ca.)**

4th Meeting of UNESCO Working Group on Ecohydrology of Estuaries and Coastal Areas - WG – Tulcea Danubio , Romania, 5-8 October 2006 (Scientific organization and Co-Chairman by L Chicharo)

1st Task forces on Ecohydrology (TF's) in the CCMAR/University of Algarve, between 7 and 10 June 2006 (Local organization by L Chícharo)

1º Workshop of Integration 4water Specific Support Action in March 2006 Kartowice (LChícharo- Moderator of Global changes Group)

2º Workshop of Integration4waterSpecific Support Action in June 2006 Varsóvia (L Chícharo Moderator of Global changes Group)

3rd meeting UNESCO Working Group on Ecohydrology of Estuaries and Coastal Areas - Split, Croatia, September 2005 (Scientific organization and Co-Chairman)

2nd meeting Working Group on Ecohydrology of Estuaries and Coastal Areas – Faro, Portugal, September 2004 (Organization and Co-Chairman)

Workshop “Managing the Guadiana estuary – the Ecohydrology and Phytotechnology approaches” University of the Algarve, Faro 1-3 Sept. 04 (Organization L Chicharo, MA Chicharo, E Wolanski)

**7. Industry contract research (2000 ca.)**

**8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

**9. Internationalization (2000 ca.)** (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

The Internationalization of the group was achieved by the leading role of Luis Chicharo on the task force of coastal systems in the Ecohydrology (EH) Programme of UNESCO Paris - IHP, on the Working Group on Ecohydrology of Estuaries and Coastal Areas within the Ecohydrology Programme of UNESCO ROSTRE and by the participation as Demosite leader of the Guadiana Estuary, EH solutions for the estuary and coastal areas. UNESCO-ICE-PAS by MA Chicharo in the framework of Ecohydrology Demosites project.

The group developed research in collaboration with abroad teams, namely in Italy, Spain, Tunisia, France, as is reflected in published papers or supervised PhD thesis and submitted proposal for collaborative projects.

Ecoreach members participate in several national and international scientific networks, mainly:

DivMar: a National Biodiversity Network for Portuguese marine and coastal biodiversity established in 2007

MedZoo: MedZoo is an association of scientists willing to collaborate on the harmonisation of methods for study of the evolution of Mediterranean and Black Sea zooplankton in time and space. Medzoo was created during the 37th CIESM congress in Barcelona in June 2004 and is functioning through the CIESM program on Zooplankton Indicators.

WssTP: The Water supply and sanitation Technology Platform (WssTP) is a European initiative for European Research and Technology Development in the water industry.

## **6e. Future research**

**1. Objectives (3000 ca.)**

Restoration techniques - To study new species and eco-technologies that can be used to restore coastal ecosystems and improve water quality

Secondary production- To study new methods for estimating secondary production and understating processes in planktonic food webs, namely by developing new production indices for copepods egg. The interaction between planktonic and benthonic food web in estuarine and coastal areas will also be investigated.

Fish larvae trophodynamics- New trophic links between fish larvae and plankton will be also a topic for research, especially alternative links between bacteria-phytoplankton and ciliates, instead of traditional phyto-nauplii-fish larvae links.

New methods for estimate secondary production, mainly zooplanktonic through copeds egg production and RNA/DNA ratios will be developped.

Global change in marine ecosystems - Impacts of alien species on coastal ecosystems, particularly for invertebrates and Effects of climate changes on coastal ecosystems, such as marine acidification on invertebrates.

The projected research is intended to be formally associated with the activity of a future International Centre for Coastal Ecohydrology (ICCE) under the UNESCO auspices, at the University of Algarve and having as an objective a close collaboration in fomenting and better disseminate research in coastal sciences with the CIMAR LA. This collaboration would enhance the internationalisation of the produced research and applications and build synergetic partnership with regional and international water related stakeholders.

## **2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

1- Project approved in March 2008- The integrated impacts of marine acidification, temperature and precipitation changes on coastal biodiversity and fisheries: how to adapt? (ACID-BIV). An ERA-NET CIRCLE-MED project Funding: 199.962,90 EUR - (FCT), Portugal; Italian Ministry for Environment, Land and Sea (IMELS), Italy; Regional Ministry of Innovation and Industry of the Galician Government trough the Directorate General for Research, Development and Innovation (XUNTA), Spain. Partners: Ecoreach, CCMAR (project leader); Consejo Superior de Investigaciones Cientificas (CSIC). Vigo, Spain; Department of Biology - University of Padova; Faculty of Science of Bizerta. Tunisia.

2- Bilateral collaborative project approved in 2008: Coastal ecology and global changes. A comparative study between Guadiana and Medjerda coastal zones (GUAMED). A bilateral FCT-MESRST project (Portugal-Tunisia). Funding: funds for exchange of researcher and graduated students approved and provided by: GRICES. Portugal; MESRST - Ministère de l'Enseignement Supérieur, de la Recherche Scientifique et de la Technologie. Tunisia. Partners: Ecoreach, CCMAR and Faculty of Science of Bizerta. Tunisia.

3- Pending funding:

- The Era-Net MarinERA call for international research project: Ecosystem indicator to reveal ecological impacts of climate change in coastal areas of the Mediterranean and Gulf of Cadiz (ECOSIND). Requested funds: 279.880 EUR. Partners: Ecoreach (CCMAR) and National Agricultural Research Foundation – Fisheries Research Institute (NAGREF-FRI) - Greece.

- Bilateral collaborative project: Coastal planktonic ecology and global. A comparative study between western Mediterranean and the Adjacent basin Gulf of Cadiz “CoPeG”. Requested



funds: exchange of researcher and graduated students under the PESSOA France (EGIDE)-Portugal (GRICES) program. Partners: Ecoreach (CCMAR) and Laboratoire d'Océanographie de Villefranche sur Mer (LOV-France).

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Garrido, S, Ben-Hamadou, R, Oliveira, PB, Cunha, ME, Chícharo, MA, van der Lingen, CD. 2008. Diet and feeding intensity of sardine *Sardina pilchardus*: correlation with satellite-derived chlorophyll data. *Marine Ecology Progress Series* 354: 245-256. IF 2007=2.32, n°C=1.

2. Garrido, S, Rosa, R, Ben-Hamadou, R, Cunha, ME, Chícharo, MA, van der Lingen, CD. 2008. Spatio-temporal variability in fatty acid trophic biomarkers in stomach contents and muscle of Iberian sardine (*Sardina pilchardus*) and its relationship with spawning. *Marine Biology* 154: 1053-1065. IF 2007=2.25, n°C=0.

3. Moschino, V, Marin, MG, Chícharo, L. 2008. Effects of hydraulic dredging on the physiological responses of the target species *Chamelea gallina* (Mollusca, Bivalvia): laboratory experiments and field surveys. *Scientia Marina* 72(3): 493-501. IF 2007=1.02, n°C=0.

4. Chicharo, MA, Chicharo, L. 2008. RNA:DNA Ratio and Other Nucleic Acid Derived Indices in Marine Ecology. *International Journal of Molecular Sciences* 9: 1453-1471; Review. IF 2007=0.75, n°C=0.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)



## **Marine Plant Ecology Research Group (ALGAE)**

### **6a. Group description**

#### **1. Group name / denomination**

**Marine Plant Ecology Research Group (ALGAE)**

#### **2. Principal investigator**

**Rui Orlando Pimenta Santos**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Carbon fluxes, Population ecology, Ecophysiology, Seaweed taxonomy

#### **5. Funding, source, dates (1000 ca.)**

Envirophyte FP6-2004-SME-COOP, 2006-2008, 113.000 EUR;

R.C. Hidromod/Electricidade de Portugal, 2006-2008, 100.000 EUR;

POCTI/MAR/58172/2004, 2005-2008, 95.000 EUR;

POCI/MAR/58427/2004, 2005-2008, 73.800 EUR;

POCTI/MAR/55377/2004, 2005-2008, 85.500 EUR;

R.C. Parque Natural da Ria Formosa, ICN, 2006-2007, 5.000 EUR;

R.C. HIDROTEC, 2005, 25.000 EUR;

POCTI/MAR/56956/2004, 2005-2008, 81.000 EUR;

EqEA - R.C. Instituto do Ambiente, 2003-2005, 50.000 EUR;

FILTRALGAE Ag de Inovação, 2004-2007, 119.428 EUR;

BISHOP PTDC/CTE-GEX/70448/2006, 2007-2010, 120.000 EUR;

CGL2006-03576/BOS, Ministerio de Educación y Ciencia, Spain, 2007-2011, 9.100 EUR;

LIFE06 NAT P 192 – BIOMARES, 2007-2011, 207.817 EUR;

CLONMACMORPH – Marie Curie Grant nr: 006385, 2004, 34.000 EUR;

Contract Instituto Portuário do Sul, 2004, 5.000 EUR;

Contract CODIF, 2004, 5.000 EUR;  
 POCTI/BSE/48918/2002, 2003-2006, 92.000 EUR;  
 GRICES/CSIC bilateral cooperation project, 2006-2007, 2.800 EUR;  
 ICCTI/CSIC, bilateral cooperation project, 2002-2003, 2.800 EUR;  
 Cooperation Agreement GRICES/CSIC, FCT, 2003-2004, 2.400 EUR;  
 ALIENS - EVK3-2001-0008, EU 2002-2005, 177.000 EUR;  
 SEAPURA - QLRT-1999-31334, EU 2001-2004, 150.000 EUR;  
 PDCT/P/Mar/15299/1999, 2000-2004, 20.000 EUR;  
 M&MS- EVK3-CT-2000-00044, 1999-2003, 330.000 EUR.

## **6b. Group team**

### **1. Researchers in the group (Include only PhD. integrated in the LA)**

- 001. Rui Orlando Pimenta Santos (Cat.: Professor Associado, Gr. Acad.: Agregação)
- 002. João Miguel Sousa da Silva (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)
- 003. Abraham Pérez Pastor (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 004. Aschwin Hillebrand Engelen (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 005. Erik-jan Malta (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

### **2. Other researchers in the group (Include here collaborators with PhD. only)**

- 001. Susana Isabel dos Santos Cabaço (Cat.: Investigador Auxiliar Gr. Acad.: Doutoramento)
- 002. Raquel Carmona Fernandez (Cat.: Outra Gr. Acad.: Doutoramento)

### **3. Other researchers in the group (non PhD.)**

- 001. Ana Isabel Delfim dos Santos Alexandre (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 002. Ana Mafalda Rocha Tavares (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 003. Andreas Guntram Jurgen Schunhoff (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 004. Carla Alexandra da Silva Monteiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 005. Cecile Jeanne Benase Godinho (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

006. Custódia Maria Luís Gago (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
007. Diogo Filipe da Silva Gonçalves Soares Paulo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
008. Diogo Manuel Rocha Tavares (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
009. Estibaliz Berecibar (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
010. Inês Paixão Nunes Figueiredo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
011. Joana Ruela Boavida (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
012. Leonardo Filipe Rodrigues da Mata (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
013. Maria Raquel Assuncao Goncalves Machas (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
014. Monya Mendes Costa (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
015. Pedro Luís Feijóo Botelho (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
016. Vasco Manuel Nobre de Carvalho da Silva Vieira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
017. Helena Maria Guerreiro Pires Barracosa (Cat.: Outra, Gr. Acad.: Mestrado)
018. Pedro António Nobre Soares Pinto das Neves (Cat.: Outra, Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

#### **1. Metabolism of coastal systems:**

The research on the metabolism of coastal systems such as Ria Formosa lagoon aimed the role of macrophytes, particularly seagrasses in the organic matter, nutrients and gas (e.g. O<sub>2</sub> and CO<sub>2</sub>) fluxes in the ecosystem. The understanding of ecosystem functioning allowed us to address important processes, such as the carbon and nitrogen budgets and the connectivity with adjacent systems. The underlying mechanisms involved in the seagrass meadows production were investigated in particular their photosynthetic ecophysiology and nitrogen uptake.

#### **2. Human impacts on coastal Systems:**

This research line focused on the effects of the human-related disturbances, including the introduction of invasive species on the coastal ecosystems of southern Portugal. Our aim was to contribute with sound scientific inputs to the conservation and management of these systems. In particular, we investigated the effects of urban effluents, sedimentation disturbances and clam harvesting on the population ecology and biology of seagrasses and the biological and population ecology traits of one of the most invasive marine algae, *Sargassum muticum*.

#### **3. Economic valorisation of seaweeds:**

The research on the economic valorisation of seaweeds focused both on the red alga *Gelidium sesquipedale*, which is harvested in Portugal for the extraction of agar and on the integrated aquaculture of seaweeds in fish farms, in order to biofiltrate their effluents and to produce economically valuable biomass. We aimed to establish an integrated cultivation system in a fish farm and to investigate the ecophysiological controls of biomass and halogen production of *Bonnemaisoniales* species. The photosynthetic mechanisms of inorganic carbon acquisition by the species were studied in order to understand if the species is limited by carbon under mass outdoor cultivation conditions.

#### 4. Effects of global change on the Portuguese marine flora:

The objective was to evaluate the long-term changes in the benthic marine flora by comparing the marine flora of the 23 sites along the Portuguese coast that were described by Ardre in the 1960's with the present situation. Range shifts of species, variations in their abundance or new introductions were analysed to test the hypotheses that the richness and abundance of warm-water species has increased, as opposed to cold-water species. Furthermore, it was hypothesized that the distribution range of the species that have their geographical limits in the coast of Portugal, has shifted northwards.

### **2. Main achievements (2000 ca.)**

Our main achievements resulted in 45 scientific publications. Nine Ph D and 10 Masters thesis were coordinated from which 3 and 8, respectively, are concluded.

We developed novel, in situ, methodologies based on continuous monitoring of CO<sub>2</sub> and O<sub>2</sub> and on chlorophyll fluorescence of photosystem II, to assess the production and respiration of biological communities of coastal systems. Whole system carbon budgets including the fluxes with adjacent systems were estimated. This involved the use of aerial photography and GIS tools to map biological communities. Using stable isotopes we traced the flow of organic matter from primary producers to consumers revealing the relative importance of primary producers and allochthonous organic matter to the food web. The underlying mechanisms involved in the production of marine plants, such as the photosynthetic rate response to daily and seasonal variation of environmental factors, the interaction between the use of light and inorganic carbon acquisition and the nitrogen uptake, were revealed.

In close collaboration with national conservation and management authorities and private companies, we have contributed with sound scientific inputs to manage the human impacts on coastal systems, such as the urban effluents, clam harvest and sediment disturbances on seagrasses and the commercial harvest of seaweed. Main contributions were done to the invasion dynamics and interactions with native biota of a seaweed invader or to the control of nuisance seaweed that causes serious fouling problems to an electrical power plant.

We developed integrated seaweed/fish cultivation systems for biofiltration of effluents and production of seaweed biomass with important cosmetic applications. A patent of this technology was submitted and a prize for a science spin-off was received. In the ambit of the global warming effects on the marine flora we revealed that the phytogeographic distribution limits along the Portuguese coast moved northwards in the last four decades. Specific changes in the marine flora were thoroughly documented. This data base and the expertise achieved on seaweed taxonomy will constitute the basis for the development of metrics for monitoring the ecological quality of coastal water bodies under the European Water Framework Directive.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Mata, L, Silva, J, Schuenhoff, A, Santos, R. 2007. Is the tetrasporophyte of *Asparagopsis armata* limited by inorganic carbon in integrated aquaculture? *Journal of Phycology* 43: 1252-1258. IF 2007=2.820, n°C=0.
2. Cabaço, S, Machás, R, Santos, R. 2007. Biomass-density relationships of the seagrass *Zostera noltii*: a tool for monitoring anthropogenic nutrient disturbance. *Estuarine Coastal and Shelf Science* 74: 557-564. IF 2007=1.799, n°C=0.
3. Cabaço, S, Santos, R. 2007. Effects of burial and erosion on the seagrass *Zostera noltii*. *Journal of Experimental Marine Biology and Ecology* 340: 204-212. IF 2007=1.750, n°C=2.
4. Carmona, R, Santos, R. 2006. Is there an ecophysiological explanation for the gametophyte-tetrasporophyte ratio in *Gelidium sesquipedale* (Rhodophyta)? *Journal of Phycology* 42: 259-269. IF 2007=2.820, n°C=2.
5. Cabaço, S, Alexandre, A, Santos, R. 2005. Population level effects of clam harvesting on the seagrass *Zostera noltii*. *Marine Ecology Progress Series* 298: 123-129. IF 2007=2.546, n°C=4.
6. Alexandre, A, Santos, R, Serrão, E. 2005. Effect of clam harvesting activity on the flowering of *Zostera noltii* in Ria Formosa (Southern Portugal). *Marine Ecology Progress Series* 298: 115-122. IF 2007=2.546, n°C=4.
7. Silva, J, Santos, R, Calleja, M, Duarte, CM. 2005. Submerged versus air-exposed intertidal macrophyte productivity: from physiological to community-level assessments. *Journal of Experimental Marine Biology and Ecology* 317: 87-95. IF 2007=1.750, n°C=3.
8. Santos, R, Silva, J, Alexandre, A, Navarro, N, Barrón, C, Duarte, CM. 2004. Ecosystem metabolism and carbon fluxes of a tidal-dominated coastal lagoon. *Estuaries* 27: 977-985. IF 2007=2.133, n°C=8.
9. Alexandre, A, Silva, J, Santos, R. 2004. The maximum nitrate reductase activity of the seagrass *Zostera noltii* (Hornem.) varies along its vertical distribution. *Journal of Experimental Marine Biology and Ecology* 307: 127-135. IF 2007=1.750, n°C=4.
10. Silva, J, Santos, R. 2003. Daily variation patterns in seagrass photosynthesis along a vertical gradient. *Marine Ecology Progress Series* 257: 37-44. IF 2007=2.546, n°C=17.

2. **Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Stigter, T, Dill, A, Malta, E, Santos, R. 2006. Quantificação da descarga de nutrientes de azoto e fósforo para a Ria Formosa por escoamento superficial. In: Proc. V Congresso Ibérico sobre Gestão e Planeamento da Água, 12 pp., CD-ROM.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Santos, R. 2004. Marine Benthic Flora. In Marine Ecology. In: Encyclopaedia of Life Support Systems (EOLSS). Carlos M Duarte (Ed), Developed under the auspices of the UNESCO, Eolss Publishers, Oxford, UK, <http://www.eolss.net>.
2. Duarte, CM, Marbà, N, Santos, R. 2004. What may cause loss of seagrasses? In: European seagrasses: an introduction to monitoring and management. Borum, J, Duarte, CM, Krause-Jensen, D, Greve, TM (Eds), pp. 24-32. EU project Monitoring and Managing of European Seagrass Beds (M&MS), EVK3-CT-2000-00044, [www.seagrasses.org](http://www.seagrasses.org).
3. Borum, J, Greve, TM, Binzer, T, Santos, R. 2004. What can be done to prevent seagrass loss? In: European seagrasses: an introduction to monitoring and management. Borum, J, Duarte, CM, Krause-Jensen, D, Greve, TM (Eds), pp. 67-71. EU project Monitoring and Managing of European Seagrass Beds (M&MS), EVK3-CT-2000-00044, [www.seagrasses.org](http://www.seagrasses.org).
4. Marbà, N, Duarte, CM, Alexandre, A, Cabaço, S. 2004. How do seagrasses grow and spread? In: European seagrasses: an introduction to monitoring and management. Borum, J, Duarte, CM, Krause-Jensen, D, Greve, TM (Eds), pp. 11-18. EU project Monitoring and Managing of European Seagrass Beds (M&MS), EVK3-CT-2000-00044, [www.seagrasses.org](http://www.seagrasses.org).
5. Cunha, AH, Duarte, CM, Krause-Jensen, D. 2004. How long it takes to recolonize seagrasses? In: European seagrasses: an introduction to monitoring and management. Borum, J, Duarte, CM, Krause-Jensen, D, Greve, TM (Eds), pp.72-76. EU project Monitoring and Managing of European Seagrass Beds (M&MS), EVK3-CT-2000-00044, [www.seagrasses.org](http://www.seagrasses.org).
6. Krause-Jensen, D, Almela, ED, Cunha, AH, Greve, TM .2004. Have seagrass distribution and abundance changed? In: European seagrasses: an introduction to monitoring and management. Borum, J, Duarte, CM, Krause-Jensen, D, Greve, TM (Eds), pp. 33-40. EU project Monitoring and Managing of European Seagrass Beds (M&MS), EVK3-CT-2000-00044, [www.seagrasses.org](http://www.seagrasses.org).
7. Krause-Jensen, D, Quaresma, AL, Cunha, AH, Greve, TM. 2004. How are seagrass distribution and abundance monitored? In: European seagrasses: an introduction to monitoring and management. Borum, J, Duarte, CM, Krause-Jensen, D, Greve TM (Eds), pp. 45-53. EU project Monitoring and Managing of European Seagrass Beds (M&MS), EVK3-CT-2000-00044, [www.seagrasses.org](http://www.seagrasses.org).
8. Silva, J, Santos, R. 2003. Comparative ecophysiology of *Gelidium sesquipedale* (Rhodophyta) erect fronds and prostrate system. In: Proceedings of the International Seaweed Symposium, Cape Town, 2001. Chapman, AR, Anderson, RJ, Vreeland, VJ, Davison IR (Eds), Oxford University Press, New York, pp. 417-424.
9. Mata, L, Santos, R. 2003. Cultivation of *Ulva rotundata* (Ulvales, Chlorophyta) in raceways, using semi-intensive fishpond effluents: yield and biofiltration. In: Proceedings of the International Seaweed Symposium, Cape Town, 2001. Chapman, AR, Anderson, RJ, Vreeland, VJ, Davison, IR (Eds), Oxford University Press, New York, pp. 237-242.

10. Santos, R, Cristo, C, Jesus, D. 2003. Stock assessment of the agarophyte *Gelidium sesquipedale* using harvest effort statistics. In Proceedings of the International Seaweed Symposium, Cape Town, 2001. Chapman, AR, Anderson, RJ, Vreeland, VJ, Davison, IR (Eds), Oxford University Press, New York, pp. 145-150.

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Cabaço, Susana. 2007. Impact of anthropogenic activities on the seagrass *Zostera noltii*. PhD Thesis, University of Algarve. Supervisor: R Santos.

Machás, Raquel. 2007. Isotopic tracking of sources in coastal systems / special emphasis to *Zostera noltii* (Horneman) food web. PhD Thesis, University of Algarve. Supervisors: R Santos, Bruce Peterson (The Ecosystems Center, Marine Biological Laboratory, MA, USA).

Silva, João. 2004. The photosynthetic ecology of the seagrass *Zostera noltii*. PhD Thesis, University of Algarve. Supervisor: R Santos.

##### **MSc THESIS**

Guimarães, Maria Helena. 2007. Contribuição para a elaboração de um plano de gestão integrada dos campos de *Z. noltii* na Ria Formosa. Master in Marine Biology, University of Algarve. Supervisors: R Santos, A Cunha (MAREE, CCMAR), J Marques (Fac. Economy, University of Algarve).

Monteiro, Carla. 2007. Reproductive ecology of *Sargassum muticum* (Yendo Fensholt) in Viana do Castelo (Northern Portugal). Master in Biology and Ecology of Marine Shoreline, University of Évora. Supervisors: R Santos, A Engelen.

Paulo, Diogo. 2007. The compared photosynthetic performances of the invasive *Sargassum muticum* and the native seagrasses of southern Portugal. Master in Marine Biology, University of Algarve. Supervisors: R Santos, J Silva, A Engelen.

Boavida, Joana Ruelva. 2007. Population model of the recent subtidal invasion of *Sargassum muticum* in southern Portugal. Master in Marine Biology, University of Algarve. Supervisors: R Santos, A Engelen.

Rosa, Ana. 2007. Estudo da flora algológica do intertidal da Praia da Vigia. Master in Biology and Geology for Teachers, University of Algarve. Supervisors: R Santos, J Silva.

Vieira, Vasco. 2005. Population modeling of bi-phasic life cycles (*Gelidium sesquipedale*). Master in Coastal Marine Studies, University of Algarve. Supervisor: R Santos.

Alexandre, Ana. 2005. Effects of clam digging on the reproduction ecology of the seagrass *Zostera noltii*. Master in Coastal Marine Studies, University of Algarve. Supervisor: R Santos.

Mata, Leonardo. 2004. Integrated aquaculture of the seaweed *Ulva rotundata* (Ulvales, Chlorophyta) using fish farm effluents. Master in Aquaculture, University of Algarve. Supervisor: R Santos.



## **5. Patents/prototypes (2000 ca.)**

Lognone, V, Patrick, D, Lunning, K, Santos, R, Mata, L, Bansemir, A, Schuenhoff, A, Lindequist, U. 2003. Procédé de production a terre des algues rouges de la famille des Bonnemaisoniacees. Submission n° 0308717, Institut National de la Propriété Industrielle, Paris, France. Later, the consortium decided to stop the application process.

## **6. Organization of conferences (2000 ca.)**

2007. Applied Phycology Symposia, European Phycological Congress (EPC).

2006. ESF LESC Exploratory Workshop. Invasion of European Shores by *Sargassum muticum*: Research Integration Towards the Future, Tavira, Portugal.

2006. 11º Encontro Nacional de Ecologia, Lisboa, Portugal.

2006. Seminário dos Equipamentos para a Educação Ambiental: qualidade e inovação. University of Algarve, Faro, Portugal.

2005. Invasive algae symposium at the 8th International Phycological Congress, Durban, África do Sul.

2004. Equipamentos para a Educação Ambiental em Portugal: Que realidades? Que perspectivas? Instituto do Ambiente, Lisboa, Portugal.

2003. Workshop on Production in Seaweeds Purifying Effluents from Marine Animal Holding Units, Universidade do Algarve, Faro, Portugal.

## **7. Industry contract research (2000 ca.)**

2006. Management of the macroalgal communities that contribute to the fouling of Sines Power Plant. Research contract for “Hidromod/EDP-Electricidade de Portugal”.

2006. Impacts of the invasive species *Sargassum muticum* in Ria Formosa lagoon. Research contract for “Parque Natural da Ria Formosa, Instituto da Conservação da natureza”.

2006. FILTRALGAE – Integrated aquaculture of seaweeds for biofiltration of nutrients from the Zoomarine aquarium. Research contract for “IDEIA/Agência de Inovação”.

2005. Impacts of Waste Water Treatment Works in seagrass meadows of southern Portugal estuaries and Ria formosa lagoon. Research contract for “HIDROTEC/Águas do Algarve”.

2004. Base-line characterization of macrophyte meadows of Arade estuary. Research contract for “Instituto Português do Sul”.

2004. Stock assessment of the seaweed *Halopteris scoparia* in southern Portugal. Research contract for “CODIF”, France.

2003. EqEA – Assessment of the Portuguese Environmental Education Centers as instruments of public participation. Research contract for “Instituto do Ambiente”.

**8. Government/organization contract research** (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

2005. Impacts of Waste Water Treatment Works in seagrass meadows of southern Portugal estuaries and Ria formosa lagoon. Research contract for “HIDROTEC/Águas do Algarve”. Publication reference:

Martins, F, Janeiro, J, Venâncio, A, Brito, A, Gabriel, S, Lopes, J, Santos, R, Cabaço, S, Nunes, S, Neves, R. 2006. Monitorização dos meios receptores na área de atendimento da AdA. Technical report, Faro.

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

Collaborative publication:

Naranjo, EM, Redondo-Gómez, S, Silva, J, Santos, R, Figueroa, ME. 2007. Effect of prolonged flooding on the invader *Spartina densiflora* Brong. *Journal of Aquatic Plant Management* 45: 121-123.

Figueroa, FL, Santos, R, Conde-Álvarez, R, Mata, L, Pinchetti, J, Matos, J, Huovinen, P, Schuenhof, f A, Silva, J. 2006. The use of chlorophyll fluorescence for monitoring photosynthetic conditions of two tank cultivated red macroalgae using fishpond effluents. *Botanica Marina* 49: 275-282.

Silva, J, Santos, R, Calleja, M, Duarte, CM. 2005. Submerged versus air-exposed intertidal macrophyte productivity: from physiological to community-level assessments. *Journal of Experimental Marine Biology and Ecology* 317: 87-95.

Santos, R, Silva, J, Alexandre, A, Navarro, N, Barrón, C, Duarte, CM. 2004. Ecosystem metabolism and carbon fluxes of a tidal-dominated coastal lagoon. *Estuaries* 27: 977-985.

Mercado, J, Niell, X, Silva, J, Santos, R. 2003. Use of light and inorganic carbon acquisition by two morphotypes of *Zostera noltii* Hornem. *Journal of Experimental Marine Biology and Ecology* 297: 71-84.

Friend, PL, Ciavola, P, Cappucci, S, Santos, R. 2003. Bio-dependent bed parameters as a proxy tool for sediment stability in mixed habitat intertidal areas. *Continental Shelf Research* 23: 1899-1917.

Collaborative research:

2007. Microben2. Ecological responses of microbenthos to accumulation of nitrophilic macroalgae on intertidal sediments: Senescence and early diagenesis. CTM2006-04015/MAR, Ministerio de Educación y Ciencia, Spain (Project participation).

2007. Conservation status of the maerl communities in the Atlantic coast of Iberian Peninsula. CGL2006-03576/BOS, Ministerio de Educación y Ciencia, Spain (Project participation).

2006-2007. Emission of volatile organic carbon by marine vegetation, with Dr. Carlos Duarte, Instituto Mediterráneo de Estudios Avanzados (IMEDEA), Mallorca, Spain, GRICES/CSIC (Collaborative research).

2006-2008. Envirophyte - Improvement of the cost effectiveness of marine land-based aquaculture facilities through use of Constructed Wetlands with *Salicornia* as an environmentally friendly biofilter and a valuable by-product FP6-2004-SME-COOP (coordination of Portuguese partner).

2004. CLONMACMORPH - Morphological responses and interactions of clonal marine macrophytes with their nutrient environment, Marie Curie Reintegration Grant, Proposal nr: 006385.

2002-2005. ALIENS - ALgal Introductions to European Shores. EVK3-2001-0008.

2003-2004. Assessment of the relationships between the stable isotope index,  $\delta^{13}\text{C}$ , and the activity of the enzyme carbonic anhydrase in primary producers of southern Iberian Peninsula, with Dr. Jesus Mercado of the Instituto Español de Oceanografía, Málaga, Spain, GRICES/CSIC (Collaborative research).

2003. DOM production from contrasting communities in the Ria Formosa (S. Portugal), and export to the Atlantic Sea, with Dr. Carlos Duarte, Instituto Mediterráneo de Estudios Avanzados (IMEDEA), Mallorca, Spain, ICCTI/CSIC (Collaborative research).

## **6e. Future research**

### **1. Objectives (3000 ca.)**

#### **1. Coastal carbon fluxes and seagrass productivity**

To implement a European Network in the ambit of a COST action, focused on how the seagrass productivity regulates the carbon fluxes in coastal ecosystems dominated by seagrasses. Contacts with research partners from Australia, Israel, and European countries are under way aiming the establishment of a consortium to apply for COST funding as well as other national and international sources to explore the following research topics:

- New methodologies for continuous monitoring of seagrass productivity in situ;
- Establishment of a broad geographical network of sites where seagrass productivity will be monitored;
- Relationships between plant productivity and net community metabolism;
- Organic and inorganic carbon fluxes between seagrass community / water column and water column / atmosphere;
- Effects of elevated temperature and  $\text{CO}_2$ , on seagrass productivity, seagrass community calcification and community metabolism.

#### **2. Use of marine macrophytes to assess and monitor the ecological quality of coastal ecosystems**

We will integrate the Portuguese consortium contracted by INAG to determine and monitor the ecological quality of Portuguese coastal ecosystems under the European Water Framework Directive (WFD). Our recent research on the long term changes of Portuguese marine flora (Global related changes in the Portuguese marine flora over a long time scale, POCTI/BSE/48918/2002) and on the biology and ecology of macrophytes in transitional waters such as Ria Formosa lagoon and estuaries of Arade and Guadiana, determined our integration in this consortium. We plan to develop new indexes based both on detailed description of species richness and ecophysiological parameters of key community species.

The group is currently applying for the European Interreg-Sudoe program together with Spanish and French partners with a project addressing the environmental management of lagoons areas used for shellfish cultivation. The proposal includes the development and use of ecophysiological and community-based markers for the evaluation of seagrass meadows status. Some of these indicators, such as the seagrass area and the shoot density, are part of the descriptors to be used in the monitoring and classification of the ecological status of the transitional or coastal water bodies under the European Water Framework Directive (WFD).

### 3. Effects of global warming on the structure of benthic ecosystems

We want to address how the distribution of marine algae will react to increasing sea surface temperature and elevated CO<sub>2</sub>. We plan to integrate molecular ecology, ecophysiology and population dynamics studies of selected species that have their distribution limits in the coasts of Europe to understand both their dispersal potential and ecophysiological constraints at the geographical distribution limits and how these will determine the population dynamics of edge populations. This will permit to preview future trends of geographical distribution limits and thus future alterations of ecosystem structure due to global warming.

## **2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

### 1. Coastal carbon fluxes and seagrass productivity

“CO<sub>2</sub> exchange between marine plant-based communities, the water column and the atmosphere in the Eastern Mediterranean. Whether or not the Mediterranean Sea can function as a sink for CO<sub>2</sub>”. Research cruise sponsored by the EcoOcean-Marine Research and Education Foundation, 2008. Current funding = 25 K USD.

### 2. Use of marine macrophytes to assess and monitor the ecological quality of coastal ecosystems

“Evaluation of the ecological quality of Portuguese coastal ecosystems under the European Water Framework Directive (WFD)”. Research contract with INAG, 2008-2010. Pending funding = 230 KEuro.

“Environmental management of lagoon areas used for shellfish cultivation”. Research proposal submitted to the European Interreg-Sudoe program, 2009-2012. Pending funding = 280 KEuro.

### 3. *Sphaerococcus coronopifolius* fouling of Sines power plant

Research contract with Hidromod. Current funding = 37.4 KEuro.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Silva, J, Feijóo, P, Santos, R. 2008. Underwater measurements of carbon dioxide evolution in marine plant communities: A new method. *Estuarine Coastal and Shelf Science* 78: 827-830. IF=1.799, n°C=0.
2. Cabaço, S, Machás, R, Vieira, V, Santos, R. 2008. Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*). *Estuarine Coastal and Shelf Science* 78: 1-13. IF=1.799, n°C=0.
3. Cabaço, S, Machás, R, Santos, R. 2007. Biomass-density relationships of the seagrass *Zostera noltii*: a tool for monitoring anthropogenic nutrient disturbance. *Estuarine Coastal and Shelf Science* 74: 557-564. IF=1.799, n°C=0.
4. Silva, J, Santos, R, Calleja, M, Duarte, CM. 2005. Submerged versus air-exposed intertidal macrophyte productivity: from physiological to community-level assessments. *Journal of Experimental Marine Biology and Ecology* 317: 87-95. IF=1.750, n°C=3.
5. Santos, R, Silva, J, Alexandre, A, Navarro, N, Barrón, C, Duarte, CM. 2004. Ecosystem metabolism and carbon fluxes of a tidal-dominated coastal lagoon. *Estuaries* 27: 977-985. IF=2.133, n°C=8.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

TOC to analyse organic and inorganic carbon in water samples;

CHN to analyze elemental composition of biological tissues;

Laboratory technician to perform analytical procedures;

Installation of mesocosm facilities in Ramalhete field station for controlled experiments;

Use of the CCMAR cabin boat for field work on coastal systems.

## **Environmental Technologies**

### **6a. Group description**

#### **1. Group name / denomination**

**Environmental Technologies**

#### **2. Principal investigator**

**Maria Clara Semedo da Silva Costa**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Bioremediation, Metals, Acid mine drainage, Sulphate-reducing bacteria

#### **5. Funding, source, dates (1000 ca.)**

- Annual Budget of CCMAR, 1.662 EUR, 2006

- Annual Budget of CCMAR, 2.000 EUR, 2007

- Project ECOTEC:

Reference and funding entity: POCI/AMB/58512/2004 (FCT)

Duration: 1st October 2005 - 31st September 2008 (3 years)

Total budget: 74.880 EUR

Total budget for University of Algarve: 52.680 EUR

Budget for University of Algarve in 2006: 19.728 EUR

Budget for University of Algarve in 2007: 14.628 EUR

- Project GERMINARE:

Reference and funding entity: POCI/AMB/60257/2004 (FCT)

Duration: 1st October 2005 - 31st September 2008 (3 years)

Total budget: 85.000 EUR

Total budget for University of Algarve: 10.625 EUR

Budget for University of Algarve in 2006: 7.764 EUR

Budget for University of Algarve in 2007: 2.081 EUR

## **6b. Group team**

### **1. Researchers in the group** (Include only PhD. integrated in the LA)

001. Maria Clara Semedo da Silva Costa (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

### **2. Other researchers in the group** (Include here collaborators with PhD. only)

001. Ana Paula Pereira Paiva (Cat.: Outra, Gr. Acad.: Doutoramento)

002. Antonio Jose Estevao Grande Candeias (Cat.: Outra, Gr. Acad.: Doutoramento)

003. José Manuel Cardoso Duarte (Cat.: Outra, Gr. Acad.: Doutoramento)

004. Maria Leonor Faleiro (Cat.: Outra, Gr. Acad.: Doutoramento)

005. Raul Jose Jorge de Barros (Cat.: Outra, Gr. Acad.: Doutoramento)

### **3. Other researchers in the group** (non PhD.)

001. Monica Sofia Furtado Martins (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives** (2000 ca.)

#### **1. General objectives**

The central aim of this research group is to contribute, with fundamental and applied research, to the development of sustainable remediation technologies for the decontamination of acid mine drainage or waters and effluents contaminated with metals and/or sulphates.

#### **2. Objectives of the Bioremediation technologies**

The main objective of this line of investigation was the objective of “ECOTEC” project which was to establish a simple, natural and low cost eco-technology, using sulphate-reducing bacteria (SRB), at bench scale, for the treatment of acid mine drainage (AMD) waters. With this eco-technology it is aimed to:

- neutralize the acidity (from pH~2 to pH~7);
- to reduce the sulphate content (from ~2500 mg/L to values below 575 mg/L);
- to remove the heavy metals (Fe, Cu, Zn and others more toxic);

in order to obtain water suitable to be used for irrigation purposes.



To develop that technology, the selection of high metals resistant (Fe, Zn, Cu) SRB inocula and low cost effective carbon sources (eg. wastes obtained at zero or at even negative cost) were two main concerns. In addition, the identification of the SRB consortium presented in the selected inoculum, using molecular biology tools, was also aimed.

The objectives of the investigation mentioned in a1.2 and in a1.3 were mainly the selection of SRB resistant to Uranium (VI) and/or Cr(VI) and with ability to reduce them to uranium (IV) and Cr(III), which are less toxic and soluble, and thus can be easily removed from solutions in the form of precipitates.

### 3. Liquid-liquid extraction of metals

The main aim of this work was to investigate whether there is a relation between the chemical structure of several malonamides derivatives and their iron(III) extraction behaviour from chloride solutions (as those obtained from hydrometallurgical processes), and particularly to try to understand the dependence of the metal extraction mechanism on the structural characteristics of the malonamides.

## **2. Main achievements (2000 ca.)**

### 1. Main achievements of the Bioremediation studies

#### 1.1. Within the framework of the ECOTEC project

- A high resistant to metals (Fe, Zn and Cu) inoculum of SRB was selected from several environmental samples to inoculate the bioreactors used in the bioremediation process for AMD treatment. Paper submitted.

- A mixture of coarse sand and calcite was selected as the best solid support of fixed bed type percolation columns as bioreactors. Those studies were important in the identification of key parameters of the bioremediation process and on the optimization of the columns design. As a result two bioremediation systems, based on solid and on liquid columns were compared. A third system with recirculation was also tested. The results demonstrated the viability of the solid and recirculation systems for AMD treatment in terms of neutralization and sulphates and metals removal. Paper published.

- A calcite tailing was successfully utilised as a neutralising and buffer material either incorporated in the solid matrix of the bioreactor or to be used in a previous stage to increase the pH of the AMD, allowing the subsequent SRB activity. Portuguese patent submitted.

- Wine wastes, largely available from local industries, were selected as a cost effective carbon and energy source for SRB. This was a very important achievement considering that the economical viability of these processes is often compromise by the cost of the substrate. In addition, the use of other materials easily obtained at low, null or even negative cost (eg. calcite tailing and coarse sand) can definitively contribute for the economical feasibility of the environmentally friendly technology that “ECOTEC” project aims to develop. Paper and patent submitted.

- The SRB consortium used to inoculate the bioreactors were identified.

#### 1.2. Other studies

Bacteria consortia resistant to uranium(VI) and chromium(VI) and with ability to remove those metals from solutions were obtained from natural environmental samples. These consortia showed potential to be used in bioremediation processes.

## 2. Main achievements of liquid-liquid extraction of metals

The introduction of an alkyl chain on the central carbon atom of several N,N,N',N'-tetrasubstituted skeleton malonamides was an important finding to increase the affinity of this organic derivatives for iron(III) at low hydrochloric acid concentrations. The collected spectroscopic results determined iron(III) extraction through, generally, as a solvation pathway. Paper published.

## 6d. Productivity

### 1. Publications in peer review journals (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Costa, MC, Pêczek, I, Sadowski, Z, Natu, S, Paiva, AP. 2007. The solvent extraction of Iron(III) from chloride solutions by N,N'-tetrasubstituted malonamides: structure-activity relationships. *Solvent Extraction and Ion Exchange* 25: 463-483. IF 2007=1.229, n°C=0.
2. Costa, MC, Duarte, JC. 2005. Bioremediation of acid mine drainage using acidic soil and organic wastes for promoting sulphate-reducing bacteria activity on a column reactor. *Water, Air and Soil Pollution* 165(1-4): 325-345. IF 2007=1.224, n°C=2.
3. Paiva, AP, Costa, MC. 2005. Application of N,N'-tetrasubstituted malonamides to the recovery of iron (III) from chloride solutions. *Hydrometallurgy* 77(1-2): 103-108. IF 2007=1.324, n°C=4.
4. Costa, MC, Martins, M, Paiva, AP. 2004. Solvent extraction of iron (III) from chloride acid media using N,N'-dimethyl-N,N'-dibutylmalonamide. *Separation Science and Technology* 39(15): 3573-3599. IF 2007=1.048, n°C=4.
5. Costa, MC, Carvalho, N, Uryga, A, Paiva, AP. 2003. Solvent extraction of iron (III) from hydrochloric acid solutions using N,N'-dimethyl-N,N'-diphenyltetradecylmalonamide. *Solvent Extraction and Ion Exchange* 21(5): 653-686. IF 2007=1.229, n°C=11.

### 2. Other publications national (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Martins, M, Faleiro, L, Barros, R, Costa, MC. 2007. Selecção de bactérias redutoras de sulfato para biorremediação de águas ácidas de mina. *Proceedings of the 9º Encontro Nacional do Ambiente, Aveiro, Portugal, April 2007*, pp. 631-636.
2. Costa, MC, Duarte, JC. 2004. Biorremediação de águas ácidas de mina com bactérias sulfato-redutoras. *Proceedings of the 8º Encontro Nacional do Ambiente, Lisboa, Portugal, October 2004*, 10 pp.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Hamidov, A, Khaydarova, V, Sharipova, S, Costa, MC, Beltrão, J. 2007. Potential of *Portulaca Oleracea* Golden Purslane as a salt removal species. Proceedings of the 3rd IASME / WSEAS International Conference on Energy, Environment, Ecosystems and Sustainable Development (EEESD'07), Crete, Greece, pp. 151-156.
2. Paiva, AP, Natu, S, Gajda, K, Costa, MC. 2006. Iron(III) removal from chloride solutions by solvent extraction. Proceedings of the Third International Symposium on Iron Control in Hydrometallurgy (36th Annual Hydrometallurgical Meeting). Dutrizac, JE, Riveros, PA (Eds). Canadian Metallurgical Society, Montreal, Canada, pp. 465-477.
3. Paiva, AP, Costa, MC. 2004. Application of N,N'-tetrasubstituted malonamides to the recovery of iron (III) from chloride solutions. Proceedings of "Quo Vadis Hydrometallurgy IV", Kosice-Herlany, Slovakia, May 2004, pp. 183-188.
4. Costa, MC, Carvalho, N, Iglesias, N, Palencia, I. 2003. Bacterial leaching studies of a Portuguese flotation tailing. Proceedings of the "15th International Biohydrometallurgy Symposium - IBS'03", Athens, Greece, September 2003.

**4. Master and Ph.D. thesis completed** (3000 ca.)

**5. Patents/propotypes** (2000 ca.)

Costa, MC, Martins, M, Jesus, C, Barros, R. 2007. Uso de lama de mármore para pré-tratamento químico de efluentes ácidos. Portuguese Patent nº 25849 G, 2007.

**6. Organization of conferences** (2000 ca.)

**7. Industry contract research** (2000 ca.)

**8. Government/organization contract research** (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

Collaboration with Prof. Zygmunt Sadoski, from Wroclaw University in Poland was done in the aim of the solvent extraction studies using malonamide derivatives. From that collaboration resulted the exchange of several Socrates/Erasmus students, in the period between 2003 and

2007. From this collaboration resulted publications n. 1, 5 (Publications in peer review journals) and n. 2 (Other publications International).

Collaboration with Prof. Inmaculada Palencia Peres (deceased), from Seville University was done to accomplish studies aiming the bioleaching of flotation tailings, from which resulted publication n. 4 (Other publications International).

Collaboration with Ludo Diels from Vito Institute in Belgium was established in 2006 in the framework of Mónica Martins PhD work, considering his large expertise in bioprecipitation with sulphate-reducing bacteria and related studies. Therefore, Mónica Martins are going to spend a training period in Vito Institute, supervised by Ludo Diels.

## **6e. Future research**

### **1. Objectives (3000 ca.)**

#### **1. Bioremediation studies**

##### **1.1. In the framework of ECOTEC proj. (2008)**

Investigation of the efficiency limits of the bioremediation systems (sulphates and metals concentration and hydraulic retention time), aiming their optimization.

Identification of the metal precipitates generated during bioremediation.

##### **1.2. Following ECOTEC**

a) Optimization of the most critical operative parameters for successful installation of the bioremediation process on situ (e.g. temperature).

Characterization of the microbiological biotome associated with the process; SRB and other microbial communities will be identified and the relationships between these diverse groups and the presence of different contaminants will be established. These results will contribute to an understanding of the overall process and the role played by SRB and other microbes, which should help improve its performance.

This work was the subject of a post-doc application of Naresh Kumar submitted to FCT in June 2008 and has the co-orientation of Prof. Deborah Power (CCMAR).

b) Bio-synthesis of nanosized semiconductors using mines wastes as materials sources: The production of nanomaterials using by-products of the bioremediation process will convert potentially harmful wastes in useful products for innovative applications. With this research is aimed to investigate the production of nanosized materials as a result of the sulphate-reducing process, developed during “ECOTEC” project, by precipitating heavy metal ions into the corresponding metal sulphides. The in situ (or ex situ) synthesis of these compounds as nanosized materials during the bioremediation processes is a main goal of this research. Because these metal sulphides have semiconductor properties their potential in environmental applications will be investigated (eg. as catalysts in the photodegradation of organic pollutants).

This work was the subject of a PhD application of João Costa submitted to FCT in June 2008 and has the co-supervision of Prof. Tito Trindade (CITMA, University of Aveiro).

c) The identification and the study of new biotechnological potentialities of SRB obtained from deep-sea hydrothermal vent sediments are aimed. The identification of specific genes presented in these SRB, responsible for the adaptation to their life in extreme conditions (pressure, temperature) is also an objective.

A part of this work will be the subject of a post-doc application of Maria de Fátima Alexandrino to be submitted to FCT in September 2008 and has the co-supervision of Prof. Adelino Canário (CCMAR). Collaboration with Matthias Koschorreck, from Helmholt Centre for Environmental Research, Germany, who works in the bioremediation of acidic lakes, is envisaged, as well as the collaboration of Rodrigo Costa a future researcher of CCMAR (Programme Science 2007, FCT).

### 1.3. Bioremediation of uranium (Mónica Martins PhD)

Identification (of the consortia) and isolation of the bacteria responsible for uranium removal

Genetic profile of the community as a function of the presence of toxic ions such as U(VI), Cr(VI) and sulphate

Comparison of the community behaviour with the behaviour of the pure culture

Studies of the mechanisms involved on the removal of U(VI) from solutions

Identification of the precipitates generated during the bioremediation process

Development of a bioreactor

The search for new bioremediation agents and the studies about their potentialities are in general priorities of the Environmental technologies group.

### 2. Liquid-liquid extraction of metals

To extend the use of malonamides, and other new amide derivatives, to the solvent extraction of platinum group metals and to study their extraction mechanisms

#### **2. Funding, source, dates** (1500 ca.) (Indicate in full including amount of current and pending funding)

- Project ECOTEC, (POCI/AMB/58512/2004), Foundation for Science and Technology (FCT).

Budget for University of Algarve in 2008: 3.600 EUR

- Project GERMINARE, (POCI/AMB/60257/2004), Foundation for Science and Technology (FCT).

Budget for University of Algarve in 2008: 2.081 EUR

- Annual budget of CCMAR

- Annual budget given by FCT for the PhD of Mónica Martins

After 2008:

The group of Environmental Technologies aims to submit several National and International research projects to Portuguese and to International entities to obtain external funds to finance the future research lines above described in the objectives.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

Publications n. 2 (Publications in peer review journals), n. 1 and 2 (Other publications national) and n. 1 (Other international publications), previously mentioned, are in the area of Bioremediation, which this group wants to pursue. The other publications mentioned are in the area of solvent extraction, that this group also wants to proceed.

In the area of bioremediation two other papers were published:

- Costa, MC, Martins, M, Jesus, C, Duarte, JC. 2008. Treatment of acid mine drainage by sulphate-reducing bacteria using low cost matrices. *Water Air and Soil Pollution* 189: 149-162. IF=1.224.

- Hamidov, A, Khaydarova, V, Sharipova, S, Costa, MC, Beltrão, J. 2008. Environmentally Useful Technique - *Portulaca Oleracea* Golden Purslane as a Salt Removal Species. *WSEAS Transactions on Environment and Development* 7(3): 117-122.

and several others are already submitted, eg.:

- Martins, M, Faleiro, L, Barros, RJ, Veríssimo, AR, Costa, MC. Biological sulphate reduction using wastes from the winery and cheese industries as carbon sources. *Biodegradation* (2008).

- Martins, M, Faleiro, ML, Barros, RJ, Veríssimo, AR, Barreiros, MA, Costa, MC. Characterization and activity studies of highly heavy metal resistant sulphate-reducing bacteria. *J. Haz. Mat.* (2008).

- Jesus, C, Martins, M, Barros, RJ, Costa, MC. Low-cost passive process to produce irrigation waters by neutralization and biological treatment of acid mine drainage. *Process Biochemistry* (2008).

In the area of liquid-liquid extraction a paper is accepted to publication:

- Paiva, AP, Malik, P, Rosa, AM, Castro, C, Costa, MC. Extraction of metal ions from chloride media: can malonamide derivatives be useful? *Proceedings of the International Solvent Extraction Conference, Tucson, USA* (2008).

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

- Atomic Absorption Spectroscopy (AAS), preferably with furnace chamber, for metals analysis with a detection limit of ppb. This equipment is essential to analyze low metals concentrations in not simple matrices, since the UV/visible spectrometer is only recommended for water analysis (low complex matrices) and for concentrations of ppm.

- Anaerobic chamber. This equipment is fundamental to isolate SRB or other anaerobes and should be acquired, particularly in the perspective of finding new SRB species or other anaerobes with bioremediation or other biotechnological potentialities.
- Auxiliary staff, especially to give assistance on the replication and maintenance of the inocula.
- High performance liquid chromatograph (HPLC) equipped with detectors for acids, alcohols and sugars analysis. This equipment is very important and widely used nowadays to the analysis of most organic compounds, as those used or metabolized by bacteria in the bioremediation processes. This equipment is needed to perform analysis that allowed the monitoring of those processes and a better understanding of the metabolisms involved.
- A protocol between CCMAR and other entity for the use of Nuclear Magnetic Resonance (NMR) spectroscopy would be welcome for this group, mainly in view of the characterization of the organic compounds synthesized for the solvent extraction purposes, and for the mechanistic studies.



## **Fisheries Biology and Hydroecology Research Group (FBHRG)**

### **6a. Group description**

#### **1. Group name / denomination**

**Fisheries Biology and Hydroecology Research Group (FBHRG)**

#### **2. Principal investigator**

**José Pedro de Andrade e Silva Andrade**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Fisheries biology, Living resources management, Cephalopod culture, Syngnathidae culture

#### **5. Funding, source, dates (1000 ca.)**

Jan 2000-Mar 2004, "Trophic interactions of the cuttlefish, *Sepia officinalis* (Cephalopoda, Sepiidae) in the Ria Formosa, the sado Estuary and the Ria de Aveiro: a tool for cephalopod resource management", Fundação para a Ciência e Tecnologia, PDCTM/C/MAR/15259/1999, Funding 180.000 EUR.

Jan 2002-Dec 2004, "Cephalopod stocks in European waters: review, analysis, assessment and sustainable management", European Union, Quality of Life and Management of Living Resources - QOL-2001-5.1.2, Funding 1.500.000 EUR.

Jan 2002-Jul 2005, "AQUASEPIA: Development of Aquaculture Practices for the Cuttlefish, *Sepia officinalis*", Agência de Inovação - P0009/POCTI 2.3/OUT.01, Funding 25.000 EUR.

Jan 2002-Dec 2006, "Towards age accreditation and certification of age determination of aquatic resources" (refª QOL-2001-01891), European Union, Quality of life and management of Living Resources, Funding 2.538.000 EUR.

Jan 2002-2004, "Feeding ecology and reproduction of little terns (*Sterna albifrons*) as ecological indicators in estuarine ecosystems", Fundação para a Ciência e a Tecnologia, POCTI/BSE/37385/2001, Funding 65.700 EUR.

May 2007-Jan 2008, Seahorse applied research (SHAR), Funded by Lusoreef Lda, 12.000 EUR.

### **6b. Group team**

#### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. José Pedro de Andrade e Silva Andrade (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

002. Eduardo Bruno Oliveira Esteves (Cat.: Professor-Adjunto, Gr. Acad.: Doutoramento)

003. António de Vilhena Andrade Ferreira Sykes (Cat.: Não aplicável (bolseiro), Gr. Acad.: Agregação)

004. Jorge Afonso Martins da Palma (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

## **2. Other researchers in the group (Include here collaborators with PhD. only)**

## **3. Other researchers in the group (non PhD.)**

001. Marta Sofia Alves Gomes (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

Since 2000, one of the objectives of the FBHRG is to establish the scientific bases for the culture of cuttlefish (*Sepia officinalis*) culture, at the commercial level. This was due to needs of the new species for aquaculture (the species short life cycle and fast growth rates imply lower production periods and associated costs) and to the fact that there is potential for further exploitation, particularly with regard to the production of undersized individuals (which would reduce the impact of illegal catches on this species from the natural environment). These smallest individuals are considered a delicacy and have the highest commercial value in Portugal.

Throughout this period the group has also focused its attention on the development and optimization of the rearing protocols of candidate species for aquaculture, namely, the Atlantic ditch shrimp, *Palaemonetes varians* and two Syngnathidae fish, the long snout seahorse, *Hippocampus guttulatus* and the broad-nosed pipefish, *Syngnathus typhle*. This shrimp species can be produced as live diet for more demanding aquaculture reared species (e.g. Syngnathidae fish) and also for human consumption, as this, is a locally valuable species both in Portugal and Spain. *H. guttulatus* is a European Syngnathidae species, protected under the CITES agreement and as other *Hippocampus* species faces the reduction of habitats with a consequent decrease of the natural populations. *Hippocampus* sp. suffer negative pressure on their wild populations due to activities such as trawling fisheries, illegal captures for commercial purposes and excessive noise produced by nautical activities. Its artificial production provides not only a valuable product for the international ornamental fish trade, but also a powerful tool for the species management. The conservation of *S. typhle* generally follows the topics mentioned above for *H. guttulatus* and its artificial production endorses the same goals.

This is in accordance to a defined strategy of the CCMAR in terms of new species to be introduced in aquaculture as well to an strategy of implementing the CCMAR as the European Reference Laboratory in terms of new species for aquaculture.

## 2. Main achievements (2000 ca.)

The FBHRG has established the culture of *Sepia officinalis* at the laboratory level and represents now the leading European laboratory regarding such technology. This is due to the following milestones:

- New life stage classifications for cultured cuttlefish – 4 stages – egg (embryonic development), hatchling, juvenile and adult. The differences between stages are based on the type of prey ingested (e.g. hatchlings require live prey while juveniles don't) and maturation (e.g. to be considered adults, cuttlefish individuals must be mature);
- Basic Adult stage - Spawner/Breeder - methodology – results indicate that both genetics and increased bottom areas may play an important role in egg quality and quantity;
- Fully developed Egg stage methodology that allows precise results;
- Basic Hatchling and Juvenile stages methodologies allow 100% survival but are still too much expensive, therefore needing further research both in the fields of nutrition and zoo-technology.

With the developed technology we are now able to culture about 5000 cuttlefish/year (2 generations).

Optimisation of the rearing protocols for *P. varians* production:

As this is a new species in the aquaculture context it was necessary to perform several experimental trials not only to determine the optimal environmental rearing conditions but also to determine the nutritional requirements of the species, which together, would lead to the establishment of a reliable protocol for its production. Firstly, wild juvenile animals were captured and adapted to captivity and as shrimp are slow feeders, a practical artificial diet was formulated and the effects of binder type and binder addition on the growth of this species was analysed. From that point on, shrimp were fed with this artificial diet easing up the all maintenance procedure and attentions could be focused on the larval production. As this protocol was mastered, attentions were then focused on the nutritional requirements of the species. The replacement of animal origin protein by vegetal protein sources, mainly, soy meal, wheat and corn gluten was analysed. Dietary requirements for protein and lipid were investigated and finally the dietary requirements for the essential amino acids, lysine, methionine and arginine was analysed.

Scientific and technical basis for the culture of *H. guttulatus*

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Palma, J, Reis, C, Andrade, J P. 2003. Impact of the bivalve dredge fishing on flatfish species in the south coast of Portugal (Algarve). *Journal of Sea Research* 50: 129-137. IF=2.204, n°C=1.

2. Chícharo, MA, Esteves, E, Santos, AMP, dos Santos, A, Peliz, Á, Ré, P. 2003. Are sardine larvae caught off northern Portugal in winter starving? An approach using nutritional condition. *Marine Ecology - Progress Series* 257: 303-309. IF 2003=2.135, n°C=8.
3. Domingues, PM, Sykes, AV, Sommerfield, A, Almansa, E, Lorenzo, A, Andrade, JP. 2004. Growth and survival of young cuttlefish, *Sepia officinalis* (Linnaeus, 1758) fed live and frozen fish and crustaceans. *Aquaculture* 229, 239-254. IF=1.735, n°C= 19.
4. Correia, M, Domingues, PM, Sykes, AV, Andrade, JP. 2005. Effects of culture density on growth and broodstock management of the cuttlefish, *Sepia officinalis* (Linnaeus, 1758). *Aquaculture* 245: 163-173. IF=1.735, n°C= 6.
5. Bargelloni, L, Alarcon, JA, Alvarez, MC, Penzo, E, Magoulas, A, Palma, J, Patarnello, T. 2005. The Atlantic-Mediterranean transition: Discordant genetic patterns in two seabream species, *Diplodus puntazzo* (Cetti) and *Diplodus sargus* (L.). *Molecular Phylogenetics and Evolution* 36: 523-535. IF=3.994, n°C=17.
6. Catry, T, Ramos, JA, Martins, J, Peste, F, Trigo, S, Paiva, VH, Almeida, A, Luís, A, Palma J, Andrade, JP. 2006. Inter-colony and annual differences in the diet and feeding ecology of Little Tern adults and chicks in Portugal. *The Condor* 108: 366-376. IF=1.448, n°C=4.
7. Sykes, AV, Domingues, PM, Correia, M, Andrade, JP. 2006. Cuttlefish culture – state of the art and future trends. *Vie et Milieu* 56(2): 129-137. IF=0.750, n°C= 6.
8. Almansa, E, Domingues, PM, Sykes, AV, Tejera, N, Lorenzo, A, Andrade, JP. 2006. The effects of feeding with shrimp or fish fry on growth and mantle lipid composition of juvenile and adult cuttlefish (*Sepia officinalis*). *Aquaculture* 256: 403-413. IF=1.735, n°C=1.
9. Silva, VS, Nunes, MA, Cordeiro, JM, Calejo, AI, Santos, S, Neves, P, Sykes, AV, Morgado, F, Dunant, Y, Gonçalves, PP. 2007. Comparative effects of aluminium and ouabain on synaptosomal choline uptake, acetylcholine release and (Na<sup>+</sup>/K<sup>+</sup>) ATPase. *Toxicology* 236: 158-177. IF=2.919, n°C=3.
10. Palma, J, Bureau, DP, Andrade, JP. In Press. Effect of binder type and binder addition to artificial diets used for the growth of *Palaemonetes varians* and *Palaemon elegans* (Crustacea: Palaemonidae) produced as aquaculture live food. *Aquaculture International*. IF=0.828. Accepted in 2007.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Esteves, E. 2006. Ecology of early-life history stages of Twaité shad *Alosa fallax fallax* (Lacépède, 1803) in the river Mira, with a note on *Alosa* sp. in the river Guadiana. PhD in Ecology, University of Algarve.

Sykes, AV. 2007. Hatchery technologies and nutritional contents of cuttlefish (*Sepia officinalis*) spawners, eggs, hatchlings and live prey associated. PhD in Aquaculture. University of Algarve.

##### **MSc THESIS**

Massei, K. 2003. A alimentação de otárias-do-Cabo, *Arctocephalus pusillus pusillus* (Schreber, 1776) num parque zoológico: aspectos calóricos, digestivos e comportamentais. MSc in Marine and Coastal Studies, University of Algarve.

Sykes, AV. 2003. On the use of live grass shrimp (*Palaemonetes varians*) as the only prey for cuttlefish (*Sepia officinalis*, Linnaeus, 1758) culture throughout the life cycle. MSc in Marine Sciences, Instituto de Ciências Biomédicas Abel Salazar, University of Porto.

Correia, M. 2006. Live diet requirements of reared juvenile cuttlefish (*Sepia officinalis* Linnaeus, 1758). MSc in Biology and Management of Marine Resources, Faculty of Sciences, University of Lisbon. Co-supervisor: Henrique Cabral (University of Lisbon).

#### **5. Patents/propotypes (2000 ca.)**

#### **6. Organization of conferences (2000 ca.)**

#### **7. Industry contract research (2000 ca.)**

From May 2007 to February 2008, a contract has been established with the enterprise Lusoreef (Lagoa, Algarve, Portugal) to carry joint research on the culture of *Hippocampus guttullatus*, under the frame of the Sea-horse applied research (SHAR). This project was used to address several basic topics on the scientific and technological basis for the production of the sea-horse species referred to above.

#### **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

#### **9. Internationalization (2000 ca.)** (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

In the last 2 years, this group established research contacts with other European and International laboratories in order to prepare a proposal to establish the European Network for Cephalopod

Aquaculture Development (ENCAD). Nationally, we have been cooperating with the IPIMAR and the University of Aveiro. We have been collaborating actively with the IFAPA – Agua del Pino Research Centre (Huelva, Spain) as well with the Laboratory of Animal Physiology from the University of La Laguna (Tenerife, Spain), exchanging both students and researchers that result in the collaborative publications that are listed above. Nonetheless, we have also established research contacts with other European and International laboratories in Spain, France, UK, Germany, Italy, Croatia, Mexico, Chile, Brasil, Argentina. These International contacts are being made for the last 2 years as part of an FP7 Project proposal to be submitted in 2009.

Regarding student exchange, we have been collaborating in the training of students from the University of Cork (since 1999, usually 2 per year) and University of Kiel (one graduation student in 2006). These training activities have been carried out under the frame of the Erasmus/Socrates exchanging programme.

## **6e. Future research**

### **1. Objectives (3000 ca.)**

This laboratory is currently establishing a worldwide network on cephalopod aquaculture to be presented in the next FP7 call in 2009. This is included in the future prospects for the next 2008-2010, where an increased cooperation on cephalopod culture development will be performed at the regional (INTERREG aquaculture project with the national IPIMAR and the Spanish IFAPA of the Junta de Andaluzia, Spain) and European (Spain, France, UK, Italy, Croacia, etc.) levels. This will allow the FBHRG not to duplicate work with other laboratories as well as to perform at the European level as the European Network for Cephalopod Aquaculture Development. Due to the innovative scientific knowledge developed, the FBHRG will then be available as a consultant in the implementation of these new technologies at both national and worldwide enterprise levels. This expansion will only be achieved by training more people at higher university levels as well as hiring technicians to perform daily routine work. In addition, to achieve these objectives there is a need for the elaboration of a cephalopod hatchery and breeding stock plan as well as the construction of seawater circuits that will allow its implementation at the all stages of the lifecycle. The development of these technologies and the extension at the infrastructure level will also allow the CCMAR to provide live animals for biological studies at the Ramalhete Field Station facilities.

Furthermore, it is planned to continue research on *P. varians*, *H. guttulatus* and *S. typhle*. The *P. varians* research will aim to establish efficient rearing systems in order to maximize larval production using a new concept in tank design and an “environmental friendly” systems that will minimize environmental impact of the plant outflows.

Regarding the Syngnathidae species, research will focus in the following areas:

1. Larval production and the diet improvement for juveniles and adults.
2. The status of seahorse populations in the Ria Formosa.
3. Variation of seahorse populations over different temporal and spatial scales

4. The use of re-stocking as a management option for the recovery of seahorse populations, using *H. guttulatus* in the Ria Formosa as a model species.

Use of Quality Index Methods (QIM): Freshness is a key element in the quality assessment of fish and seafood products by consumers. QIM procedures for several commercially fished, adult fish and cephalopods have now been developed. It is planned to use, eventually after adaptation, the QIM schemes already developed for cuttlefish, together with microbiological and chemical/nutritional analyses, to study the deterioration of specimens cultured in different conditions of temperature, feed and/or ration, up to a certain size/age and compare the process(es) with those of wild-caught, fishery-originated individuals. This line of research will be developed in collaboration with the DITVPP of the INIAP/IPIMAR which has been collaborating with the group for the last 4 years.

**2. Funding, source, dates** (1500 ca.) (Indicate in full including amount of current and pending funding)

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Sykes, AV, Oliveira, AR, Domingues, PM, Cardoso, CM, Andrade, JP, Nunes, ML. 2008. Assessment of European cuttlefish (*Sepia officinalis*, L.) nutritional value and freshness under ice storage using a developed Quality Index Method (QIM) and biochemical methods. LWT - Food Science and Technology. DOI: 10.1016/j.lwt.2008.05.010. IF 2007=1.589, n°C=0.
2. Correia, M, Palma, J, Andrade, JP. 2008. Effects of live prey availability on growth and survival in early stages of cuttlefish *Sepia officinalis* (Linnaeus, 1758) life cycle. Aquaculture Research 39: 33-40. IF=1.067.
3. Sykes, AV, Almansa, E, Lorenzo, A, Andrade, JP. 2008. Lipid characterization of both wild and cultured eggs of cuttlefish (*Sepia officinalis*) throughout the embryonic development. Aquaculture Nutrition. DOI: 10.1111/j.1365-2095.2008.00566.x. IF 2007=1.642, n°C=0.
4. Palma, J, Stockdale, J, Correia, M, Andrade, JP. 2008. Growth and survival of adult long snout seahorse (*Hippocampus guttulatus*) using frozen diets. Aquaculture 278: 55-59. IF=1.735.
5. Palma, J, Bureau, DP, Andrade, JP. In Press. Effect of binder type and binder addition to artificial diets used for the growth of *Palaemonetes varians* and *Palaemon elegans* (Crustacea: Palaemonidae) produced as aquaculture live food. Aquaculture International. DOI: 10.1007/s10499-007-9155-5. IF=0.828. Accepted in 2007.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

This expansion will only be achieved by training more people at higher university levels as well as hiring technicians to perform daily routine work. We must stress that, due to the growing importance of the research field of expertise, within 5 years the CIMAR will have to hire an Auxiliary Researcher for the field of Cephalopod Aquaculture.



In addition, to achieve these objectives there is a need for the elaboration of a cephalopod hatchery and breeder stock plan as well as the construction of seawater circuits that will allow its implementation at the all stages of the lifecycle. The development of these technologies and the extension at the infrastructure level will also allow the CIMAR to provide live animals for biological studies at the Ramalhete Field Station facilities. In order to achieve the proposed objectives we will need to expand the team at least with one technician to maintain the cuttlefish stock and perform routine work at the Ramalhete Field Station. Due to the nature of this type of work, we will require an additional technician/student that may perform routine work during weekends. In terms of facilities, the laboratory at the FCMA building will need restructuration for the establishment of new nutritional methods and newly design tanks and seawater circuits will be needed for the cuttlefish culture at their different life stages in the Ramalhete Field Station. There will be a need to upgrade some of the computing equipment and the image analysis system.

## **Biophysics**

### **6a. Group description**

#### **1. Group name / denomination**

#### **Biophysics**

#### **2. Principal investigator**

**Maria Leonor Nunes Ribeiro Cruzeiro**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Protein folding, Protein function, Misfolding diseases, VES hypothesis

#### **5. Funding, source, dates (1000 ca.)**

70.000 EUR - PI for two tasks in the research project "Nonlinear waves in discrete, periodic and quasiperiodic systems", from the Foundation for Science and Technology, Portugal (2005-2008).

### **6b. Group team**

#### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Maria Leonor Nunes Ribeiro Cruzeiro (Cat.: Professor Associado, Gr. Acad.: Doutoramento)

#### **2. Other researchers in the group (Include here collaborators with PhD. only)**

#### **3. Other researchers in the group (non PhD.)**

001. Paulo Alexandre dos Santos Silva (Cat.: Assistente de Investigação, Gr. Acad.: Mestrado)

### **6c. Objectives & achievements**

#### **1. Objectives (2000 ca.)**

The ultimate aim of the Biophysics Group is to understand how proteins fold and also how they function. Proteins are the machines of life, they mediate most processes that take place in a cell, and understanding how they function is not only of interest for Marine Biology but also for Biology in general, as well as for Biomedicine, for Biotechnology and for the Pharmaceutical Sciences. Our scientific approach is based on two fundamental assumptions: a) the free energy

landscape of proteins is multi-funnel shaped and b) protein folding and function involve the storing and propagation of energy in the form of vibrational excited states (VES), something that we have started to designate as the VES hypothesis. The vibrational state we are mostly interested in is the amide I vibration, whose storing and transport from carbonyl to carbonyl is described by the Davydov/Scott model.

In the period 2003-2007 the research work was developed along four lines:

- 1) application of the Davydov/Scott model for energy transfer in proteins to the interaction of proteins with electromagnetic radiation;
- 2) application of the VES hypothesis to the structural instability of prions and other proteins involved in misfolding diseases;
- 3) extension of the Davydov/Scott model to a model that does not conserve the amide I number, in order to explain the generation of conformational changes;
- 4) testing of the assumption a) above through computer simulations.

An extra line pursued was the investigation of the stabilization of electron pairs by vibrational modes of nonlinear lattices, a topic that mixes the Davydov/Scott model with the Hubbard model that describes electron correlations in condensed matter systems. Although the direct interest here is the mechanism of High Temperature Superconductivity, these models may in the future prove useful also for electron transfer in proteins.

## **2. Main achievements (2000 ca.)**

Concerning the first research line specified in item Objectives, the Davydov/Scott model was extended to account for the full three dimensional structure of proteins and applied to the calculation of the amide I band of a thermalized alpha-helix. A comparison with experimentally obtained spectra led to the estimation of most controversial parameter in the Davydov/Scott model, that represents the interaction of the amide I excitation with the mechanical vibrations of the peptide groups, which was found to be approximately -30 pN (see ref 5. in Publications in peer review journals).

Concerning the second research line specified in item Objectives, the extended Davydov/Scott model was applied to the transfer of energy from the bending mode of water to the amide I vibrations of proteins. It was found that proteins with large amounts of glutamine and asparagine can extract three to fifteen times more energy from neighbouring water molecules, something that may explain the greater instability of the proteins associated with misfolding diseases (see refs. 4 in Publications in peer review journals and ref. 4 in Previous publications in the area).

Concerning the third research line specified in item Objectives, a non-conservative Davydov/Scott-like Hamiltonian was considered in which the number of amide I excitations can vary. The transfer of energy from the amide I vibrations to the mechanical vibrations of the lattice was studied and found to be greater when the lattice vibrations induced by the amide I excitations are in resonance with the natural vibrations of the lattice. Also a fully quantum formalism for the thermalization of these systems, based on the Quantum State Diffusion equation and Lindblad operators, is being developed (see refs. 1 and 6-8 in item Publications in peer review journals)

Concerning the fourth research line, four proteins, representative of the four CATH classes, were selected and the fold of each was threaded on the sequence of the others. Comparing the energy of the native conformation of each of these four proteins with the energy of the three alternative and highly artificial conformations shows that they are all very similar, something that supports assumption a) in item Objectives (see ref. 4 in item Previous publications in the area).

## 6d. Productivity

### 1. Publications in peer review journals (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Silva, PAS, Cruzeiro-Hansson, L. 2003. A reduced set of exact equations of motion for a non-number-conserving Hamiltonian. *Physics Letters A* 315/6: 447-451. IF 2007=1.711, n°C=3.
2. Brizhik, LS, Cruzeiro-Hansson, L, Eremko, AA, Olkhovska, Yu, V. 2003. Dynamical properties of Davydov solitons in molecular chains. *Ukrainian Journal of Physics* 48: 667-671. n°C=0.
3. Cruzeiro, L, Eilbeck, JC, Marín, JL, Russell, FM. 2004. Dynamical two electron states in the Hubbard-Davydov model. *The European Physical Journal B* 42: 95-102. IF 2007=1.356, n°C=0.
4. Cruzeiro, L. 2005. Why are proteins with Glutamine- and Asparagine-rich regions associated with protein misfolding diseases?. *Journal of Physics: Condensed Matter* 17: 7833-7844. IF 2007=1.886, n°C=3.
5. Cruzeiro, L. 2005. Influence of the nonlinearity and dipole strength on the amide I band of protein alpha-helices. *Journal of Chemical Physics* 123: 234909. IF 2007=3.044, n°C=1.
6. Silva, PAS, Cruzeiro, L. 2005. How does a protein function? *FEBS Journal* 272: 19. IF 2007=3.396, n°C=0.
7. Silva PAS, Cruzeiro, L. 2005. Storage and conversion of energy by proteins. *European Biophysics Journal* 34: 625. IF 2007=2.238, n°C=0.
8. Silva, PAS, Cruzeiro, L. 2006. Dynamics of a nonconserving Davydov monomer. *Physical Review E* 74: 021920. IF 2007=2.483, n°C=1.
9. Cuevas, J, Silva, PAS, Romero, FR, Cruzeiro, L. 2007. Dynamics of the Davydov-Scott monomer in a thermal bath: Comparison of the full quantum and semiclassical approaches. *Physical Review E* 76: 011907. IF 2007=2.483, n°C=0.
10. Silva, PAS, Cruzeiro, L. 2007. Vibrational excited states and protein conformational changes. *European Biophys Journal* 36: S61. IF 2007=2.238, n°C=0.

### 2. Other publications national (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Cruzeiro-Hansson, L. 2003. What drives protein folding and protein function ? In: Proceedings of the Third Conference on Localization and Energy Transfer in Nonlinear Systems. Vázquez, L, Mackay, RS, Paz, MP (Eds). World Scientific, New Jersey-Hong Kong, pp. 169-176.
2. Cruzeiro, L. 2005. The Davydov model for Energy Transfer in Proteins. In: Encyclopedia of Nonlinear Science. Scott, A (Ed), Routledge, New York and London, pp. 187-189.

**4. Master and Ph.D. thesis completed** (3000 ca.)

**5. Patents/propotypes** (2000 ca.)

**6. Organization of conferences** (2000 ca.)

**7. Industry contract research** (2000 ca.)

**8. Government/organization contract research** (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

The Biophysics group has an on-going collaboration with the Nonlinear Physics Group of the University of Seville, Seville, Spain and in particular Prof. Francisco Romero Romero (the leader) and Dr. Jesús Cuevas Maraver, on the general theme of protein folding and function. In particular we have been studying quantum thermalization schemes based on Lindblad operators. This collaboration has already led to the following publication:

Cuevas, J, Silva, PAS, Romero, FR, Cruzeiro, L. 2007. Dynamics of the Davydov-Scott monomer in a thermal bath: Comparison of the full quantum and semiclassical approaches. Physical Review E 76: 011907.

Leonor Cruzeiro is also an honorary research fellow at the Mathematics Department of Heriot-Watt University, Edinburgh, Scotland, UK, where the Biophysics group collaborates with Prof. Chris Eilbeck on the interaction of quantum particles with mechanical vibrations, with models that are an extension of those applied to energy transfer in proteins. In this period, this collaboration has resulted in the following publication:

Cruzeiro, L, Eilbeck, JC, Marín, JL, Russell, FM. 2004. Dynamical two electron states in the Hubbard-Davydov model. *European Physical Journal B* 42: 95-102.

The Biophysics group has also collaborated with Profs. Larissa Brizhik and Alexander Eremko, from the Bogolyubov Institute for Theoretical Physics, Kiev, Ukraine, on the influence of electromagnetic radiation on energy transfer in proteins, using a model developed by Prof. A.S. Davydov, who was a leader there (now deceased). In this period, this collaboration has resulted in the following publication:

Brizhik, LS, Cruzeiro-Hansson, L, Eremko, AA, Olkhovska, Yu V. 2003. Dynamical properties of Davydov solitons in molecular chains. *Ukrainian Journal of Physics* 48: 667-671.

## 6e. Future research

### 1. Objectives (3000 ca.)

Building on the previous research on vibrational energy transfer in proteins and on classical dynamics of biomolecules, in the last five years the Biophysics Group of CCMAR has launched four new lines of research around the main theme of protein folding and function (see the second paragraph of item 6c. Objectives and also item 6c. Achievements above). The objective for the next five years is to develop further and consolidate each of those four new lines.

Concerning the first research line specified in item 6c. Objectives, work has already begun on a method to calculate the temperature dependence of absorption spectra (see refs. 2 and 5 in item 3, below). This method will be applied to the amide I band of alpha-helices and beta-sheets, as well as to the organic crystal of acetanilide and to full proteins. Dr. Holly Freedman, from the U.S.A, has successfully applied for an individual postdoctoral grant of the Foundation for Science and Technology, FCT, Portugal to perform this research and is due to start in January 2009.

Concerning the second research line specified in item 6c. Objectives, work has already begun on further applications of the VES hypothesis to protein misfolding diseases, namely, to explain the instability of proteins with large polyglutamine tracts (see refs. 1 and 4 in item 3, below). The aim is now to perform further simulations of energy transfer from the hydration waters to proteins with a varying number of glutamine residues in order to try to understand why huntingtins with more than 36 successive glutamines are particularly pathogenic.

Concerning the third research line specified in item 6c. Objectives, further work on extensions of the Davydov/Scott model to Hamiltonians that do not conserve the amide I quanta will be pursued. The general aim is to find a mechanism by which the release of the energy stored in the amide I vibrations can lead to a conformational change. Because the amide I excitation is a quantum particle and because proteins change conformation at finite temperature (typically 298-310 K) it is also necessary to include the thermal bath within a full quantum mechanical framework. To this end we have already started to collaborate with the Nonlinear Physics Group of the University of Seville (see ref. 3 in item Previous publications below).

Concerning the fourth research line specified in item 6c. Objectives, the aim is to perform further simulations to characterize better the free energy landscape of proteins (see assumption a) in item 6c. above). A criticism that has been made by referees of ref. 4 in item Previous publications below and of another article currently submitted is that the simulations made so far have been too

short and do not allow for definitive conclusions. Profiting from the development of potentials that include the interaction of the protein with water molecules without the use of an explicit bath, simulations, at least hundreds of nanoseconds long (and a few microseconds, in some cases), will be performed to determine whether proteins can have structures that are very different from the native ones and that are equally stable from a thermodynamic point of view.

**2. Funding, source, dates** (1500 ca.) (Indicate in full including amount of current and pending funding)

As mentioned in item just above, Dr. Holly Freedman has successfully applied to the Foundation for Science and Technology, Portugal (FCT) for an individual postdoctoral grant and will be joining the Biophysics Group of CCMAR in January 2009.

The project "Nonlinear waves in discrete, periodic and quasiperiodic systems", from the Foundation for Science and Technology, Portugal (amount: 70.000 EUR), has just finished.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Cruzeiro, L. 2005. Why are proteins with Glutamine- and Asparagine-rich regions associated with protein misfolding diseases? *Journal of Physics: Condensed Matter* 17: 7833-7844. IF=1.886, n°C=3.

2. Cruzeiro, L. 2005. Influence of the nonlinearity and dipole strength on the amide I band of protein alpha-helices. *Journal of Chemical Physics* 123: 234909. IF=3.044, n°C=1.

3. Cuevas, J, Silva, PAS, Romero, FR, Cruzeiro, L. 2007. Dynamics of the Davydov-Scott monomer in a thermal bath: Comparison of the full quantum and semiclassical approaches. *Physical Review E* 76: 011907. IF=2.483, n°C=0.

4. Cruzeiro, L. 2008. Protein's multi-funnel energy landscape and misfolding diseases. *Journal of Physical Organic Chemistry* 21: 549-554. IF=1.594, n°C=0.

5. Cruzeiro, L. Influence of the sign of the coupling on the temperature dependence of optical properties of one dimensional exciton models. *Journal of Physics B* (accepted for publication). IF=2.012.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

All the research lines of the Biophysics Group involve computer simulations and owe a lot to the free operating systems and software that have gradually been made available in the internet. On the other hand, some of the simulations (for example, the classical dynamics of proteins, however small, and some of the quantum energy transfer simulations) can only be done in especially designed clusters or in supercomputers. The Biophysics Group has at the moment only four operating personal computers and thus those requirements have only been very partially met by the use of the supercomputer facilities in the Laboratory of Advanced Computing of the University of Coimbra, which started in the beginning of 2007, as well as by a collaboration with the Informatics Group of CITI, at the New University of Lisbon (Prof. Pedro Medeiros and Paulo Afonso Lopes), which started towards the end of 2007. It must be emphasized that the successful



completion of fourth line of research (see the last paragraph in item 6e. Objectives above) will require a dedicated cluster with 50 CPUs of at least 2 GHz and with fast connections between them, with an estimated cost of some 50.000 EUR.

## Group of Synthesis and Organic Reactivity

### 6a. Group description

#### 1. Group nName / denomination

#### Group of Synthesis and Organic Reactivity

#### 2. Principal investigator

**Maria Lurdes Santos Cristiano**

#### 3. Location of group (Host institution)

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### 4. Keywords

Drug design, Structure-reactivity-activity correlations, Bioactive compounds, New synthetic methods

#### 5. Funding, source, dates (1000 ca.)

Project POCTI/FCB/33580/2000, from 2002 to 2005, 64.843,73 EUR.

Project PTDC/SAU-FCF/71345/2006, from 2007 to 2010, 20.400 EUR.

### 6b. Group team

#### 1. Researchers in the group (Include only PhD. integrated in the LA)

001. Maria Lurdes Santos Cristiano (Cat.: Professor Associado, Gr. Acad.: Agregação)

002. Nuna Cláudia Peixoto de Araújo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

#### 2. Other researchers in the group (Include here collaborators with PhD. only)

#### 3. Other researchers in the group (non PhD.)

001. Rui Filipe de Ascensao Almeida (Cat.: Assistente de Investigação, Gr. Acad.: Licenciatura)

002. Andreia Cristina Domingues Bringela (Cat.: Estagiário de Investigação, Gr. Acad.: Ensino Secundário)

003. Lília Isabel Lameirinhas Cabral (Cat.: Estagiário de Investigação, Gr. Acad.: Ensino Secundário)

004. David José Pereira Gago (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

005. Edite Manuela da Graça Veríssimo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

006. Emanuel Fernando Branco Ochoa Morgado (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

007. Luís Miguel Teodoro Frija (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

## 6c. Objectives & achievements

### 1. Objectives (2000 ca.)

Research activities may be divided in two areas, which interact synergistically:

#### A- Structural Analysis and Studies on the Reactivity of Bioactive Compounds

The investigation of structure-reactivity relationships is essential for the interpretation and prediction of the outcome of reactions. This general rule may be applied to all reacting systems, including the interpretation of interactions between chemicals (e.g. drugs) and their biological targets.

The Group has been investigating in the field of Reactivity, within the broad research area of Physical Organic Chemistry. The work involved the synthesis, structural analysis and investigation of the reactivity of derivatives of heterocyclic bioactive compounds. The aim is the improvement and fine-tuning of properties, for instance biological activity, and also the development of new synthetic methods applicable to the preparation of new bioactive compounds and to the modification of bioactive natural products. Structural elucidation and mechanistic studies were approached, in catalysed and non-catalysed reactions: (i) reductive cleavage of carbon-oxygen bonds catalysed by transition metals, (ii) acid-catalysed oxidation of unsaturated compounds with nitroarenes, (iii) sigmatropic isomerisations (iv) photoreactivity.

#### B- Design and Synthesis of DNA-Directed Endoperoxides

During the last years, the Group has been investigating in the field of Medicinal Chemistry. We aim at the design and synthesis of new antimalarial and anti-neoplastic agents. The idea is to develop synthetic routes to DNA-directed endoperoxides, combining a DNA binding moiety with endoperoxide-based antimalarials. The rationale relies on the ability of Fe(II) to selectively activate peroxide-type drugs, generating radical species that damage key biomolecules. The compounds produced should be capable of simultaneous selective oxidative damage to a complementary strand of DNA and liberation of a protein inhibitor, in a combination chemotherapy-like approach. Because tumour cells are known to have higher Fe(II) concentrations than normal cells, the DNA-directed peroxide compounds designed may also act as potential antiproliferative agents. In vitro and in vivo tests are conducted at the Liverpool School of Tropical Medicine and the Department of Pharmacology of the University of Liverpool.

### 2. Main achievements (2000 ca.)

During the period under evaluation, the scientific production of the Group was:

Papers in international (ISI) journals – 15

Papers in international (non ISI) journals – 1

Invited lectures in conferences – 3

Oral presentations – 8

Poster presentations – 37

Poster prizes – 3

Supervision

PhD students – 3

MSc students – 3

Final year students – 9

The successful organization of the 11th European Symposium of Organic Reactivity was an important achievement in 2007, bringing further international recognition to the group in the area of Physical Organic Chemistry. As a result MLS Cristiano became the portuguese representative in the International Advisory Board for ESOR Symposia, was invited to join the IUPAC subcommittee on Structural and Mechanistic Chemistry and was also invited as Editor of Journal of Physical Organic Chemistry. This Journal dedicated 2 Issues to the ESOR meeting organised by us, within CCMAR.

The major achievement, however, was the involvement in the area of Medicinal Chemistry that let to our participation in the FP7 Project “The safety pharmacology of Artemisinins when used to reserve pathophysiology of malaria in pregnancy (ARTEMIP). The funds now available will enable more research in this area.

The group has increased from 3 to 8 members, more collaborations were established and there is some optimism concerning future achievements in research, provided that we can gather the necessary resources.

Considering the lack of basic equipment in UALG, namely NMR and MS facilities, the small amount of money that we were granted for this period, and the fact that a major part of the research involves preparative organic chemistry, we think that we can be somehow proud with what we achieved.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Cristiano, MLS, Gago, DJP, d'A Rocha Gonsalves, AM, Johnstone, RAW, McCarron, M, Varejão, JMTB. 2003. Investigations into mechanism of action of nitrobenzene as a mild dehydrogenating agent under acid-catalysed conditions. Journal of Organic and Bioorganic Chemistry: 565-574. IF 2007=3.167, n°C=4.

2. Araújo, NCP, Brigas, AF, Cristiano, MLS, Frija, LMT, Guimarães, EMO, Loureiro, RMS. 2004. Heteroaromatic benzyl ethers as intermediates for palladium-catalysed transfer hydrogenolysis of benzyl alcohols. *Journal of Molecular Catalysis A: Chemical* 215: 113. IF 2007=2.707, n°C=6.
3. Gomez-Zavaglia, A, Reva, ID, Frija, LMT, Cristiano, MLS, Fausto, R. 2005. Molecular structure, vibrational spectra and photochemistry of 2-methyl-2H-tetrazol-5-amine in solid argon. *Journal of Physical Chemistry A* 109: 7967. IF 2007=2.918, n°C= 10.
4. Frija, LMT, Khmelinskii, IV, Cristiano, MLS. 2005. Novel efficient synthesis of 3,4-dihydro-6-substituted-3-phenylpyrimidin-2(1H)-ones. *Tetrahedron Letters* 46: 6757. IF 2007=2.615, n°C=6.
5. Frija, LMT, Cristiano, MLS, Guimarães, EMO, Martins, NC, Loureiro, RMS, Bickley, J. 2005. Palladium-catalysed reduction of heteroaromatic naphthyl ethers: structural effects on reactivity. *Journal of Molecular Catalysis A: Chemical* 242: 241. IF 2007=2.707, n°C=3.
6. O'Neill, PM, Verissimo, E, Ward, SA, Davies, J, Bachi, MD, Korshin, EE, Araujo, N, Pugh, M, Cristiano, MLS, Stocks, PA. 2006. Diels-Alder/Thiol-olefin co-oxidation approach to the antimalarial 2,3-dioxabicyclo [3.3.1] nonane pharmacophore. *Bioorganic and Medicinal Chemistry Letters* 16(11): 2991-2995. IF 2007=2.604, n°C=1.
7. Frija, LMT, Khmelinskii, IV, Cristiano, MLS. 2006. Mechanistic investigations into the photochemistry of 4-allyltetrazolones in solution; a new approach to the synthesis of 3,4-dihydropyrimidinones. *Journal of Organic Chemistry* 71(9): 3583-3591. IF 2007=3.959, n°C=3.
8. Gomez-Zavaglia, A, Reva, ID, Frija, LMT, Cristiano, MLS, Fausto, R. 2006. Photochemistry of 1-phenyl-tetrazolone isolated in solid argon. *Journal of Photochemistry and Photobiology A: Chemistry* 179: 243-255. IF 2007=1.911, n°C=6.
9. Frija, LMT, Reva, ID, Gómez-Zavaglia, A, Cristiano, MLS, Fausto, R. 2007. Photochemistry and vibrational spectra of matrix-isolated 5-ethoxy-1-phenyl-1H-tetrazole. *Journal of Physical Chemistry A* 111: 2879-2888. IF 2007=2.918, n°C=1.
10. Frija, LMT, Reva, ID, Gómez-Zavaglia, A, Cristiano, MLS, Fausto, R. 2007. UV-induced photochemistry of matrix-isolated 1-phenyl-4-allyl-tetrazolone. *Photochemical & Photobiological Sciences* 61(11): 1170-1176. IF 2007=2.208, n°C=1.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Araújo, N. 2006. The Drug-hybrid approach to antimalarial chemotherapy. *Drugs of the Future* 31(A): 36-37.

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Veríssimo, Edite. 2007. New approaches to antimalarial combination chemotherapy: Aminoquinoline-peroxide hybrids. Financed by FCT, SFRH/BD/6176/2001. Supervisor: MLS Cristiano; co-supervisor: PM O'Neill (Univ. of Liverpool, UK).

##### **MSc THESIS**

Coelho, Daniela. 2008. Estudos de Reactividade em Derivados de Compostos Heterocíclicos.

#### **5. Patents/propotypes (2000 ca.)**

#### **6. Organization of conferences (2000 ca.)**

1. ESOR XI, European Symposium on Organic Reactivity, Faro, Portugal, July 2007, 1-6.

Chair of the Organizing Committee – Maria de Lurdes Cristiano

All members in the research group were involved in this event that gathered around 250 scientists from 34 countries.

Organized outside CCMar

2. 8º Encontro Nacional de Química Física da SPQ, Luso, June 2007.

Maria de Lurdes Cristiano - Member of the Scientific Committee.

3. GLUPOR VI/3rd Iberian Carbohydrate Meeting, Lisboa, September 2007.

Maria de Lurdes Cristiano - Member of the Scientific Committee.

4. ESOR X, European Symposium on Organic Reactivity, Rome, July 2005, Maria de Lurdes Cristiano - Chair of a scientific session.

5. Química ao Sul, Faro, 20 de Maio de 2005, Maria de Lurdes Cristiano - Chair of the Organizing Committee and member of the Scientific Committee.

6. I Encontro de Professores do Sul, Faro, 18 e 19 de Abril de 2005,

Maria de Lurdes Cristiano - Chair for the workshop “Debate dos novos programas curriculares de Física e Química.”

7. Reaction Mechanisms VII, Dublin, 4-8 July 2004,

Maria de Lurdes Cristiano - Chair of a scientific session.

8. AIDA, 29274-IC-2-2003-AT-ERASMUS-IPUC16, University of Malta, 2004, Maria de Lurdes Cristiano - Chair for the workshop “Chemical aspects of pollution control”.

**7. Industry contract research (2000 ca.)**

**8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

**9. Internationalization (2000 ca.)** (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

The major collaborations of the Group are (institution/team leader):

Department of Chemistry, University of Liverpool, UK (Profs Paul M. O'Neill and Robert A.W. Johnstone).

Department of Pharmacology, University of Liverpool, UK (Prof. Kevin Park)

Liverpool School of Tropical Medicine, UK (Prof. Stephan Ward).

Department of Chemistry, University of Coimbra (Profs. Rui Fausto and Ermelinda Eusébio).

Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina (Dr Andrea Gomez-Zavaglia).

Faculty of Chemistry, Jagiellonian University at Krakow, Poland (Dr. Agnieszka Kakzor).

**6e. Future research**

**1. Objectives (3000 ca.)**

The Group aims at pursuing research in the areas of Organic Reactivity and Medicinal Chemistry. These areas are broad and interdisciplinary, and the work already carried out granted us some international recognition. Now, more efforts have to be made towards a stronger collaboration within CIMAR, so that the research skills of the group in these areas contribute to/benefit from the skills of other groups.

The group has joined CIMAR in 2006. So far, contacts have already been made with the group of Professor Cancela, whereby we will synthesize fosmidomycin derivatives that will be tested for biological activity in marine species. Also, it was agreed that some of the endoperoxide and cysteine-protease inhibitors with antimalarial activity, produced by us, are tested on organisms under study in her group (EDGE group).

In the last decades, research in marine ecology has boosted, and uncovered secondary metabolites from marine organisms that are active in the treatment of various diseases (e.g. antibiotic, antiviral and antineoplastic activity). Some of them are in clinical trials (e.g. Bryostatin 1 and Dolastatin 10, for the treatment of several cancer types). Thus, the sea offers entirely new classes of organic compounds, that need to be studied from the view-point of their structure and



reactivity, and that should be explored as drug candidates. Accordingly, research in the group, at a medium term, should be directed towards applications in marine biomedical prospecting. However, in order to ensure a sound and safe strategic switch into this area, better analytical facilities in the Campus should be made available.

In the area of medicinal chemistry, we are now involved in an European Network that includes WHO, financed within FP7. The aim of the research is to address some toxicological aspects related to the presently available chemotherapeutic solutions for malaria. Since May 2008, a post-doctoral researcher and 2 graduate researchers are working in this project and paid by EC funds. In parallel, the group is working on alternative solutions, with the aim of producing antimalarials with increasing potency and selectivity. Some of the endoperoxide-based compounds have shown activity in cancer cell lines, so there is an increased interest in developing them as antineoplastic agents. Specifically, we are trying to produce DNA-directed drugs, so the drug design incorporates minor groove binders. The major aim of the group, at the moment, is to boost research in this field: we have some funds for this research at the moment, and mostly because of humanitarian issues. Malaria is an endemic disease that affects mostly children and pregnant woman, in underdeveloped countries.

**2. Funding, source, dates (1500 ca.)** (Indicate in full including amount of current and pending funding)

2007-2010, Studies on the Reactivity of Derivatives of Tetrazole and Isothiazole; Isothiazolyl - Tetrazoles as potential Bidentate Ligands and their Application in Catalysis, Funding: 20.400 EUR.

Project PTDC/SAU-FCF/71345/2006

2008-2011, The Safety Pharmacology of Artemisinins when used to reverse pathophysiology of Malaria in Pregnancy (ARTEMIP). Funding: 172.480 EUR.

FP7 Cooperation Work Programme: Theme 1 – Health. Health-2007-2.3.2-4: Addressing Knowledge Gaps in Pregnancy Malaria Participants as below.

**3. Previous publications in the area (1500 ca.)** (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. O'Neill, PM, Stocks, PA, Pugh, MD, Araujo, NC, Korshin, EE, Bickley, JF, Ward, SA, Bray, PG, Pasini, E, Davies, J, Verissimo, E, Bachi, MD. 2004. Design and Synthesis of Endoperoxide Antimalarial Prodrug Models: Prototypes for Selective Intraparasitic Generation of Cysteine Protease Inhibitors and Other Parasitocidal Species. *Angewandte Chemie International Edition* 43: 4193. IF=10.031, n°C=12.

2. Stocks, PA, Bray, PG, Barton, VE, Al-Helal, M, Jones, M, Araújo, NCP, Gibbons, P, Ward, SA, Hughes, RH, Biagini, GA, Davies, J, Amewu, R, O'Neill, PM. 2007. Evidence for a common non-heme chelatable-iron-dependent activation mechanism for semisynthetic and synthetic endoperoxide antimalarial drugs. *Angewandte Chemie International Edition* 119: 1-7. IF=10.031, n°C=2.

3. Veríssimo, E, Berry, N, Gobbons, P, Cristiano, MLS, Rosenthal, P, Gut, J, Ward, S, O'Neill, PM. 2008. Design and synthesis of novel 2-pyridone peptidomimetic falcipain 2/3 inhibitors. *Bioorganic and Medicinal Chemistry Letters* 18: 4210-4214. IF=2.604, n°C= 0.
4. Frija, LMT, Khmelinskii, IV, Serpa, C, Reva, ID, Fausto, R, Cristiano, MLS. 2008. Photochemistry of 5-allyloxy-tetrazoles: steady state and laser flash photolysis study. *Journal of Organic and Biomolecular Chemistry* 6(6): 1046-1055. IF=3.167, n°C=0.
5. Almeida, R, Gómez-Zavaglia, A, Kaczor, A, Cristiano, MLS, Eusébio, MES, Maria, TMR, Fausto, R. 2008. First observation of a Chapman rearrangement of a pseudosaccharyl ether in the solid-state: the thermal isomerisation of 3-(methoxy)-1,2-benzisothiazole 1,1 dioxide revisited. *Tetrahedron* 64(15): 3296-3305. IF= 2.869, n°C=0.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

The work in synthetic organic chemistry requires an NMR service for routine analysis available in the Campus. This is urgent, and the lack of basic NMR facilities has severely hindered the development of research by the group. The same applies to Mass Spectrometry. We have been using the equipment for compound analysis and characterization at the University of Liverpool and, recently, the NMR service at New University of Lisbon. However, this is a poor solution, because we have to wait for results, sometimes during several weeks, before proceeding with the synthesis.

We have a good collaboration with Centro de Química of Universidade de Coimbra, that provides us with facilities in Matrix isolation coupled to FT-IR Spectroscopy and Differential Scanning Calorimetry analysis. Also, we use computer centers located in Krakow for high level Molecular Orbital calculations. For the moment, this is a very good compromise and is specially valuable for the work in Reactivity.

Considering the projects we have in hands in the area of Medicinal Chemistry, a preparative HPLC would be a great help, so we could conveniently purify the target compounds (drugs) before sending them for biological assays. Also, It would be important for the development of the group to have a post-doctoral researcher in the area of reactivity.

## **BioVanadium Research Group** (only at CCmar in 2006 and 2007)

### **6a. Group description**

#### **1. Group name / denomination**

**BioVanadium Research Group**

#### **2. Principal investigator**

**Manuel Aureliano Pereira Martins Alves**

#### **3. Location of group** (Host institution)

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Decavanadate, Oxidative stress, Vanadium toxicology, Muscle contraction and regulation

#### **5. Funding, source, dates** (1000 ca.)

98.000 Euros, POCTI program financed through FEDER for the research project 38191/QUI/2001 (to M.Alves), “Cadmium and vanadium compounds interactions with calcium pump”, 2002-2005.

### **6b. Group team**

#### **1. Researchers in the group** (Include only PhD. integrated in the LA)

001. Manuel Aureliano Pereira Martins Alves (Cat.: Professor Associado, Gr. Acad.: Doutoramento)

002. Teresa Paula Martins Tiago (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

#### **2. Other researchers in the group** (Include here collaborators with PhD. only)

#### **3. Other researchers in the group** (non PhD.)

001. Ana Margarida Pereira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

002. Maria Joao Rodrigues Pereira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

003. Sandra Sofia Ganchas Soares (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

004. André Gonçalo Antunes dos Santos (Cat.: Outra, Gr. Acad.: Bacharelato)

005. Fábio Levi Piedade Neto Guerra Fontes (Cat.: Outra, Gr. Acad.: Bacharelato)

006. Rosa Maria Faustino Brissos (Cat.: Outra, Gr. Acad.: Ensino Secundário)

## 6c. Objectives & achievements

### 1. Objectives (2000 ca.)

Research topics: 1) Interaction of vanadate with myosin/actin; 2) Insulin-mimetic vanadate complexes effects on calcium pump; 3) Cellular responses induced by vanadate.

Environmental contamination by toxic metals, in particular by vanadium, has increased dramatically during the last decades due to the use of fossil fuels. In spite of the emerging interest in the pharmacological effects of some vanadium compounds, for instance as an insulin-mimetic, in the treatment of diabetes, the toxicology of vanadium constitutes a area of increasing interest. Vanadium is one of the important transition elements in biology. In aqueous solution vanadium (V) occurs in a number of oxometalates called vanadates. At neutral pH and through the range of concentrations most frequently employed in biochemical studies, the predominant vanadate species are the monomer ( $\text{H}_2\text{VO}_4^-$ ), dimer ( $\text{H}_3\text{V}_2\text{O}_7^-$ ) and tetramer ( $\text{V}_4\text{O}_{12}^{4-}$ ). These species reach chemical equilibrium on a millisecond time scale making difficult the identification of the oligomers responsible for the effects promoted in biological systems.  $^{51}\text{V}$  NMR spectroscopy studies have proved to be highly informative in systems of biological relevance. The rapid exchange between vanadate oligomers and the changes in the  $^{51}\text{V}$  NMR signal line-widths at different conditions makes this spectroscopy a sensitive tool to examine interactions between proteins and vanadate species. However, the disappearance of one of the vanadate species resonance upon binding to protein is dictated by the total population of free exchanging oxovanadates and would result whether this or another vanadate species were bound to the protein. 1) Myosin is a highly specialised protein involved in the process of muscle contraction, which along with actin, converts the chemical energy of ATP hydrolysis to mechanical work. Although the actomyosin ATPase activity has been described to be about 90% inhibited by  $\text{V}_1$ , it was suggested that this inhibition might be due in part to the presence of polymeric vanadate ions. 2) Sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$ -ATPase is a transmembrane transport system, which accumulates  $\text{Ca}^{2+}$  at expense of ATP splitting during the process of muscle relaxation. ATP is used in a process involving the transfer of the phosphoryl group to the  $\text{Ca}^{2+}$ -ATPase with subsequent breakdown of the phosphorylated enzyme. The mechanism by which the  $\text{Ca}^{2+}$  pumping is associated with ATP hydrolysis is not fully understood in clear molecular terms and is usually summarized by a cycle of sequential reaction steps with two major states of the enzyme,

### 2. Main achievements (2000 ca.)

Vanadium can be used as a tool in the study of biochemical mechanisms such as: muscle contraction, calcium homeostasis, cytoskeleton structure and function, in vitro and in vivo oxidative and nitrosative stress, cell death and cell signalling. Others research lines in the Group of BioVanadium includes the study of reactive and nitrosative oxygen species in the structure and function of contractile proteins and the study of the molecular mechanisms involved in insulin resistance and type-2 diabetes.

The degree of toxicity depends on the mode of administration such as intraperitoneal or intravenous, vanadium concentration, time of exposition and is also dependent; at some extend, of the vanadate species such as decavanadate that may induce several cellular effects (Figure 1).

Figure 1. Scheme of the proposed cellular targets of decavanadate (V10): 1) V10 uptake through anionic channels; 2) V10 binding to membrane proteins; 3) formation of V10 upon intracellular acidification; 4) reduction of monomeric vanadate by antioxidant agents; 5) reduction of decameric vanadate by enzymes; 6) binding of V10 to target proteins preventing its reduction; and 7) accumulation V10 into subcellular organelles such as mitochondria. V10, decameric vanadate; V1, monomeric vanadate; E, enzyme; P, protein; AC, anionic channel.

1) It has been suggested that V4 interacts with myosin, being responsible for the vanadate-induced photolytic cleavage of myosin subfragment-1 (S1). There are also some indications that decameric vanadate (V10O<sub>28</sub>6-), a large polymeric anion formed under mildly acidic conditions, interacts to S1 as well as to affect the actomyosin ATPase activity although little is known about its putative binding sites. In order to gain a deeper knowledge of decavanadate-myosin interactions, the 51V NMR approach was applied to S1, taking advantage of the fact that contrarily to the labile oxovanadates, V10 is sufficiently long-lived that the broadening of NMR signals can be attributed exclusively to protein binding. 51V NMR spectra of 5 mM decavanadate solution (containing 148 mM V1 and 485 mM V10 species) were obtained at several conditions and the relative order of line broadening upon protein addition (reflecting the interaction of vanadate species with myosin S1) was analyzed. To study a possible competition between polymeric vanadate species and V10 we have also used a 2 mM metavanadate solution mixed to 5 mM decavanadate solution, containing in total 760 mM V1, 146 mM V2, 268 mM V4 and 487 mM V10. The relative order of line broadening of 51V NMR signals.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Tiago, T, Ramos, S, Aureliano, M, Gutiérrez-Merino, C. 2006. Peroxynitrite induces F-actin depolymerization and blockade of myosin ATPase stimulation. *Biochemical and Biophysical Research Communications* 342: 44-49.

2. Tiago, T, Simão, S, Aureliano, M, Martín-Romero, FJ, Gutiérrez-Merino, C. 2006. Inhibition of skeletal muscle S1-myosin ATPase by peroxynitrite. *Biochemistry* 45: 3794-3804.

3. Ramos, S, Manuel, M, Tiago, T, Duarte, RO, Martins, J, Gutiérrez-Merino, C, Moura, JJG, Aureliano, M. 2006. Decavanadate interactions with actin inhibit G-actin polymerization and stabilize decameric vanadate species. *Journal of Inorganic Biochemistry* 100(11): 1734-1743.

4. Soares, S, Martins, H, Aureliano, M. 2006. Vanadium distribution dependence on decavanadate administration. *Archives of Environmental Contamination and Toxicology* 50: 60-64.

5. Soares, SS, Gutiérrez-Merino, C, Aureliano, M. 2007. Mitochondria as a target for decavanadate toxicity in *Sparus aurata* heart. *Aquatic Toxicology* 83: 1-9.

6. Soares, SS, Gutiérrez-Merino, C, Aureliano, M. 2007. Decavanadate induces mitochondrial membrane depolarization and inhibits oxygen consumption. *Journal of Inorganic Biochemistry* 101: 789-796.

7. Soares, SS, Martins, H, Duarte, RO, Moura, JJG, Coucelo, J, Gutiérrez-Merino, C, Aureliano, M. 2007. Vanadium distribution, lipid peroxidation and oxidative stress markers upon decavanadate in vivo administration. *Journal of Inorganic Biochemistry* 101: 80-88.
8. Tiago, T, Matel, P, Gutiérrez-Merino, C, Aureliano, M. 2007. Binding modes of decavanadate to myosin and inhibition of the actomyosin ATPase activity. *Biochimica et Biophysica Acta* 1771: 474-480.
9. Aureliano, M, Gândara, R. 2005. Decavanadate contribution to vanadium toxicity. *Journal of Inorganic Biochemistry* 99: 979-985.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Aureliano, M, Soares, SS, Tiago, T, Ramos, S, Gutiérrez-Merino, C. 2007. Biological effects of decavanadate: Muscle contraction, in vivo oxidative stress, and mitochondrial toxicity. In *Vanadium: The versatile metal*. Kustin, K, Pessoa, JC, Crans, DC (Eds), pp. 249-263. ACS Symposium Series, Washington, DC.
2. Aureliano, M, Soares, SS, Heras, F, et al. 2007. The pathways of cell death in cardiomyocytes induced by vanadate. *Free Radical Research* 41: S17-S17.
3. Tiago, DM, Laize, V, Cancela, ML, et al. 2007. Vanadate and bone metabolism: Effect on proliferation and mineralization of fish bone-derived cells. *Free Radical Research* 41: S57-S58.
4. Tiago, T, Silva, D, Santos, A, et al. 2007. Actomyosin modulation by peroxynitrite. *Free Radical Research* 41: S58-S58.

**4. Master and Ph.D. thesis completed** (3000 ca.)

Soares, Sandra. 2007. FCMA, University of Algarve. Co-supervisors: M Aureliano, J Coucelo and C Gutiérrez-Merino.

Contribution of decameric vanadate to vanadate toxic effects was studied not only in vitro but also in vivo. Following an intravenous administration of a decavanadate solution in *Sparus aurata* it is suggested that decameric vanadate has a different response pattern on oxidative stress markers of cardiac tissue than monovanadate, pointing out that the differential contribution of vanadate oligomers should be taken into account to rationalize in vivo vanadate toxicity. Moreover, vanadium accumulation in mitochondria, in particular when decameric vanadate is administered, indicates this subcellular organelle as a potential target of vanadate toxicity effects. In fact, in vitro studies showed that decameric vanadate is a stronger mitochondrial depolarization agent as well as inhibitor of oxygen consumption, besides affecting mitochondrial membrane repolarization, in fish cardiac and rat hepatic isolated mitochondria. It was also observed that decameric vanadate alters mitochondrial bioenergetics by inducing changes in the redox steady-

state of b-type cytochromes and blocking mitochondrial electron transport chain. In cardiomyocytes, mitochondrial membrane depolarization is reported as an early event of vanadate-induced necrotic cell death. It is concluded that, when compared to monovanadate, decameric vanadate affects differently biological systems by different mechanisms and contributes, at least in part, for vanadate toxic effects on mitochondria.

**5. Patents/prototypes (2000 ca.)**

**6. Organization of conferences (2000 ca.)**

**7. Industry contract research (2000 ca.)**

**8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

**9. Internationalization (2000 ca.)** (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

**6e. Future research**

**1. Objectives (3000 ca.)**

1) Toxicity effects of vanadium compounds with insulin mimetic properties in calcium regulation. 2) Mechanisms of cell death induced by vanadate oligomers; 3) Reactive oxygen and nitrogen oxygen species in muscle proteins and cytoskeleton structures. 4) Signaling pathways for vanadate toxicity.

**2. Funding, source, dates (1500 ca.)** (Indicate in full including amount of current and pending funding)

Only from the Plurianual of CCMAR.

**3. Previous publications in the area (1500 ca.)** (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Tiago, T, Aureliano, M, Gutiérrez-Merino, C. 2002. Quenching of Myosin Intrinsic Fluorescence Unravels the Existence of a High Affinity Binding site for Decavanadate. Journal of Fluorescence 12: 87-90.



2. Tiago, T, Aureliano, M, Duarte, RO, Moura, JGG. 2002. Vanadate Oligomers Interaction with Phosphorylated Myosin. *Inorganica Chimica Acta* 339C: 317-321.
3. Tiago, T, Aureliano, M, Gutiérrez-Merino, C. 2004. Decavanadate Binding to a High Affinity Site near the Myosin Catalytic Centre Inhibits F-Actin-Stimulated Myosin ATPase Activity. *Biochemistry* 43: 5551-5561.
4. Tiago, T, Aureliano, M, Moura, JGG. 2004. Decavanadate as a Biochemical Tool in the Elucidation of Muscle Contraction Regulation. *Journal of Inorganic Biochemistry* 98: 1902-1910.
5. Aureliano, M, Tiago, T, Gândara, RMC, Sousa, A, Duarte, RO, Kaliva, M, Salifoglou, A, Moura, JGG. 2005. Interactions of vanadium(V)-citrate complexes with the sarcoplasmic reticulum calcium pump. *Journal Inorganic Biochemistry* 99: 2355-2361.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

**Molecular Biology of Marine Organisms (MBMO), now consolidated as group EDGE (Evolution, Development and Gene Expression)**

**6a. Group description**

**1. Group name / denomination**

**Molecular Biology of Marine Organisms (MBMO), now consolidated as group EDGE (Evolution, Development and Gene Expression)**

**2. Principal investigator**

Maria Leonor Quintais Cancela Fonseca

**3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

**4. Keywords**

Molecular determinants of tissue mineralization, Host-parasite interaction, Transcriptional regulation, Molecular evolution

**5. Funding, source, dates (1000 ca.)**

EDGE research activities have been funded through:

12 national projects (1.277.443 EUR)

7 international projects (391.241 EUR)

6 collaborative actions with academic labs (12.194 EUR)

3 contracts with industrials (72.286 EUR)

4 prizes (52.000 EUR)

FCT Pluriannual (87.625 EUR)

Total funding: 1.892.789 EUR

National:

PDCTM/P/MAR/15308/1999

POCTI/C/BIO/12143/1998

POCTI/FIS/34668/1999

POCTI/BCI/48748/2002

POCTI/CVT/42098/2001

POCTI/BIA-BCM/58677/2004

POCTI/CVT/57982/2004

POCI/MAR/57921/2004

POCI/MAR/61623/2004

POCI/MAR/60883/2004

PTDC/CVT/72083/2006

22-05-01-FDR-00020

International:

Spanish Funding Agency/CICYT64

EU-FP6/GOCE-CT-2004-505403

INTERREG IIIA/SP5.P117/03

EU-FP6/SSA-FOOD-044442

EU-FP7/FP6-2005-SSP5A

NRC/172483/S40

NRC/165203/S40

Collaborative actions:

Luso-Espanholas/AI-E-423/02

EU-FP6/COST Action B23

Treaty of Windsor/B-72/04

Luso-Espanholas/AI-E-104/05

Treaty of Windsor B-47/07

PESSOA/DEEPBONE

Industrial contracts:

Transgás Atlântico SA

Novozymes

Águas do Algarve

Prizes:

Ceratonia 2006 & 2007

MGE-ImaGene2 2006 (2 prizes)

## **6b. Group team**

### **1. Researchers in the group (Include only PhD. integrated in the LA)**

- 001. Maria Leonor Quintais Cancela Fonseca (Cat.: Professor Catedrático, Gr. Acad.: Agregação)
- 002. Dina Cristina Fernandes Rodrigues da Costa Simes (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)
- 003. Pedro Miguel Leal Rodrigues (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)
- 004. Paulo Jorge Travessa Gavaia (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)
- 005. Vincent Laizé (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)
- 006. Natércia Maria da Silva Conceição (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 007. Sara Maria Mira da Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

### **2. Other researchers in the group (Include here collaborators with PhD. only)**

- 001. Ivar Ronnestad (Cat.: Professor Associado, Gr. Acad.: Doutoramento)
- 002. Vanesa Robles Rodríguez (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)
- 003. Idílio Jorge Matias Pereira Pinto (Cat.: Ciência 2007, Gr. Acad.: Doutoramento)
- 004. Juan Bosco Ortiz Delgado (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 005. Laurence Myriam Elandalloussi (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 006. Vijayakumar Parameswaran (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 007. Sandra Maria Pinto Marques (Cat.: Outra, Gr. Acad.: Doutoramento)

### **3. Other researchers in the group (non PhD.)**

- 001. Marta Sofia Mendes Valente Bernardo (Cat.: Assistente de Investigação, Gr. Acad.: Licenciatura)
- 002. Ricardo Manuel Rafael Afonso (Cat.: Assistente de Investigação, Gr. Acad.: Licenciatura)
- 003. Ricardo Mario Bastos Leite (Cat.: Assistente de Investigação, Gr. Acad.: Mestrado)
- 004. Anabela Ben-Simon Brito (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

- 005. Brigitte Sandra Nunes Simões (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 006. Carla Alexandra São Bento Viegas (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 007. Cátia Andreia Lourenço Marques (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 008. Céline Rodrigues Madeira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 009. Cindy Vitória Fazenda (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 010. Daniel António Martins Tiago (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 011. Gisela João Ribeiro Lemos Dionisio (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 012. Ines Costa de Carvalho (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 013. Joana Alexandra Teixeira Rosa (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 014. João Pedro Gonçalves Cardeira da Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 015. Marta Isabel da Silva Rafael (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 016. Michael Nogueira Viegas (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 017. Miguel Alexandre Medeiros Vidigal Caldeira Pais (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 018. Odete Dmingues Cordeiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 019. Rita Margarida Teixeira Ascenso (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 020. Rui Pedro Peres dos Santos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 021. Sandra Maria Sengo Mesquita (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 022. Sofia Isabel Franco Cavaco (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 023. Tomé Pereira de Azevedo Santos Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 024. Vânia Cristina Palma Roberto (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 025. Vera Alexandra Garcia da Fonseca Batista (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

#### **I. MOLECULAR DETERMINANTS OF TISSUE MINERALIZATION**

- Develop fish & amphibians in vitro systems to analyse mineralization-related pathways, gene expression & regulation, and test mineralogenic effects of drugs
- Identify orthologs of mammalian bone Gla-proteins and other bone- & cartilage-related proteins in fish, study their spatial-temporal expression & regulation to bring insight into their function and evolution
- Identify genes & regulatory pathways involved in normal vertebrate skeletogenesis
- Implement strong genomic/proteomic capabilities within the group suitable to unveil key genes/proteins involved in abnormal skeletal development and mineralization
- Implement a suitable zebrafish facility for in vivo studies
- Develop know-how, through training and external collaborations, for developmental studies related to skeleton formation
- Establish purification methodologies/tools to identify new proteins involved in calcium modulation

## II. PERKINSUS-BIVALVE INTERACTIONS

- Ascertain the degree of Perkinsus infection prevalence in clams and other co-habiting bivalves along Portuguese coast by performing an extended and continued survey. Identify permissive/non-permissive bivalve species
- Identify environmental conditions affecting Perkinsus survival/proliferation
- Develop a specific & sensitive diagnostic tool to follow Perkinsus infection of bivalves
- Develop genomic tools for Perkinsus (e.g. EST, cDNA and genomic libraries) to identify genes & metabolic pathways. Tools will also be developed with the ultimate objective of developing integrated high throughput analysis for Perkinsus functional genomics
- Analysis of Perkinsus-clam interactions through the use of subtractive hybridization & differential display approaches to unveil genes & metabolic pathways involved in parasite infection

## III. POPULATION GENETICS

- Development of molecular tools suitable for paternity/sexing and genotyping wild populations for conservation & management purposes, including Portuguese populations of Bonelli's eagle and otters, and economically relevant marine crustaceans and fish

### **2. Main achievements (2000 ca.)**

#### I. MOLECULAR DETERMINANTS OF TISSUE MINERALIZATION

- Development of FIRST fish & Xenopus bone-derived cell lines and fish cell line database (within FP6 project)

- Purification & cloning of bone-related Gla proteins (OC, MGP) in a variety of fish (including an ancestral OC in sturgeon)
- Characterization of OC/MGP evolutionary relationship and identification of domains/amino acids essential for protein function
- Cloning of mineralization-related gene promoters (e.g. MGP, OC, OP, SDR, BMP2, FHL2 and colX) from fish/amphibians, analysis of promoter activity and identification of transcription factor binding sites
- Proteomic analysis of oyster nacre & identification of proteins with osteoinductive effect
- Implementation of zebrafish facility (3 independent systems, 147 aquaria, microinjection equipment)
- Development of new techniques for introduction of DNA into zebrafish embryos (lipid based and laser-mediated transfections) & bone-derived fish cells
- Establishment of a comparative proteomics platform for fish protein identification
- Identification of proteins related with bone deformities in fish, using 2D techniques

## II. PERKINSUS-BIVALVE INTERACTIONS

- Development of a clonal culture of *Perkinsus olseni* (PO) in 2002 allowed in vitro testing of drugs (i.e. antimalarial) and ex vivo clam infection to identify environmental conditions affecting parasite proliferation and survival
- Development of a PCR-ELISA-base assay to determine infecting *Perkinsus* levels & species
- Extended survey of PO prevalence along Portuguese coast. Infection found to be linked to water temperature/salinity/pollution, iron-dependent, and inhibited by glyphosate & pyrimethamine
- Identification through functional genomics of target metabolic pathways (i.e. shikimate, folate) to control *Perkinsus* infection in clam hatcheries

## III. POPULATION GENETICS

Development & optimization of:

- Microsatellites for Bonelli's eagle towards conservation studies
- Techniques to preserve/extract DNA from faecal samples suitable to estimate effective population size (now offered as an external service)
- Paternity/sexing and genetic diversity analyses to determine differential contribution from brooders in specific populations (for management and aquaculture)



## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

The results obtained from EDGE research activities have been published in 38 peer reviewed papers from 2003 to 2007. A selection of 10 of these publications is presented below. In 2008, EDGE has already published 13 peer reviewed papers and 2 book chapters.

1. Simes, DC, Williamson, MK, Ortiz-Delgado, JB, Viegas, CSB, Price, PA, Cancela, ML. 2003. Purification of matrix Gla protein from a marine teleost fish, *Argyrosomus regius*: Calcified cartilage and not bone as the primary site of MGP accumulation in fish. *Journal of Bone and Mineral Research* 18(2): 244-259. IF=6.004, n°C=14.
2. Pinto, JP, Conceição, N, Gavaia, PJ, Cancela, ML. 2003. Matrix Gla protein gene expression and protein accumulation colocalize with cartilage distribution during development of the teleost fish *Sparus aurata*. *Bone* 32(3): 201-210. IF=3.966, n°C=13.
3. Pombinho, AR, Laizé, V, Molha, DM, Marques, SMP, Cancela, ML. 2004. Development of two bone-derived cell lines from the marine teleost *Sparus aurata*; evidence for extracellular matrix mineralization and cell-type-specific expression of matrix Gla protein and osteocalcin. *Cell and Tissue Research* 315(3): 393-406. IF=2.613, n°C=16.
4. Elandalloussi, LM, Leite, RM, Afonso, R, Nunes, PA, Robledo, JAF, Vasta, GR, Cancela, ML. 2004. Development of a PCR-ELISA assay for diagnosis of *Perkinsus marinus* and *Perkinsus atlanticus* infections in bivalve molluscs. *Molecular and Cellular Probes* 18(2): 89-96. IF=2.364, n°C=5.
5. Leite, RB, Afonso, RM, Cancela, ML. 2004. A Comprehensive survey of *Perkinsus* sp. (Apicomplexa) infestation in Carpet Shell clams, *Ruditapes decussates* (L) along the Portuguese coast. *Aquaculture* 240: 39-53. IF=1.735, n°C=5.
6. Laizé, V, Martel P, Viegas, CSB, Price, PA, Cancela, ML. 2005. Evolution of matrix and bone  $\gamma$  carboxyglutamic acid proteins in vertebrates. *Journal of Biological Chemistry* 280(29): 26659-26668. IF=5.581, n°C=6.
7. Conceição, N, Silva, AC, Fidalgo, J, Belo, JA, Cancela, ML. 2005. Identification of alternative promoter usage for the matrix Gla protein gene - Evidence for differential expression during early development in *Xenopus laevis*. *FEBS Journal* 272(6): 1501-1510. IF=3.396, n°C=2.
8. Pinto, JP, Conceição, N, Viegas, CSB, Leite, RB, Hurst, LD, Kelsh, RN, Cancela, ML. 2005. Identification of a new pebp2 $\alpha$ A2 isoform from zebrafish *runx2* capable of inducing osteocalcin gene expression in vitro. *Journal of Bone and Mineral Research* 20: 1440-1453. IF=6.004, n°C=2.
9. Fonseca, VG, Laizé, V, Valente, MS, Cancela, ML. 2007. Identification of an osteopontin-like protein in fish associated with mineral formation. *FEBS Journal* 274(17): 4428-4439. IF=3.396, n°C=3.

10. Laize, V, Viegas, CSB, Price, PA, Cancela, ML. 2006. Identification of an osteocalcin isoform in fish with a large acidic prodomain. *Journal of Biological Chemistry* 281(22): 15037-15043. IF=5.581, n°C=1.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

The results obtained from EDGE research activities have been published in 6 national papers from 2003 to 2007 (see list below). In addition, research activities of the lab have been divulged in national journals (Portuguese and French) such as *Expresso* (A Última Fronteira, November 2004), *Focus* (Lontras Debaixo de Olho, October 2004) and *Pública* (Lontra - Vida Animal, February 2004), *Télégramme* (Cinq Photos Scientifiques Lauréates d'un Concours Européen, October), *Ouest-France* (Jury Onternational au Concours de Photos à La Station Biologique de Roscoff, October 2006), *Ushuaia* (Plongée dans les Abysses, November 2006), *Digital Journals*, Local Journals such as *Jornal Académico* (Interview of ML Cancela, December 2004) and *Public Radio Stations* (TSF, Interview to ML Cancela, March 2004).

1. Leite, RB, Rodrigues, PM, Elandalloussi, LM, Afonso, RM, Nunes, PA, Cancela, ML. 2003. *Perkinsus Atlanticus* – Desenvolvimento de um método de diagnóstico para detecção da infecção em moluscos bivalves. *Boletim de Biotecnologia* 75, pp. 8-10.

2. Dias, PJ, Leite, RB, Afonso, R, Cancela, ML. 2005. Can experimental infection contribute to study mollusk diseases? Perkinsiosis as a case study. *Acta Parasitológica Portuguesa* 12(1-2): 413-414, Lisboa, Portugal.

3. Leite, RB, Elandalloussi, L, Afonso, R, Rodrigues, P, Ascenso, R, Cancela, ML. 2005. An overview of the metabolic pathways in the protozoan parasite *Perkinsus atlanticus/olseni*: Identification of potential targets for therapeutic drugs. *Acta Parasitológica Portuguesa* 12(1-2): 405-406, Lisboa, Portugal.

4. Dias, E, Cancela, L, Fonseca, L, Beja, P, Dentinho, T. 2006. *Gestão de Recursos Marinhos: Teses em Gestão e Conservação da Natureza, Vol I*. Principia: Scientific and University Publications, Cascais, Portugal.

5. Dias, E, Cancela, L, Fonseca, L, Beja, P, Dentinho, T. 2007. *Gestão da Água: Teses em Gestão e Conservação da Natureza, Vol II*. Principia: Scientific and University Publications, Cascais, Portugal.

6. Marques, CL, Rafael, MS, Tiago, DM, Cancela, ML, Laizé, V. 2007. Desenvolvimento de sistemas celulares de peixe adequados ao estudo da mineralização in vitro. *Boletim de Biotecnologia*.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Gomes, HL, Leite, RB, Afonso, R, Stallanga, P, Cancela, ML. 2004. A microelectrode impedance method to measure interaction of cells. *Proceedings of the IEEE Sensors 2004*, 24-27 October, Vienna, Austria, pp. 1011-1013.

2. Cancela, ML, Laizé, V. 2005. Replicação do DNA genómico. In: *Biologia Celular e Molecular*. Azevedo, C (Ed), 4ª edição, LIDEL-Edições Técnicas, Lisboa, pp. 141-152.
3. Sarrazin, J. et al. including Gavaia, P. 2006. MoMARETO: A cruise dedicated to the spatio-temporal dynamics and the adaptations of hydrothermal vent fauna on the mid-Atlantic ridge. *InterRidge News* 15: 24-33.
4. Cancela, ML, Laizé, V. 2007. Transcrição alternativa e edição do RNA: uma contribuição essencial na evolução do genoma. In: *O Mundo do RNA: Novos desafios e Perspectivas Futuras*. Arraiano, CM, Fialho, AM (Eds). LIDEL-Edições Técnicas, Lisboa, pp. 83-96.
5. Tiago, DM, Laizé, V, Cancela, ML, Aureliano, M. 2007. Vanadate and bone metabolism: effect on proliferation and mineralization of fish bone derived cells. *Proceedings of the European meeting of the Society for Free Radical Research International*, 10-13 October, Vilamoura, Portugal, pp. 183-186.
6. Pittman, K, Rønnestad, I, Gavaia, PJ, Cancela, ML, Guerreiro, P, Ribeiro, L, Aragão, C, Hamre, K, Moren, M, Yúfera, M, Conceição, L. 2007. FISH LARVAL RESEARCH: A tool for sustainable food production and understanding environmental impacts on developing organisms. *Aquaculture Europe* 32(4): 5-10 (Outcomes of the LARVAR 06 - Workshop in Fish Larval Research held at the CCMAR in 2006).

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Simes, Dina. 2003. Co-supervisor: ML Cancela (CCMAR), PA Price (UC San Diego, USA)

Gavaia, Paulo. 2005. Co-supervisor: ML Cancela (CCMAR), MC Sarasquete (ICMAN-CSIC, Spain)

Mira, Sara. 2006. Co-supervisor: ML Cancela (CCMAR), P Beja (ERENA), P Dias (CNRS, France)

Tiago, Daniel. 2008. Co-supervisor: ML Cancela, V Laizé and MA Alves (CCMAR)

Ascenso, Rita. 2008. Co-supervisor: ML Cancela (CCMAR), G Vasta (COMB-Univ of Maryland, USA)

##### **MSc THESIS**

Pais, Susana. 2003. Co-supervisor: ML Cancela (CCMAR), J Fernandes (DGA), C Rocha (CIMA)

Inácio, Ana. 2004. Co-supervisor: ML Cancela (CCMAR), P Beja (ERENA)

Fidalgo, João. 2005. Supervisor: ML Cancela (CCMAR)

Leite, Ricardo. 2005. Supervisor: ML Cancela (CCMAR)

Dias, Patrícia. 2005. Supervisor: ML Cancela (CCMAR)

- Fonseca, Vera. 2005. Co-supervisor: ML Cancela (CCMAR), V Laizé (CCMAR)
- Pombinho, António. 2005. Co-supervisor: ML Cancela (CCMAR), V Laizé (CCMAR)
- Roberto, Vânia. 2006. Co-supervisor: ML Cancela (CCMAR), P Gavaia (CCMAR)
- Barbosa, Vera. 2007. Co-supervisor: ML Cancela (CCMAR), V Robles (CCMAR, now at the Center of Regenerative Medicine in Barcelona, Spain)
- Cepo, Susana. 2007. Co-supervisor: ML Cancela (CCMAR), R Costa (Independent University), A Portugal (University of Coimbra)
- Santos, Erika. 2007. Co-supervisor: ML Cancela (CCMAR), MM Abreu (ISA), C Nabais (University of Coimbra)
- Silva, Conceição. 2007. Co-supervisor: ML Cancela (CCMAR), C Rocha (CIMA)
- Rainha, Rita. 2008. Co-supervisor: ML Cancela, S Mira (CCMAR)
- Afonso, Ricardo. 2008. Co-supervisor: ML Cancela and R Leite (CCMAR)
- Silva, João. 2008. Co-supervisor: ML Cancela and P Gavaia (CCMAR)
- Santos, Rui. 2008. Co-supervisor: ML Cancela and P Gavaia (CCMAR)
- Madeira, Celine. 2008. Co-supervisor: ML Cancela and S Mira (CCMAR)

## **5. Patents/propotypes (2000 ca.)**

MBMO-EDGE lab is involved in several patents (provisional U.S. Patent number 61/136.315; other patents are pending and therefore not described here) related to the discovery of proteins of medical and biotechnological interest.

MBMO-EDGE lab is also involved in numerous entries of NCBI databases, including:

- 90751 Expressed Sequence Tag (EST) records
- 143 core nucleotide sequence records
- 103 protein records
- 1 three dimension macromolecular structure
- 3 three dimensional domains from Entrez Structure
- 3 population study data sets

## **6. Organization of conferences (2000 ca.)**

EDGE Lab has been involved from 2004 to 2007 in the organization of 5 Conferences, Symposia and Workshops related to its research activities.

Cancela, ML. 2004. (President of Organizing committee) XIV National congress of Biochemistry, 4-6 December, Vilamoura, Portugal. (approx. 700 participants)

Cancela, ML. 2006. (Co-organizer of symposium at ASBMR 28th Annual Meeting). Symposium A: New Advances in Bone Evolutionary Biology. 18 September, 2006, Philadelphia, PA, USA. (approx. 3000 participants)

Cancela, ML, Leite, RB. 2007. Members of Organizing Committee of WOPER – Workshop for the analysis of the impact of Perkinsosis to the European shellfish industry, 10-12 September, Vigo, Spain. (approx. 120 participants)

Leite, RB, Cancela, ML. 2007. (R.Leite: President of Organizing Committee). RNA extraction workshop in collaboration with the Max Planck Institute (Berlin) and Pádua University, 5-8 May, Faro, Portugal. (12 participants)

Cancela, ML. 2007. (Member of Organizing Committee). VIII International Symposium on Experimental Techniques. 2-3 November, Faro, Portugal. (approx. 100 participants)

#### **7. Industry contract research (2000 ca.)**

Transgás Atlântico, SA. LONTRAS (47.386 EUR). 01/2001-12/2004, Genotyping and sexing of Otters from Costa Vicentina/SW coastal region of Portugal.

Novozymes. CEPHOZYMES (11.900 EUR). 05/2007-02/2008, DNA extraction, cloning and gene analysis of specific enzymes from marine invertebrates.

EWOS (3.000 EUR), 02/2005-06/2005.

Águas do Algarve (~10.000 EUR). 2005/06, DNA extraction from fresh water reservoirs and implementation of PCR-based detection assay for microorganisms (cryptosporidium).

#### **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

Cancela, ML. 2005-present. Presidency of the Portuguese Biochemistry Society, a non-profit, nationally recognized organization promoting the development and dissemination of biochemistry in Portugal. Of particular relevance has been the organization of National Biochemistry meetings and special interest meetings (i.e. Clinical Biochemistry, Biochemistry of RNA, training courses), joint meetings with other Biochemistry societies (Spanish, English), promoting education in Biochemistry.

Cancela, ML. 2005-2007. Portuguese delegate to the Research council of FEBS (Federation of European Biochemistry Societies) to participate in decisions to affect European Biochemistry programs for advanced training, fellowship awarding, education in biochemistry, science dissemination, participation of women in science. Of particular relevance has been the success in promoting the election of various Portuguese scientists to FEBS committees in the last 3 years (2 for fellowship committee, 1 for education in biochemistry committee, 1 for advance course committee).

Cancela, ML. 2007. Portuguese delegate to the Research Council of PABMB (Federation of Pan American Societies of Biochemistry and Molecular Biology) to participate in decisions to affect Ibero-American joint Biochemistry programs for advanced training, fellowship awarding, education in biochemistry, science dissemination. Of special relevance has been the implementation of a program to develop a joint Portuguese-Brazilian biochemistry dictionary and strengthen Brazilian interactions at National Biochemistry Meetings.

Cancela, ML, Leite, RB. 2007. EU/FP7/SSA/FOOD/044442 WOPER - Report from workshop for analysis of the impact of Perkinsosis in European shell fish industries. To develop coordinated actions for analysis of the impact of Perkinsosis in Europe. Coordinator: Antonio Villalba (CETMAR, Spain). Other participant institutions: University of Bretagne Occidentale (France), IFREMER (France), IZS Venice (Italy), CSIC-Madrid (Spain).

#### **9. Internationalization (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)**

Internationally recognized achievements of EDGE group shown through project and authorship collaborations, graduate training and networking, involving 19 countries from Europe, America, Africa and Asia.

#### **RESEARCH-RELATED**

1) NoE Marine Genomics Europe (MGE): Fish & Shellfish node partner, coordination of flagship project FICEL (270 kEuro, 4 institutes). Outcomes include projects FP7 I3-infrastructure “Assemble” (10 MEuro, 8 institutes, approved) and Small Collaborative Project “Microshell” (2.5 MEuro, 8 institutes, pending). Major role in developing cDNA libraries from seabream and clam (within MGE consortium) developed at the Max Plank Institute-Berlin.

2) COST Actions B23 (New Frontiers in Oral Facial Regeneration, L Cancela national representative) and Larvanet (Critical Success factors for European aquaculture, a multidisciplinary approach - accepted).

3) Project evaluations (e.g. European Commission, Treaty of Windsor, INRA, French National Research Agency-ANR, Dutch Research Council-NWO, INTAS, etc.), journal editor (J Bone Mineral Research, IF 2007=6.004) and referees (15 journals).

4) Bilateral collaborative actions with groups in Spain, UK, France, USA (FCT, GRICES, Treaty of Windsor, British Council, FLAD).

5) Oral communication+plenary lectures at international conferences (~19).

#### **TRAINING**

MSc (7), PhD student (9) and post doctoral (5) training of international students and/or with international collaboration, teaching of undergraduate ERASMUS/SOCRATES students in ongoing University degrees.

Hosting visiting researchers: sabbaticals (2), short term training (~25 from Norway, Germany, Spain, France, USA).

#### **RESEARCH DISSEMINATION**

International publications (38) communications in international meetings (74), organization of international symposiums (3)

## **PUBLIC AWARENESS**

IMAGENE photo exhibition within MGE network ([www.marine-genomics-europe.org](http://www.marine-genomics-europe.org)), oceanographic cruises ([www.ifremer.fr/momareto/](http://www.ifremer.fr/momareto/))

## **6e. Future research**

### **1. Objectives (3000 ca.)**

For the next 5 yr period, based on current members' interests, achievements, collaborations, funding and PhD projects:

#### **I. MOLECULAR DETERMINANTS OF MINERALIZATION/CALCIUM MOBILIZATION (ML Cancela, V Laizé, P Gavaia, D Simes, N Conceição, PM Rodrigues)**

**GENES & REGULATORY PATHWAYS INVOLVED IN SKELETOGENESIS, ENVIRONMENTAL ADAPTATIONS, TISSUE REGENERATION (ML Cancela, V Laizé, P Gavaia, N Conceição)**

Using zebrafish & seabream as model systems, integrated approach, functional genomics, proteomics, bioinformatics, in-house developed tools including new cell lines, microarrays, antibodies, cDNA and genomic libraries. Collaboration with 18 groups from 9 countries.

#### **FAMILY OF GLA-CONTAINING PROTEINS: NEW MEMBERS, EVOLUTION, FUNCTION IN HEALTH & DISEASE (ML Csncela, D Simes)**

Development of immuno-based diagnostic assays for new antigen detection in biological samples relevant for human health (Viegas et al, 2008; patent pending). Collaboration with USA & Portuguese hospitals.

#### **NEW OSTEOCALCIN ISOFORMS (V Laizé, D Simes)**

Being first to identify existence of osteocalcin isoforms (Laizé et al, 2006), our goal is to complete this study by identifying their function & evolution. Collaboration with USA.

#### **GENE EXPRESSION & SKELETAL DEFORMITIES (P Gavaia, D Simes, PM Rodrigues)**

Development of diagnostic tools suitable to detect skeletal malformations in fish through genomic, proteomic, microarray, histological-based tools (Roberto et al, submitted). Collaboration with 7 groups (including CCMAR-Aquaculture group) from 6 countries.

#### **PROTEINS FROM OYSTER NACRE WITH OSTEOINDUCTIVE FUNCTION (D SIMES)**

Following our identification of nacre proteins as in vitro mineralization promoters their molecular structure, regulation & function will be analysed.

#### **NEW CELL LINES FROM MODEL FISH SPECIES (V Laizé, ML Cancela)**



Following development of FIRST fish bone-derived cell lines (seabream; Pombinho et al. 2004), we now seek at developing additional lines from other fish (e.g. zebrafish, sole, salmon) requiring in vitro homologous systems (FP7 project ASSEMBLE, funded).

## II. UNVEILING PERKINSUS SPP (PE) METABOLIC PATHWAYS SUITABLE AS THERAPEUTIC TARGETS TO LIMIT INFECTION AND FOR BIOTECHNOLOGICAL APPLICATIONS (ML Cancela, R Leite)

Study of PE intracellular machinery, PE as model for toxicological/metabolic/genomic studies, expression/silencing of PE genes involved in virulence, PE potential as unicellular factory to synthesize bio-molecules of economical interest. Collaboration with 7 groups from 5 countries.

## III. ACCESSING WILD POPULATION'S VARIABILITY, SEX, PARENTAL GAMETE CONTRIBUTION, GENETIC DIVERSITY (S Mira, ML Cancela)

Goals based on previous achievements including: i) Identification of multiple paternity in Norway lobster and correlation with degree of stock exploitation, ii) Differential contributions of fish brood stock for progeny and implementation of selective breeding programs, and iii) Survey/sexing/assessment of Bonelli's eagle and otter populations for management of wild populations. Collaboration in LIFE & FCT projects with 6 groups from 4 countries.

## IV. ORGANIZATION OF THE FIRST INTERNATIONAL WORKSHOP ON FISH SKELETOGENESIS (EDGE LAB)

To be held April 27-29, 2009 in Tavira, Portugal (by invitation only) ~100 CONFIRMED participants from 12 countries.

### 2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)

Projects accepted (9)

PTDC/MAR/70855/2006-FATTYBONE (total, 197.453 EUR/EDGE 83.450 EUR), FCT, 06/2008-05/2011

PTDC/MAR/71685/2006-HYDRAA (total: 190.802 EUR/EDGE: 60.000 EUR), FCT, 04/2008-03/2011

INFRA-2008-1.1.1-ASSEMBLE (total: 13.971.049 EUR/CCMAR: 2.668.249 EUR), EU-FP7, 2008

PTDC/MAR/70858/2006-TEXBREAM (total 188.327 EUR/EDGE: 60.000 EUR), FCT, 06/2008-05/2011

PTDC/MAR/64533/2006-CRYOSPERM (total: 189.775 EUR/EDGE: 10.000 EUR), FCT

COST Action FA0801-LARVANET - Critical success factors for fish larval production in European Aquaculture: a multidisciplinary network (budget: not applicable)

MGE Consumable grant (5.750 EUR) Application of next-generation DNA sequencing to the production of Expressed Sequence Tags (ESTs) in the carpet-shell clam (*Ruditapes decussatus*) and Manila clam (*R. philippinarum*)

MGE Consumable grant (5.000 EUR) Development of microsatellites from AFLP-redived enriched libraries in the Manila clam *Ruditapes philippinarum*

MGE Consumable grant (5.000 EUR) EST sequencing of the *Perkinsus olseni*: A model for alveolata/apicomplexa evolution

Projects submitted / final evaluation pending (9)

PTDC/BIA-BCM/65634/2006-EVOC (total: 140.801 EUR/EDGE: 124.709 EUR), FCT

PTDC/BIA-BCM/67035/2006-RUNX3 (124.012 EUR), FCT

PTDC/CVT/65074/2006-BIOFISH (total: 200.000 EUR/EDGE: 64.000 EUR), FCT

PIC/IC/82807/2007-OSTEOFISH (159.820 EUR), FCT

PIC/IC/82997/2007-CHONDROMEF (124.949 EUR), FCT

KBBE-2007-3-3-04-WASTEUP (total: 8.283.883 EUR/EDGE: 514.667 EUR), European Commission-FP7

KBBE-2008-1-2-04-MICROSHELL (total: 2.772.578 EUR/EDGE: 328.886 EUR), European Commission-FP7

ECOAQUA (total: 492.400 EUR/EDGE: 40.000 EUR), INTERREG

FP7-SME-2008-1-PROSPAWN (total: 960.000 EUR/CCMar: 99.000 EUR), European Commission

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Viegas, CSB, Simes, DC, Laizé, V, Williamson, MK, Price, PA, Cancela, ML. 2008. Gla-rich protein (GRP): a new vitamin K-dependent protein identified from sturgeon cartilage and highly conserved in vertebrates. *Journal of Biological Chemistry*. In press. IF 2007=5.581.

2. Leite, RB, Brito, AB, Cancela, ML. 2008. An oxygen molecular sensor, the HIF prolyl-4 hydroxylase, in the marine protist *Perkinsus olseni*. *Protist* 159: 355-368. IF 2007=3.102.

3. Tanguy, A, Bierne, N, Saavedra, C, Pina, B, Bachère, E, Kube, M, Bazin, E, Bonhomme, F, Boudry, P, Boulo, V, Boutet, I, Cancela, L, Dossat, C, Favrel, P, Huvet, A, Jarque, S, Jollivet, D, Klages, S, Lapègue, S, Leite, R, Moal, J, Moraga, D, Reinhardt, R, Samain, J-F, Zouros, E, Canario, A. 2008. Increasing genomic information in bivalves through new EST collections in four species: Development of new genetic markers for environmental studies and genome evolution. *Gene* 408: 25-30. IF 2007=2.871.

4. Tiago, DM, Cancela, ML, Aureliano, M, Laizé, V. 2008. Vanadate proliferative and anti-mineralogenic effects are mediated by MAPK and PI-3K/Ras/Erk pathways in a fish chondrocyte cell line. *FEBS Letters* 582: 1381-1385. IF 2007=3.263.
5. Conceição, N, Laizé, V, Simões, B, Pombinho, AR, Cancela, ML. 2008. Retinoic acid is a negative regulator of matrix Gla protein gene expression in teleost fish *Sparus aurata*. *Biochimica Biophysica Acta* 1779: 28-39. IF 2007=2.371.
6. Kohli, V, Robles, V, Cancela, ML, Acjer, JP, Waskiewicz, AJ, Elezzabi, AY. 2007. An alternative method for delivering exogenous material into developing zebrafish embryos. *Biotechnology and Bioengineering* 98(6): 1230-1241. IF 2007=3.037.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

To continue to implement research lines within both accepted/submitted projects and new collaborations (Training and research), we require:

1. Electroporator for DNA delivery (transfection) into *Perkinsus* and fish cells to develop transgenic clones
2. Plate reader equipped for fluorescence, luminescence and absorbance for functional assays in cells and fish larvae
3. Expanded cell culture facilities (new incubators & safety cabinets to replace those existing and heavily used, and to increase cell culture capacities) in order to reach the objectives proposed with recently accepted projects (e.g. ASSEMBLE, FATTYBONE)
4. Microscopy room to fit the various microscopes (i.e. stereo microscopes, inverted fluorescence microscope) recently acquired, and a place for a confocal microscope, which is becoming an urgent need (see below)
5. Confocal microscope with dedicated technician for proper imaging analysis to investigate spatial-temporal gene expression in cultured cells and fish embryos, to be shared with other groups from CCMAR and UALG
6. Adequate Zebrafish facilities (present facility was implemented in a “contentor” outside the building) with additional aquariums & filtration system to replace those existing and heavily used, and to increase zebrafish culture capacities, in order to fulfill objectives proposed in new projects (e.g. CRYOSPERM)
7. Two research positions to (1) strengthen research activity related to *Perkinsus* and (2) develop the research activities related to marine genomics
8. One technician position to take into charge the general maintenance/running of the laboratory, now fulfilled by temporary positions within projects

## **Fisheries, Biodiversity and Conservation**

### **6a. Group description**

#### **1. Group name / denomination**

**Fisheries, Biodiversity and Conservation**

#### **2. Principal investigator**

**Karim Erzini**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Fisheries, Biodiversity, Conservation, Management

#### **5. Funding, source, dates (1000 ca.)**

Total of 20 projects in 2002-2007 (7 international, 13 national, 15 co-ordinations), with total funding for the group of 1.612.328 EUR (not counting pluriannual funding and not counting funding from the Ocean Tracking Network, which is still being negotiated).

BIOMARES, EU LIFE /Secil, 254.388 EUR;

OTN , Canada Foundation for Innovation, being negotiated;

RENSUB III, CCDR Algarve, 103.279 EUR;

PROMOPESCA, INTERREG IIIA, 53.333 EUR;

SPORTFISH, FCT, 76.500 EUR;

TELEMETRIA, FCT, 86.500 EUR;

RENSUB II, CCDR Algarve, 123.458 EUR;

BIOPECAS, MARE, DGPA, 246.052 EUR;

GESTPESCA II, INTERREG IIIA, 26.666 EUR;

RENSUB I, CCDR Algarve, 85.702 EUR;

ARADE, DGPA - MARE , 139.946 EUR;

POLVARTE, MARE, DGPA, 105.053 EUR;

GESTPESCA I, INTERREG IIIA, 52.500 EUR;

BRD, FCT, 73.001 EUR;  
 CEPHSTOCK, EC DG XII, 27.600 EUR;  
 CORRAME, FCT, 10.500 EUR;  
 STRESS , EU DG Research, 27.830 EUR;  
 LOBASSESS, FCT, 9.300 EUR;  
 SAGRES, FCT, 50.000 EUR;  
 SURVIVAL, FCT, 60.720 EUR.

## **6b. Group team**

### **1. Researchers in the group (Include only PhD. integrated in the LA)**

- 001. Karim Erzini (Cat.: Professor Associado, Gr. Acad.: Agregação)
- 002. Maria Margarida M Castro (Cat.: Professor Associado, Gr. Acad.: Agregação)
- 003. Margarida de Lurdes de Jesus Bastos Cristo (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)
- 004. Maria Teresa Calvinho Cerveira Borges (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)
- 005. Jorge Manuel dos Santos Goncalves (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)
- 006. Alexandra Paula Mimoso Henriques Cunha (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 007. José Carlos Caetano Xavier (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

### **2. Other researchers in the group (Include here collaborators with PhD. only)**

- 001. William Lawrence Silvert (Cat.: Outra, Gr. Acad.: Doutoramento)

### **3. Other researchers in the group (non PhD.)**

- 001. Maria Margarida Oliveira Maló Machado (Cat.: Assistente de Investigação, Gr. Acad.: Licenciatura)
- 002. Carlos Manuel Loureco Afonso (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 003. Cheila Pereira Almeida (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 004. Daniel Miranda Machado (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 005. David Maria Aguiar Abecasis (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 006. Frederico Miguel Cunha de Oliveira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

007. Joaquim Miguel Neves Ribeiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
008. Luis Manuel Correia Ferreira Bentes (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
009. Mafalda Rangel Malheiro Dias de Oliveira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
010. Maria Esmeralda de Sá Leite Correia da Costa da Fonseca (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
011. Pedro Alexandrino Silva Ferreira Martins Monteiro ( Cat.: Não aplicável (bolseiro) Gr. Acad.: Mestrado )
012. Pedro Filipe Duarte Alves da Veiga (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
013. Rui Pedro Andrade Coelho (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
014. Sonia Maria De Sousa Olim (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

Fishing and fishing related activities are of considerable importance in the Algarve (southern Portugal), which is the Portuguese region with the highest percentage of the working population directly or indirectly involved in fisheries. The main objectives of the group are to contribute to the improved management and sustainable exploitation of the multi-species resources of the Algarve coastal waters. In recent years, the fisheries sector has been under considerable pressure, with a decline in landings of many of the most important species. Within this context, the main objectives of the group are to contribute to the improved management and sustainable exploitation of the multi-species resources of the Algarve coastal waters. Specific objectives include:

- Studying the impact of commercial fishing activity and the implications for biodiversity: species and size selectivity of different gears, quantification of the by-catch and discards of different gears, and evaluation of ghost fishing;
- Studying the impact of recreational fishing activity;
- Finding ways to mitigate by-catch, discarding and to propose management measures and changes in legislation based on the above mentioned studies;
- Biology, population dynamics, and ecology of fish and invertebrates: growth and mortality parameters, maturity and reproduction, feeding ecology;
- Identification of essential fish habitat, by classification of the bottom, mapping of the biodiversity and distribution and abundance of key species;
- Long-term monitoring of key habitats and recruitment of fish and invertebrates;

- Understanding the spatio-temporal dynamics of key commercial species by means of tagging and telemetry studies;
- Modeling and simulation studies for spatio-temporal dynamics, eco-trophic modeling, evaluating the effects of different management measures, and understanding the influence of environmental and fisheries factors.

## 2. Main achievements (2000 ca.)

A full inventory of the biodiversity associated with fisheries in the Algarve has been completed, resulting in a book published in 2007. A significant part of the Algarve coast to a depth of 30m has been mapped, with classification of the bottom and identification of fish, invertebrates and algae (1137 species, including new species and new records for Portugal and Europe), and data implemented in a Geographic Information System (GIS).

With a project on purse seines finalised, the group has now completed a series of projects spanning a period of more than 10 years on the impacts in terms of by-catches and discarding, species and size selectivity of all the main gears used in the Algarve. For some gears (deepwater hake longline and demersal purse seine), gear modifications for the reduction by-catch have been successfully tested, with the collaboration of commercial fishermen.

The biology, population dynamics and ecology of a number of species of fish, cephalopods and crustaceans have been studied, namely commercially important sea breams (Sparidae), deepwater sharks, octopus and Norway lobster. Estimated parameters have been used in stock assessments for some species and for simulation based studies to evaluate different management measures.

A project on recreational / sport fishing was completed, resulting in the first estimates of the impacts (total catches, species composition, catch rates, sizes of fish) of this activity in the south of Portugal.

Acoustic telemetry has been used to study the spatio-temporal dynamics of sea bream species within the Ria Formosa, and to evaluate the artificial reef use by wild and cultured (restocked) sea breams.

Annual monitoring of key nursery habitats (Ria Formosa lagoon - since 2001 and Arade river estuary - since 2003) has been carried out. These time series will provide important information on recruitment variability and will be useful for evaluating climate change effects.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n°C=). Give title and full citation in original language. DO NOT translate)

The group published 71 papers (SCI) in 2003-2007. The 10 publications were selected to illustrate the diversity of research within the group. Group members are in bold. (JR = journal rank, IF = Impact Factor, n°C = number of citations).



1. Campos, A, Fonseca, P, Erzini, K. 2003. Size selectivity of diamond and square mesh cod ends for four by-catch species in the crustacean fishery off the Portuguese south coast. *Fisheries Research* 60: 79-97. IF 2007=1.196, n°C=12.
2. Castro, M, Encarnação, P, Henriques, P. 2003. Increment at molt for the Norway lobster (*Nephrops norvegicus*). *ICES Journal of Marine Sciences* 60: 1159-1164. IF 2007=1.934, n°C=1.
3. Erzini, K. 2005. Trends in NE Atlantic landings (southern Portugal): identifying the relative importance of fisheries and environmental variables. *Fisheries Oceanography* 14: 195-209. IF 2007=2.348, n°C=11.
4. Fonseca, P, Campos, A, Larsen, R, Borges, T, Erzini, K. 2005. Using a modified Nordmøre grid for by-catch reduction in the Portuguese crustacean trawl fishery. *Fisheries Research* 71: 223-239. IF 2007=1.196, n°C =10.
5. Gallucci, VF, Taylor, I, Erzini, K. 2006. Conservation and management of exploited shark populations based on reproductive value. *Canadian Journal of Fisheries and Aquatic Science* 63: 931-942. IF 2007=2.058, n°C=4.
6. Gonçalves, JMS, Bentes, L, Coelho, R, Correia, C, Lino, PG, Monteiro Ribeiro, J, Erzini, K. 2003. Age and growth, maturity, mortality and yield per recruit for two banded bream (*Diplodus vulgaris* Geoffr.) from the south coast of Portugal. *Fisheries Research* 62: 349-359. IF 2007=1.196, n°C=10.
7. Morais, P, Borges, TC, Carnall, V, Terrinha, P, Cooper, C, Cooper, R. 2007. Trawl-induced bottom disturbances off the south coast of Portugal: direct observations of the “Delta” manned-submersible on the Submarine Canyon of Portimão. *Marine Ecology* 28: 112-122. IF 2007=0.970, n°C=0.
8. Ribeiro, J, Bentes, L, Coelho, R, Gonçalves, JMS, Lino, PG, Monteiro, P, Erzini, K. 2006. Seasonal, tidal and diurnal changes in fish assemblages in the Ria Formosa lagoon (Portugal). *Estuarine, Coastal and Shelf Science* 67: 461-474. IF 2007=1.733, n°C=8.
9. Stergiou, KI, Moutopoulos, DK, Hernando Casal, JA, Erzini, K. 2007. Trophic signatures of small-scale fishing gears and their implications for conservation and management. *Marine Ecology Progress Series* 333: 117-128. IF 2007=2.546, n°C=2.
10. Vasconcelos, P, Gaspar, M, Castro, M. 2006. Imposex in *Hexaplex* (*Trunculariopsis*) *trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve coast - southern Portugal). *Marine Pollution Bulletin* 52: 337-341. IF 2007=2.334, n°C=6.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Borges, TC. (coordinator). 2007. Biodiversidade nas pescas do Algarve (Sul de Portugal) / Biodiversity in the fisheries of Algarve (South Portugal). University of the Algarve. 685p.
2. Santos, MN, Erzini, K (eds). 2007. Catálogo de espécies de peixes de interesse comercial da costa sul atlântica da Península Ibérica). Projecto Gestpesca II, Manual 1. Junta de Andalucia, 127 p.

3. Erzini, K, Zuur, AF, Ieno, EN, Pierce, GP, Tuck, I, Smith, GM. 2007. Estimating common trends for Portuguese fisheries landings, Chapter 34. In: Zuur, AF, Ieno, EN and Smith, GM. *Analysing Ecological Data*, Springer. Series: Statistics for Biology and Health, XXVI, 672 p.
4. Teixeira, SB, Fernandes, J, Gaspar, P, Gonçalves, J, Bentes, L, Ribeiro, J, Lino P. 2007. *Percorso subaquático da Praia da Marinha*. Comissão de Coordenação e Desenvolvimento Regional do Algarve, Faro, 30p.
5. Erzini, K. 2006. Future of fisheries: prospects for economic diversification in fishery-dependent areas. pp. 18-24, In: *Uma Visão Marítima Europeia, Conferência da Regiões Periféricas Marítimas da Europa, Celula de Prospectiva das Periferias Marítimas da Europa. Encontros do Porto, 8-9 de Dezembro 2005*. 122 p.
6. Stobberup, KA, Ramos, VM, Coelho, ML, Erzini, K. 2004. Changes in the coastal ecosystem of the Cape Verde archipelago over the last two decades: a simulation study using ECOSIM. In: Chavance, P, Ba, M, Gascuel, D, Vakily, M, Pauly, D (eds), *Pêcheries maritimes, écosystèmes et sociétés en Afrique de l'Ouest: un demi-siècle de changement. Actes du symposium international, Dakar – Sénégal, 24-28 juin 2002*. Collection des rapports de recherche halieutique A.C.P. – EU, numero 15, Bruxelles.
7. Tenório, J M, Afonso, C M L. 2004. Description of four new species of *Conus* from the Cape Verde Islands (Gastropoda, Conidae). *Visaya* 1(2): 24-37.
8. Brander, K, Blom, G, Borges, MF, Erzini, K, Henderson, G, MacKenzie, BR, Mendes, H, Santos, AMP, Toresen, R. 2003. Changes in fish distribution in the eastern North Atlantic; are we seeing a coherent response to changing temperature? *ICES Marine Science Symposium* 219: 261-270.
9. Blot J.-Y, Fraga, T, Caleja, P, Bispo, J, Silva, JA, Galvão, M, Worthington, A, Martins, R, Sasaki, R, Gonçalves, J, Coelho, R, Monteiro, P, Gonçalves, P, Tissot, IM. 2003. Faro A-, um sítio de naufrágio ao largo do Algarve. Estudo das biocenoses marinhas associadas ao naufrágio Faro A-. *Actas 2º Encontro de Arqueologia do Algarve*. Silves 18/X/2003 Tissot, 18 p.
10. Pita, C, Pintassilgo, P, Erzini, K, Dinis, MT. 2003. A coastal community in the Algarve (south of Portugal): staying or exiting from fisheries? *Proceedings of the MARE Second International Conference "People and the Sea II: Conflicts, threats and opportunities"*. Centre for Maritime Research (MARE), Amsterdam, Netherlands, 4-6 September, 10 p.

**3. Other publications international (3000 ca.)** (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**4. Master and Ph.D. thesis completed (3000 ca.)**

## PhD THESIS

Coelho, R. 2007. Biology, population dynamics, management and conservation of deep water lantern sharks, *Etmopterus spinax* and *Etmopterus pusillus* (Chondrichthyes: Etmopteridae) in

southern Portugal (northeast Atlantic). PhD Dissertation, University of Algarve. 264 p. Supervisor: K Erzini.

Vasconcelos, P. 2007. Biology, ecology and fishery of the banded murex, *Hepaplex* (*Trunculariopsis*) *trunculus*, in the Ria Formosa lagoon. PhD Dissertation, University of Algarve. 245 p. Supervisor: Miguel Gaspar (IPIMAR CRIP-Sul); Co-supervisor: M Castro.

Beldade, MRO. 2006. Patterns and processes of variation of a rocky bottom cryptobenthic fish assemblage. PhD. Dissertation, Universidade do Algarve. 192 p. Supervisor: E Gonçalves (ISPA), Co-supervisor: K Erzini.

Hazin, HG. 2006. Influência das variáveis oceanográficas na dinâmica populacional do espadarte, *Xiphias gladius* (Linnaeus, 1758), capturados pela frota brasileira (Influence of oceanographic variables on the population dynamics of the swordfish, *Xiphias gladius* (Linnaeus, 1758), captured by the Brazilian fleet). PhD. Dissertation, University of Algarve. 202 p. Supervisor: K. Erzini.

Stobberup, KA. 2005. Study of community structure, trophic interactions and exploitation pattern in the Cape Verde coastal ecosystem. PhD. Dissertation, University of Algarve. 144 p. Supervisor: K Erzini.

Campos, A. 2003. Selectivity and by-catch reduction devices: the trawl fisheries of Portugal. PhD. Dissertation, University of Algarve. Supervisor: K Erzini.

## **MSc THESIS**

Selection of Master of Science thesis (of 17 completed in 2003-2007)

Conduto, T. 2007. Ecological indicators and gear based management of Algarve fisheries. MSc in Aquaculture and Fisheries. Supervisor: K Erzini, Co-supervisor: T Borges.

Costa, R. 2007. Reproduction, age and growth of four coastal fish species in the NE Atlantic. MSc, Estudos Marinhos e Costeiros, University of Algarve. Supervisor: K Erzini, Co-supervisor: E Isidro (D.O.P Açores).

Jesus, A. 2007. Follow up on the local implementation of a MPA in the small fishing village of San Felipe, Yucatan: environmental changes and community-based management issues. MSc in Marine Biology, University of Algarve. Supervisor: J Fraga and J Euán, CINVESTAV, Yucatán, México; Co-supervisor: M Castro.

Marques, AM. 2007. European-eel (*Anguilla anguilla*, Linnaeus 1758) in Tagus River. MSc, Gestão e Conservação da Natureza, University of Algarve, University of Açores, Instituto Politécnico de Tomar. Supervisor: M Castro.

Santos, SV. 2004. The demise of the pearl fisheries in Tuticorin, Tamil Nadu (India): social and biological aspects and their application towards a sustainable management for the chank fisheries and condition improvement of the associated diver fisherfolk. MSc, Estudos Marinhos e Costeiros, University of Algarve. Supervisor: K Erzini, Co-supervisor: M Bavinck (University of Amsterdam).

## **5. Patents/propotypes (2000 ca.)**

The group has developed the following prototypes:

A sledge mounted underwater fish-eye camera, linked by cable to a video recorder on board the research vessel. The sledge mounted camera is towed behind the boat and has been used in sea bed mapping projects to quantify bottom living organisms and to classify bottom type.

Lobster larvae collectors were built and deployed on the bottom off the SW coast. The initial prototype was modified to include a cheaper and more resistant frame in PVC and is at this moment being tested in the sea.

A new design of plastic octopus pot was developed and tested. The objective was to increase catch rates while decreasing negative environmental impact as much as possible.

## **6. Organization of conferences (2000 ca.)**

Seabed Classification Products, by Chris Elliott of QTC – Quester Tangent Corporation, Canada. Universidade do Algarve, 27 January, 2006.

IV Encontro das Pescas. 4 of June 2005. Universidade do Algarve. This was the fourth in the series organised by the group, where the objective is to present the research carried out to the commercial fishing community and to fisheries managers. A total of 110 people attended, with 10 oral communications given by the group and 12 posters presented.

## **7. Industry contract research (2000 ca.)**

In 2007 this group participated in an environmental impact assessment of dredging along the Portuguese continental coast entirely financed by the industry (Amb & Veritas/Marinertes). Our main role within the project was to evaluate the impacts of dredging on the marine life, namely the benthic organisms.

In 2007 our group was contracted by the Municipality of Vila Real de Santo António (VRSA) to develop a small research project in order to help the fish canning industry in producing a regional food product (“Muxama”) based on tuna fish according to environmental criteria and to develop sustainable fisheries based tourism. This study was already used by the municipality on several occasions namely in European fisheries fund meetings. A special publication about “fisheries touristic routes in VRSA” dedicated to the general public is also being produced.

The group is participating in a LIFE project (2007-2010), partly funded by SECIL, the cement manufacturer.

## **8. Government/organization contract research (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)**

With the objective of contributing to the management of the underwater (from 0 to 30 m depth) component of the National Ecological Reserve, the subtidal marine communities of the central Algarve were mapped based on an annual sampling strategy (RENSUB: 2003-2007). This study is being done under the contract with the regional administration (Comissão de Coordenação e Desenvolvimento Regional do Algarve, CCDR Algarve) and is intended to help implementing a

spatial planning of the marine environment which could minimise potential conflicts between the variety of activities taking place in this particular coastal zone.

In 2003/2004 an applied research project on evaluation of the dredging on the marine communities and habitats, phase 1 (biological baseline study), was carried out. The second phase started in 2007 and is ongoing. This study was requested and financed by the regional administration (CCDR - Algarve) and aimed to evaluate the impacts of an increasing activity in the Algarve coast: dredging for beach nourishment.

In 2007 five underwater routes were developed at well known beaches in the Algarve. The goal of this work was to describe a touristically sustainable use of the Central Algarve National Underwater Ecological Reserve (REN). This project was financed by the Regional Development and Coordination Commission of the Algarve (CCDR - Algarve) and provides the tools that allow the definition of underwater routes, with accurate scientific information on the fauna, flora and their geographic location.

In 2004-2005, K. Erzini was part of the team that prepared the PROT - Algarve (plan for the management of the territory of the Algarve, including the coastal zone). Specifically, he contributed to the fisheries section of the PROT.

The research carried out resulted in or contributed to 6 major reports, such as:

- CCDRA. 2005. Plano Regional de Ordenamento do Território do Algarve (PROT - Algarve). 305 p. + anexos.

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

Collaborative publication

Of the 71 papers (SCI) published in 2003-2007, 23 involved international collaborations. The international partners included: AZTI (Basque country), University of Cádiz (Spain), Aristotle University (Hellas), University of British Columbia (Canada), University of Washington (U.S.A.), Norwegian Fisheries Institute, IMROP (National Fisheries Institute, Mauritania), British Antarctic Survey (U.K.), Birdlife International, CSIC (Spain), and the Fisheries Institute of Cape Verde.

International Collaboration in Research projects

From 2003 to 2007, the group was involved in a number of projects with international partners, such as the GESTPESCA I and GESTPESCA II projects (Spanish partners), the Ocean Tracking Network (multi-national, global), the BIOMARES LIFE project (Spanish and US partners), the CEPHSTOCK project (European partners), and SEMAPP (US partners). A member of the group has participated in the MEDITS cruises (International Trawl Survey in the Mediterranean) in 2006 and 2007, as an elasmobranch expert.

Evaluation of International Projects

A member of the group has been regularly invited to evaluate research proposals: evaluation of 6th Framework proposals in 2005 and 2006; evaluation of 7th framework proposals in 2007.

### Graduate training – international students

The group received three PhD students from Spain, Greece and Brazil for training in gear selectivity and modeling. A Spanish research assistant from Palma de Mallorca also received training in selectivity of static fishing gear in 2005.

A member of the group is one of the coordinators of the International Masters in Aquaculture and Fisheries, and of the Erasmus Mundus Masters in Biodiversity and Conservation. Both Masters have international students.

### Visits by international scientists

The group received at least 39 international scientists (Europe, US, South America, and Africa for meetings, training, collaboration, and sabbaticals. The visits ranged from one day to a six month sabbatical.

## 6e. Future research

### 1. Objectives (3000 ca.)

#### Marine Protected Areas (MPA)

Within the context of the BIOMARES project (2007-2010), the group will focus on evaluating the effects of different levels of protection within the Arrábida marine park (total, partial and complementary). Regular monitoring using a variety of gears is being carried out. Indicators will be calculated from these data (e.g. density of key species, mean sizes). Acoustic telemetry will be used to study spillover or the contribution of the MPA to the adjacent non-protected areas. Key species to study include several sea breams, soles, bass and cuttlefish. The role of herbivory within the MPA will also be studied, focusing on the biology and ecology of the dominant herbivore fish species, *Sarpa salpa*.

#### Spatio-temporal dynamics and habitat use

In addition to the telemetry studies in the MPA, the group will continue acoustic telemetry studies in Algarve coastal waters, with emphasis on artificial reef related work in collaboration with IPIMAR. Specifically, the goals are to evaluate the use of artificial reefs (the largest area in European waters) by wild and cultured (stocked) sea breams. Through the global Ocean Tracking Network project, the group will participate in the deployment of arrays of hydrophones across the Strait of Gibraltar and the continental shelves of southern Portugal and north-western Morocco. These arrays will eventually allow the monitoring of movements of highly migratory species.

#### Sea bed classification, mapping and essential fish habitat

In the next few years the group will finish mapping the entire Algarve coastal waters to a depth of 30m using divers, video camera mounted on a towed sledge and beam trawl. The data is being implemented in GIS and will be used develop indicators and for modeling (e.g. GAMs), allowing the identification of biodiversity hotspots and essential fish habitat. The group has been invited to participate in a major pan-European proposal focusing on sea bed mapping and biodiversity.



## Monitoring of key habitats

In addition to the monitoring in the Arrábida MPA, the group will continue their long-term annual monitoring programs of the ichthyofauna of the Ria Formosa lagoon (since 2001) and the Arade River estuary (since 2003).

## Sport fishing

Having completed a study of shore-based rod and line sport fishing, along 250 km of coastline from Sines to Vila Real de Stº António, the group will now focus on boat-based sport fishing and underwater spear fishing.

## Fisheries management and conservation

The group will continue work contributing to ensuring the sustainability of Portuguese fisheries. In this context, ongoing studies are evaluating of alternative, less damaging fishing gears, in specific fisheries. Simulation and modeling studies will also be used to evaluate different management scenarios. Ongoing bio-economic studies will also contribute to providing the basis for improved management and conservation of fisheries resources.

### **2. Funding, source, dates** (1500 ca.) (Indicate in full including amount of current and pending funding)

The group has 7 ongoing projects, with funding until 2010 in the case of the BIOMARES project. In the case of the Ocean Tracking Network (OTN), the group will only be receiving equipment. The total value of the equipment (mainly hydrophones, costing approximately \$10,000) has not been decided, but could be more than \$500,000.

The group is negotiating a fourth RENSUB project (RENSUB IV) that will finish the mapping and sea bed classification of the entire Algarve coast to a depth of 30m. This project will last two years (2009-2010) and the total value will be approximately 100.000 EUR.

The group has submitted a number of proposals and is awaiting the outcome of the evaluations (e.g. Programa de Estímulo à Investigação 2008, Fundação Calouste Gulbenkian). These proposals are on the use of telemetry to study connectivity and spatio-temporal dynamics of some of the most important species in the Arrábida marine park, and the role of herbivory in the marine park.

A contract (2008-2011) will be signed with INAG (the national water institute). The group will receive 71.710 EUR (+ VAT) for monitoring of the fish fauna of the Guadiana and Arade estuaries, and for data analysis and development of indicators of ecological health and potential.

The group will submit a seventh framework proposal for Call: FP7-KBBE-2009-3: Sustainable use of seas and oceans: integration of aquaculture and fisheries in the coastal zone and will be submitting proposals for National funding (FCT) in 2008/2009.

### **3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (nº C=). Give title and full citation in original language)

## Acoustic telemetry:



1. Abecasis, D, Erzini, K. 2008. Site fidelity and movements of gilthead seabream (*Sparus aurata*) in a coastal lagoon (Ria Formosa, Portugal). *Estuarine, Coastal and Shelf Science* (in press). IF=1.799, n°C=0.

Modeling, habitat use;

2. Hazin, H, Erzini, K. 2008. Spatial prediction of swordfish (*Xiphias gladius*) in the south Atlantic. *Fisheries Research* 90: 45-55. IF=1.196, n°C=0.

Predator-prey interactions:

3. Leitão, F, Santos, MN, Erzini, K, Monteiro, CC. 2008. The effect of predation on artificial reef juvenile demersal fish species. *Marine Biology* 153: 1233-1244. IF=2.215, n°C=0.

Indicators:

4. Erzini, K, Inejih, CAO, Stobberup, KA. 2005. An application of two techniques for the analysis of short, multivariate non-stationary time series of Mauritanian trawl survey data. *ICES Journal of Marine Science* 62: 353-359. IF=1.934, n°C=3.

Management:

5. Castro, M, Araújo, A, Monteiro, P, Madeira, AM, Silvert, W. 2003. The efficacy of releasing caught lobsters as a management measure. *Fisheries Research* 65: 475-484. IF=1.196, n°C=4.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

Most of the research involves field work (diving, sampling, fishing). The group has two small boats for inshore work. The delivery of the 9m research vessel, hopefully in 2008, will greatly increase the research capabilities, allowing the group to work along the whole Algarve coast and continental shelf. The group has always had excellent relations with the commercial fishing sector, with much of the research over the years carried out using commercial fishing vessels.

A bio-economics researcher would be a valuable addition to the group. In fact, the group has proposed the opening of such a research position within the framework of the Ciência 2008 program (hiring of highly qualified researchers with at least 3 years post-doc experience). Such a researcher will be able to contribute to many aspects of the research of the group.

## Hydrobiology

### 6a. Group description

#### 1. Group name / denomination

### Hydrobiology

#### 2. Principal investigator

**Adriano Agostinho Donas Bôto Bordalo e Sá**

#### 3. Location of group (Host institution)

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### 4. Keywords

Estuarine ecology, Tropical water ecology, Nutrient cycles, Bioremediation

#### 5. Funding, source, dates (1000 ca.)

Ciimar Multi-Annual funding (2004, 2005, 2006, 2007):

Adriano A. Bordalo - 3.440 EUR, 3.400 EUR, 2.000 EUR, 2.500 EUR

Ana Paula Mucha - 3.440 EUR, 3.400 EUR, 2.000 EUR, 2.500 EUR

Catarina Magalhães - 0 EUR, 3.400 EUR, 2.000 EUR, 2.500 EUR

FCT Projects:

PTDC/AMB/64441/2006 (2007-2010) - 25.800 EUR

POCTI/CTA/48386/2002 (2005-2008) - 5.040 EUR

POCTI/CTA/39034/2001 (2003-2005) - 75.000 EUR

POCTI/MGS/45533/2002 (2003-2005) - 105.266 EUR

POCTI/BSE/38615/2001 (2001-2004) - 30.000 EUR

FCT Scholarships:

Cristina Rigaud Abreu: 2003 PRAXIS XXI/BD/19991/99 - 2.750 EUR

Catarina Magalhães: 2003-2004 SFRH/BD/1397 - 5.500 EUR

Sandra Ramos: 2003-2005 SFRH/BD/6968/2001 - 8.250 EUR

Isabel Azevedo: 2003-2005 SFRH/BD/4660/2001 - 8.250 EUR

Liliana Carvalho: 2003-2005 SFRH/BD/7073/2001 - 8.250 EUR

Catarina Teixeira: 2006-2007 SFRH/BD/22317/2005 - 5.500 EUR

FLAD grants:

Rita Teixeira: 2004 IMAR / FLAD Grant Program - 1.200 EUR

Others:

Ciência Viva- Biologia no Verão 2003. Bactérias na Praia - 20.000 EUR

University of Porto. Escola de Ciências da Vida e da Saúde. Summer course 2006 - 500 EUR

University of Porto. Escola de Ciências da Vida e da Saúde. Summer course 2007 - 1.000 EUR

## **6b. Group team**

### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Adriano Agostinho Donas Bôto Bordalo e Sá (Cat.: Professor Associado, Gr. Acad.: Agregação)

002. Ana Paula de Campos Mucha (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

003. Catarina Maria Pinto Mora Pinto de Magalhães (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

004. Sandra Cristina da Costa e Silva Ramos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

### **2. Other researchers in the group (Include here collaborators with PhD. only)**

001. Corália Maria Fortuna de Brito Vicente (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

002. José Manuel Lage Campelo Calheiros (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

003. Rui Manuel Vitor Cortes (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

004. Pedro Manuel Silva Duarte (Cat.: Professor Associado, Gr. Acad.: Doutoramento)

005. Pedro Miguel Alfaia Barcia Re (Cat.: Professor Associado, Gr. Acad.: Doutoramento)

006. Cristina Isabel Ramires Rigaud de Abreu (Cat.: Assistente, Gr. Acad.: Doutoramento)

007. Raquel Beatriz Ribeiro de Mesquita (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

### **3. Other researchers in the group (non PhD.)**

001. Ana Margarida Pinto Henrique Machado (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

002. Catarina Fernanda de Carvalho Pinheiro Teixeira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

003. Eva Catarina Costa Amorim (Cat.: Outra, Gr. Acad.: Licenciatura)

004. Joana Savva Bordalo e Sa (Cat.: Outra, Gr. Acad.: Licenciatura)

005. Liliana Isabel Queirós de Almeida Carvalho (Cat.: Outra, Gr. Acad.: Mestrado)

006. Maria Isabel da Silva Costa Azevedo (Cat.: Outra, Gr. Acad.: Mestrado)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

The activities within the lab are spread over three main areas: (i) research; (ii) consulting; and (iii) outreach. The lab aims to contribute to the aquatic research from a multidisciplinary point of view, dealing from water quality monitoring to modelling, from early life stages of fish to denitrification and global warming and epidemiology. Research is carried out in temperate (Portugal) and tropical environments (Thailand – SE Asia, Guinea-Bissau– W Africa, S. Tomé and Príncipe – W Africa). The main research areas are (a) monitoring estuarine water quality in terms of physical (conductivity, salinity, temperature, turbidity), chemical (dissolved oxygen, inorganic carbon, pH, nitrate, nitrite, ammonium, phosphate, silicate) and biological (bacteria, viruses, fecal indicators, primary production) parameters, (b) estuarine primary productivity, (c) nitrogen cycle and global warming, (d) anthropogenic impact in estuarine communities, (e) phytoremediation and bioremediation, (f) estuarine fish assemblages, with special emphasis to the estuarine nursery role for early life stages of fishes (g) watershed restoration, (h) ecological modelling, (i) tropical water quality and cholera, (j) water management and public health.

### **2. Main achievements (2000 ca.)**

Monitoring estuarine water quality: research in the River Douro estuary involving monthly water column (since 2005) key environmental data.

Estuarine primary productivity: research in the River Douro estuary has been performed in the water column, intertidal sediments and hard surfaces.

Nitrogen cycle and global warming: research has been carried out in the water, sediments and hard surfaces in several estuaries in Portugal (Douro, Cávado, Sado and Ave) as well as in Bangpakong estuary, Thailand. Nitrogen fixation, nitrification, denitrification, annamox and nitrous oxide production, a powerful green-house gas were evaluated. In addition, the structure of the microbial populations involved in nitrification and denitrification are also studied.

Anthropogenic impact on estuarine communities: research performed in Douro and Lima estuaries in order to understand the effect of heavy metals and other pollutants on macrobenthic and microbial communities and on the denitrification process.

Phytoremediation and bioremediation: the role of the interaction between plants and microorganisms in the removal of heavy metals and other pollutants from sediments has been studied in several estuaries in Portugal (Douro, Cávado and Sado).

Estuarine fish assemblages: research conducted in the Lima River estuary to understand the dynamics of fishes, focusing on the potential nursery role of estuaries and ultimately assess the carrying capacity of these ecosystems for fish species, some with high economical important such as sardines and flatfishes.

Watershed restoration: using the River Febros watershed (a tributary of the Douro) as a model, different tools for water quality, sediment quality and river bank quality have been employed.

Ecological modeling: by means of an object-oriented model several key parameters, including primary production, have been modelled in the River Douro estuary.

Tropical water quality, cholera and public health: research done in Thailand and Guinea-Bissau. In Thailand the water quality of the Bangpakong River watershed and subtidal sediments has been carried out. In Guinea-Bissau, water quality and its relation to disease including cholera has been evaluated and sustainable measures do increase water quality have been designed.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

The Laboratory of Hydrobiology members have published a total of 23 ISI-listed peer-reviewed articles in the period 2003-2007. The mean Impact Factor (based on ISI JCR 2007) for those 23 publications was 2.53. Each PhD member as a score of 1.9 publications (ISI) per year.

1. Bordalo, AA, Savva-Bordalo, J. 2007. The quest for safe drinking water: an example from Guinea-Bissau (West Africa). *Water Research* 41: 2978-2986. IF 2007=3.427, n°C=9.
2. Magalhães, CM, Costa, J, Teixeira, C, Bordalo, AA. 2007. Impact of trace metals on denitrification in estuarine sediments of the Douro River estuary, Portugal. *Marine Chemistry* 107: 332-341. IF 2007=3.805, n°C=9.
3. Magalhães, CM, Bano, N, Wiebe, WJ, Hollibaugh, J, Bordalo, AA. 2007. Composition and activity of betaproteobacteria ammonia-oxidizing communities associated with intertidal rocky biofilms and sediments of the Douro River estuary, Portugal. *Journal of Applied Microbiology* 103: 1239-1250. IF 2007=2.501, n°C=0.
4. Azevedo, IC, Duarte, PM, Bordalo, AA. 2006. Pelagic metabolism of the Douro estuary (Portugal) – Factors controlling primary production. *Estuarine Coastal and Shelf Science* 69: 133-146. IF 2006/2007=1.733/1.799, n°C=2.
5. Ramos, S, Cowen, RK, Paris, C, Ré, P, Bordalo, AA. 2006. Environmental forcing and larval fish assemblage dynamics in the Lima River estuary (northwest Portugal). *Journal of Plankton Research* 28: 275-286. IF 2006/2007=1.619/1.897, n°C=1.
6. Magalhães, CM, Joye, SB, Moreira, R, Wiebe, WJ, Bordalo, AA. 2005. Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification rates in intertidal sediments

and rocky biofilms of the Douro River estuary, Portugal. *Water Research* 39: 1783-1794. IF 2005/2007=3.019/3.427, n°C=11.

7. Mucha, AP, Vasconcelos, MTSD, Bordalo, AA. 2003. Macrobenthic community in the Douro estuary: relations with trace metals and natural sediment characteristics. *Environmental Pollution* 121: 169-180. IF 2003/2007=2.002/3.135, n°C=46.

8. Mucha, AP, Bordalo, AA, Vasconcelos, MTSD. 2003. Comparison of the response of three micro-algae species exposed to elutriates of estuarine sediments based on growth and chemical speciation. *Environmental Toxicology and Chemistry* 22: 576-585. IF 2003/2007=2.429/2.309, n°C=5.

9. Bordalo, AA. 2003. Microbiological water quality in urban coastal beaches: the influence of water dynamics and optimization of the sampling strategy. *Water Research* 37: 3233-3241. IF 2003/2007=1.812/3.427, n°C=4.

10. Magalhães, CM, Bordalo, AA, Wiebe, WJ. 2003. Intertidal biofilms on rocky substratum can play a major role in estuarine carbon and nutrient dynamics. *Marine Ecology Progress Series* 258: 275-281. IF 2003/2007= 2.135/2.546, n°C=19.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Bordalo, AA. 2005. O Rio inconformado. In: O Rio. Carvalho, A, Lopes, A, Wellenkamp, M (Eds), Panmixia, Porto, Portugal.

2. Bordalo, AA. 2005. A escassez de água. Resumos do Encontro Nacional de Biotecnologia, 19 de Maio, Porto, Portugal.

3. Bordalo, AA. 2005. Estuário do Douro e saúde ambiental. Resumos da 1ª Conferencia sobre Saúde. Instituto Superior de Ciências da Saúde do Norte, 12-13 de Maio, Porto, Portugal.

4. Bordalo, AA. 2005. O Estuário do Douro. A qualidade e o futuro. Resumo da Encontro Nacional dos Estudantes de Engenharia do Ambiente, 20- 22 de Abril, Porto, Portugal.

5. Bordalo, AA. 2005. Estuário do Douro poluído por linhas de água contaminadas. Sustentabilidade ecológica em risco. Água em Revista, 14-15 de Maio, Porto, Portugal.

6. Bordalo, AA. 2004. O futuro com a Directiva da Água. Fórum Ambiente – Anuário 2004.

7. Bordalo, A.A. 2004. O Douro, o estuário e o futuro. O Tripeiro, Janeiro de 2004.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Mucha, AP, Almeida, CMR, Machado, AM, Magalhães, CM, Vasconcelos, MTSD, Bordalo, AA. 2007. In vitro response of salt marsh microbial community to mixed contamination, Proceedings of the International Meeting Soil and Wetland Ecotoxicology, 26-27 November 2007, Barcelona, Spain.

2. Mucha, AP, Almeida, CMR, Bordalo, AA, Vasconcelos, MTSD. 2007. Influence of salt marsh plants on trace metal levels and bioavailability in water column during sediment re-suspension occurrences in dredging. SETAC Europe 17th Annual Meeting , 20-24 May 2007, Porto, Portugal.
3. Magalhães, C, Kiene, RP, Buchan, A, Machado, A, Teixeira, C, Wiebe, WJ, Bordalo, AA. 2007. A novel inhibitory interaction between marine nitrogen and sulfur cycles. 10th Symposium on Aquatic Microbial Ecology, Faro, Portugal.
4. Teixeira, C, Magalhães, C, Boaventura, R, Bordalo, AA. 2006. Importance of denitrification in subtidal sediments of an urban polluted estuary (Douro, Portugal). 9th International Estuarine Biogeochemistry Symposium, Warnemünde, Germany.
5. Costa, J, Magalhães, C, Teixeira, C, Bordalo, AA. 2006. Heavy metals effects on sediments denitrification and N<sub>2</sub>O production: Douro estuary, Portugal. 5th Symposium on the Atlantic Iberian margin. Aveiro, Portugal.
6. Ramos, S., Santos, JS, Cowen, RK, Ré, P, Bordalo, AA. 2005. Is Lima River Estuary (NW Portugal) acting as a nursery area for *Sardina pilchardus* (WALBAU, 1792)? 29th Annual Larval Fish Conference, July 2005, Barcelona, Spain.
7. Magalhães, C, Bano, N, Wiebe, WJ, Hollibaugh, JT, Bordalo, AA. 2005. Comparison of ammonium oxidizing bacterial phylotypes and function between intertidal rocky biofilms and sediments of the Douro River Estuary, Portugal. ASLO Summer Meeting. Santiago de Compostela, Spain.
8. Azevedo, I, Bordalo, AA. 2004. River flow and tidal influence on the water quality of an urban estuary: Douro, Portugal. ECSA 37 – ERF 2004 Estuaries and Change, 21-24 June, Balina, Australia.
9. Ramos, S, Ré, P, Bordalo, AA. 2003. Fish larvae in a seasonally stratified temperate estuary: Lima estuary (Portugal). 27th Annual Larval Fish Conference. August 2003, Santa Cruz, USA.
10. Magalhães, C, Moreira, R, Wiebe, WJ, Bordalo, AA. 2003. Salinity and inorganic effects on nitrification and denitrification rates in intertidal sediments and rocky biofilms. 7th International Specialised Conference on Diffused Pollution and Basin Management; IWA DipCon 2003, August 2003, Dublin, Ireland.

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Ramos, Sandra. 2007. Ichthyoplankton of the Lima estuary (NW Portugal): ecology of the early life stages of Pleuronectiformes. PhD in Aquatic Sciences by Instituto de Ciências Biomédicas Abel Salazar, University of Porto. Supervisor: Adriano Bordalo, Co-supervisor: Pedro Ré (Faculty of Sciences, University of Lisbon).

Magalhães, Catarina. 2006. Inorganic nitrogen dynamics in intertidal rocky biofilms and sediments of the Douro Estuary: processes and communities. PhD in Aquatic Sciences by Instituto de Ciências Biomédicas Abel Salazar, University of Porto. Supervisor: Adriano Bordalo, Co-supervisor: William J. Wiebe (University of Georgia, USA).



Abreu, Cristina. 2005. Application of Molecular Techniques to the Study of Bacteria and Archaea Populations in the River Douro Estuary. PhD in Biomedical Sciences by Instituto de Ciências Biomédicas Abel Salazar, University of Porto. Supervisor: Adriano Bordalo, Co-supervisor: Paolo De Marco (IBMC, University of Porto).

Onrassami, Ratiwun. 2003. Survival of Fecal Indicator Bacteria in Bangpakong estuary (Eastern Thailand). PhD in Aquatic Sciences by Instituto de Ciências Biomédicas Abel Salazar, University of Porto. Supervisor: AA Bordalo, Co-supervisor: Chutiwan Dechsakulwatana (Thailand).

Nilsumranchit, Wandee. 2003. Application of a water quality index to the Bangpakong (Eastern Thailand). PhD in Aquatic Sciences by Instituto de Ciências Biomédicas Abel Salazar, University of Porto. Supervisor: AA Bordalo; Co-supervisor: Kashane Chalermwat (Thailand).

## **MSc THESIS**

Teixeira, Catarina. 2006. Desnitrificação em sedimentos subtidais do Rio Douro. MSc in Environmental Engineering by the Faculty of Engineering, University of Porto. Supervisor: AA Bordalo, Co-supervisor: R Boaventura (Faculty of Engineering, University of Porto).

### **5. Patents/prototypes (2000 ca.)**

Prototype – 2D numerical model of the Crestuma-Reservoir (Douro River)

Prototype – 3D numerical model of the Douro River estuary

### **6. Organization of conferences (2000 ca.)**

Collaboration with INAG in the organization of the northern estuaries workshops for the implementation of the Water Framework Directive – May 2007 (ICBAS, Porto)

### **7. Industry contract research (2000 ca.)**

Pavilhão da Água. 2007. Recuperação e manutenção de experiências biológicas no pavilhão da água.

SMAS/Porto. 2006. Monitorização da qualidade química e bacteriológica da água do estuário do Rio Douro Fase III.

Truticultura do Minho. 2005. Qualidade da água e sedimentos no Rio Coura na envolvente da Truticultura do Minho – FASE I.

Truticultura do Minho. 2005. Qualidade da água e sedimentos no Rio Coura na envolvente da Truticultura do Minho – FASE II.

Truticultura do Minho. 2005. Qualidade da água e sedimentos no Rio Coura na envolvente da Truticultura do Minho – FASE II.

SMAS/Matosinhos. 2005. Distribuição espacial da contaminação fecal na praia de Matosinhos e zona costeira envolvente.

SMAS/Porto. 2004-2005. Monitorização da qualidade química e bacteriológica da água do estuário do Rio Douro Fase II.

Turbogás/RWE. 2001-2004. Possible effects of cooling water discharge on the water quality of the Crestuma reservoir. Phase 2.

Águas do Douro e Paiva SA. 2003. Avaliação da qualidade da água no estuário médio e superior do Rio Douro no trecho Ponte D. Maria II – Ponte do Freixo.

**8. Government/organization contract research** (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

FCT (2007-2010). AQUASENSE – Development of miniaturised flow techniques for investigating dynamic environmental systems: river, estuarine and coastal waters (PTDC/AMB/64441/2006).

FCT (2005-2008). Rhizosphere Biogeochemistry and its Relevance for Endpoints and Phytoremediation (POCTI/CTA/48386/2002).

FCT (2003-2005). Importância do interface superfície rochosa/coluna de água nos ciclos do carbono e azoto de um sistema estuarino eutrófico (POCTI/CTA/39034/2001).

FCT (2003-2005). ECODOURO - Modelação do efeito da redução de caudal e de descargas intermitentes sobre a dinâmica e processos na albufeira de Crestuma (POCTI/MGS/45533/2002).

FCT (2001-2004). Estudo de populações de Archaea no Douro inferior. (POCTI/BSE/38615/2001).

Câmara Municipal de Viana do Castelo (2007) - Cenários para a instalação de um porto de recreio no estuário do Rio Lima.

Ciência Viva- Biologia no Verão 2003. Bactérias na Praia.

University of Porto. Escola de Ciências da Vida e da Saúde. Summer course 2006.

University of Porto. Escola de Ciências da Vida e da Saúde. Summer course 2007.

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

Networks or other forms of participation of the Research Group at the international level)

United States of America

• Dep. of Marine Science, UGA: Prof. W Wiebe, Prof. J Hollibaugh, Prof. S Joye. Structure and function of microbial communities; biogeochemical cycling of nutrients.

Project POCTI/CTA/39034/01; POCTI/MGS/45533/2002

PhD prog: C Magalhães; C Teixeira

Pub: Magalhães 2006 PhD thesis, Magalhães et al. 2003 Mar Ecol Prog Ser: 258: 275-281; Magalhães et al. 2005 Wat Res. 39: 1783-1794; Magalhães et al. 2005 Estuaries 28: 592-607;

Bordalo et al. 2006 Environ Manage 38: 910-920; Magalhães et al. 2007 J Appl Microbiol 103: 1239-1250; Magalhães et al. 2008 Microb Eco 55: 259-268.

- RSMAS, Univ. Miami: Prof. R Cowen, Prof. C Paris, Fisheries oceanography and larval ecology.

PhD prog: S Ramos

Pub: Ramos et al. 2006 Est Coast and Shelf Sci 66: 303-314; Ramos et al. 2006 J Plankton Res 28: 275-286 and Ramos 2007 PhD thesis.

- Dauphin Island Sea Lab, Univ. S Alabama. Prof. R Kiene. Cycling of organic sulfur in aquatic systems.

Post-Doc prog: C Magalhães.

- USNA Prof. M Vieira. Estuarine hydrodynamics and interactions with biota.

Project POCTI/MGS/45533/2002

Pub: Vieira & Bordalo 2000 Oceanol Acta 23: 585-594 and Bordalo & Vieira 2005 Est Coast and Shelf Sci 63: 143-154.

United Kingdom

- IECS, Univ. Hull. Prof. M Elliott. Derive and test a quantitative indicator of ecosystem functioning and health for use in management plans and the future policies for the estuarine management.

Post-Doc prog: S Ramos

Thailand

- Fac. Science, Univ. Burapha. Prof. K Chalermwat, Water quality indexes, biogeochemical and microbial estuarine dynamics.

Pub: Bordalo et al. 2001 J Appl Microbiol 35: 3635-3642, Bordalo et al. 2002 J Appl Microbiol 93:864-871; W. Nilsumranchit 2003 and R. Onrassami 2003 PhD thesis.

Guinea-Bissau

- Research with personal funds (A. A. Bordalo)- Variability of well water quality dry/wet seasons, outbreak of intestinal diseases and cholera,.

Pub: Bordalo and Savva-Bordalo 2007 Wat Res 41: 2978-2986.

New Zealand

- U. Waikato. Prof. C Cary, Prof. I R McDonald. Microbial diversity along latitudinal gradient of Antarctica.

Post-Doc prog: C Magalhães.

S.Tomé and Principe

- Contacts have been established with the International Committee of the Red Cross and the local Ministry of Natural Resources to evaluate the water quality.

## 6e. Future research

### 1. Objectives (3000 ca.)

The lab intends to pursue its multidisciplinary approach, with special emphasis in the following research areas: estuarine and coastal dynamics, watershed management, Water Framework Directive implementation, microbial diversity and function, nitrogen and sulphur cycles and global warming, bioremediation, cholera and tropical water quality and public health.

Since 1985, research has been focussed on the Douro estuary and the coastal zone of Porto. Progressively, research has expanded into other Portuguese estuaries (Lima, Cavado, Ave, Leça), as well as to tropical estuaries (Bangpakong, Thailand) and freshwater (Bolama, Guinea-Bissau). In 2008, the evaluation of freshwater quality in S. Tomé Island (W Africa) will be carried out. Besides basic knowledge of each system, the attention of the researchers has been devoted to processes and functions and their implications to the health of the ecosystems as well as to human health.

The current management of aquatic ecosystems relies on a good knowledge of their structure and functioning and of the way in which the habitat status, use, loss and gain has been influenced by human activities. The EU Water Framework Directive requires the classification of the ecological status of all water bodies. Thus, research in this area will deal with development of tools that allow the assessment of ecological status of these ecosystems, the evaluation of its response to natural and man-induced stressors and the design of mitigation measures to fulfil legislation requirements. Those tools will include the design of conceptual models of ecosystems function, development of new environmental indicators and methodologies for health assessment and restoration of small watersheds.

Specifically, ongoing research in nitrogen cycle will proceed in order to investigate the role of denitrification, anammox, dissimilatory nitrate reduction to ammonium and nitrification as fates for nitrogen transformations in estuarine sediments. Another goal will be the evaluation of new interactions between organic sulfur degradation compounds and the last step of denitrification, including processes (involved in global warming) and microbial communities. These studies represent a new perspective of the interaction between marine nitrogen and sulfur cycles, giving a new understanding about nitrous oxide production in the marine environment. The dynamics of microbial communities and of key nitrogen processes with main relevance to potential release of N<sub>2</sub>O (with high impact in the global warming), in tropical estuaries will also be addressed. In future, we also intend to extend studies to the Antarctica environments, in order to understand how microbial diversity and function changes along latitudinal gradients.

Another challenging area is bioremediation. The studies will be carried out in coastal ecosystems, in order to develop methodologies for the cleanup of oil contaminated sediments, based on the stimulation of the degradation processes of microbial communities. These also involve the isolation of autochthonic hydrocarbon biodegrading microorganism, not genetically modified.

Finally, the research in Guinea-Bissau which pursues the amelioration of the living conditions of the population including the decrease of child mortality (presently 204/1,000 births against 3/1,000 births in Portugal), through the implementation of simple and sustainable measures based in the results of the ongoing studies will be monitored on site. Similar studies will be carried out in S. Tomé and Príncipe islands also in W Africa.

**2. Funding, source, dates** (1500 ca.) (Indicate in full including amount of current and pending funding)

OILDEBEACH - Buried oil in the intertidal beach zone: coupling between beach morphodynamic, natural degradation, forcing mechanisms and biological activity (2008-2011). Accepted by the AMPERA Joint Management Committee. Partners: University of Vigo/GEOMA (Galicia/ES); CIIMAR (PT); University of Montpellier II / GLADYS (FR); University of Porto/ICBAS (PT). Total funding: 199,994 euros; CIIMAR: 30.000 EUR.

BIOREM - Desenvolvimento de um guia de metodologias de biorremediação para aplicar em zonas estuarinas e costeiras contaminadas por hidrocarbonetos (0167\_BIOREM\_1\_P). Submitted to “Programa Operacional de Cooperacao Transfronteiriça Espanha-Portugal 2007-2013”. Partners: ICBAS, Universidade do Porto, Instituto Português e dos Transporte Marítimos (IPTM), CIIMAR, GEOMA, Universidade de Vigo. (Requested funding budget: 42.9127 EUR).

Phytoremediation and Bioremediation: potentialities of plant - microorganisms interactions in the rhizosphere of salt-marsh plants (PTDC/MAR/72029/2006). PI: Ana Paula Mucha. Submitted to FCT. (Requested funding budget: 16.2321 EUR)

Novel interaction between marine biogeochemical nitrogen and sulfur cycles: characterization and ecological implications. (PTDC/MAR/66292/2006) PI: Catarina Magalhães. Submitted to Portuguese Science Foundation. Classified as Very Good is currently in Revaluation procedure. Partners: CIIMAR, University of Georgia, USA, University of Tennessee, USA, Dauphin Island Sea Lab, USA. (Requested funding budget: 98.898 EUR)

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

Beside the above mentioned references, several other publications should be mentioned:

1. Ramos, S, Cowen, RK, Ré, P, Bordalo, AA. 2006. Temporal and spatial distribution of larval fish assemblaged in the Lima River estuary (Portugal). *Estuarine Coastal and Shelf Science* 66: 303-314. IF 2006/2007=1.633/1.799, n°C=3.
2. Mucha, AP, Vasconcelos, MTSD, Bordalo, AA. 2005. Spatial and seasonal variations of the macrobenthic community and metal contamination in the Douro estuary (Portugal). *Marine Environmental Research* 60: 531-550. IF 2005/2007=1.611/1.930, n°C=5.
3. Magalhães, C, Wiebe, WJ, Joye, SB, Bordalo, AA. 2005. Inorganic nitrogen dynamics in intertidal rocky biofilms and sediments of the Douro River estuary (Portugal). *Estuaries* 28: 592-607. IF 2005/2007=1.284/2.133, n°C=6.

4. Mucha, AP, Almeida, M, Bordalo, AA, Vasconcelos, TSD. 2005. Exudation of organic acids by a marsh plant and implications on trace metal availability in the rhizosphere of estuarine sediments. *Estuarine, Coastal and Shelf Science* 65: 191-198. IF 2005/2007=1.633/1.799, n°C=5.

5. Bordalo, AA, Vieira, MEC. 2005. Spatial variability of phytoplankton, bacteria and viruses in the mesotidal salt wedge Douro Estuary (Portugal). *Estuarine, Coastal and Shelf Science* 63: 143-154. IF 2005/2007=1.633/1.799, n°C=5.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

The acquirement of several equipment will be crucial for the progress of future research in: (i) estuarine ecology – AUV with GPS and CTD including oxygen, pH, chlorophyll and turbidity probes; (ii) molecular microbial ecology – vertical laminar airflow workbench, ultra-low temperature freezer, high capacity autoclave, GelCompar II software, Real-time PCR and Automatic Sequencer for genotyping and sequencing analysis; (iii) biogeochemistry of nitrogen and sulphur elements - membrane inlet mass spectrophotometre for isotope paring analysis; (iv) biorremediation – mesocosms facilities for bioremediation experiments; (v) estuarine and coastal management – plankton nets, trawling nets, flowmeters, current meters, multiparametric probes, research vessel, light Incubator for primary production measurement, fuorescence microscopy.

## **Ecophysiology**

### **6a. Group description**

#### **1. Group name / denomination**

#### **Ecophysiology**

#### **2. Principal investigator**

**João José Oliveira Dias Coimbra**

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Physiology, Environment, Adaptation, Migration

#### **5. Funding, source, dates (1000 ca.)**

During the period 2003-2008, the Ecophysiology group has obtained funding through competitive research grants at both national (FCT) and European levels as well as basic funding through the FCT pluriannual program. Post doctoral and PhD students are supported by national (FCT) scholarships. Collectively the group has led nine FCT projects within the PTDC, POCI and POCTI programs totaling over 653.000,00 € and group members have participated in another four FCT projects worth over 253.000 EUR. Members of the group have participate in international collaborations on projects funded by the European Commission (INTERREG, EROCIPS AMPERA/016165) and European Space Agency (STRAPLEX), Biotechnology and Biological Sciences Research Council (UK), Natural Science and Engineering Research Council (Canada) and European bilateral exchange programs with Germany and Spain (GRICES/DAAD and CRUP) involving over 12.2 M EUR.

### **6b. Group team**

#### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. João José Oliveira Dias Coimbra (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

002. Jorge Pereira Machado (Cat.: Professor Associado, Gr. Acad.: Doutoramento)

003. Alberto Teodorico Rodrigues Moura Correia (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

004. Jose Carlos Fernandes Antunes (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

005. José Fernando Magalhães Gonçalves (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

006. Sergio Reis Cunha (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)



007. Jonathan Mark Wilson (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

008. Alfredo Nuno Damasceno Pinto de Oliveira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

009. Joana Ferreira Marques Ferreira Cardoso (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

## **2. Other researchers in the group (Include here collaborators with PhD. only)**

001. Mathilakath M. Vijayan (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

002. Dr. Roderic William Wilson (Cat.: Professor Associado, Gr. Acad.: Doutoramento)

003. Christopher Anthony Cooper (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

## **3. Other researchers in the group (non PhD.)**

001. Ana Filipa dos Santos Gonçalves (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

002. Ana Paula Magalhaes Lima (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

003. Begona Fernandez Duran (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

004. Diana Catarino das Neves Viegas (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

005. Joana Catarina Rocha Moreira da Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

006. Joana Helena Goncalves da Costa Padrao (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

007. João Filipe Pereira Vilares Neves (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

008. Maria Inês de Almeida Páscoa (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

009. Odete Marinho Gonçalves (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

010. Patrick Neil Bowskill Reis dos Santos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

011. Pedro Miguel Coutinho Victorino Borges Morais (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

012. Ronaldo Gomes de Sousa (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

013. Sérgio Catarina de Amorim Costa Dias (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

014. Sofia Gabriel Garcia Santos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

015. Tânia Marisa Ramos Pipa (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

016. Mariana Fonseca Hinzmann (Cat.: Outra, Gr. Acad.: Ensino Secundário)

017. Ricardo Manuel Campinho Capela (Cat.: Outra, Gr. Acad.: Ensino Secundário)

## 6c. Objectives & achievements

### 1. Objectives (2000 ca.)

1. Investigate the physiological challenges faced by migratory fishes (*Anguilla anguilla*, *Platichthys flesus*, *Petromyzon marinus*, *Scyliorhinus cannicula*, *Pagellus bogaraveo*), focusing on the abiotic factors of salinity and hydrostatic pressure. The species that are investigated are of commercial and/or biological significance.
2. Determine migratory patterns of fishes (*A. anguilla*, *Conger conger*, *P. flesus*) at different life history stages using otolith fingerprinting and complementary techniques. Establishing techniques and continuing monitoring of European eel (*A. anguilla*) recruitment levels.
3. Defining the mechanisms for dealing with physiological constraints and challenges of ammonotelism in fishes. Ammonia can be highly toxic to animals yet is a natural byproduct of metabolism.
4. Elucidate the mechanism of shell calcification in freshwater bivalves (*Anodonta cygnea*). In addition, the definition of culture conditions for freshwater bivalves (*A. cygnea*, *Hyriopsis myersiana*).
5. Technological advances to aid in biological discovers and monitoring of the environment. Specifically the development of methods for the non-invasive monitoring of physiological parameters and the development of hyperbaric systems for the maintenance of fish and other aquatic organisms for investigating hydrostatic pressure effects on physiology.

### 2. Main achievements (2000 ca.)

1. Development of a hyperbaric system for maintaining aquatic organisms (marine or fresh water) for prolonged periods at constant or fluctuating levels of hydrostatic pressure (up to 8 MPa). This system has been used to successfully study the effects of diurnal changes in hydrostatic pressure on gonadal development in flounder on their spawning migration. In addition technological advances have also been made in the area of bio and environmental monitoring.
2. Elucidation of the early life cycle of conger eels (*Conger conger* and *Conger oceanicus*) using otolith microstructure and elemental and isotopic analyses of microchemistry. Establishing techniques and continuing monitoring of European eel (*A. anguilla*) recruitment levels.
3. Identification of ammocoete metamorphosis as the critical time in the acquisition of marine ionoregulatory competence in the anadromous marine lamprey (*Petromyzon marinus*).
4. Finding that catadromous juvenile estuarine glass eel (*Anguilla anguilla*) do not require changes in branchial ion transport proteins to cope with direct transfer to freshwater. Change in expression levels of these proteins require long term acclimation.
5. Identify and characterize the principal biomineralization events on freshwater bivalve *Anodonta cygnea*. This includes the identification and characterization of mantle cell proteins involved in intercellular calcium binding and transport, extrapallial fluid macromolecules involved in the shell calcification mechanisms and the identification of the physical site (tissue) associated with the active calcium uptake from the environment.

6. Improvement of freshwater bivalve larval glochidia culture and juvenile production. Freshwater pearl mussel *Hyriopsis myersiana* and *Anodonta cygnea* for culture and conservation purposes.
7. Inclusion in EU level networks and participation in other national and international collaborative projects.
8. Dissemination of results generated by the group. Over the period 2003-2007 we collectively have 70 international peer reviewed publications in addition to three book chapters. We also have in excess of 100 conference presentations. Group members are also actively involved in education of the public.
9. Research training at the undergraduate and graduate levels (12MSc, 9 PhD completed).

#### 6d. Productivity

- 1. Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Bleher, R, Machado, J. 2004. Properties of the paracellular pathway of the outer mantle epithelium. *Journal of Experimental Zoology* 301A: 419-427. IF 2004/2007: 0.588/1.238, n°C=6.
2. Cardoso, J.F.M.F., Witte, J.IJ., van der Veer, H.W. 2006. Intra- and interspecies comparison of energy flow in bivalve species in Dutch coastal waters by means of the Dynamic Energy Budget (DEB) theory. *Journal of Sea Research* 56: 182-197. IF 1.765 n°C=5
3. Correia, AT, Able, KW, Antunes, C, Coimbra, J. 2004. Early life history of the American conger eel (*Conger oceanicus*) as revealed by otolith microstructure and microchemistry of metamorphosing leptocephali. *Marine Biology* 145: 477-488. IF 2004/2007=1.772/2.215, n°C=6.
4. Correia, AT, Antunes, C, Isidro, EJ, Coimbra, J. 2003. Changes in otolith microstructure and microchemistry during the larval development of the European conger eel (*Conger conger*). *Marine Biology* 142: 777-789. IF 2003/2007=1.841/2.215. n°C=6.
5. Damasceno-Oliveira, A, Fernandez-Duran, B, Goncalves J, Serrao P, Soares-da-Silva P, Reis-Henriques MA, Coimbra J. 2007. Effects of cyclic hydrostatic pressure on the brain biogenic amines concentrations in the flounder, *Platichthys flesus*. *General and Comparative Endocrinology* 153: 385-389. IF=2.562, n°C=0.
6. Damasceno-Oliveira, A, Gonçalves, J, Silva, J, Fernández-Durán, B, Coimbra, J. 2004. A pressurizing system for long-term study of marine or freshwater organisms enabling the simulation of cyclic vertical migrations. *Scientia Marina* 68(4): 615-619. IF 2004/2007=0.527/0.945, n°C=3.
7. Ip, YK, Randall, DJ, Kok, TKT, Barzaghi, C, Wright, PA, Ballantyne, JS, Wilson, JM, Chew, SF. 2004. The giant mudskipper *Periophthalmodon schlosseri* facilitates active NH<sub>4</sub><sup>+</sup> excretion by increasing acid excretion and decreasing NH<sub>3</sub> permeability in the skin. *Journal of Experimental Biology* 207: 787-801. IF 2004/2007=2.679/2.029, n°C=18.

8. Sousa, R, Antunes, C, Guilhermino, L. 2007. Species composition and monthly variation of the Molluscan fauna in the freshwater subtidal area of the River Minho estuary. *Estuarine, Coastal and Shelf Science* 75: 90-100. IF 2007=1.799, n°C= 2.

9. Wilson, JM, Leitão, A, Gonçalves, AF, Ferreira, C, Reis-Santos, P, Fonseca, A.V, Da Silva, JM, Pereira, CM, Coimbra, J. 2007. Modulation of branchial ion transport protein expression by salinity in glass eels (*Anguilla anguilla* L.). *Marine Biology* 151: 1633-1645. IF 2007=2.215, n°C=2.

10. Wilson, JM, Antunes, JC, Bouça, PD, Coimbra, J. 2004. Osmoregulatory plasticity in glass eels (*Anguilla anguilla*): Freshwater entry and changes in branchial ion transport protein expression. *Canadian Journal of Fisheries and Aquatic Sciences* 61: 432-442. IF 2004/2007=1.972/2.058, n°C=15.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Chew, SF, Wilson, JM, Ip, YK, Randall, DJ. 2005. Nitrogen excretion and defence against ammonia toxicity In: *Fish Physiology Vol. 22 Tropical Fishes*. Val, D, Randall, DJ (Eds), Academic Press, San Diego.

2. Ferreira da Silva, Sousa, R, Antunes, C, Santos, S, Gonçalves, P, Patinha, C. 2003. Avaliação do grau de stress ambiental do estuário do rio Lima devido às actividades de dragagem. IV Congresso Ibérico de Geoquímica / XIII Semana de Geoquímica, 14 a 18 Julho 2003, Coimbra, pp. 340-342.

3. Ip, Y K, Chew, S F, Wilson, JM, Randall, DJ. 2004. Defences against ammonia toxicity in tropical air-breathing fishes exposed to high concentrations of environmental ammonia: A review. *Journal of Comparative Physiology B* 174: 565-575.

4. Letão, A, Damasceno-Oliveira, A, Pereira, CM, Coimbra, JC, Wilson, JM. 2008. Transport stress in glass eels. In *Avanços em Endocrinologia Comparativa*, Vol4. Cadiz (in press).

5. Moura, G, Coimbra, J, Machado, J. 2004. Insights on nacre formation in the freshwater clam, *Anodonta cygnea* (L.): an overview. In: *Biom mineralization (BIOM2001)-Formation, diversity, evolution, and application*. Kobayashi, I, Ozawa, H (Eds), Tokai University Press, Japan pp. 129-132.

6. Pratoomchat, B, Sawangwong, P, Machado, J. 2004. The identification of molt stages in *Scylla serrata* based on cuticle morphology. In: *Biom mineralization (BIOM2001)-Formation, diversity, evolution, and application*. Kobayashi, I, Ozawa, H (Eds), Tokai University Press, Japan, pp. 98-102.

7. Randall, DJ, Ip, YK, Chew, SF, Wilson, JM. 2004. Air breathing and ammonia excretion in the mudskipper, *Periophthalmodon schlosseri*. *Physiological and Biochemical Zoology* 77(5): 783-788.

8. Silva, SR, Cunha, S, Matos, A, Cruz, N. 2008. Synthetic Aperture Techniques for Sonar Systems. Chapter of book “Advances in Sonar Technology”, I-Tech Online (in press).

9. Sousa, R, Dias, S, Antunes, C, Guilhermino, L. 2007. Fauna exótica presente no estuário do rio Minho: distribuição e impactos potenciais. Actas III Simpósio Ibérico sobre a bacia hidrográfica do rio Minho, pp. 58-65.

10. Wilson, JM. 2007. The use of immunochemistry in the study of branchial ion transport mechanisms. In: Fish Osmoregulation. Baldisserotto, B, Mancera, JM, Kapoor, BG (Eds). Science Publisher Inc., Enfield, pp.359-394.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**4. Master and Ph.D. thesis completed** (3000 ca.)

### PhD THESIS

Damasceno-Oliveira, Alfredo. 2007. Regulação hormonal da reprodução na solha (*Platyichthys flesus* L.); relação com o ciclo migratório. PhD Aquatic Sciences, ICBAS-UP. Supervisor J Coimbra, Co-supervisor MA Reis-Henriques (CIMAR).

Kovitvadhi, Satit. 2007. In vitro Culture of Freshwater Mussel Juveniles *Hyriopsis myersiana*. U. Burapha, Thailand. Supervisor J Machado.

Lima, Ana Paula. 2007. Aperfeiçoamento da cultura in vitro de larvas e juvenis de *Anodonta cygnea*. ICBAS-UP. Supervisor J Machado.

Martins, José Júlio. 2007. Estudo sobre o ciclo de vida do camarão (*Macrobrachium rosenbergii*): aspectos ligados à Fisiologia da Reprodução e Regulação Osmótica. U. Trás-os-Montes e Alto Douro. Supervisor J Coimbra.

Coimbra, Ana Maria . 2006. Efeito de poluentes organoclorados no comportamento reprodutivo e no desenvolvimento larvar da tilápia nilótica, *Oreochromis niloticus*. PhD in Aquatic Sciences, ICBAS, University of Porto. Supervisor: MA Reis-Henriques (ICBAS/CIIMAR), Co supervisor: J Coimbra (ICBAS/CIIMAR).

Moncaut, Natalia 2006. Isolation and functional characterization of gonadotrophin releasing hormone receptors. ICBAS, University of Porto. Supervisor: Adelino Canário (CCMAR), Co supervisor: João Coimbra (CIIMAR).

Correia, Alberto Teodorico. 2004. Early life history of the European conger eel (*Conger conger*) as revealed by otolith microstructure and microchemistry of leptocephali. ICBAS-UP. Supervisor J Coimbra, Co-supervisor JC Antunes.

Rocha, Ana Maria. 2008. Clonagem, caracterização funcional e expressão dos receptores das gonadotrofinas (FSH e LH) no robalo europeu (*Dicentrarchus labrax* L.). CSIC, U. Sevilha. Co-supervisor J Coimbra.

Sousa, Ronaldo. 2008. Factors contributing to the invasive success of *Corbicula fluminea* (Müller, 1774) PhD in Aquatic Sciences, ICBAS, University of Porto. Supervisor: L Guilhermino (ICBAS/CIIMAR), Co supervisor: C Antunes (CIIMAR).

## **MSc THESIS**

Maia-Mendes, Mónica. 2007. Estrutura da comunidade de macroinvertebrados bentónicos do estuário do rio Minho. U Porto. Supervisor JC Antunes.

Lyra, Frederico. 2007. Caracterização da comunidade de macroinvertebrados bentónicos do estuário do rio Minho - sua relação com a distribuição de poluentes no sedimento. FCUP. Supervisor C Antunes.

Picanço, Thais. 2007. Caracterização da comunidade de macroinvertebrados bentónicos do sapal dos rios Minho e Coura. ICBAS-UP. Supervisor C Antunes.

Mota, Micaela. 2007. Comunidade de macroinvertebrados bentónicos do rio Coura como indicador biológico da qualidade da água. FCUP. Supervisor C Antunes.

Dias, Ester. 2007. Estudo da dieta do corvo-marinho-de-faces-brancas (*Phalacrocorax carbo* Linnaeus, 1758) no estuário do rio Minho (NO-Portugal). FCUP. Supervisor C Antunes.

Neves, João. 2005. Caracterização molecular e funcional do gene *Nramp* em robalo (*Dicentrarchus labrax*). ICBAS-UP. Supervisor P Rodrigues (IBMC), Co-supervisor J Wilson.

Viegas, Diana. 2005. Sound Wave Phase Processing for Precise Positioning. MSc Computational Methods in Sciences & Engineering, FEUP. Supervisor S Cunha.

Lopo, Miguel. 2004. Avaliação do impacto da extracção de inertes em populações naturais de peixes. ICBAS-UP. Supervisor L Guilhermino, Co-supervisor C Antunes.

Lima, Ana Paula. 2004. Culture in vitro of glochidium *Anodonta cygnea* (Linnaeus, 1758). ICBAS-UP. Supervisor J Machado.

Sousa, Ronaldo. 2004. As comunidades de macroinvertebrados bentónicos como indicadores ecológicos, no estuário do rio Lima. ICBAS-UP. Supervisor C Antunes.

Santos, Sofia. 2004. Efeitos da exposição ao cádmio em parâmetros bioquímicos e na histologia da brânquia de tilápia. UTAD. Supervisor J Wilson, Co-supervisor A Fontainhas Fernandes (UTAD).

Catita, Eduardo. 2003. Projecto para a criação de uma área de protecção marinha na zona envolvente à Ínsua de Caminha. ICBAS-UP. Supervisor C Antunes.

## **5. Patents/propotypes (2000 ca.)**

1. Hyperbaric system for the long-term study and conservation of intermediate- and deep-depth aquatic organisms (Sistema hiperbárico para o estudo e conservação por longos períodos de organismos aquáticos de média/grande profundidade). CIMAR/INEGI. Portuguese invention patent nº103774 and International patent pending. Seafloor: Portuguese trade mark nº 423824. CIMAR/INEGI.

2. Dispositivo portátil e método para medição e cálculo de parâmetros dinâmicos da locomoção pedestre, National patent request no. 103933, Jan 2008. M Correia, S Cunha.

#### **6. Organization of conferences (2000 ca.)**

2008. International Congress Seasink 2008. University Fernando Pessoa. Porto, Portugal. 26-28 July.

2007. Workshop “Comunicar: A Ciência Trocada por Miúdos”. University Fernando Pessoa, 27 June, Porto, Portugal.

2007. Seminário "Novas tecnologias no estudo de otólitos e suas aplicações no âmbito da biologia marinha e pesqueira". CIMAR, Porto, Portugal, 22 January.

2006. III Simpósio Ibérico Sobre a Bacia Hidrográfica do Rio Minho. Vila Nova de Cerveira, Portugal, 1-2 July.

2006. II INDICANG: towards the definition and the development of indicators. Porto, Portugal, 6-7 June.

#### **7. Industry contract research (2000 ca.)**

#### **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

J Coimbra coordinated the task force of CIIMAR regarding the environmental impact of the Prestige oil spill for the Intersectoral Oceanographic Commission (Ministry of Science, Technology and Higher Education). The following report was produced:

- Coimbra, J, Guilhermino, L, Santos, P, Sousa-Pinto, I, Santos, M, Pereira, R, Lima, I. 2004. Relatório do Grupo de Trabalho Sobre o Derrame do Prestige. CIIMAR, Porto, 109 p.

Members of the research group participated in the project EROCIPS (Emergency Response to Coastal Oil, Chemical and Inert Pollution; Interreg IIIB) with the objective of defining protocols, tools and guidance material to improve existing coastal pollution response plans. The following report was produced and disseminated to local authorities such as the Portuguese Maritime Authority:

- Moreira, S, Santos, M, Cunha, I, Sousa, A, Lima, D, Coimbra, J, Reis-Henriques, MA, Guilhermino, L. 2007. Environmental monitoring report. Deliverable 7.3.5, 102 p.

Members of the research group participated in meetings organized by the Comissão de Coordenação para o Desenvolvimento da Região Norte (CCDR-N) for the elaboration of the Regional Agenda for the Sea, setting priorities for the sustainable development of marine research in the North of Portugal region.



## **9. Internationalization (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)**

The Ecophysiology group is involved internationally through the bilateral exchanges of students and researchers with Germany, and Spain using grants supported by GRICES and CRUP, respectively. In addition through collaborations on British Biological Sciences Research Council (BBSRC, UK) grants with Dr. RW Wilson (University of Exeter), we have been involved in the advanced training of post-doctoral researchers in Porto. We also currently have a post-doctoral researcher (Dr. A Damasceno-Oliveira) with a cross appointment to the Liverpool Microarray Facility and Centre for BioArray Innovation, University of Liverpool with Dr. A Cossins. Researchers within the group are also involved in EU INTERREG projects with partners in the south of Europe (see project listing). We are also in collaboration with the Education Department of the European Space Agency (ESA), under the scope of the STRAPLEX programme. Through provisions in their FCT fellowships, PhD students have worked abroad (R. Sousa: Cambridge UK; J.M. Moreira Silva: CityU HongKong, U.Waterloo Canada; U. Copenhagen, Denmark; AF Gonçalves: U. Waterloo; SG Santos: U.Cadiz).

Through international collaborative research we have publications with colleagues in Germany (R Bleher and A Ziegler, U. Ulm; M Hafner, Institut fur Molekularbiologie und Zellkulturtechnik, Mannheim; H Frank, Bayreuth U), U.K. (RW Wilson U.Exeter; A Cossins, U.Liverpool), Spain (AG Checa and A Rodrigues Navarro, U. Granada; R Maria, U. Valladolid), Canada (CM Wood, McMaster University; PA Wright, U.Guelph; CJ Brauner, U. British Columbia; JD Morgan, Vancouver Island U.), the United States (KW Able, Rutgers; SD McCormick USGS), Brasil (T Almeida Silva and F Brito, U. Federal Paulo Afonso; JCN Barros and TVS Alves, U. Federal Pernambuco; Dept. Cartografia, U. Estadual S. Paulo) Singapore (YK Ip, National U.; SF Chew, NanYang Tech U.), and Thailand (B Pratoomchat, Burapha U.; K Uthaiwan and K Satit, Kasetsart U). Between 2003 and 2008 collectively the group has over fifty collaborative publications.

## **6e. Future research**

### **1. Objectives (3000 ca.)**

1. The group will continue to address the questions that arise from the physiological adaptation of fishes in future work, however, with a clear shift to using transcriptomics. This approach will be used to study changes in the transcriptome of maturing (silver) European eel and rainbow trout during hydrostatic pressure adaptation. The European eel is reasonably pressure tolerant while the rainbow trout is shallow water species that is relatively pressure intolerant. The physiological study of the ontogeny of ion regulation will continue in lamprey as well as mechanisms of ammonia tolerance in teleost fishes.

2. Develop further otolith finger printing (use of elemental and isotopic techniques) as biological tracers of fish migration and for stock discrimination in the lab. Otolith strontium: Calcium ratio analysis is being used to infer the migratory pattern of the European flounder (*Platichthys flesus*) in the Minho River estuary. ICPMS (SB and LA) stock discrimination techniques are also being developed for two sparidae species (*Diplodus vulgaris* and *Spondylus cantharus*).

3. Further elucidate the calcification processes in freshwater bivalves. This includes the identification and characterization of mantle cell proteins involved in intercellular calcium binding and transport, extrapalial fluid macromolecules involved in the shell calcification

mechanisms and the identification of the physical site (tissue) associated with the active calcium uptake from the environment.

4. Further develop in vitro culture conditions for freshwater bivalves for species conservation and aquaculture.

5. Related to future advances will be the technological aspect of the group. This will involve improvements and technological developments to the hydrostatic chamber system (high pressure levels) and for remote monitoring systems.

**2. Funding, source, dates** (1500 ca.) (Indicate in full including amount of current and pending funding)

PTDC/MAR/64016/2006. FCT, Life under pressure: transcriptomics of the hydrostatic pressure acclimation in a shallow water teleost. July 2008 to June 2011. Funding CIMAR 132.640,00 EUR.

PTDC/AMB/70431/2006, FCT, BiOtoMetal - Multidisciplinary assessment of aquatic environment by anthropogenic contaminants (METALs), using a combined BIOmarker - OTolith chemical analysis approach in fishes. Jan 2008 – Jan 2011. Total funding 112.833,00 EUR, Funding CIMAR 31.800,00 EUR.

BB/F009364/1. Novel driving forces for water transport & osmoregulation: carbonate precipitation and osmotic coefficients. Biotechnology and Biological Sciences Research Council (BBSRC), 2007-. Total funding 420.000,00 £, Funding CIMAR 5.000 EUR.

NSERC (Canada), Strategic Projects Grants Supplemental Competition 2007. Juvenile pink salmon and the health of the Broughton Archipelago Ecosystem. Natural Sciences and Engineering Research Council (Canada). Mar 2008-. Total funding 195.000,00\$cdn, funding CIMAR no direct funding.

Pending funding

Integrated Actions Luso-Espanholas. João Coimbra (ICBAS, UP), Juan Miguel Mancera (Faculty of Sciences, University of Cadiz). Stress in glass eels. CRUP pending funding: 2.500 EUR.

Programa Operacional de Cooperação Transfronteiriça Espanha-Portugal 2007-2013. Valorización de los recursos naturales de la cuenca hidrográfica del Miño-Minho. NATURA MIÑO-MINHO. Total funding: 2.200.000,00 EUR, CIIMAR Funding: 590.546,00 EUR.

Fundo EDP para a Biodiversidade 2008. Promoção da colonização da enguia europeia (*Anguilla anguilla*) no rio Coura. Funding: 111.250,00 EUR.

The members of the laboratory will actively participate in the next call for national projects (expected at the end of 2008). A continuation of the INDICANG program is being proposed (INDICANG-2) which will include more catchment areas.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Cunha, S, Gonçalves, R, Silva, S, Correia, A. 2008. An automated marine biomonitoring system for assessing water quality in real-time. *Ecotoxicology* 17: 558-564. IF 2007=2.405, n°C=0
2. Lopes-Lima, M, Bleher, R, Forg, T, Hafner, M, Machado, J. 2008. Studies on a PMCA-like protein in the outer mantle epithelium of *Anodonta cygnea*: insights on calcium transcellular dynamics. *Journal of Comparative Physiology B* 178: 17-25. IF 2007=2.029, n°C=1.
3. Reis-Santos, PN, McCormick, SD, Wilson, JM. 2008 Ionoregulatory changes during metamorphosis and salinity exposure of juvenile sea lamprey (*Petromyzon marinus* L.). *Journal of Experimental Biology* 211: 978-988. IF 2007=2.972, n°C=1.
4. Nawata, CM, Hung, CCY, Tsui, TKN, Wilson, JM, Wright, PA, Wood, CM. 2007. Ammonia excretion in rainbow trout (*Oncorhynchus mykiss*): Evidence for Rh glycoprotein and H<sup>+</sup>-ATPase involvement. *Physiological Genomics* 31: 463-474. IF 2007=3.493, n°C=5.
5. Kadar, E, Checa, AG, Damasceno-Oliveira, A, Machado, JP. 2008. Shell nacre ultrastructure and depressurisation dissolution in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus*. *Journal of Comparative Physiology B*: 178: 123-130. IF 2007=2.029, n°C=0.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

It is important to invest in new hyperbaric systems and also to adapt/improve the existing ones. A larger room and also a technician for the control/servicing of the hyperbaric systems would greatly benefit the Ecophysiology group.

## **Environmental Toxicology**

### **6a. Group description**

#### **1. Group name / denomination**

#### **Environmental Toxicology**

#### **2. Principal investigator**

Maria Armanda Reis Henriques

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Endocrine disruption, Aquatic ecotoxicology, Environmental and Human health, Emergent pollutants

#### **5. Funding, source, dates (1000 ca.)**

The group has been able to attract a wide range of financing sources in the period of 2003-2007:

- European projects:

- acronym ACE, Contract nº EVK1(31/12/2002–31/05/2005), Funding for Letox 203.697,00 EUR;

- acronym EROCIPS, INTERREG IIIB, Code: 168 (01/11/2004-01/11/2007), Funding for Letox 250.000,00 EUR.

- National projects from FCT:

- 6 projects (PDCTM/PP/MAR/15280/99; PDCTM/MAR/99; POCI/MAR/59462/2004; POCI/MAR/56964/2004; POCI/MAR/56964/2004; POCI/MAR/60895/2004) have been financed with a total budget for Letox of 299.330,00 EUR.

- Undergraduated project from University of Porto/ Ilídio Pinho Foundation (1/10/2005-30/12/2005) total budget for Letox of €3.350 EUR.

- GRICES:

- Indo-Portuguese Research Cooperation and Scientific Cooperation Portugal/Spain, total budget for Letox of 13.500 EUR.

- Other funding:

- pluriannual FCT support, total budget for Letox of 4.900 EUR

- student's scholarships, total budget for Letox of 20.400 EUR

- The laboratory available budget in the period 2003-2007 was approximately 650.000,00 EUR.

## **6b. Group team**

### **1. Researchers in the group (Include only PhD. integrated in the LA)**

- 001. Maria Armanda Reis Henriques (Cat.: Professor Catedrático, Gr. Acad.: Agregação)
- 002. Miguel Alberto Fernandes Machado e Santos (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)
- 003. Ana Maria Monteiro Paiva Coimbra (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 004. Justyna Kopecka-Pilarczyk (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 005. Marta Sofia Sa Ferreira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

### **2. Other researchers in the group (Include here collaborators with PhD. only)**

- 001. Ana Dulce de Ascensao Almeida Correia (Cat.: Não aplicável (bolseiro) Gr. Acad.: Doutoramento)

### **3. Other researchers in the group (non PhD.)**

- 001. Daniela da Silva Lima (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 002. Filipa Margarida Barroso Ferreira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 003. Joana Filipa de Sousa Micael Pereira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 004. Joana Silveira Soares (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 005. Marisa Sárria Pereira de Passos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 006. Paulo Sandro Quintal de Freitas (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 007. Renata Isabel de Sousa Gonçalves (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

The research of Letox in the period of 2003-2007 focused in two main areas:

a) In the scope of endocrine disruption (ED) we pursued several complementary aims. In the aquatic environment endocrine disrupting chemicals (EDCs) do not exist alone. Instead, there is a complex mixture of estrogenic, androgenic, anti-estrogenic and anti-androgenic compounds. Therefore, single ecotoxicological evaluations may not reflect the multitude of antagonistic or synergistic stimulus that wildlife animals may face. This clearly indicates that there is the need for more robust methodological designs that takes in consideration these uncertainties. Therefore,

under controlled laboratory conditions we aimed at evaluating the effects and the molecular and biochemical mechanisms of disruption of fish and marine invertebrate's exposure to EDCs. Studies aimed at evaluating both single and complex mixtures of EDCs with similar mode of action (estrogenic) and antagonist mode of action (estrogenic and androgenic). Additionally, we also aimed to understand the impact of EDCs acting in thyroid hormones metabolism. In parallel, because of the inexistence of data on the effects of estrogenic chemicals in Portuguese aquatic ecosystems, screening of ED in estuarine and costal fish was performed.

b) The second area of research aimed at the development of a novel holistic approach based on the use of a battery of sensitive biological tests for diagnosis, monitoring and remediation of oil pollution and other organic chemicals. In the scope of this line, one of the aims was to develop a multiparametric monitoring system to monitor the quality and control assessment of oil pollution and to improve our understanding of the mechanisms of toxicity and detoxification of organic contaminants in aquatic organisms. In parallel, we also aimed at evaluating the presence of persistent organic pollutants, POPs (PCBs and DDT) and the more representative metals, Cd, Hg, Pb and As, in two farmed fish, sea bream and sea bass along the various stages of the production chain, until the market size. We intended to establish the impact of fish feeds on aquaculture production systems on the sea food quality and safety and the contribution to the control of flesh quality and oxidative stability, together with health benefits for consumer.

## **2. Main achievements (2000 ca.)**

a) In the scope of ED, we have initiated a innovative approach which aims the understanding of the effects of EDCs under complex mixtures. We have demonstrated that xenoestrogens act in an additive way in vitellogenin induction in marine fish, further validating the concentration addition methodology for chemicals with a similar mode of action. These findings will be essential for the risk assessment of EDCs in the aquatic environment. In a step further to understand the effects of more complex mixture of chemicals with an antagonist mode of action, we have demonstrated, for the first time, that the masculinising effects of one of the main environmental xenoandrogens can be blocked or ameliorated by concomitant exposure to estrogenic chemicals, both in fish and gastropods. This highlights the challenge that we face when trying to integrate all the mechanistic findings in hazard assessment procedures. We have also contributed to a better understanding of the molecular mechanisms underlying imposex, the best documented example of ED in wildlife. In our field approach, we have demonstrated the presence of fish feminisation in estuarine and coastal areas along the Portuguese coast thus highlighting the need for action from local authorities.

b) We have developed a multiparametric monitoring system for the quality and control assessment of oil pollution in estuarine and marine areas. A new laboratory Multispecies Marine Biomonitor was developed for recording the behavioural responses of fish and mussel to oil. Identically were identified a set of biomarkers for an easily warning detection of oil toxicity. In the scope of seafood quality, a comparison between quality of wild and farmed fish was done. Based on the results of environmental and feed contamination of individual PCBs and the resulting levels in the different organs of fish it was possible to estimate, using a single model, the relative importance of the different sources of contamination. In parallel, we continued our research on the mechanisms of detoxification of organic contaminants including a new topic of research on membrane transporters in fish, the multidrug resistant pump P-glycoprotein (P-gp) and the multidrug resistance-associated proteins (MRPs).

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Antunes, P, Gil, O, Ferreira, M, Vale, C, Reis-Henriques, MA. 2007. Depuration of PCBs and DDTs in mullet under captivity conditions. *Chemosphere* 67: S58-S64. IF 2007=2.739, n°C=6.
2. Castro, LFC, Melo, C, Guillot, R, Mendes, I, Queirós, S, Lima, D, Reis-Henriques, MA, Santos, MM. 2007. The estrogen receptor of the gastropod *Nucella lapillus*: Modulation following exposure to an estrogenic effluent? *Aquatic Toxicology* 84: 465-468. IF 2007=2.975, n°C=3.
3. Castro LFC, Lima D, Machado, A, Melo, C, Hiromori, Y, Nishikawa, J, Nakanishi, T, Reis-Henriques, MA, Santos, MM. 2007. Imposex induction is mediated through the Retinoid X Receptor signalling pathway in the neogastropod *Nucella lapillus*. *Aquatic Toxicology* 85: 57-66. IF 2007=2.975, n°C=6.
4. Correia, AD, Freitas, S, Scholze, M, Gonçalves, JF, Booj, P, Lamoree, MH, Mañanos, E, Reis-Henriques, MA. 2007. Mixtures of estrogenic chemicals enhance vitellogenic response in sea bass. *Environmental Health Perspectives* 115(1): 115-121. IF 2007=5.636, n°C=0.
5. Ferreira, M, Moradas-Ferreira, P, Reis-Henriques, MA. 2006. The effect of long-term depuration on phase I and phase II biotransformation in mullets (*Mugil cephalus*) chronically exposed to pollutants in River Douro Estuary, Portugal. *Marine Environmental Research* 61(3): 326-338. IF 2006/2007=2.106/1.930, n°C=9.
6. Santos, MM, Micael, J, Carvalho, AP, Morabito, R, Booy, P, Massanisso, P, Lamoree, M, Reis-Henriques, MA. 2006. Estrogens counteract the masculinizing effect of tributyltin in zebrafish. *Comparative Biochemistry and Physiology C* 142: 151-155. IF 2006/2007=1.991/2.345, n°C=9.
7. Castro, LFC, Santos, MM, Reis-Henriques, MA. 2005. The genomic environment around the Aromatase gene: evolutionary insights. *BMC Evolutionary Biology* 5: 43. IF 2005/2007=4.447/4.091, n°C=4.
8. Ferreira, M, Moradas-Ferreira, P, Reis-Henriques, MA. 2005. Oxidative stress biomarkers in two resident species, mullet (*Mugil cephalus*) and flounder (*Platichthys flesus*), from a polluted site in River Douro Estuary, Portugal. *Aquatic Toxicology* 71(1): 39-48. IF 2005/2007=2.719/2.975, n°C=14.
9. Santos, MM, Castro, LFC, Vieira, MN, Micael, J, Morabito, R, Massanisso, P, Reis-Henriques, MA. 2005. New insights into the mechanism of imposex induction in the dogwhelk *Nucella lapillus*. *Comparative Biochemistry and Physiology C* 141: 101-109. IF 2005/2007=1.456/2.345, n°C=20.
10. Ferreira, M, Antunes, P, Gil, O, Vale, C, Reis-Henriques, MA. 2004. Organochlorine contaminants in flounder (*Platichthys flesus*) and mullet (*Mugil cephalus*) from Douro estuary, and their use as sentinel species for environmental monitoring. *Aquatic Toxicology* 69: 347-357. IF 2004/2007=2.418/2.975, n°C=17.



**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Reis-Henriques, MA, Ferreira, M. 2006. Application of biological tools in estuarine and coastal waters monitoring. Research Highlights at CIMAR Associate Laboratory, pp. 47-49.
2. Ferreira, M, Costa, J, Reis-Henriques, MA. 2007. Impacts of environmental pollution in fish: a molecular, biochemical and cellular approach. Research Highlights at CIMAR Associate Laboratory, pp. 52-53.
3. Castro, LF, Santos, MM, Lima, D, Reis-Henriques, MA. 2007. Of snails and fishes: an evolutionary framework for the study of endocrine disruption. Research Highlights at CIMAR Associate Laboratory, pp. 52-53.
4. Santos, MM, Castro, LF. 2007. From the environment to the gene: endocrine disruption in marine invertebrates and fish. The associated laboratories in Portugal Highlights, pp. 18-19.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Antunes, P, Gil, O, Reis-Henriques, MA. 2005. Kinetics of PCB accumulation and elimination in sea bass (*Dicentrarchus labrax*) of two size class. ECOTOX 2005 – Advances and Trends in Ecotoxicology, pp. 16-18.
2. Antunes, P, Ferreira, M, Gil, O, Reis-Henriques, MA. 2005. Effects of PCBs in selected metabolic enzymes in juvenile and adult sea bass (*Dicentrarchus labrax*). ECOTOX 2005 – Advances and Trends in Ecotoxicology, pp. 99-102.
3. Reis-Henriques, MA, Rodrigues, P, Ferreira, M, Santos, MM. 2006. Endocrine disruption in Portuguese aquatic ecosystems. Learned Discourses, SETAC Globe, volume 7, number 2, pp. 31-32.

**4. Master and Ph.D. thesis completed** (3000 ca.)

**PhD THESIS**

Batista, Frederico. 2007. Avaliação do potencial aquícola da ostra Portuguesa. Supervisor: Pierre Boudry (IFREMER), Co-supervisor: MA Reis-Henriques (ICBAS/CIIMAR).

Damasceno-Oliveira, Alfredo. 2007. Regulação hormonal da reprodução na solha (*Platyichthys flesus* L.); relação com o ciclo migratório. PhD in Aquatic Sciences, ICBAS, University of Porto. Supervisor: J Coimbra (ICBAS/CIIMAR), Co supervisor: MA Reis-Henriques (ICBAS/CIIMAR).

Coimbra, Ana Maria. 2006. Efeito de poluentes organoclorados no comportamento reprodutivo e no desenvolvimento larvar da tilápia nilótica, *Oreochromis niloticus*. PhD in Aquatic Sciences, ICBAS, University of Porto. Supervisor: MA Reis-Henriques (ICBAS/CIIMAR), Co supervisor: JCoimbra (ICBAS/CIIMAR).

Ferreira, Marta. 2006. Alterações endócrinas e enzimáticas da solha (*Platyichthys flesus* L.) e na tainha (*Mugil cephalus*) expostas a contaminantes orgânicos no estuário do Rio Douro. PhD in Aquatic Sciences, ICBAS, University of Porto. Orientadora: MA Reis-Henriques (ICBAS/CIIMAR). Co orientador: P Moradas Ferreira (ICBAS/IBMC).

## **MSc THESIS**

Micael, Joana. 2006. Estudo dos efeitos de disruptores endócrinos com actividade androgénica e estrogénica na integridade de ADN de *Danio rerio*. Master in Sciences of the Sea – Sea Resources, ICBAS, University of Porto. Supervisor: MM Santos (CIIMAR), Co-supervisor: MA Reis-Henriques (ICBAS/CIIMAR).

Rodrigues, Pedro. 2006. Estudo da feminização da papila urogenital masculina no Caboz d'areia (*Pomatoschistus minutus*) nos estuários dos Rios Minho e Lima. Master in Sciences of the Sea – Sea Resources, ICBAS, University of Porto. Supervisor: MM Santos (CIIMAR), Co-supervisor: MA Reis-Henriques (ICBAS/CIIMAR).

### **5. Patents/propotypes (2000 ca.)**

### **6. Organization of conferences (2000 ca.)**

### **7. Industry contract research (2000 ca.)**

### **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

- Members of the research group participated in the project EROCIPS (Emergency Response to Coastal Oil, Chemical and Inert Pollution; Interreg IIIB) with the objective of defining protocols, tools and guidance material to improve existing coastal pollution response plans. The following reports were produced and disseminated to local authorities such as the Portuguese Maritime Authority:

- Cunha, I, Sousa, A, Lima, D, Santos, M, Reis-Henriques, MA, Guilhermino, L. 2006. Environmental monitoring: protocols for type of monitoring and seasonality. Deliverable 7.1.1b, 74 p.

- Moreira, S, Santos, M, Cunha, I, Sousa, A, Lima, D, Coimbra, J, Reis-Henriques, MA, Guilhermino, L. 2007. Environmental monitoring report. Deliverable 7.3.5, 102 p.

- Members of the research group integrated a task force on the scope of the Prestige oil spill:

- Coimbra, J, Guilhermino, L, Santos, P, Sousa-Pinto, I, Santos, MM, Pereira, R, Lima, I. 2004. Relatório elaborado por uma equipa do CIMAR, no âmbito da "Task-Force" sobre o derrame do

Prestige para análise no grupo de trabalho sobre monitorização da zona costeira, da COI - Comissão Oceanográfica Intersectorial, Porto, 110 p.

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

- France

- Conseil Régional d'Aquaculture
- Conseil Régional de Bretagne
- Conseil Régional des Pays de la Loire
- Conseil Régional de Poitou Charentis

- Germany

- Galablaboratories
- Departement of Biology and Chemistry, Institute of Cell Biology, Biochemistry and Biotechnology, University of Bremen

- India

- National Institute of Oceanography (NIO), Goa

- Italy

- Dipartimento di Scienze Ambientali, Università Ca' Foscari Venezia
- ENEA Centro Ricerche Casaccia

- Japan

- Osaka University

- Spain

- Instituto Tecnológico para o Control do Medio Mariño de Galicia (INTECMAR)
- Conselleria de Pesca y Asuntos Maritimos
- CETMAR
- Conselleria de Medio Ambiente
- Institut de Ciències del Mar – CSIC
- Department of Chemistry and Science of the Materials, University of Huelva

- The Netherlands

- Netherlands institute for Sea Research (NIOZ)
- Institute for Environmental studies (IVM), Amsterdam University
- Department of Pharmacochimistry, Vrije Universiteit Amsterdam
- United Kingdom
- Department of Biological Sciences, Brunel University
- Centre for Toxicology, The School of Pharmacy, University of London
- Devon Country Council
- Dorset Country Council
- Pembrokeshire County Council
- Environmental and Heritage Services
- Devon Wildlife Trust
- Croatia
- Rudjer Boskovic Institute, Zagreb
- Belgium
- Zoological Institute K.U. Leuven
- Members of the research group taught the course “Advanced course in monitoring and management of pollution in coastal and estuarine areas”, National Institute of Oceanography (NIO), Goa, India, 5-17 March 2007.
- During 2003-2007 Letox has been involved in two European projects: ACE “Analysing combination effects of mixtures of estrogenic chemicals in marine and freshwater organisms” and “EROICIPS – The Emergence Response to coastal Oil, Chemical and Inert Pollution from Shipping”. It has also been involved in one Grices collaboration with India and two Grices collaborations with Spain (Galicia and Barcelona).
- The collaborations with international groups resulted in the publication of 12 articles in international ISI journals (some of these publications can be found above).

## **6e. Future research**

### **1. Objectives (3000 ca.)**

In the field of endocrine disruption (ED), we will continue the research involving the study of chemical mixtures with additive and antagonistic effects. In particular, we will focus in changes at different levels of biological organization, i.e., from changes in gene expression up to

alterations on population dynamics, such as impact in the reproductive output and animal behaviour. One of the major challenges in the field of ED is the understanding of the population dynamic impact of these chemicals, together with the understanding of the chemicals mode of action. This will be pursued with our approach. In the past, we have shown the importance of the modulation of the retinoic acid signalling pathways in the development of ED disruption in gastropods. We will continue the study of the impact of modulation of retinoic acid signalling pathways in gastropods, and start a similar study with fish. In fact, one of the innovative aspects of our research is the study of disruption mechanisms in phylogenetically distinct groups thus taking into account an evolutionary approach perspective in endocrine disruption.

A new research line will focus in the aquatic ecotoxicology of pharmaceuticals including the assessment of combination effects. Most of these compounds are by nature biologically active and hydrophobic. Therefore, aquatic animals and humans can take them up easily. As they are continuously discharged to the environment via a number of routes, it is urgent to increase the knowledge on the potential impact of these drugs in aquatic organisms. Those chemicals acting through receptor-mediated mechanisms, for instance drugs that bind to steroid, thyroid or peroxisome proliferator-activated receptor are of particular concern. Our main aim is to help both nature resource managers and the scientific community in the hazard assessment of these chemicals.

On the mechanisms of detoxification of organic contaminants we will pursue the development of tools for better clarify the role of the “phase III” of detoxification, i.e., the transport proteins involved in the elimination of xenobiotics. The research will focus on P-glycoprotein (P-gp) and the multidrug resistance-associated proteins (MRPs) in fish in order to better understand the physiological and toxicological detoxification functions. The work will be performed with aquatic animal populations from polluted habitats and under controlled laboratory conditions (a pharmacological approach) by exposing fish to selected pollutants, such as PAHs. The role of these membrane proteins on survival, reproduction and detoxification upon chemical insult will be investigated.

In the future, we intend to strengthen the present collaboration with national and local authorities such as the national Environmental agency in support of policy developments and hazard assessment of chemicals. Similarly, we will seek to establish future collaborations with the industry.

## **2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

- FCT Project, POCI/MAR/59462/2004: The molecular mechanisms of sexual differentiation in gastropode and the role of vertebrate – like steroid hormones. Project coordinator: Filipe Castro. 01/10/2005-01/10/2008. Funding for Letox 32.280,00 EUR. (Available budget 4.000,00 EUR)
- FCT Project, POCI/MAR/59094/2004: SEAQUA – Quality of seabass and seabream; accumulation of organochlorine compounds and heavy metals and biomarkers of exposition. Coordinator of the project: Maria Armada Henriques. 2/11/2005-30/11/2008. Funding for Letox 49.944,00 EUR. (Available budget 10.000,00 EUR)
- FCT Project, POCI/MAR/60895/2004: Effects of complex mixtures of antagonistic endocrine disrupting chemicals in two fish species with different life histories. Project Coordinator at CIIMAR: Miguel Santos. 1/10/2005-30/03/2009. Funding Letox 48.810,00 EUR (Available budget 8.000,00 EUR)

- FCT project, PTDC/MAR/68106/2006: The modulations of retinoic acid signalling pathways by environmental pollutants in teleosts. Project Coordinator: Miguel Santos. 1/09/2008-30/08/2011. Funding for Letox 71.000,00 EUR.

- 1.825.596,00 EUR from EU, INTERREG IVC: Decision-making tools for Endocrine dISruption in Europe – DENISE. Partner Scientific Leader: Miguel Santos. Funding for Letox 283.000,00 EUR. Pending.

- 3.072.233,49 EUR from EU, INTERREG IV, Atlantic Area: ARCOPOL – The Atlantic Regions' COastal POLLution Response. Partner Scientific Leader: Maria Armanda Henriques. 01/01/2009-31/12/2011. Funding for Letox 200.00,00 EUR. Pending.

- 1.286.948,00 EUR from EU, INTERREG Area – Trans-national Programme España/Portugal: INCAGES – Indices de Calidad Ambiental para la GESTión Integral en Áreas Costeras Transfronterizas del Sur de Galicia y Norte de Portugal. Partner Scientific Leader: Maria Armanda Henriques. 01/01/2009-31/12/2011. Funding for Letox 152.600,00 EUR. Pending.

**3. Previous publications in the area (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)**

1. Antunes, P, Gil, O, Reis-Henriques, MA. 2007. Evidence for higher magnification factors of lower chlorinated PCBs in cultivated seabass. *Science of the Total Environment* 377: 36-44. IF 2007=2.182, n°C=0.

2. Correira, AD, Gonçalves, R, Scholze, M, Ferreira, M, Reis-Henriques, MA. 2007. Biochemical and behavioral responses in gilthead seabream (*Sparus aurata*) to phenanthrene. *Journal of Experimental Marine Biology and Ecology* 347: 109-122. IF 2007=1.750, n°C=2.

3. Coimbra, AM, Reis-Henriques, MA, Darras, VM. 2005. Circulating thyroid hormone levels and iodothyronine deiodinase activities in Nile tilapia (*Oreochromis niloticus*) following dietary exposure to Endosulfan and Aroclor 1254. *Comparative Biochemistry and Physiology C* 141: 8-14. IF 2005/2007=1.456/2.345, n°C=7.

4. Micael, J, Reis-Henriques, MA, Carvalho, AP, Santos, MM. 2007. Genotoxic effects of binary mixtures of xenoandrogens (Tributyltin, Triphenyltin) and a xenoestrogen (Ethinylestradiol) in a partial life-cycle test with zebrafish (*Danio rerio*). *Environmental International* 33: 1035-1039. IF 2007=2.797, n°C=0.

5. Rodrigues, P, Reis-Henriques, MA, Campos, J, Santos, MM. 2006. Urogenital Papilla feminization in male *Pomatoschistus minutus* from two estuaries in northwestern Iberian Peninsula. *Marine Environmental Research* 62: S258-S262. IF 2006/2007=2.106/1.930, n°C=4.

**4. Special requirements (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)**

No special requirements.

## Chemical Speciation and Bioavailability (CS&B)

### 6a. Group description

#### 1. Group name / denomination

**Chemical Speciation and Bioavailability (CS&B)**

#### 2. Principal investigator

Maria Teresa Sa Dias de Vasconcelos

#### 3. Location of group (Host institution)

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### 4. Keywords

Chemical speciation, Biogeochemical processes, Interactions

#### 5. Funding, source, dates (1000 ca.)

In the period 2003-2007 the CS&B Lab was involved in seven financed projects/protocols at the National level, either as a coordinator (in five projects/protocols) or as a collaborator (in two projects/protocols). Three of those projects were financed by private companies (Aquaculture Company, Amorim Revestimentos S.A. and Petróleos de Portugal S.A., Refinaria do Porto) whereas the other four were financed by FCT (projects POCTI/QUI/15089/1999, POCTI/CTA/38411/2001, POCTI/CTA/48386/2002, POCI/AMB/60267/2004). Another source of financing was the “Plurianual FCT funds” attributer to PhD researchers. Total funds involved in the mentioned period was ca. 290.000 EUR. A majority of those funds (ca. 46 %) were obtained through research projects financed by FCT, whereas contract-research activities (in cooperation with private companies) accounted for ca. 31 % and “Plurianual FCT funds” represented ca. 23 % of the total funds.

Additionally, an FCT financing of 150.000 EUR was obtained through “Programa Nacional de Re-equipamento Científico – PNRC” (REEQ/304/QUI/2005). The proposal was submitted through LAQUIPAI – a small research centre already extinct. The responsible and respective team transferred to CIIMAR in 2003.

### 6b. Group team

#### 1. Researchers in the group (Include only PhD. integrated in the LA)

001. Maria Teresa Sa Dias de Vasconcelos (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

002. Maria Antonia Santos Mendes Salgado (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

003. Cristina Marisa Ribeiro de Almeida (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)



004. Teodor Ludmilov Stoichev (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

## **2. Other researchers in the group (Include here collaborators with PhD. only)**

001. Maria Clara Ramalho Monteiro Pires Basto (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

002. Maria da Conceicao Constantino Fernandes (Cat.: Outra, Gr. Acad.: Mestrado)

## **3. Other researchers in the group (non PhD.)**

001. Ana Cristina Silva Rocha (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

002. Ana Mafalda Saraiva Baptista (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

003. Maria da Nazaré Parada Figueiredo de Sousa Couto (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

004. Pedro Neves de Carvalho (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

005. Pedro Nuno da Costa Leão (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

006. Rafael Domingues Evangelista (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

007. Ana Cláudia Moreira Dias (Cat.: Outra, Gr. Acad.: Licenciatura)

008. Manuela Fernanda Gomes Moreira da Silva (Cat.: Outra, Gr. Acad.: Licenciatura)

009. Pedro Nuno Ribeiro Rodrigues (Cat.: Outra, Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

The CS&B Lab is focused on studies of chemical speciation (differentiation and quantification of different chemical forms of contaminants and natural constituents) in environmental systems.

The multiplicities of chemical species that can be found in the environment react with each other and interact with the biota present in the media they are in. The physical and chemical properties, as well as the organisms involved, determine how a certain chemical is going to be reflected on the environment.

The final goals of the Lab activities are to get answers for the following questions: (1) how does the chemical composition of a medium influence the responses of an organism in contact with it; and (2) how do organisms from a given system affect the chemical composition of the surrounding medium?

Therefore, research carried out at CS&B Lab intends to give a contribution for the comprehensiveness of biogeochemical processes and may be also relevant for risk assessment purposes.

The strategy of CS&B Lab has been pioneer in integrating the study of inorganic and organic chemicals as well as live organisms. Indeed, traditionally, most studies have been addressed to only inorganic (particularly heavy metals) or organic contaminants/nutrients, each one carried out by a different research team with distinct expertise, who ignored eventual antagonistic or synergetic effects caused by factors out of the study. However, there is increasing evidence that such mutual effects occur and can influence markedly the biological response of the organisms in natural systems.

The strategy of the Lab is based on the following working methodologies:

I- Observation of each sample through different analytical windows, by using independent analytical techniques: spectrophotometric, electrochemical, chromatographic and spectrophotometric, to obtain as much information as possible on sample composition;

II- Development of different projects with complementary specific aims, which converge to the same final goal;

III- Studies in situ and ex situ which are complemented with studies in vitro, to have a better control of certain parameters, which facilitates the interpretation of the results.

## **2. Main achievements (2000 ca.)**

### **A - Research on water and sediment/soil**

A1 - A line focused on mutual influence of trace metals and algae/cyanobacteria involved 2 concluded Pos Doc (one initiated before 2003), 2 PhD in progress and a concluded MSc. Seven papers were published after 2003 in international journals with peer review (IJPR), 1 is submitted and 2 are in preparation. Recently, studies were extended to mutual interactions among phytoplankton and emerging contaminants (endocrine disrupters and antibiotics) involving a Pos Doc, a PhD and an under graduated student, with 1 paper published in IJPR and 2 in preparation.

A2 - Research on chemical pollution and biological effects, namely on water quality of aquaculture enterprises (financed by an Aquaculture Company) and on metals bioaccumulation in fish organs (a FCT project) involved 1 concluded PhD and 3 papers published in IJPR.

A3 - A research line, started in 2003, on application of vascular plants for rhizoremediation, taking emphasis on the plant role in conditioning the nearby environment (2 financed projects: one from FCT and another from Petróleos de Portugal S.A., Refinaria do Porto) involved 2 concluded Pos-Doc, 2 PhD in progress and 1 concluded and 3 in progress MSc. Ten papers were published in IJPR, 1 submitted and 7 are in preparation.

A4 - Optimization of analytical methods for metal determinations in water, biological and soil matrixes with 3 papers published in IJPR.

### **B - Research on atmospheric environment**

B1 - Indoor air quality, in collaboration, financed by Amorim Revestimentos S.A., produced 2 concluded and 2 in progress MSc, 2 papers published in IJPR and 3 papers published in conference proceedings;

B2 - Outdoor air quality (2 FCT financed projects): on chemical characterization of PM<sub>2.5</sub> and PM<sub>10</sub> fractions of urban aerosol and on biomonitors. The last produced a concluded MSc and 7 papers were published in IJPR.

C - Before 2003 the CS&B lab was involved still in other projects, which were concluded after 2003:

C1 - Metal and organometal speciation in wines and their precursors, with 6 papers published after 2003 in IJPR;

C2 - Metals and macrobenthic fauna interactions with 4 papers published after 2003 in IJPR.

All reported studies were presented in ca. 50 national and international conferences, including invited and keynote lectures and oral and poster presentations (some in proceedings).

## 6d. Productivity

### 1. Publications in peer review journals (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n°C=). Give title and full citation in original language. DO NOT translate)

1. Almeida, CMR, Mucha, AP, Bordalo, AA, Vasconcelos, MTSD. 2004. Influence of the sea rush *Juncus maritimus* on metal concentration and speciation in estuarine sediment colonized by the plant. *Environmental Science and Technology* 38: 3112-3118. IF 2004/2007=3.554/4.363, n°C=14.

2. Azenha, MA, Evangelista, R, Martel, F, Vasconcelos, MT. 2004. Estimation of the human intestinal permeability of butyltin species using the Caco-2 cell line model. *Food and Chemical Toxicology* 42: 1431-1442. IF 2004/2007=2.341/2.186, n°C=6.

3. Baptista, MS, Vasconcelos, MT, Cabral, JP, Freitas, MC, Pacheco, AMG. 2006. The ability of biological and organic synthetic materials to accumulate atmospheric particulates containing copper, lead, nickel and strontium. *Journal of Environmental Monitoring* 8: 147-152. IF 2006/2007=1.523/1.833, n°C=3.

4. Fachini, A, Vasconcelos, MTSD. 2006. Enhancing diatom growth by using zeolites to change seawater composition. *Environmental Science and Pollution Research* 13: 238-241. IF 2006/2007=1.980/3.894, n°C=4.

5. Fernandes, C, Fontaínhas-Fernandes, A, Peixoto, F, Salgado, MA. 2007. Bioaccumulation of heavy metals in *Liza saliens* from the Esmoriz-Paramos coastal lagoon, Portugal, *Ecotoxicology and Environmental Safety* 66: 426-431. IF 2007=2.014, n°C=6.

6. Leão, PN, Vasconcelos, MTSD, Vasconcelos, VM. 2007. Role of marine cyanobacteria in trace metal bioavailability in seawater. *Microbial Ecology* 53: 104-109. IF 2007=2.558, n°C=0.

7. Mucha, AP, Leal, MFC, Bordalo, AA, Vasconcelos, MTSD. 2003. Comparison of the response of three micro-algae species exposed to elutriates of estuarine sediments, based on growth and

chemicals speciation. *Environmental Toxicology and Chemistry* 22: 576-585. IF 2003/2007=2.429/2.309, n°C=5.

8. Mucha, AP, Bordalo, AA, Vasconcelos, MTSD. 2003. Macrobenthic community in the Douro Estuary: Relations with trace metals and natural sediment characteristics. *Environmental Pollution* 121: 169-180. IF 2003/2007=2.002/3.135, n°C=46.

9. Mucha, AP, Almeida, CMR, Bordalo, AA, Vasconcelos, MTSD. 2005. Exudation of organic acids by a marsh plant and implications on trace metal availability in the rhizosphere of estuarine sediments. *Estuarine, Coastal and Shelf Science* 65: 191-198. IF 2005/2007=1.633/1.799, n°C=5.

10. Santos, R, Machado, MJC, Ruiz, I, Sato, K, Vasconcelos, MTSD. 2007. Space charge and mass discrimination effects on lead isotope ratio measurements by ICP-QMS in environmental samples with high uranium content. *Journal of Analytical Atomic Spectrometry* 22: 783-790. IF 2007=3.269, n°C=3.

The CS&B Lab has published, after 2003, 43 papers in international journals with peer review, with several publications resulting from national and international collaborations, including collaborations with other Labs from CIMAR LA. Impact factor (based on ISI JCR 2007) for those publications ranged from 0.499 to 4.363. During the period 2003-2007, the CS&B Lab has published an average of 1.8 publications (ISI) per year per present member with 1 PhD degree.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

Papers in conference proceedings

1. Almeida, CMR, Mucha, AP, Delgado, MFC, Caçador, MI, Bordalo, AA, Vasconcelos, MTSD. 2007. Influence of organic pollutants in the Cu phytoremediation potential of *Halimione portulacoides*. *Soil and Wetland Ecotoxicology (SOWETOX)*, Barcelona, Spain, 26-27 November 2007 (Oral presentation), CD of SOWETOX.

2. Almeida, CMR, Mucha, AP, Delgado, MFC, Caçador, MI, Bordalo, AA, Vasconcelos, MTSD. 2007. Influence of mixed contaminants in the rhizoremediation potential of *Halimione portulacoides*. 2nd International Symposium on Wetland Pollutant Dynamics and Control (WETPOL), Tartu, Estonia, 16-20 September 2007 (Oral presentation), pp. 33-34.

3. Lyra, F, Carvalho, P, Antunes, C, Vasconcelos, MT, Basto, MC. 2006. Resultados preliminares sobre contaminação de sedimentos do rio Minho por metais vestigiais e pesticidas organoclorados. III Simpósio Ibérico Sobre a Bacia Hidrográfica do Rio Minho, Vila Nova de Cerveira, Portugal, 1-2 June 2006 (Oral presentation), pp. 98-109.

4. Mucha, AP, Almeida, CMR, Vasconcelos, MTSD. 2006. Resposta de uma planta de sapal (*Juncus maritimus*) a contaminação por cobre. CiFyQA 2006 - IV Congreso Iberoamericano de Física y Química Ambiental, Cáceres, Spain, 22-26 May 2006 (Oral presentation), pp. 347-348.
5. Mucha, AP, Almeida, CMR, Machado, AM, Magalhães, CM, Vasconcelos, MTSD, Bordalo, AA. 2007. In vitro response of salt marsh microbial community to mixed contamination. Soil and Wetland Ecotoxicology (SOWETOX), Barcelona, Spain, 26-27 November 2007 (Oral presentation), CD of SOWETOX.
6. Reis, PA, Almeida, CMR, Antunes, C. 2006. Estudo geoquímico de metais em sedimentos do sapal do Estuário do Minho. III Simpósio Ibérico Sobre a Bacia Hidrográfica do Rio Minho, Vila Nova de Cerveira, Portugal, 1-2 June 2006, pp. 89-97 (Oral presentation).
7. Samúdio, MJ, Silva, GVA, Oliveira Fernandes, E, Guedes, J, Vasconcelos, MTSD. 2006. A detailed indoor air study in a school of Porto. Proceedings of Healthy Buildings 2006 Conference, Lisboa, Portugal (Oral presentation), pp. 345-349.
8. Silva, GV, Fernandes, EO, Vasconcelos, MTSD, Santos, AM. 2003. Adsorption/desorption of volatile organic compounds by uncoated cork parquet. Healthy Buildings 2003, 7th International Conference, Singapore, 7-11 December 2003 (Oral presentation), pp. 414-419.
9. Silva, GVA, Martins, AO, Oliveira Fernandes, E, Guedes, J, Vasconcelos, MTSD. 2006. VOCs in indoor air in several schools of Porto. Proceedings of Healthy Buildings 2006 Conference, Lisboa, Portugal (Oral presentation), pp. 227-230.

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Almeida, CMR. 2003. Isotopic and multi-element characterisation of wine for identification of lead contamination sources and of the provenance region. PhD in Chemistry, Faculty of Sciences, University of Porto. Supervisor: MTSD Vasconcelos (CIIMAR).

Fernandes, MCC. 2007. Contaminação da Barrinha de Esmoriz/Lagoa de Paramos por metais pesados. Efeitos bioquímicos e histológicos em *Liza saliens*. PhD in Exact, Natural and Technological Sciences - Environmental Sciences, University of Trás-os-Montes e Alto Douro. Supervisor: MA Salgado (CIIMAR), Co-supervisor: Fontainhas-Fernandes (University of Trás-os-Montes e Alto Douro).

##### **MSc THESIS**

Martins, AO. 2003. Influência de materiais de revestimento de pavimentos na qualidade do ar interior em termos de compostos orgânicos voláteis. MSc in Chemistry, Faculty of Sciences, University of Porto. Supervisor: MTSD Vasconcelos (CIIMAR).

Martins, JPMM. 2003. Identificação e quantificação de exsudados libertados pelas raízes da planta *Cyperus eragrostis* Lamark. MSc in Chemistry, Faculty of Sciences, University of Porto. Supervisor: MTSD Vasconcelos (CIIMAR).

Santos, RMP. 2003. Espectrometria de massa com plasma acoplado por indução ICP-MS - uma nova janela analítica para estudo dos metais ultra-vestigiários em águas naturais. MSc in Chemistry, Faculty of Science, University of Porto. Supervisor: MTSD Vasconcelos (CIIMAR).

Alves, F. 2005. Investigation of organo-chlorine contaminants in Douro river estuarine sediments and rhizosediments. MSc in Chemistry, Faculty of Sciences, University of Porto. Supervisor: MCP Basto (CIIMAR), Co-supervisor: MTSD Vasconcelos (CIIMAR).

Pereira, AJAC. 2005. Estabelecimento de métodos para investigar a presença de compostos orgânicos voláteis e semi-voláteis (pesticidas) em espécies biológicas com implicações na saúde pública. MSc in Chemistry, Faculty of Sciences, University of Porto. Supervisor: MTSD Vasconcelos (CIIMAR).

Leão, PNC. 2006. Interactions between marine cyanobacteria and trace metals in seawater. MSc in Hidrobiology, Faculty of Sciences, University of Porto. Supervisor: MTSD Vasconcelos (CIIMAR), Co-supervisor: V Vasconcelos (CIIMAR).

Lyra, FJPCF. 2007. Caracterização da comunidade de macroinvertebrados bentónicos do estuário do Rio Minho – sua relação com a distribuição de poluentes no sedimento. MSc in Applied Ecology, Faculty of Sciences, University of Porto. Supervisor: C Antunes (CIIMAR), Co-supervisor: MCP Basto (CIIMAR).

Reis, PA. 2007. Estudo Geoquímico de Metais em Sedimentos do Sapal dos Rios Minho e Coura. Master in Sea Sciences and Marine Resources, ICBAS, University of Porto. Supervisor: CMR Almeida (CIIMAR), Co-supervisor: C Antunes (CIIMAR).

An average of 2 to 4 graduate students per year were included in research projects in progress at CS&B Lab which resulted in identical number of completed graduation theses.

It must be referred that during the period 2003-2007, besides the completed theses indicated above, seven more PhD theses and eight MSc theses were initiated in the CS&B Lab, some of them in collaboration either with another CIIMAR Lab or with national and international institutions.

## **5. Patents/propotypes (2000 ca.)**

## **6. Organization of conferences (2000 ca.)**

Membership in organization teams of scientific international meetings (MTSD Vasconcelos):

- Member of the Technical Program Committee of the 8th Healthy Buildings Conference - HB 2006, Lisbon, Portugal, 2006.

Member of the Scientific Committee of the:

- 9th Conference on Chemistry in the Environment of the Federation of the European Chemical Societies (FECS) on: "Behaviour of Chemicals in the Environment", Bordeaux, France, 2004.

- 10th Conference on Chemistry in the Environment of the European Chemical Association for Molecular Sciences, (EuCheMS), Rimini, Italy, 2005.
- 11th Conference on Chemistry in the Environment of the European Chemical Association for Molecular Sciences, (EuCheMS), Torun, Poland, 2007.
- 4th Ibero-American Conference on Environmental Physics and Chemistry, Cáceres, Spain, 2006.

#### **7. Industry contract research (2000 ca.)**

Over the years, the scientific expertise and reliable experimental facilities associated to the CS&B Lab have gained recognition by several private companies in need of different environmental studies and cooperation protocols were established with several of those companies.

Collaborations:

- Cooperation with Amorim Revestimentos S.A. and IDMEC on Indoor Air Quality
- Cooperation with the Portuguese Association of Small and Media Companies on Environmental Diagnostics in the Industry
- Cooperation with the UNIMED on Chemical Characterization of Occupational Environmental
- Cooperation with the Real Companhia Velha on Characterization of Port Wine
- Cooperation with the SMGP – Consultores, Lda on Chemical Characterization of Occupational Environment
- Cooperation with Petróleos de Portugal S.A. Refinaria do Porto, GALP Energy Group - Petróleos de Portugal S.A on Rizoremediação de Hidrocarbonatos de Petróleo em Solos da Refinaria do Porto
- Cooperation with Paracelsia Industria Farmacêutica S.A. on Aluminum Determination on Serum Samples
- Cooperation with Hovione FarmaCiencia S.A. on Survey of some pharmaceuticals effects on freshwater cyanobacteria. Does good manufacturing practice (GMP) during pharmaceuticals manufacture influence the biological response?
- Cooperation with Department of Production and Systems, School of Engineering, University of Minho
- Cooperation with several Small and Media Companies, like Sondar Company and ECOINSIDE - Soluções em Ecoeficiência e Sustentabilidade, Lda., and Centres, like Centro de Apoio Tecnologia à Indústria Metalomecânica (CATIM), for training courses for under-graduate and graduate students, namely for development of MSc theses.

#### **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)



**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

• Collaborative publications

Buszewski, B, Ligor, T, Filipiak, W, Vasconcelos, MTSD, Pompe, M, Veber, M. Toxicological and Environmental Chemistry, doi: 10.1080/02772240701384418.

Santos, R, Machado, MJC, Ruiz, I, Sato, K, Vasconcelos, MTSD. 2007. Journal of Analytical Atomic Spectrometry 22: 783-790.

Vanhaecke, F, Balcaen, L, Deconinck, I, De Schrijver, I, Almeida, CMR, Moens, L. 2003. Journal of Analytical Atomic Spectrometry 18: 1060-1065.

• Socrates/Erasmus Bilateral Agreements in Environmental Chemistry (MTSD Vasconcelos)

- Swiss Federal Institute of Technology, K. Hungerbuehier

- SECETOX, Masaryk University, Brno, Czech Republic, Ivan Holoubek

- Dep Environmental Chemistry and Ecoanalytics, Nicolas Copernicus University, Torun, Poland, Boguslaw Buszewski

- Lab of Physic-Toxic-Chemistry of Natural Systems, Bordeaux University, Bordeaux, France, Philippe Garrigues

- Environmental Analysis Group, National and Kapodistrian Athens University, Greece, Panayotis Siskos

- Biology Faculty, Environmental Sciences and Biochemistry, Barcelona University, Spain, Anna Riera

- Universidad del País Vasco - Bilbao, Spain

- Universidad Politecnica de Valencia, Spain

• Cooperation in International Organizations (MTSD Vasconcelos):

- Member of the Editorial Board of:

ESPR, Springer

Chemistry Central Journal

- Portuguese Chemical Society Delegate in "Division for Chemistry and the Environment (DCE)" of the Federation of the European Chemical Societies, presently European Chemistry and Molecular Science (EuCheMS)

- Member of the Steering Committee of DCE / EuCheMS

- International programs of Quality Control:

Lead in Blood. Instituto Nacional de Seguridad e Higiene en el Trabajo (INSHT), Zaragoza

Metals in Air. INSHT, Barcelona

COVs in indoor air. Berufsgenossenschaftliches Institut für Arbeitsschutz – Round Robin Test “Organic substances with thermodesorption” 2003

- Prizes and Honours (MTSD Vasconcelos):

- 2007, Medal for foreign personality given by “Divisão de Química do Ambiente e Bens Culturais” from Italian Chemical Society “In recognition for her outstanding scientific contributions in developing methodologies for environmental control and her activity devoted to the European Community”

- Review of scientific papers:

- CS&B members are reviewers for several international scientific journals

## **6e. Future research**

### **1. Objectives (3000 ca.)**

CS&B Lab will continue its previous lines of research pursuing the final goal of gathering as much information as possible on mutual interaction between organisms and the medium they are living in. Several areas previously identified as strategic priorities for consolidation and growth of the group are already supported by on going projects, whereas other will be initiated.

Studies on mutual interactions of phytoplankton and compounds with endocrine disrupting effects and pharmaceuticals will prosecute. Presently (end of 2007) are in progress 1 under-graduation, 1 PhD and 1 Pos Doc projects. Collaboration with CIIMAR Lab LEAN will provide different phytoplanktonic species and with Hovione FarmaCiencia S.A. (a pharmaceutical Company) will provide required chemicals, in addition to relevant specific knowhow. Collaboration with LEAN also includes an PhD project in progress on freshwater cyanobacteria allelopathic properties.

Studies regarding optimization of analytical methods for determination of pharmaceuticals will be initiated as well as investigation of suitable biological remediation processes for their reduction/elimination from residual waters released from waste water treatment plants (WWTP). Not only aquatic medium but also soils/sediments will be considered. New PhD and Pos Doc projects are planned to be started. Work will be carried out in cooperation with a company devoted to construction and management of water and WWTP (a project is in preparation).

Studies on Esmoriz/Paramos coastal lagoon sediments and fish will prosecute focused on organic compounds, namely PAHs. Water quality of aquaculture enterprises monitoring will go on within a collaboration protocol.

Studies focused on vascular plants role in conditioning the nearby environment and on their role in rhizoremediation processes will prosecute, including study of antagonisms or synergisms among organic and inorganic concomitant pollutants. A financed project, a PhD (in collaboration with Algarve University) and 3 MSc are in progress. Investigation of the influence of compounds

released by the studied plants into the medium (exudates) will be intensified. For this, optimization of methodologies for detection and quantification of these ultratrace substances in water, biological and soil/sediment matrixes are crucial, requiring research. A PhD was launched recently and 1 Pos Doc project is prepared to be submitted in 2008. New MSc projects can also be included in these studies. Collaboration with CIIMAR Lab Hydrobiology will allow integrating and complementing the results to be obtained with microbiological information.

Studies of suitable biological remediation processes concerning soil contaminated with petroleum hydrocarbons (PHC) will prosecute (a PhD is in progress), focused also in optimization of expeditious analytical methods to detect degradation. A new line of research will be initiated (an international interdisciplinary project) concerning bioremediation of PHC in contaminated beaches in collaboration with Hydrobiology Lab. New MSc and under graduate students will be integrated in these studies.

Studies on atmospheric environment will prosecute with a financed project, a collaboration protocol and 2 MSc theses in progress. In addition, collaboration with several small and medium companies and centres will go on on several environmental subjects for under-graduate and graduate students training. Three MSc theses are in progress and new MSc and under-graduate students will be integrated in future studies.

## **2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

- PhD members “Plurianual”.

- “Rhizosphere biogeochemistry ...”, POCTI/CTA/48386/2002. Project leader: Vasconcelos MTSD. Other participants: IPIMAR, FCUL, ICBAS. FCT financing: 50.738 EUR. Ends in 2008.

- “Chemical characterization of PM10 e PM2,5 fractions of urban aerosol” POCI/AMB/60267/2004. FCUP/CIIMAR responsible: Vasconcelos MTSD. Other participants: CESAM/UA. FCT financing: 17.280 EUR. Ends in 2008.

- “Indoor air quality and emissions from flooring materials”. Cooperation protocol with Amorim Revestimentos S.A. ADFCUP/CIIMAR responsible: Vasconcelos MTSD. Other participants: FEUP/IDMEC. Financing: 10.000 EUR / year.

- “Rizoremediação de hidrocarbonatos de petróleo ...”. Cooperation protocol with Petróleos de Portugal S.A., Refinaria do Porto. ADFCUP/CIIMAR responsible: Vasconcelos MTSD. Financing: 64.071 EUR. Ends in 2009.

- “Buried oil in the intertidal beach zone: ...”. CIIMAR responsible: Almeida CMR. Other participants: Vigo University (Spain), Montpellier University (France), ICBAS (Portugal). ERA NET AMPERA financing: 49.500 EUR. From 2008-2011.

- “Remoção de vestígios de fármacos em águas residuais”. CIIMAR Responsible: Vasconcelos MTSD. Other participants: CQO Plus - Engenharia, Ambiente e Energia Lda. Submitted in 2008 to Vale IDT, Quadro de Referência Estratégico Nacional (QREN).

- “Desenvolvimento de um guia de metodologias de bioremediação para aplicar em zonas estuarinas e costeira contaminadas por hidrocarbonetos”, Submitted in 2008 to Programa Tranfronteiriço Espanha-Portugal 2007-2013. Participants: ICBAS, Instituto Portuário e dos

Transportes Marítimos – Delegação Norte and CIIMAR (CS&B and Hydrobiology Labs) from Portugal, Vigo University from Spain.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Almeida, CMR, Mucha, AP, Bordalo, AA, Vasconcelos, MTSD. 2006. Comparison of the role of the sea club-rush *Scirpus maritimus* and the sea rush *Juncus maritimus* in terms of concentration, speciation and bioaccumulation of metals in the estuarine sediment. *Environmental Pollution* 142: 151-159. IF 2006/2007=2.769/3.135, n°C=2.
2. Baptista, MS, Vasconcelos, MTSD. 2006. Cianobacteria metal interactions: requirements, toxicity and ecological implications. *Critical Reviews in Microbiology*, 32: 127-137. IF 2006/2007=3.829/3.368, n°C=1.
3. Carvalho, PN, Pinto, LF, Basto, MCP, Vasconcelos, MTSD. 2007. Headspace solid-phase micro-extraction and gas chromatography-ion trap tandem mass spectrometry method for butyltin analysis in sediments: Optimization and validation. *Microchemical Journal* 87: 147-153. IF 2007=1.800, n°C=0.
4. Fernandes, C, Fontainhas-Fernandes, A, Monteiro, S, Salgado, MA. 2007. Histopathological gill changes in wild leaping grey mullet (*Liza saliens*) from the Esmoriz-Paramos coastal lagoon, Portugal. *Environmental Toxicology* 22: 443-448. IF 2007=1.728, n°C=1.
5. Mucha, AP, Almeida, CMR, Bordalo, AA, Vasconcelos, MTSD. (in press). Salt marsh plants (*Juncus maritimus* and *Scirpus maritimus*) as sources of strong complexing ligands. *Estuarine, Coastal and Shelf Science*. doi:10.1016/j.ecss.2007.09.011. IF 2007=1.799, n°C=1.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

The recent re-equipment financing project allowed the CS&B Lab to have proper equipment for the future research being planned and there will be no need for more equipment or facilities.

PhD, MSc and under-graduate students, as well as PhD members and Pos-Doc researchers will continue prosecuting the projects described in the objectives of the future research. The projects to be initiated in a near future will require under graduate, MSc and PhD students as well as Pos Doc researchers, as also described above in the objectives of the future research.

## **Ecotoxicology (ECOTOX)**

### **6a. Group description**

#### **1. Group name / denomination**

#### **Ecotoxicology (ECOTOX)**

#### **2. Principal investigator**

Lúcia Maria das Candeias Guilhermino

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Ecotoxicology, Biomonitoring and ecological risk assessment, Ecosystem evolution, Functional ecology

#### **5. Funding, source, dates (1000 ca.)**

From 2003 to 2007, the Ecotoxicology laboratory (ECOTOX) contributed to obtaining at least a total of 1.713.073,00 EUR from FCT and other national projects (715.761,20 EUR), international projects (697.067,40 EUR), pluriannual funding (88.500,00 EUR), students fees (53.680,00 EUR) and other sources, including industry, organization of events and others (158.064,00 EUR). Total amount for ECOTOX: 1.060.009,00 EUR from national projects (474.199,00 EUR), international projects (308.630,00 EUR), pluriannual funding (88.500,00 EUR), students fees (53.680,00 EUR) and others (135.000,00 EUR).

ECOTOX participated in 10 national projects (L. Guilhermino as Scientific Coordinator - SC of 9, 5 from FCT). ECOTOX was involved in 16 international projects, including 1 EU project (INTERREG IIIB, Atlantic Area, 168-EROCIPS, Guilhermino was SC of CIIMAR team), 7 bi-lateral cooperation projects (U.K., France, Spain, Slovenia, Mexico, Brazil), 8 projects in the scope of cooperation between the University of Porto and other Universities in Mexico, Costa Rica and Brazil. The team also participated in projects of science divulgation, including one coordinated by L. Guilhermino and funded with 104.800,00 EUR.

### **6b. Group team**

#### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Lúcia Maria das Candeias Guilhermino (Cat.: Professor Catedrático Gr. Acad.: Agregação)

002. Laura Maria Simões Coutinho Guimarães (Cat.: Investigador Auxiliar Gr. Acad.: Doutoramento)

003. Carlos Alexandre Sarabando Gravato (Cat.: Não aplicável (bolseiro) Gr. Acad.: Doutoramento)

004. Joana Fernades da Fonseca da Costa Martins Osswald (Cat.: Não aplicável (bolseiro) Gr. Acad.: Doutoramento)

## **2. Other researchers in the group (Include here collaborators with PhD. only)**

001. Jaime Rendón von Osten (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

002. Maria Margarida da Fonseca e Castro Cardoso (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

003. Manuela Maria Teixeira Basto de Faria Frasco (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

004. Maria Teresa Garrett Silveirinha Sottomayor Neuparth (Cat.: Outra, Gr. Acad.: Doutoramento)

## **3. Other researchers in the group (non PhD.)**

001. André Miguel Moura da Costa e Sousa (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

002. Andreia Monteiro da Cruz Ribeiro Gouveia (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

003. Inês Marrazes de Lima (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

004. Joana Reis de Almeida (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

005. Luis Miguel dos Santos Russo Vieira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

006. Marcos Rubal García (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

007. Ronaldo Gomes de Sousa (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

008. Ana Rita Neves Sousa (Cat.: Outra, Gr. Acad.: Bacharelato)

009. Maria Alexandra Santos Martins (Cat.: Outra, Gr. Acad.: Licenciatura)

010. Maria Fernanda Ferreira Marques (Cat.: Outra, Gr. Acad.: Licenciatura)

011. Maria Manuel Valente Correia da Silva Moreira (Cat.: Outra, Gr. Acad.: Licenciatura)

012. Nuno Filipe Alexandre Henriques (Cat.: Outra, Gr. Acad.: Bacharelato)

013. Ricardo Estêvão Oliveira Almeida (Cat.: Outra, Gr. Acad.: Bacharelato)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

The central objective of ECOTOX is to investigate the evolution and functioning of aquatic ecosystems under anthropogenic and natural stress, with emphasis on estuarine and coastal ecosystems. We are particularly interested in the assessment of long-term alterations induced by chemical contamination, biological invasions and climate changes on both biotic and abiotic

components of ecosystems, in understanding how and why these alterations may affect their structure and functioning and how to achieve a compromise between biodiversity conservation and sustainable development in global change scenarios. For this, we have been working in several Portuguese ecosystems and in tropical selected areas, using 3 complementary approaches: the comparison of similar ecosystems under different types and/or levels of pollution (or other stressors), the study over time of the same ecosystem and the comparison of similar problems in temperate and tropical regions. In all cases, integrated strategies including methods and tools from ecotoxicology, ecology, genetics and chemistry have been used.

Specifically we intend to:

- Characterize relevant ecosystems of Portuguese coast and selected case studies in tropical areas, including the identification of key-species and main problems (e.g. chemical pollution, eutrophication, landscape alterations, climate changes, biological invasions), and to study their evolution over time.
- Assess the impact of stressors on organisms, populations and on the whole ecosystem by using conventional approaches such as ecological and ecotoxicological monitoring, bioaccumulation and biomagnifications studies, ecological risk assessment and new methods developed by the group (e.g. in situ and laboratory bioassays with native species).
- Understand the mechanisms of action of stressors at sub-individual, individual and population levels and how the presence of stressors can influence species relationships (e.g. predation, competition), biotic/abiotic interactions, energy transfer and biomass in the ecosystem.
- Contribute for the achievement of a compromise between biodiversity conservation and sustainable development by providing tools, results and recommendations to decision-makers supporting scientific-based environmental policies and ecosystem management.

## **2. Main achievements (2000 ca.)**

57 papers published in ISI journals.

5 new in situ ecotoxicity bioassays for estuarine ecosystems with native species;

2 in situ ecotoxicity bioassays for freshwater ecosystems with native species;

4 new ecotoxicity lab bioassays;

5 new ecotoxicity assays and an integrated approach (water quality index, biomarkers and in situ assays) for use in the tropics;

Validation of 21 native species (macroalgae, crustaceans, bivalves, gastropods, sea-urchins, annelids, fishes, reptiles, birds) biomarkers for use in biomonitoring studies in temperate and tropical ecosystems.

Cholinesterases (ChE) characterization in 12 species (tropical and temperate regions) and their responses to reference chemicals and pollutants.

Effects of PAHs, pesticides, metals and pharmaceuticals on several marine and freshwater species of temperate and tropical areas, including mechanisms of toxicity and detoxification.



Mechanisms of genetic resistance to metals and pesticides in cladocerans (molecular and population levels).

Mechanisms of ChE inhibition by metals (e.g structure of human butyrylcholinesterase in complex with Hg (2007, gil145579736|pdb|2J4CIA[145579736], databases: Protein, Structure, 3D Domains).

190 gene sequences in GenBank (accession n°s: FG548387-FG548414 and GD180689-GD180850).

DQ305041, *Mytilus galloprovincialis* ras (ras) mRNA, gil83777077|gb|DQ305041.1|[83777077], Core nucleotide sequence database).

Knowledge about contamination levels in NW Portuguese coast (Aveiro lagoon – Spanish border), Minho, Lima, Cávado and Douro Rivers estuaries and Aveiro lagoon, using integrated approaches including water quality variables, chemical analysis (e.g. metals, PAHs, pesticides), bioaccumulation factors, biomarkers and condition indexes.

Characterization of freshwater tidal area of Minho estuary, mainly of the macrobenthic community, population dynamics of key-species, impact of 2005 heat wave on the community and populations recovery.

Knowledge on the invasions of Minho and Lima rivers by the non-native invasive species *Corbicula fluminea*, including its impact on native species and on ecosystems.

Functional characterization of the community of copepods of Cávado and Minho estuaries and knowledge about resistance to pollution and microevolution due to pollution in selected species.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Frasco, M, Colletier, J-P, Weik, M, Carvalho, F, Guilhermino, L, Stojan, J, Fournier, D. 2007. Mechanisms of cholinesterase inhibition by inorganic mercury. *FEBS Journal* 274: 1849-1861. IF 2007=3.396, n°C=3.

2. Frasco, MF, Fournier, D, Carvalho, F, Guilhermino, L. 2006. Cholinesterase from the common prawn (*Palaemon serratus*) eyes: Catalytic properties and sensitivity to organophosphate and carbamate compounds. *Aquatic Toxicology* 77: 412-421. IF 2006/2007=2.964/2.975, n°C=5.

3. Lima, I, Moreira, SM, Rendon-Von Osten, J, Soares, AMVM, Guilhermino, L. 2007. Biochemical responses of the marine mussel *Mytilus galloprovincialis* to petrochemical environmental contamination along the North-western coast of Portugal. *Chemosphere* 66: 1230-1242. IF 2007=2.739, n°C=7.

4. Nunes, B, Carvalho, F, Guilhermino, L. 2006. Effects of widely used pharmaceuticals and a detergent on oxidative stress biomarkers of the crustacean *Artemia parthenogenetica*. *Chemosphere* 62: 581-594. IF 2006/2007=2.442/2.739, n°C=9.
5. Cairrão, E, Couderchet, M, Soares, AMVM, Guilhermino, L. 2004. Glutathione-S- transferase activity of *Fucus* spp. as a biomarker of environmental contamination. *Aquatic Toxicology* 70: 277-286. IF 2004/2007=2.418/2.975, n°C=9.
6. Nunes, B, Carvalho, F, Guilhermino, L. 2004. Acute and chronic effects of clofibrate and clofibric acid on the enzymes acetylcholinesterase, lactate dehydrogenase and catalase of the mosquitofish, *Gambusia holbrooki*. *Chemosphere* 57: 1581-1589. IF 2004/2007=2.359/2.739, n°C=8.
7. Osten, RVJ, Ortíz-Arana, A, Guilhermino, L, Soares, AMVM. 2005. In vivo evaluation of three biomarkers in the mosquito fish (*Gambusia yucatana*) exposed to pesticides. *Chemosphere* 58: 627-636. IF 2005/2007=2.297/2.739, n°C=1.
8. Nunes, B, Carvalho, F, Guilhermino, L. 2005. Acute toxicity of widely used pharmaceuticals in aquatic species: *Gambusia holbrooki*, *Artemia parthenogenetica* and *Tetraselmis chuii*. *Ecotoxicology and Environmental Safety* 61: 413-41. IF 2005/2007=2.022/2.014, n°C=10.
9. Castro BB, Guilhermino, L, Ribeiro, R. 2003. In situ bioassay chambers and procedures for assessment of sediment toxicity with *Chironomus riparius*. *Environmental Pollution* 125: 325-335. IF 2003/2007=2.002/3.135, n°C=20.
10. Frasco, M, Fournier, D, Carvalho, F, Guilhermino, L. 2005. Do metals inhibit acetylcholinesterase (AChE)? Implementation of assay conditions for the use of AChE activity as a biomarker of metal toxicity. *Biomarkers* 10: 360-375. IF 2005/2007=1.662/1.978, n°C=14.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Lima, I, Soares, AMVM, Guilhermino, L. 2005. Effects of fuel oil water accommodated fraction on key enzymatic systems of the marine mussel *Mytilus galloprovincialis*. *Revista de Toxicología* 22: 138.
2. Paiva, C, Guimarães, L, Sieuve Monteiro, L, Guilhermino, L. 2005. Efeitos crónicos do pesticida clorpirifos na actividade da enzima acetilcolinesterase em fêmeas de *Daphnia magna* e na sua descendência. *Revista de Toxicología* 22: 139.
3. Martínez-Lopez, E, Gravato, C, Guilhermino, L, García-Fernández, AJ. 2007. Evaluación de los efectos ecotoxicológicos del cobre en lubina (*Dicentrarchus labrax*) mediante el uso de biomarcadores e otros parámetros ecológicos. *Revista Española de Toxicología* 24: 82-83.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Afonso, MJ, Marques, JM, Guimarães, L, Costa, I, Teixeira, J, Seabra, C, Rocha, FT, Guilhermino, L, Chaminé, HI. 2007. Urban hydrogeology of the Paranhos sector, Porto city (NW

Portugal): a geoenvironmental perspective. In: *Aquifer Systems Management: Darcy's Legacy in a World of Impending Water Shortage*. Chery, L, De Marsily, G (Eds). Selected papers on Hydrogeology, IAH Special Publications. Taylor & Francis Group, London, 596 pp. ISBN 9780415443555.

2. Rendón-von Osten, J, Memije, M, Guilhermino, L. 2007. Sperm counts, haematological and biochemical parameters changes in agricultural workers of the Bonfil region (Campeche State, Mexico). In: *Environmental Fate and Ecological Effects of Pesticides*. Del Re, AAM, Capri, E, Fragoulis, G, Trevisan, M (Eds). La Goliardica Pavese, Pavia, pp. 864-868. ISBN 978-88-7830-473-4.

3. Gravato, C, Faria, M, Alves, A, Guilhermino, L. 2008. Biomonitoring studies performed with European eel populations from the estuaries of Minho, Lima and Douro rivers (NW Portugal). In: *Advanced Environmental Monitoring*. Kim JY, Platt, U (Eds). Springer, Dordrecht. ISBN 978-1-4020-6363-3 (HB), ISBN 978-1-4020-6364-0 (e-book).

4. Rendon von-Osten, J, Ortiz-Arana, A, Memije, M, Soares, AMVM, Guilhermino, L. 2004. Atividades humanas e contaminação química de ecossistemas aquáticos. *Actas 2º Seminário de Engenharia Ambiental e dos Recursos Naturais, Desenvolvimento e Ambiente. Novos Desafios para o Século XXI*, p. 1-6.

5. Afonso, MJ, Chaminé, HI, Guimarães, L, Marques, JM, Carreira, P, Paiva, C, Costa, I, Teixeira, J, Martins Carvalho, J, Guilhermino, L, Rocha, FT. 2006. Hydrochemical, ecotoxicological and isotopical approach from the Paranhos spring galleries catchworks from Porto urban area. *Proceedings of the International Water Conference, Centro de Estudos de Água-ISEP. Porto, Portugal, 12-14 Junho*, p. 213-222.

6. Afonso, MJ, Chaminé, HI, Gomes, A, Fonseca, PE, Marques, JM, Guimarães, L, Guilhermino, L, et al. 2006. Urban hydrogeomorphology and geology of the Porto metropolitan area (NW Portugal): a multidisciplinary approach. *Pre-congress Proceedings of the 10th IAEG International Congress-Engineering Geology for Tomorrow's Cities. IAEG2006, The Geological Society of London*, paper 92, 9 p (CD-ROM).

7. Guilhermino, L. 2007. The use of biomarkers to assess the effects of environmental contamination in coastal and estuarine ecosystems: what questions remain? An example of the Portuguese NW coast. *ICES CM Documents, CD, document I: 17*, 4 p.

8. Gravato, C, Guimarães, L, Guilhermino, L. 2007. Effects of pollutants on estuarine and coastal animals: from genes to populations. *ICES CM Documents, CD, document I:18*, 10 p.

9. Afonso, MJ, Marques, JM, Guimarães, L, Costa, I, Teixeira, J, Seabra, C, Rocha, FT, Guilhermino, L, Chaminé, HI. 2006. Urban hydrogeological mapping of the Porto area (NW Portugal): a geoenvironmental perspective. *Proceedings of the International Symposium Aquifer Systems Management, CD with Full Texts, Theme 4*, pp. 65-77.

10. Guilhermino, L. 2008. Biomarkers for the assessment of effects and/or exposure to environmental contaminants: a special reference to pesticides. *Proceedings Summer School Pesticides – Environment 2007. Quaderno Grifa, nº 26*: 407-416. ISBN 978-88-95221-01-4.

#### 4. Master and Ph.D. thesis completed (3000 ca.)

##### PhD THESIS

Rendón von Osten, J. 2004. Environmental impact of anthropogenic activities on human and animal populations from Palizada, Campeche, México. PhD in Biology, University of Aveiro. Supervisor: AMVM Soares (CESAM, University of Aveiro), Co-Supervisor: L Guilhermino (CIIMAR).

Nunes, B. 2005. Development of assays for the assessment of the toxicity of xenobiotics in salt-water. PhD in Biomedical Sciences, ICBAS, University of Porto. Supervisor: L Guilhermino (CIIMAR), Co-Supervisor: F Carvalho (Faculty of Pharmacy, University of Porto).

Moreira, SM. 2006. An integrated approach for the improvement of ecological relevance and cost-effectiveness in toxicity assessments of estuarine sediments. PhD in Biomedical Sciences, ICBAS, University of Porto. Supervisor: L Guilhermino (CIIMAR), Co-Supervisor: R Ribeiro (IMAR, University of Coimbra).

Frasco, M. 2007. Effects of environmental contaminants on the cholinesterasic function of an estuarine species (*Palaemon serratus*). PhD in Biomedical Sciences, ICBAS, University of Porto. Supervisor: Lúcia Guilhermino (CIIMAR), Co-Supervisor: Félix de Carvalho (Faculty of Pharmacy, University of Porto).

##### MSc THESIS

Assunção, MGL. 2003. Cytochrome P450 1A enzymes in Pacific harbour seals (*Phoca vitulina richardsi*) from British Columbia, Canada: non-invasive biomarkers of contaminant exposure. Master in Sea Sciences and Marine Resources, ICBAS, University of Porto. Supervisor: Peter S Ross (Institute of Ocean Sciences, Canada), Co-Supervisor: L Guilhermino (CIIMAR).

Antunes, CPQ. 2003. Viabilidade de populações naturais em estudos ecotoxicológicos: caso de *Crago crangon*. Master in Toxicology, University of Aveiro. Supervisor: F Morgado (CESAM, University of Aveiro), Co-Supervisor: L Guilhermino (CIIMAR).

Monteiro, MSCA. 2003. Avaliação da toxicidade de contaminantes ambientais em populações de *Pomatoschistus microps* (Krøyer, 1938). Master in Advanced Molecular Methods, University of Aveiro. Supervisor: AMVM Soares (CESAM, University of Aveiro), Co-Supervisor: L Guilhermino (CIIMAR).

Lopo, M. 2004. Avaliação do impacto da extracção de inertes em populações naturais de peixes. Master on Sea Sciences and Marine Resources, ICBAS, University of Porto. Supervisor: L Guilhermino (CIIMAR), Co-Supervisor: C Antunes (CIIMAR).

#### 5. Patents/prototypes (2000 ca.)

##### Gene Sequences and Protein Data Bases

- 2J4CA, Structure of human butyrylcholinesterase in complex with 10 mM HgCl<sub>2</sub>. Abril 2007 (Colletier, JP, Frasco, M F, Carvalho, F, Guilhermino, L, Stojan, J, Fournier, D, Weik, M) gil145579736|pdb|2J4CA|A[145579736], databases: Protein, Structure, 3D Domains.

- DQ305041, *Mytilus galloprovincialis* ras (ras) mRNA, partial cds. Julho 2006.

(Lima, IM, Peck, MR, Soares, AMVM, Guilhermino, LM, Rotchell, J) gi83777077|gb|DQ305041.1|[83777077], databases: Core nucleotide sequence database (includes GenBank), Protein.

- 28 gene sequences with accession numbers from FG548387 to FG548414, Liver sea bass cDNA library, EST. (Gravato, C, Van der Ven, K, Vandenbrouck, T, De Coen, WM, Guilhermino, L), databases: GenBank.

- 162 gene sequences with accession numbers from GD180689 to GD180850 Liver sea bass cDNA library, EST. (Gravato, C, Almeida, JR, Van der Ven, K, Vandenbrouck, T, De Coen, WM, Guilhermino, L), databases: GenBank.

## **6. Organization of conferences (2000 ca.)**

- 2003 - 9th International Congress of the European Association for Veterinary Pharmacology and Toxicology. Lisbon, Portugal, 13-18 July 2003. Lúcia Guilhermino was member of the Local Organising Committee. More than 600 participants from different countries around the world.
- 2003 - 5th Iberian and 2nd Iberoamerican Congress of Environmental Contamination and Toxicology – Environmental Problems in an Iberoamerican Context. Porto, Portugal, 22-24 September 2003. Lúcia Guilhermino was Chair of the Organising Committee and member of the Scientific Committee. 230 participants from 15 countries.
- 2006 - 1st Portuguese and Iberian Meeting of Veterinary Toxicology for students – VETOX I. 3-4 June 2006, Porto, Portugal. Lúcia Guilhermino promoted the event and supervised the students who organised it. 72 participants from Portugal, Spain and France.
- 2007 - II Encontro Nacional de Estudantes de Pós-graduação. Ronaldo Sousa and Inês Lima were members of the Organising Committee. Lúcia Guilhermino promoted the event and coordinated the organisation. Porto, Portugal, 4-6 June 2007.
- 2007 - 17th Annual Meeting of SETAC - Europe – Multiple stressors for the environment – present and future challenges and perspectives. Porto, Portugal, 20-24 May 2007. Lúcia Guilhermino was Vice-chair of the Organising Committee and Chair of the Scientific Committee. 1967 participants from 65 countries.

## **7. Industry contract research (2000 ca.)**

The laboratory of Ecotoxicology provides consulting and other services to industry, including toxicity tests and support for REACH, the new European Community regulation on chemicals and their safe use (EC 1907/2006). For example, in 2007, the laboratory routinely performed bioassays with fish for the “GROUP EIBOL” (Pucol, Valencia, Spain, [www.eibol.com](http://www.eibol.com)) to assess the toxicity of new pesticides. In addition, the laboratory has been also performing toxicity assays, environmental studies and consulting services regarding the management of residuals, sediments removal and other environmental matters for national industry (e.g. NOSTOC – Laboratório de Investigação biológica, LDA, <http://www.nostoc.pt>), local and national Authorities (e.g. Municipality of Fafe, Portugal). In addition, the laboratory has been also involved in several environmental studies performed by CIIMAR to industry (e.g. ARNOR – Dragagens e Comercialização de Inertes, LDA, 2000-2003), and institutions (e.g. local and

national authorities). In 2007, a technical workshop in cooperation with a private company has held. Furthermore, Lúcia Guilhermino has been participating in projects of the Autonomous University of Campeche, Mexico, in cooperation with oil and pesticide companies operating in the Gulf of Mexico to reduce the environmental and human health impact of the oil extraction and transformation, and of the use of pesticides in agriculture.

**8. Government/organization contract research** (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

Lúcia Guilhermino and Inês Lima participated in the “task force” of CIIMAR regarding the environmental impact of the ‘Prestige’ oil spill for the Intersectoral Oceanographic Commission (Ministry of Science, Technology and Higher Education). The following report was produced:

- Coimbra, J, Guilhermino, L, Santos, P, Sousa-Pinto, I, Santos, M, Pereira, R, Lima, I. 2004. Relatório do Grupo de Trabalho Sobre o Derrame do Prestige. CIIMAR, Porto, 109 pp.

• Members of the research group participated in the project EROCIPS (Emergency Response to Coastal Oil, Chemical and Inert Pollution; Interreg IIIB) with the objective of harmonizing strategies and procedures for emergency response to coastal oil, chemical and inert pollution from shipping. The following reports were produced and disseminated to local authorities (e.g. Portuguese Maritime Authority):

- Cunha, I, Guilhermino, L. 2006. Environmental monitoring: protocol for selection of the monitoring area and sites. Deliverable 7.1.1a, pp. 4.

- Cunha, I, Sousa, A, Lima, D, Santos, M, Reis-Henriques, MA, Guilhermino, L. 2006. Environmental monitoring: protocols for type of monitoring and seasonality. Deliverable 7.1.1b, pp. 74.

- Cunha, I, Guilhermino, L. 2006. Environmental monitoring: protocols for selection of sentinel species and collection of specimens. Deliverable 7.2.1, pp. 3.

- Moreira, S, Cunha, I, Guilhermino, L. 2007. Guidelines for a long-term monitoring programme. Deliverable 7.3.1, pp. 13.

- Moreira, S, Santos, M, Cunha, I, Sousa, A, Lima, D, Coimbra, J, Reis-Henriques, MA, Guilhermino, L. 2007. Environmental monitoring report. Deliverable 7.3.5, pp. 102.

- Moreira, S, Mota, M, Guilhermino, L. 2007. Environmental monitoring: protocols for monitoring pollution damage to different types of sensitive habitats. Deliverable 7.4.2, pp. 19.

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

• Main networks:

SPAIN – CSIC Torre La Sal (Navarro), CSIC Barcelona (Barata), INIA (Tarazona), Univs Coruna (Mendez), Extremadura (Soler), Murcia (García-Fernández), Valencia (TorreBlanca) and Basque Country (Cajaraville).



UK: Univ Sussex (Rotchell).

BELGIUM: Univ Antwerp (De Coen).

FRANCE: Univ Paul Sabatier (Fournier).

FINLAND: Finish Inst Marine Research (Lehtonen);

SLOVENIA: Univ Ljubljana (Stojan);

BRAZIL: Univ Federal Baía (da Silva),

MEXICO: Autonomous Univ Campeche (Rendón von Osten), CIAD Mazatlán (Betancourt-Lozano, García de la Parra); Benemerita Univ Puebla (Mangas-Ramirez);

COSTA RICA: Univ Nacional (Castillo);

USA: Univ New Hampshire (Byers), Western Washington Univ (Harper).

• L. Guilhermino is/was evaluator for International Foundation for Science, INTAS, Fondo Investigacion Científica de Argentina, Fund for Scientific Research – Fladers (Belgium), European Commission. She is Associate Editor of Biomarkers member of the Science Committee of World Council of Society of Toxicology and Environmental Chemistry (SETAC) and SETAC Europe Council and was President of the Iberoamerican Society of Environmental Contamination and Toxicology. The lab is involved in several networks and initiatives for international cooperation.

Domingues; Guilhermino; Soares; Nogueira; Monaghan 2007 *Ecotoxicol Environ Saf.* (in press)

Lima; Peck; Rendón-Von Osten; Soares; Guilhermino; Rotchell 2008 *Mar Pollut Bul* 56: 633-640.

Damásio; Guilhermino; Soares; Riva; Barata 2007 *Chemosphere* 70: 74-82.

Sousa; Freire; Rufino; Méndez; Gaspar; Antunes; Guilhermino 2007 *Estuarine, Coastal & Shelf Science* 74: 166-174

Varó; Nunes; Amat; Torreblanca; Guilhermino; Navarro 2007 *Aquat Living Resour* 20: 263-270

Nunes; Carvalho; Guilhermino; Van Stappen 2006 *Environ Pollut* 144: 453-462

Cunha; Hoff; Van de Vijver; Guilhermino; Esmans; De Coen 2005 *Mar Pollut Bul.* 50: 1128-1132

Cunha; Mangas-Ramirez; Guilhermino 2007 *Comp Biochem Physiol C* 145: 648-657

Moreira-Santos; Fonseca; Moreira; Rendón von Osten; da Silva; Soares; Guilhermino; Ribeiro 2005 *Environ Toxicol Chem* 24: 2234-2242.

García de la Parra; Bautista-Covarrubias; Rivera-de la Rosa; Betancourt-Lozano; Guilhermino 2006 *Ecotoxicol Environ Saf* 65: 372-380



Rendón-von Osten; Soares; Guilhermino 2005 Environ Toxicol Chem 24: 313-317

## 6e. Future research

### 1. Objectives (3000 ca.)

1. Understanding how the presence of chemical pollution drives the evolution of populations of key estuarine species (e.g. *Carcinus maenas*): by studying populations developing in estuaries with different levels of pollution and performing laboratory assays with individual chemicals and mixtures, relevant molecular genetics alterations will be identified; working with selected polymorphic genes, the genetic diversity and possible differential selection pressures in populations developing in different estuaries will be studied.

2. Based on the already existing knowledge about several ecosystems of the NW coast of Portugal and the effects of both chemical and natural stressors on individuals, populations and on the abiotic component, the effects of chemical pollution and natural stressors on ecosystem functioning will be investigated using both in situ and laboratory experiments. For example, the effects on species competition (including competition between native and NIS), predation, primary production and energy transfer will be studied using approaches already in development. Particular attention will be given to “ecological engineer” species, such as *Corbicula fluminea*.

3. Linking “omics”, biomarkers, behaviour and intrinsic population growth rate: in the continuation of the work that has been performed in the last year, efforts will be put in establishing relationships between different biological organization levels to take advantage of the new technology in understanding complex ecological and ecotoxicological problems.

4. In the scope of an already funded international project and in the continuation of work that has been performed by the group in previous projects (e.g. CONTROL, RISK, EROCI), methodologies for risk assessment of oil and chemical spills will be standardized and validated in the Atlantic coast of Portugal and Galicia, with particular focus on planktonic and benthonic organisms.

5. Following the important results that have been obtained in the estuary of the Minho river about the impact of the 2005 heat wave on the community of mollusks and the results from the comparison between the effects of pollution in temperate and tropical ecosystems, efforts in investigating potential effects of global changes will be considerably increased. We will investigate questions such as: what are the predicted changes on the abiotic component? How these changes will affect different populations and the relationships among them? Will these changes modify the toxicity of individual pollutants and their toxicological interactions in complex mixtures? How they will influence the evolution of communities under chemical stress?

6. In Mexico, all the work performed in the Palizada river ecosystem is being integrated in relation to land use and anthropogenic main activities in the area. It will be sent to the Authorities of the Campeche State with recommendations regarding ecosystem management, pesticides interdiction, conservation measures, etc. Recently, a project with endangered species (e.g. *Crocodylus moreletii*) was initiated and will be continued. In Mexico (Campeche, Sinaloa, Puebla), Brazil (Baia state) and Costa Rica, the research already going on about the effects of pollution, climate changes, biodiversity conservation and sustainable development will be continued.

**2. Funding, source, dates (1500 ca.)** (Indicate in full including amount of current and pending funding)

- 89.354,00 EUR, FCT POCTI/BIA-BDE/59037/2004 Searching for Evidence of Microevolution due to Pollution in Estuarine Copepod Populations. Project coordinator (PI): L Guilhermino. 2005 - 2008. Funding ECOTOX 81.576,00 EUR.
- 75.000,00 EUR, FCT POCI/MAR/58244/2004, PPCDT/MAR/58244/2004 What Type of Contamination by Metals is Efficiently Detected by the Cholinesterase from the Marine Prawn *Palaemon serratus*? PI: L Guilhermino. 2006 - 2009. ECOTOX: 57.861 EUR.
- 189.814,00 EUR, FCT PTDC/MAR/71143/2006 Molecular, biochemical and genetic variability in estuarine populations of shore crab (*Carcinus maenas*) exposed to different levels of contamination. PI: L Guimarães. 2008 - 2011. ECOTOX: 189.814,00 EUR.
- 90.384,00 EUR, EU ERA 6th FP, AMPERA (ERAC-CT2005-016165) Implementation of risk assessment methodologies for oil and chemical spills in the European marine environment. Partner Leader: L Guilhermino. 2008 - 2011. ECOTOX: 90.384,00 EUR.
- 196.250,00 EUR, FCT (PENDING appeal in evaluation) PTDC/BIA-BDE/67557/2006: Factors contributing to different invasive behaviour of a non-indigenous species in two Portuguese estuaries and ecological impact on native key species. PI: L Guilhermino. 2009 - 2011. ECOTOX: 196.250,00 EUR.
- 130.914,00 EUR, FCT (PENDING appeal in evaluation) PTDC/MAR/64074/2006: What's the ecological relevance of molecular and sub-cellular alterations induced by polycyclic aromatic hydrocarbons on seabass juveniles (*Dicentrarchus labrax* L.)? PI: C Gravato. 2009 - 2011. ECOTOX 130.914,00 EUR.
- 274.644.40 EUR, EU, TCP, INTERREG IV B - SUDOE (SOE1/P2/E130) (PENDING, submitted): POLCLEAN - Improvement of the cleaning of hydrocarbon pollution and the response by the intervention teams and volunteers. 2009 - 2011. ECOTOX: 205.883,00 EUR.

**3. Previous publications in the area (1500 ca.)** (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Sousa, R, Freire, R, Rufino, M, Méndez, J, Gaspar, M, Antunes, C, Guilhermino, L. 2007. Genetic and shell morphological variability of the invasive bivalve *Corbicula fluminea* (Müller, 1774) in two Portuguese estuaries. *Estuarine, Coastal & Shelf Science* 74: 166-174. IF 2007=1.799, n°C=3.
2. Elumalai, M, Antunes, C, Guilhermino, L. 2007. Enzymatic biomarkers in the crab *Carcinus maenas* from the Minho River estuary (NW Portugal) exposed to zinc and mercury. *Chemosphere* 66: 1249-1255. IF 2007=2.739, n°C=2.
3. Monteiro, M, Quintaneiro, C, Nogueira, A, Morgado, F, Soares, AMVM, Guilhermino, L. 2007. Impact of chemical exposure on the fish *Pomatoschistus microps* Krøyer (1838) in estuaries of the Portuguese Northwest coast. *Chemosphere* 66: 514-522. IF 2007=2.739, n°C=4.

4. Rendón-von Osten, J, Memije, M, Ortiz, A, Soares, AMVM, Guilhermino, L. 2006. An integrated approach to assess water quality and environmental contamination in the fluvial-lagoon system of Palizada River, Mexico. *Environmental Toxicology and Chemistry* 25: 3024-3034. IF 2006/2007=2.202/2.309, n°C=1.

5. Sousa, R, Antunes, C, Guilhermino, L. 2006. Factors influencing the occurrence and distribution of *Corbicula fluminea* (Müller, 1774) in the River Lima estuary. *Annales de Limnologie – International Journal of Limnology* 43: 165-171. IF 2006/2007=0.579/0.482, n°C=4.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

- Two additional rooms with control of temperature: one for ecological and ecotoxicological assays (competition and predation experiments); the other for molecular genetics (the room already available is too small)
- One technician for maintaining the cultures of animals in laboratorial conditions
- Microarray spotter and microarray scanner, UV stratalinker
- One boat for estuarine ecotoxicological work

## **Ecotoxicology, Genomics and Evolution (LEGE)**

### **6a. Group description**

#### **1. Group name / denomination**

**Ecotoxicology, Genomics and Evolution (LEGE)**

#### **2. Principal investigator**

Vitor Manuel Oliveira Vasconcelos

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Ecotoxicology, Cyanotoxins, Genomics, Evolution

#### **5. Funding, source, dates (1000 ca.)**

One of our main concerns in terms on funding is to diversify the inputs. The activity of LEGE has being financed from different sources, not only using the plurianual budget from FCT (52.500 EUR) but also from national projects (304.000 EUR) and international cooperation projects (20.000 EUR). LEGE provides services that are an important part of our final budget, diversifying the inputs and opening the laboratory to the exterior (60.000 EUR) by contracts with different entities, environmental and health authorities, private laboratories. We also do some technology transfer by contracts with the Water Industry (37.000 EUR). Our total budget for the period 2003-2007 was 473.500 EUR.

### **6b. Group team**

#### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Vitor Manuel Oliveira Vasconcelos (Cat.: Professor Associado, Gr. Acad.: Agregação)

002. Luis Filipe Pereira de Oliva Teles (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

003. Maria do Rosário Fidalgo Martins (Cat.: Professor-Adjunto, Gr. Acad.: Doutoramento)

004. Agostinho Antunes Pereira (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

005. Isabel Cristina Guimarães Nogueira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

006. Susana Rodrigues Pereira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

#### **2. Other researchers in the group (Include here collaborators with PhD. only)**

001. Olga Maria Oliveira da Silva Lage (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

002. Martin Leslie Saker (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

003. Rute Andreia Rodrigues da Fonseca (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

### **3. Other researchers in the group (non PhD.)**

001. Ana Maria dos Santos Rocha (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

002. Cristiana Ivone Tavares Moreira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

003. Joana do Passo Carneiro Azevedo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

004. Joana Isabel Correia Bondoso (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

005. João Paulo Rodrigues Machado (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

006. José Carlos de Moraes Martins (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

007. Pedro Nuno da Costa Leão (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

008. Rui Seabra Alves Martinho (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

009. Vitor Manuel Capela Ramos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

010. Viviana Raquel Vieira da Silva Lopes (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

011. Bárbara Bilreiro de Mendôça Frazão (Cat.: Outra, Gr. Acad.: Licenciatura)

012. Joana Soares de Oliveira Martins (Cat.: Outra, Gr. Acad.: Licenciatura)

013. Susana Micaela Machado Ferreira do Vale (Cat.: Outra, Gr. Acad.: Mestrado)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

Ecotoxicological study of cyanobacteria and other potentially toxic organisms in marine, brackish and freshwaters. Occurrence of the main species and of the main toxins, and production of bioactive compounds with potential pharmacological interest.

Ecotoxicological assays regarding the effects of neurotoxins and hepatotoxins in different organisms. Use of behavioral and histopathological alterations as endpoints. Studies on the bioaccumulation of toxins and research of possible toxin vectors. Human health risk assessment of toxin occurrence in drinking, recreational waters. Impact of contamination of toxins in water used for agriculture.

Use of molecular and computational genomics/proteomics in the characterization of toxin metabolism of genes/proteins in humans and other animal species, allowing an understanding of ecotoxicological adaptation mechanisms.

Evolution of aquatic systems in what concerns with eutrophication processes and development of prediction models for application by water management authorities and water treatment plants. Development of alert systems using ecotoxicological responses of organisms in different aquatic contamination scenarios.

## **2. Main achievements (2000 ca.)**

During the past years we assessed the increased diversity of cyanobacteria species and of cyanotoxins in Portuguese ecosystems. Joining the worldwide spread of microcystin producing species we detected species, such as *Cylindrospermopsis raciborskii*, known to produce the cytotoxin cylindrospermopsin- CYL and detected for the first time the presence of anatoxin-a in strains isolated from Portuguese freshwaters. A diversity of cyanotoxins with hepatotoxic, neurotoxic and cytotoxic effects are commonly found in our fresh and brackish waters. Most of the strains isolated from marine environments do not produce the common toxins found in the freshwater specimens but they are toxic to some invertebrates and can also produce secondary metabolites that may be used as pharmaceutical tools. Some strains isolated from Portuguese marine systems produced apoptotic activity in neuroblastoma cells. CYL can be accumulated in freshwater mussels such as *Anodonta cygnea* without causing damage to this organism and to a lesser extent, *Daphnia magna* can also accumulate CYL Neurotoxins of the group of the saxitoxins – PST are accumulated by *A. cygnea* up to 26 µg PST/100 g and a similar situation occurred with *D. magna*. By the use of immunoassays (ELISA) and molecular methods such as a sensitive multiplex PCR we showed that many dietary supplements are contaminated with the hepatotoxic microcystin. Finally, genomic studies across organisms (notably mammals) have allowed valuable insights to understand the functional changes of various gene/proteins involved in important cellular functions, notably the metabolism of xenobiotic compounds such as drugs and toxins.

During the period 2003-2007 we published a total of 42 papers in SCI journals.

## **6d. Productivity**

### **1. Publications in peer review journals (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)**

1. Saker, ML, Nogueira, IR, Vasconcelos, VM, Neilan, BA, Eaglesham, GK, Pereira, P. 2003. First report and toxicological assessment of the cyanobacterium *Cylindrospermopsis raciborskii* from Portuguese freshwaters. *Ecotoxicology and Environmental Safety* 55(2): 243-250. IF 2003/2007=0.983/2.014, n°C=31.
2. Pereira, P, Dias, E, Franca, S, Pereira, E, Carolino, M, Vasconcelos, V. 2004. Accumulation and depuration of cyanobacterial paralytic shellfish toxins by the freshwater mussel *Anodonta cygnea*. *Aquatic Toxicology* 68: 339-350. IF 2004/2007=2.418/2.975, n°C=14.
3. Rohrlack, T, Christoffersen, K, Dittmann, E, Nogueira, I, Vasconcelos, V, Börner, T. 2005. Ingestion of microcystins by *Daphnia*: Intestinal uptake and toxic effects. *Limnology and Oceanography* 50(2): 440-448. IF 2005/2007=3.249/3.277, n°C=17.

4. Selheim, F, Herfindal, L, Martins, R, Vasconcelos, V, Doskeland, S-O. 2005. Neuro-apoptogenic and thrombocyte function modulating toxins in non-blooming marine cyanobacteria from the Portuguese coast. *Aquatic Toxicology* 74: 294-306. IF 2005/2007=2.719/2.975, n°C=2.
5. Saker, M, Welker, M, Vasconcelos, VM. 2007. Multiplex PCR for the detection of cyclic heptapeptides (microcystins) in dietary supplements produced for human consumption. *Applied Microbiology & Biotechnology* 73(5): 1136-1142. IF 2007=2.475, n°C=2.
6. Martins, J, Oliva Teles, L, Vasconcelos, V. 2007. Assays with *Daphnia magna* and *Danio rerio* as alert systems in aquatic toxicology. *Environment International* 33: 417-425. IF 2007=2.797, n°C=0.
7. Rellán Piñeiro, S, Osswald, J, Vasconcelos, V, Gago-Martinez, A. 2007. Analysis of anatoxin-a in biological samples using liquid chromatography with fluorescence detection after SPME extraction. *Journal of Chromatography A* 1156(1-2): 134-140. IF 2007=3.641, n°C=2.
8. Vasconcelos, VM, Wiegand, C, Pflugmacher, S. 2007. Dynamics of glutathione-s-transferases in *Mytilus galloprovincialis* exposed to toxic *Microcystis aeruginosa* cells, extracts and pure toxins. *Toxicon* 50: 740-745. IF 2007=2.246, n°C=0.
9. Johnson, WE, Eizirk, E, Murphy, WJ, Pecon-Slaterry, J, Antunes, A, Teeling, E, O'Brien, SJ. 2006. The late Miocene radiation of the Felidae: a genetic assessment. *Science* 311: 73-77. IF 2006/2007=30.028/26.372, n°C=55.
10. Pontius, JU, Mullikin, JC, Smith, D, Agencourt Sequencing Team, Lindblad-Toh, K, Gnerre, S, Clamp, M, Chang, J, Stephens, R, Neelam, B, Volfovsky, N, Schäffer, AA, Agarwala, R, Narfström, K, Murphy, WJ, Giger, U, Roca, AL, Antunes, A, Menotti-Raymond, M, Yuhki, N, Pecon-Slaterry, J, Johnson, WE, Bourque, G, Tesler, G, NISC Sequencing Program, O'Brien, SJ. 2007. Initial sequence and comparative analysis of the cat genome. *Genome Research* 17: 1675-1689. IF 2007=11.224, n°C=8.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Azevedo, S, Vasconcelos, VM. 2006. Toxinas de cianobactérias: causas e consequências para a saúde pública. In: *Ecotoxicologia Aquática: Princípios e Aplicações*. Pedro A Zagatto and Eduardo Bertolotti (Eds), Rima Editora, S. Paulo, Brasil, pp. 433-452.

**4. Master and Ph.D. thesis completed** (3000 ca.)

## PhD THESIS



Pereira, Paulo. 2004. Ecotoxicologia de cianobactérias produtoras de PST – *Aphanizomenon* sp. PhD at Faculty of Sciences, University of Porto. Supervisor: V Vasconcelos, Co-supervisor: S Franca (INSA, Lisboa).

Nogueira, Isabel. 2004. Efeitos ecotoxicológicos de cianobactérias em cladóceros. Faculty of Sciences, University of Porto. Supervisor: V Vasconcelos.

Martins, Rosário. 2006. Ecotoxicologia de cianobactérias marinhas. PhD at Faculty of Sciences, University of Porto. Supervisor: V Vasconcelos.

Da Fonseca, Rute. 2006. Computational studies on cytochromes P450. PhD, Faculty of Sciences, University of Porto. Supervision: MJ Ramos and A Melo (Chemistry Department, FCUP), Co-supervision: A Antunes.

Osswald, Joana. 2007. Produção de neurotoxinas por cianobactérias de água doce em Portugal continental. PhD at Faculty of Sciences, University of Porto. Supervisor: V Vasconcelos.

### **MSc THESIS**

Xavier, Luis. 2004. Ecologia e gestão de lagos urbanos. Master in Hydrobiology, Faculty of Sciences, University of Porto. Supervisor: V Vasconcelos.

Caeiro, Joaquim. 2004. Dinâmica de toxinas de cianobactérias em albufeiras eutrofizadas do Alentejo. Master in Hydrobiology, Faculty of Sciences, University of Porto. Supervisor: V Vasconcelos.

Vale, Micaela. 2005. Eutrofização da albufeira do Torrão. Faculty of Sciences, University of Porto. Supervisor: V Vasconcelos.

Raposo, Filomena. 2005. Avaliação das cianobactérias, incluindo outras microalgas, existentes nos vários tanques de uma ETAR, ao longo de um ano. Detecção e quantificação de cianotoxinas extracelulares e intracelulares. Master in Applied Ecology, Faculty of Sciences, University of Porto. Supervisor: VM Vasconcelos, R Morais (School of Biotechnology, Universidade Católica Portuguesa).

Leão, Pedro. 2006. Interações entre Cianobactérias e Metais em Água do Mar. Master in Hydrobiology, Faculty of Sciences, University of Porto. Supervisor: V Vasconcelos, Co-supervisor: MTSD Vasconcelos (FCUP/CIIMAR).

Soares, Marlene. 2006. Uso de macrófitas aquáticas na remoção de nutrientes num lago urbano. Master in Hydrobiology, Faculty of Sciences, University of Porto. Supervisor: V Vasconcelos.

Araújo, Sofia. 2007. Contributo para um plano de monitorização do meio receptor do litoral de Matosinhos. Master in Sea Sciences - Marine Resources, ICBAS, University of Porto. Supervisor: V Vasconcelos, Co-supervisor: F Veloso Gomes (Faculty of Engineering, University of Porto).

Osório, Daniel. 2007. Structural and functional implications of positive selection at the primate angiogenin gene. Master at University of Minho. Co-supervision: A Antunes with MJ Ramos (Chemistry Department, FCUP).

Fernandes, Sandra. 2007. Estudo dos mecanismos de destoxificação de microcistinas em bivalves. Master in Applied Ecology, Faculty of Sciences, University of Porto. Supervisor: V Vasconcelos.

## **5. Patents/propotypes (2000 ca.)**

## **6. Organization of conferences (2000 ca.)**

- President of the organizing committee of the 4th Iberian Limnology Conference, 5 to 9 July 2004, Porto. (V Vasconcelos)
- Organizer and chairman of the round table “The Biology of Drought”, Bibliotheca FCUP, 31 May 2005, Porto. (V Vasconcelos)
- Organizer of the Conference “Ecology in the City Park”, 24 to 27 November 2005, Porto. (V Vasconcelos)
- Member of the organizing committee of the VII SETAC Europe 2007 - Society of Environmental Toxicology and Chemistry, 20-24 May 2007, Porto. (V Vasconcelos)
- Member of the national organizing committee of the 10th Symposium of Aquatic Microbial Ecology- SAME, 2-7 September 2007, University of Algarve, Faro. (V Vasconcelos)
- President of the Advanced Workshop Toxic Cyanobacteria. Tools for toxin analysis and effects on aquatic organisms. CIIMAR, 16-20 April 2007, Porto. (V Vasconcelos)

## **7. Industry contract research (2000 ca.)**

The laboratory LEGE has been working with the water industry (Água do Douro e Paiva, S.A.) in projects related to early warning of cyanobacteria blooms; use of bioindicators to evaluated possible contamination of water and development of fast methods for toxicity evaluation of cyanobacteria blooms.

Projects:

- Bioindicadores na gestão da qualidade da água no rio Douro. Duration: 2002-2004. Funding: Águas do Douro e Paiva, S.A.. Principal Investigator: V Vasconcelos.
- Gestão (vigilância, previsão e controlo) da ocorrência de florescências fitoplanctónicas no rio Douro (troço Entre-os-rios/Crestuma), Duration: 2002-2004. Funding: Águas do Douro e Paiva, S.A. Principal Investigator: V Vasconcelos.
- Toxicologia de estirpes de cianobactérias isoladas das albufeitas de Crestuma-Lever (Rio Douro) e Torrão (Rio Tâmega). Duration: 2003-2004. Funding: Águas do Douro e Paiva, S.A. Principal Investigator: V Vasconcelos.

The following reports were published:

- Vasconcelos, V, Oliva Teles, L. 2003. Bioindicadores na gestão da qualidade da água do rio Douro. Projecto Alerta. Relatório Final para a Empresa Águas do Douro e Paiva, Porto.

- Vasconcelos, VM, Oliva Teles, L, Martins, J. 2003. Gestão (vigilância, previsão e controlo) da ocorrência de florescências fitoplanctónicas no rio Douro (troço Entre-os-Rios – Crestuma). Relatório Final para a empresa Águas do Douro e Paiva, Porto.
- Vasconcelos, V, Oliva Teles, L, Martins, J. 2004. Bioindicadores na gestão da qualidade da água do rio Douro. Projecto Biomonitorização. Relatório Final para a empresa Águas do Douro e Paiva, Porto.
- Vasconcelos, VM. 2004. Águas do Douro e Paiva, ADP, Porto – Elaboração de parecer e reunião com administradores, relativo à utilização da água da albufeira do Torrão (Rio Tâmega) como origem para abastecimento ao Grande Porto.

**8. Government/organization contract research** (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

LEGE has been responsible for the analysis of water and counseling to many public health departments in what concerns the environmental and human health risk management associated with toxic cyanobacteria and other phytoplankton.

V Vasconcelos and LO Teles joined the team of Quaternaire in order to elaborate the Management Plan for the Reservoir of Crestuma-Lever (2003-2004) and later V. Vasconcelos was the responsible for the Water quality chapter of the revision of the management plan of the Alqueva reservoir (2005-2006). Crestuma reservoir is the main water source for the Porto area (1 million inhabitants) being also located in Douro river used for transportation, recreation and electricity production. Alqueva reservoir is the largest reservoir in Europe and is mainly used for agricultural and recreational purposes. V Vasconcelos also acted as consultant for the Aguas de Douro e Paiva enterprise, responsible for the uptake, treatment and distribution of drinking water in Porto area.

- Vasconcelos, V, Oliva Teles, L. 2004. Caracterização da albufeira de Crestuma-Lever tendo em vista a elaboração dos estudos de base. Qualidade da água. Plano de Ordenamento da Albufeira de Crestuma-Lever. Relatório para a empresa Quaternaire, Lisboa.
- Vasconcelos, V. 2006. Revisão do Plano de Ordenamento das albufeiras do Alqueva e Pedrógão. V Vasconcelos - Responsável pela Qualidade da água, integrado na equipa da Quaternaire.

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

Collaboration with international groups resulting in publication in peer review journals:

V Vasconcelos: Stephan Pflugmacher, Leibniz Institut of Freshwater Ecology-IGB, Berlin, Germany, Stein-Oven Døskeland, U. of Bergen, Norway, Brahim Oudra -U. Cadi Ayyad, Marrakesh, Morocco; Ana Gago, U. de Vigo, Spain; Amel Jenhani, Institut National Agronomique de Tunisie, Tunis- Tunisia.

A Antunes: Stephen J O'Brien, Warren E Johnson, Shu-Jin Luo, Jennifer Troyer, Jill Pecon-Slaterry, Melody E Roelke, Joan Pontius, Laboratory of Genomic Diversity, NCI, NIH, USA; Philippe Gaubert, Muséum National d'Histoire Naturelle, Paris, France; Li Zhang from the

Beijing Normal U, Beijing, P. R China; Paolo Martelli Ocean Park, Aberdeen, Hong Kong; Shih-Chien Chin, Taipei zoo, Taipei, Taiwan; Norman T-L Lim, National U. of Singapore, Singapore.

External evaluation of projects: Canadian Aquatic Invasive Species Network, Toxicology of invasive cyanobacteria. 2006; Ministerio de Educación, Ciencia Y Tecnología de la República Argentina - Toxicology of invasive cyanobacteria in Argentina. 2007 (V Vasconcelos); National Science Foundation, USA (2006) Evolutionary genetics (A Antunes).

National Contact (Portugal) of CYANONET- Global Network for the Hazard Management of Cyanobacterial Blooms and Toxins in Water Resources, of the International Hydrology Programme of UNESCO (2005) (V Vasconcelos).

Member of the Task Force - Marine and Freshwaters Toxins – of the Association of Official Analytical Chemists- AOAC International (2007) (V Vasconcelos).

Member of scientific committees of international conferences:

4th Iberian Conference on Limnology, 5-9 de July 2004, Porto, Portugal. (V Vasconcelos)

VII bi-annual short course on "Recent Advances In Conservation Genetics", 7-20 January 2007, The Hawaii Institute of Marine Biology, Honolulu, Oahu Hawaii, USA (A Antunes).

VII SETAC Europe 2007 , 20-24 May 2007, Porto (V asconcelos).

2nd Spanish Meeting on Cyanotoxins, U. Autónoma de Madrid, 11-13 de Julho 2007, Madrid, Spain (V Vasconcelos).

V Vasconcelos acted as external referee of PhD thesis in Ecotoxicology: U. de Vigo, Spain; Humboldt-University, Berlin, Germany; U. of Helsinki, Finland; U. of Copenhagen, Denmark; U. of Cape Town, South Africa

## 6e. Future research

### 1. Objectives (3000 ca.)

Development of early warning methods for the detection and quantification of cyanobacteria and cyanotoxins using molecular approaches (RT PCR) and ecotoxicological approaches. Search for new bioactive compounds including new toxins from marine environments using marine invertebrates as sources. Risk analysis of the BMMA in marine and freshwater environments including development of new analytical techniques. Study of action of known and unknown toxins from cyanobacteria using a proteomic approach.

Evolutionary genomics/proteomics studies of cellular protein-coding genes operating in detoxification, development, immune system, and genetic disease onset; and the inference of the comparative genomic principles from various organisms (from microorganisms to mammals) that participate in these processes. The patterns of genomic variation across organisms are of critical value in interpreting the structural dynamics and the functional diversifications that occurred in organismal genomes over millions of years of evolution. Such knowledge also provides reliable insight for biodiversity studies of various species, from microorganisms to mammals.

**2. Funding, source, dates** (1500 ca.) (Indicate in full including amount of current and pending funding)

On going projects

- POCTI/MAR/61569/2004, FCT. Coordination: V Vasconcelos, 2005-2008, 77.072 EUR.
- POCI/BIA-BDE/61719/2004, FCT, Coordination: V Vasconcelos, 2005-2008, 54.880 EUR.
- POCTI/CTA/46733/2002, FCT, Biodiversidade e toxicidade de cianobactérias marinhas. FCT-CIMAR. Coordination: V Vasconcelos, 2006-2009, 60.000 EUR.
- Survey of some pharmaceuticals effects on freshwater cyanobacteria. Does good manufacturing practice (GMP) during pharmaceuticals manufacture influence the biological response? University of Porto, Pre-graduation projects 2007 - CIMAR. Coordination: Teresa Vasconcelos, Researcher: V Vasconcelos. 4.000 EUR
- Allelopathic effect of water contaminated with cyanobacteria on the germination and growth of several plants species. University of Porto, Pre-graduation projects 2007 - CIMAR. Coordination: V Vasconcelos. 4.000 EUR.
- PTDC/AMB/67075/2006, FCT, CIMAR. FCT. Coordination: V Vasconcelos, 2008-2011, 140.818EUR.

The following projects have been funded and some others are being evaluated by FCT:

PTDC/AMB/67075/2006, FCT, Application of real time quantitative PCR in studies on the occurrence and toxicity of freshwater cyanobacteria in drinking and recreational waters, 2008-2010, 140.818 EUR. Principal Investigator: V Vasconcelos.

PTDC/MAR/68106/2006, FCT, The modulation of retinoic acid signalling pathways by environmental pollutants in teleosts, 2008-2011, 188.980 EUR. Principal researcher: M Santos, Researcher: A Antunes.

PTDC/BIA-BDE/70982/2006, FCT, Phenogenetic drift in evolution: insights into the genetic basis of vertebrate developmental genes, 2008-2010, 148.000 EUR. Principal Researcher: A Antunes.

LEGE will continue to do some effort to maintain and possibly increase the amount of services and contracts with industry.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Saker, ML, Fastner, J, Dittmann, E, Christiansen, G, Vasconcelos, VM. 2005. Variation between strains of the cyanobacterium *Microcystis aeruginosa* isolated from a Portuguese river. *Journal of Applied Microbiology* 99: 749-757. IF 2005/2007=2.127/2.501, n°C=9.

2. Martins, J, Soares, ML, Saker, ML, OlivaTeles, L, Vasconcelos, VM. 2007. Phototactic behaviour in *Daphnia magna* Straus as an indicator of chemical stress in the aquatic environment. *Ecotoxicology and Environmental Safety* 67: 417-422. IF 2007=2.014, n°C=0.
3. Morais, J, Augusto, M, Carvalho, AP, Vale, M, Vasconcelos, VM. 2008. Microcystins - cyanobacteria hepatotoxins- bioavailability in contaminated mussels exposed to different environmental conditions. *European Food Research & Technology* 227: 949-952. IF 2007=1.159, n°C=0.
4. Da Fonseca, R, Johnson, WE, O'Brien, SJ, Ramos, MJ, Antunes, A. 2008. The adaptive evolution of the mammalian mitochondrial genome. *BMC Genomics* 9: 119. IF 2007=4.180, n°C=0.
5. Luo, SJ, Johnson, WE, Martnenson, J, Antunes, A, Martelli, P, Uphyrkina, O, Traylor-Holzer, K, Smith, JLD, O'Brien, SJ. 2008. Subspecies genetic assignments of worldwide captive tigers increase conservation value of captive populations. *Current Biology* 18: 592-596. IF 2007=10.539, n°C=0.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

A researcher in the proteomics area is needed to accomplish the ongoing and future objectives of the laboratory. Recently CIIMAR acquired a LC-MS that will enable us to better characterize proteins and peptides, not depending on external services. LEGE has an adequate amount of equipment for genomic analyses and will use common CIIMAR equipment such as RT-PCR, ultracentrifuge, HPLC. A new microplate reader with temperature control, kinetic readings and UV and Fluorometric detection is needed and will be bought as soon as possible depending on financial support. LEGE staff will hopefully be increased by some Post Doc and PhD students pending on FCT funding. Graduate and undergraduate students are quite attracted by the themes LEGE develops.

## Pathology

### 6a. Group description

#### 1. Group name / denomination

## Pathology

#### 2. Principal investigator

Jorge Guimaraes da Costa Eiras

#### 3. Location of group (Host institution)

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### 4. Keywords

Pathology, Fish, Mollusc, Crustaceans

#### 5. Funding, source, dates (1000 ca.)

The funding for the research performed within the period was provided mainly by FCT (Foundation for the Science and Technology). In other cases, funding was provided by private institutions, and also by cooperative research programs between Portugal and other countries. Furthermore, part of the funding (pluriannual) was provided by FCT as an annual support of the research group. In all, the funding for the five years period was of about 99.000 EUR.

### 6b. Group Team

#### 1. Researchers in the group (Include only PhD. integrated in the LA)

001. Carlos José Correia de Azevedo (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

002. Jorge Guimaraes da Costa Eiras (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

003. Fernanda Russell Pinto (Cat.: Professor Associado, Gr. Acad.: Doutoramento)

004. Aurélia Maria de Pinho Marques Saraiva ( Cat.: Professor Auxiliar, Gr. Acad.: Agregação)

005. Cristina Maria Bravo de Faria Cruz (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

006. Maria João Faria Leite Dias dos Santos ( Cat.: Professor Auxiliar Gr., Acad.: Doutoramento)

#### 2. Other researchers in the group (Include here collaborators with PhD. only)

001. José Américo Pereira de Sousa (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)



### **3. Other researchers in the group (non PhD.)**

- 001. Claire Juliana Francisco (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 002. Custodio Pedro Simao Boane (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 003. Graça Maria Figueiredo Casal (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 004. Joana Isabel Ferreira Marques (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 005. Susana Maria Rocha Pina (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 006. Carla Helena Dias Ventura (Cat.: Outra, Gr. Acad.: Licenciatura)
- 007. Daniella Luiza Antunes de Campos Duarte (Cat.: Outra, Gr. Acad.: Licenciatura)
- 008. Francisca Isabel Merino Nunes Cabral Cavaleiro (Cat.: Outra, Gr. Acad.: Licenciatura)
- 009. João Paulo Soares (Cat.: Outra, Gr. Acad.: Licenciatura)
- 010. Luis Filipe da Cunha Melo Silva Rangel (Cat.: Outra, Gr. Acad.: Licenciatura)
- 011. Margarida Dulce da Conceição Aragão Hermida (Cat.: Outra, Gr. Acad.: Licenciatura)
- 012. Ricardo Bruno de Araújo Severino (Cat.: Outra, Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

The main objectives of the group are:

- Contribute to the knowledge of fish parasites specially that ones with greater impact in aquaculture and public health
- Use parasites as biological tags for marine and estuarine fish population's discrimination of commercially important Portuguese, Brazilian and Angolan fishery species,
- Contribute to the knowledge of bacterial fish diseases
- Perform studies on the detection and isolation of bacteriophages from the environment and their use as prophylactic and therapeutic agents against fish pathogenic bacteria
- Contribute to the knowledge of Digenea parasite species that infect several hosts collected in different shallow-water ecosystems; perform a morpho-anatomic and molecular characterization of the different parasitic stages allowing their identification; contribute to the ecoparasitological characterization and clarification of digenean life cycles; develop a database of Digenea ITS1 sequences.

## 2. Main achievements (2000 ca.)

Several fields of research were pursued in the last 5 years and the results obtained allowed the publication of a significant number of papers (71, plus 18 published or in press in 2008) in international referred journals, the presentation of a number of communications in international (75) and national (46) meetings, as well as the publication of several textbooks on fish diseases and fish parasitology.

During this period 4 PhD and 11 MSc students performed their research in the Laboratory and 2 PhD and 9 MSc thesis were completed with success.

## 6d. Productivity

### 1. Publications in peer review journals (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Azevedo, C, Matos, E. 2003. *Amazonspora hassar* n. gen. and n. sp. (Phylum Microsporidia, fam. Glugeidae), a parasite of the Amazonian teleost, *Hassar orestis* (fam. Doradidae). *Journal of Parasitology* 89: 336-341. IF 2003/2007=1.137/1.129, n°C=4.
2. Azevedo, C, Matos, E. 2004. *Nematopsis gigas* n. sp. (Apicomplexa), a parasite of *Nerita ascencionis* (Gastropoda, Neritidae) from Brazil. *Journal of Eukaryotic Microbiology* 51: 214-219. IF 2004/2007=1.403/1.525, n°C=0.
3. Azevedo, C, Conchas, RF, Tajdari, J, Montes, J. 2006. Ultrastructural description of new *Rickettsia*-like organisms in the commercial abalone *Haliotis tuberculata* (Gastropoda: Haliotidae) from the NW of Spain. *Diseases of Aquatic Organisms* 71: 233-237. IF 2006/2007=1.509/1.598, n°C=0.
4. Casal, G, Costa, G, Azevedo, C. 2007. Ultrastructural description of *Ceratomyxa tenuispora* (Myxozoa), a parasite of the marine fish *Aphanopus carbo* (Trichiuridae), from the Atlantic Coast of Madeira Island (Portugal). *Folia Parasitologica* 54: 165-171. IF 2007=1.00, n°C=1.
5. Cruz, C, Barbosa, C, Saraiva, A. 2007. Distribution of larval anisakids in blue whiting horse off Portuguese fish market. *Helminthologia* 44: 21-24. IF 2007=0.373, n°C=0.
6. Eiras, JC. 2006. Synopsis of the species of *Ceratomyxa* Thélohan, 1892 (Myxozoa, Myxosporidia, Ceratomyxidae). *Systemic Parasitology* 65: 49-71. IF 2006/2007=0.856/1.125, n°C=5.
7. Eiras, JC, Joaber, P Júnior, Sampaio, LA, Robaldo, R, Abreu PC. 2007. *Myxobolus* sp. can cause in vivo myoliquefaction in the host *Paralichthys orbignyanus* Valenciennes, 1893 (Osteichthyes, Paralichthyidae). *Diseases of Aquatic Organisms* 77: 255-258. IF 2007=1.598, n°C=0.
8. Russell-Pinto, F, Gonçalves, JF, Bowers, E. 2006. Digenean Larvae Parasitizing *Cerastoderma edule* (Bivalvia) and *Nassarius reticulatus* (Gastropoda) From Ria de Aveiro, Portugal. *Journal of Parasitology* 92: 319-332. IF 2006/2007=1.300/1.129, n°C=2.

9. Santos, MJ, Karvonen, A, Pedro, JC, Faltýnková, A, Seppälä, O, Valtonen, ET. 2007. Qualitative and quantitative behavioral traits in a community of furcocercariae trematodes: tools for species separation? *Journal of Parasitology* 93(6): 1319-1323. IF 2007=1.129, n°C=0.

10. Saraiva, A, Rosim, DF, Silva-Souza, AT. 2006. Nematode parasites of characoid fishes from Brazil. *Bulletin of the European Association of Fish Pathologists* 26 (6): 271 -274. IF 2006/2007=0.581/0.449, n°C=0.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Azevedo, C. 2005. *Biologia Celular e Molecular* (4ª edição), LIDEL – Edições Técnicas, Lisboa.

2. Azevedo, C. 2007. *Bioquímica* (1ª edição) (direcção), LIDEL - Edições Técnicas, Lisboa.

3. Azevedo, C. 2007. A Célula: Matriz Estrutural dos Sistemas Biológicos. In: *Bioquímica. Organização Molecular da Vida*. Quintas, Freire e Halpern (Eds), Lidel, Lisboa, pp. 121-138.

4. Azevedo, C. 2005. Nucléolo e Transcrição dos rRNA. In: *Biologia Celular e Molecular* (4ª edição). C Azevedo (Coord.), Lidel, Lisboa, pp. 193-213.

5. Azevedo, C, Azevedo, J. 2005. Retículos e Ribossomas. In: *Biologia Celular e Molecular* (4ª edição). C Azevedo (Coord.), Lidel, Lisboa, pp. 215-232.

6. Azevedo, C, Sunkel, CE. 2005. Mitose. In: *Biologia Celular e Molecular* (4ª edição). C Azevedo (Coord.), Lidel, Lisboa, pp. 371-395.

7. Azevedo, C. 2005. Espermatozóides e Óvulos. In: *Biologia Celular e Molecular* (4ª edição). C Azevedo (Coord.), Lidel, Lisboa, pp. 431-445.

8. Sousa, M, Azevedo, C. 2005. Especialização da Membrana Celular. In: *Biologia Celular e Molecular* (4ª edição). C Azevedo (Coord.), Lidel, Lisboa, pp. 53-74.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Eiras, JC, Segner, H, Wahli, T, Kapoor, B. (Editors). 2008. *Fish Diseases*. Science Publishers Inc., Enfield, NH, USA. Two Volumes, 1312 pp.

2. Eiras, JC. 2004. Aspectos gerais da patologia das parasitoses de peixes marinhos. In: *Sanidade de Organismos Aquáticos*. Ranzani-Paiva, MJT, Takemoto, RM, Lizama, MAP (Eds). Editora Varela, São Paulo, Brasil, pp. 145-158.

3. Matos, E, Casal, G, Matos, P, Corral, L, Azevedo, C. 2004. Microorganismos parasitos de animais aquáticos da Amazônia. In: *Sanidade de Animais Aquáticos*. Ranzani-Paiva, MJT, Takemoto, RM, Lizama, MAP (Eds), Editora Varela, São Paulo, pp. 159-178.

4. Saraiva, A. 2006. Aspectos gerais de histologia e histopatologia de peixes. In: Sanidade de organismos aquáticos no Brasil. Silva-Souza, AT (Eds), Abrapoa, Maringá, pp. 239-252.

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Marques, Joana. 2007. Parasitoses de peixes chatos (Pisces: Pleuronectiformes) da costa Portuguesa e suas relações com a história vital, utilização do habitat, filogenia e estrutura populacional dos hospedeiros. PhD in Biology, Faculty of Sciences, University of Lisbon.

##### **MSc THESIS**

Antão, Ana Maria. 2003. Estudo comparativo da comunidade de endohelmintas parasitas do tubo digestivo de diferentes populações de *Anguilla anguilla* (L.). Master in Applied Ecology, Faculty of Sciences, University of Porto.

Desclaux, Céline. 2003. Interactions hotes-parasites: diversité, mecanismes d'infestation et impacte des Trematodes digenes sur les coques *Cerastoderma edule* (Mollusce bivalve) en milieu lagunaire macrotidal. Master, Bordeaux 1 University. Co-supervisor: F Russel-Pinto (CIIMAR).

Candoso, Mónica Emília. 2003. Variação sazonal das principais parasitoses do robalo (*Dicentrarchus labrax* L.) de aquacultura da Ria de Aveiro. Master in Applied Ecology, Faculty of Sciences, University of Porto.

Barbosa, Maria Cristina. 2005. Parasitose provocada por larvas de ANISAKIDAE no verdinho (*Micromesistius poutassou*) em Portugal. Master in Biology, Faculty of Sciences, University of Porto.

Ventura, Carla Helena. 2006. Parasitas do Peixe-espada preto (*Aphanopus carbo*, Lowe 1839) de Sesimbra e Madeira. Master in Applied Ecology, Faculty of Sciences, University of Porto.

Hermida, Margarida Dulce. 2006. Estudo de parasitas metazoários de uma população de enguia Europeia (*Anguilla anguilla*) em meio salobro. Master in Marine Sciences – Marine Resources, Instituto de Ciências Biomédicas de Abel Salazar, University of Porto.

Ramos, Miguel Filipe. 2006. Caracterização bioquímica e molecular de estirpes de *Aeromonas salmonicida* subsp. *salmonicida* isoladas em Portugal. Master in Applied Ecology, Faculty of Sciences, University of Porto.

Cavaleiro, Francisca Isabel. 2007. Dinâmica populacional dos Copepoda parasitas da solha *Platichthys flesus* (Linnaeus, 1758) da região noroeste da costa portuguesa. Master in Applied Ecology, Faculty of Sciences, University of Porto.

Soares, João Paulo. 2007. Contributos para a Parasitofauna dos Peixes de Profundidade da Costa Portuguesa. Master in Applied Ecology, Faculty of Sciences, University of Porto.

#### **5. Patents/prototypes (2000 ca.)**

## 6. Organization of conferences (2000 ca.)

## 7. Industry contract research (2000 ca.)

During the last years the research performed was, in most cases, done in collaboration with several institutions from different countries: University of Kingston (U.K.), Natural History Museum, London (UK), University of Maringá (Brazil), University of Rio Grande (Brazil), University of Londrina (Brazil), Fisheries Institute of São Paulo (Brazil), National Institute of Amazonian Research (Brazil), Hungarian Academy of Science (Hungary), Czech Academy of Sciences (Czech Republic), Department of Biological and Environmental Sciences, University of Jyväskylä (Finland), Federal Rural University of Amazonia, (Carlos Azevedo Research Laboratory), Belém (Brazil); Federal University of Pará, Belém (Brazil); Instituto de Investigaciones Marinas, Vigo (Spain); “Centro de Investigaciones Marinas”, (CIMA), Vilanova de Arousa (Spain); Humboldt State University, California (USA); Federal University of Pernambuco, Recife (Brazil); Federal University of Piauí, Teresina (Brazil); Federal University of Santa Catarina, Florianópolis (Brazil); Federal University Fluminense (Laboratory of Pathology), Niterói (Brazil); Federal University of Paraná (Lab. of Pathology), Curitiba (Brazil); EMBRAPA (Lab. of Parasitology), Corumbá (Brazil); National Institute of Fishery Research of Luanda (INIP) (Angola); Centro de Investigaciones Mariñas, Vilanova de Arousa (Spain); Laboratoire d’Océanographie Biologique, Bordeaux (France).

## 8. Government/organization contract research (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

## 9. Internationalization (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

## 6e. Future research

### 1. Objectives (3000 ca.)

The objective for the next few years is to continue the research on fish parasitology and fish bacteriology, as well as to increase the international cooperation.

In the first case it is intended to continue the study of the systematic of Myxozoan fish parasites, as well as the host-parasite relationship. The identification of new species shall involve traditional and molecular methods. On the other hand, we intend to intensify the study of the alternate stages of the life cycle of Myxozoans, the actinospore stages infecting oligochaetes and polichaetes, once this subject is practically unknown in Portugal. It is also intended to perform experimental laboratory studies on the life cycle of these parasites.

Present research interests are also to study parasites of the Phyla Microsporidia, Apicomplexa and Haplosporidia in freshwater and marine animals, such as fishes, crustaceans and molluscs from Ibero-Atlantic and Brazilian fauna. Morphological and ultrastructural characterization of the

parasites by light microscopy, scanning (SEM) and transmission electron microscopy (TEM). Studies of the life cycle, pathology associated to the parasites and studies of cytochemistry. Molecular studies to identify the parasites by phylogenetic characterization of the conserved genes.

An important field of study is the characterization of parasites in commercially important fish species and the use of parasites as biological tags. Several studies were already done, and this research will be increased in the next years.

Studies on the detection and isolation of bacteriophages from the environment and their use as prophylactic and therapeutic agents against fish pathogenic bacteria will continue.

Concerning the the studies of Digeneans it is intended to continue the search and identification of Digenea parasites from hosts collected in several shallow-water ecosystems and perform the characterization of their larval stages morpho-anatomically, by light and scanning electron microscopy, and with the use of molecular data; clarify more digenean life cycles; study the effects that parasites exert on their hosts and its influence in the ecosystems dynamics; study spatial heterogeneity (based on molecular methods) between sites (local parasite faunas) and within sites (individual parasite faunas); increase the digenean species molecular database and perform phylogenetic studies.

It is important to stress that most of the research to be done shall be in collaboration with researchers from other countries. The intensification of the international cooperation is an important objective. It is intended to prepare a number of cooperative research programmes for submitting for funding, all of them integrating researchers from different countries, including both MSc and PhD students.

**2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

3.400 EUR. Project: Amoeba gill disease and sucoticiatosis of aquaculture sea bass. Funded by Fundação Ilídio Pinho.

750 EUR. Project: Analyses Methods of Fish Parasites Molecular and Ecology Techniques. Funded by FCT (SFRH/BSAB/492/2005).

1500 EUR. Project: Phylogenetic position and fine anatomy of *Didymobothrium* sp., a non-segmented tapeworm pathogen of sole with important implications for the evolution of the higher Cestoda (Platyhelminthes). Funded under the Treaty of Windsor Anglo-Portuguese Joint Research Programme. Funded by British Council and CRUP.

2.020 EUR. Project: Survey on myxozoan and apicomplexan parasites in Portugal and Hungary with special emphasis on their alternative and paratenic hosts. Funded under the Scientific and technological Cooperation between Portugal and the Hungarian National Office for Research and Technology.

21.389 EUR. Project: Black scabbard fish in the Portuguese waters: conservation measures and fish quality control. Funded by FCT (Project APHACARBO – POCTI/CVT/46851/2002).

60.000 EUR. Project: Caracterização filogenética do parasita *Perkinsus atlanticus* pelo número de cromossomas e identificação de genes conservados. Funded by FCT (POCTI/CTA/47583/2002).

Other funding:

Project: Parasitoses of Amazonian Fauna. Funded by CNPq- Brazil, Carlos Azevedo Research Laboratory, UFRA – Belém, Brasil.

Project: Estudo de Parasitoses da Fauna do Rio Paraguaia- Corumbá- Pantanal do Sul (2008-2010). Funded by EMBRAPA-Corumbá, Mato Grosso do Sul, Brasil.

Project: Estudo de Parasitoses da Fauna da Costa Atlântica de Portugal e Brasil, Funded by Fundação Engº António de Almeida, Porto.

Project: Parasitoses da Fauna Angolana da Região de Luanda (2008- 2010). Funded by Ministério das Pescas - Instituto de Investigação Pesqueira de Luanda, Angola.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (nº C=). Give title and full citation in original language)

1. Casal, G, Matos, E, Azevedo, C. 2003. Light and electron microscopic study of the myxosporean, *Henneguya friderici* n. sp. from the Amazonian teleostean fish, *Leporinus friderici*. *Parasitology* 126: 313-319. IF 2003/2007=1.821/2.081, nºC=6.

2. Tuntiwaranuruk, C, Chalermwat, K, Upatham, ES, Kruatrachue, M, Azevedo, C. 2004. Investigation of *Nematopsis* spp. oocysts in 7 species of bivalves from Chonburi Province, Gulf of Thailand. *Diseases of Aquatic Organisms* 58: 47-53. IF 2004/2007=1.263/1.598, nºC=1.

3. Marques, JF, Santos, MJ, Cabral, HN, Palm, H. 2005. First record of *Progrillotia dasyatidis* Beveridge (Cestoda: Trypanorhyncha) plerocerci from Teleost fishes off the Portuguese coast, with a description of the surface morphology. *Parasitology Research* 96: 206-211. IF 2005/2007=1.226/1.512, nºC=1.

4. Marques, JF, Santos, MJ, Cabral, HN. 2006. Soleidae macroparasites along the Portuguese coast: latitudinal variation and host-parasite associations. *Marine Biology* 105: 185-198. IF 2006/2007=1.756/2.215, nºC=1.

5. Marques, J, Santos, MJ, Gibson, D, Cabral, H, Olson, P. 2007. Cryptic species of *Didymobothrium rudolphii* (Cestoda: Spathebothriidea) from the sand sole, *Solea lascaris*, off the Portuguese coast, with an analysis of their molecules, morphology, ultrastructure and phylogeny. *Parasitology* 134: 1057-1072. IF 2007=2.081, nºC=0.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)



## **Nutrition, Growth and Quality of Fish (LANUCE)**

### **6a. Group description**

#### **1. Group name / denomination**

**Nutrition, Growth and Quality of Fish (LANUCE)**

#### **2. Principal investigator**

Luísa Maria Pinheiro Valente

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Nutrition, Growth, Flesh quality, Muscle

#### **5. Funding, source, dates (1000 ca.)**

- TOTAL FCT Pluriannual (2004-2007): 75.450 EUR

- PROJECTS (2003-2007): 9 projects, 5 coordination and a total of 563.186,45 EUR

2002-2004: Pulse-fish: Craft-1999-72052. CIIMAR 118.000 EUR. Partners: SOTEXPRO, PROFAT, INRA (Fr), CTC (Sp).

2002-2006: POCTI/CVT/Nº 39239. Total 100.000 EUR; CIIMAR 52.537 EUR. Partners: CCMAR and Direcção Regional das Pescas.

2003-2006: Project POCTI/CVT Nº 39237. Total 80.000 EUR; CIIMAR 30.300 EUR. Partners: UTAD and IPIMAR.

2002-2005: POCTI/CVT/42426/2001. Partners: ICETA, IPIMAR.

2002-2005: POCTI/BSE/37978/2001. Partners: ICETA.

2003-2005: Integrated action Luso-Britânica Project nº B-17/03-British Council. Partners: GKT School of Biomedical Sciences, King's College London.

2005-2008: Optidietas, IDEIA 70/00073 consort with SORGAL sa, financed by AdI. Total 380.343,48 EUR; CIIMAR 312.379,45 EUR. Partners: CCMAR and SORGAL.

2005-2008: POCTI/MAR/57022/2004.

2007-2009: SEACASE. FP6-2005-SSP5A, financed by EU. Total 239.1401 EUR; CIMAR-LA 312.486 EUR, CIIMAR 49.970 EUR. Partners: LANUCE, IPIMAR and involving researchers of five European Countries (Portugal, Spain, France, Italy and Greece).

## 6b. Group team

### 1. Researchers in the group (Include only PhD. integrated in the LA)

- 001. Emidio Ferreira Santos Gomes (Cat.: Professor Catedrático, Gr. Acad.: Agregação)
- 002. Luísa Maria Pinheiro Valente (Cat.: Professor Associado, Gr. Acad.: Doutoramento)
- 003. Paulo Manuel Rodrigues Vaz-Pires (Cat.: Professor Associado, Gr. Acad.: Doutoramento)
- 004. Paulo José de Azevedo Pinto Rema (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)
- 005. Paulo Manuel Rodrigues Martins da Costa ( Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)
- 006. Rodrigo Ozorio (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

### 2. Other researchers in the group (Include here collaborators with PhD. only)

### 3. Other researchers in the group (non PhD.)

- 001. Alexandra Ernestina Fernandes Barbosa (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 002. Amélia Cláudia Figueiredo Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 003. Begona Fernandez Duran (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 004. Carlos Manuel Lourenço Cardoso (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 005. Joana Margarida Guimarães e Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 006. Paula Cristina Paulo Videira da Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 007. Pedro Alexandre Coelho Borges (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 008. Pedro Fernandes Seixas (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 009. Ricardo do Amaral Ribeiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 010. Sara Isabel da Silva Pires Marques Barrento (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 011. Bruno Graziano da Silva Turini (Cat.: Outra, Gr. Acad.: Licenciatura)
- 012. Catarina da Rocha Cruzeiro (Cat.: Outra, Gr. Acad.: Licenciatura)
- 013. Célia Cristina de Aguiar Carvalho (Cat.: Outra, Gr. Acad.: Licenciatura)
- 014. Claudia de Fátima Escórcio Pereira (Cat.: Outra, Gr. Acad.: Licenciatura)
- 015. João da Mota e Silva Rodrigues de Carvalho (Cat.: Outra, Gr. Acad.: Licenciatura)

016. José Miguel Pereira Gomes (Cat.: Outra, Gr. Acad.: Licenciatura)

017. Katia Pinto Pedrosa (Cat.: Outra, Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

The central aim of the group is to contribute to the sustainable development of the aquaculture industry, through basic and applied research directed to the optimisation of diets and cultivation techniques in order to improve growth and muscle quality. Special attention has been given to the introduction of new species to aquaculture, namely the blackspot seabream (*Pagellus bogaraveo*), the sole (*Solea senegalensis*), the bream (*Diplodus sargus*) and the octopus (*Octopus vulgaris*). Ongoing projects deal with the following aspects:

1) Nutritional requirements and optimization of dietary regimes in important species for aquaculture: Formulation and production of fish diets which promotes maximum growth performance and better final fish quality; low pollution diets are also considered. Dietary nutrient metabolism studies involve standard laboratory facilities for proximate analysis of feed and fish tissues and activity and regulation of key enzymes involved in lipid metabolism.

2) Growth characterization and flesh quality: Biochemical, histological and histochemical parameters, namely the tissue fat composition, number and size of muscle fibres and capacity of recruitment of new fibres are analysed under different rearing conditions. The laboratory not only uses recent sensory, physical and microbiological analysis to characterize the “freshness level” of several kinds of seafood, but also is responsible for the creation of new sensory schemes for cephalopods under the recently developed Quality Index Method. In the physical methods, this laboratory is using Torrymeter and Freshmeter instrumental analysis. In the microbiological area, the use of counts of specific spoilage organisms was developed in practical terms.

### **2. Main achievements (2000 ca.)**

Nutrient requirements, dietary formulations and feeding practices were optimized for blackspot seabream, Senegalese sole and bream. Blackspot seabream has a 50% dietary protein requirement but low lipid tolerance (<10%). Optimal growth of sole is obtained with 8% lipids; the estimated lysine requirement (4.68 g lysine / 16 g N) was used to design diets using plant protein sources. Protein requirement for *Diplodus vulgaris* was 7.8 g /kg body weight, and this species performed better than *D. sargus*. Output: 8 papers (+ 3 submitted); 2 PhD; 1 MSc.

The bottleneck of Octopus culture is the rearing of its paralarval. Microalgal species were used to improve the biochemical composition of juvenile *Artemia* as prey for paralarvae. Output: 1 paper, 1 PhD.

The production of ornamental aquatic species has now evolved into an industrial activity. Information regarding nutritional requirements, food utilization and behaviour of ornamental fish were considered. Output: 3 papers, 2 submitted;

Understanding the environmental factors that regulates muscle differentiation and growth is important to select the best strategy to optimize growth and meat quality a) High-protein diets

favoured muscle hyperplasia; b) High temperature accelerates development and muscle growth promoting hyperplastic growth. Output: 2 papers, 3 submitted; 1 PhD.

Valorisation of aquaculture products. Production of functional foods. The use of dietary conjugated linoleic acid (CLA) by fish has a potential benefit for human health. Being fish an important source of protein and polyunsaturated fatty acids, a further increase in its CLA content could enhance the nutritional value of fish meat for human consumption. When supplemented, fish show the highest muscle CLA deposition of any other animal. CLA can be incorporated up to 1 % in both rainbow trout and sea bass diets contributing to the production of a functional food. Output: 5 papers, 2 MSc.

Sensory, physical and microbiological characterization of freshness in sea products: New sensory schemes for cephalopods were developed under the Quality Index Method that was compared and confirmed using other methods, namely physical methods (Torrymeter and Freshmeter) and counts of specific spoilage organisms. Output: 4 papers.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

LANUCE has published 37 full papers and 4 abstracts in international journals in the SCI (1.5 papers/researcher/year). We expect to improve the figures in the next few years due to the large number of PhD students in our laboratory at the moment and also by the inclusion of a new member (Paulo Costa) since in December 2007. Herewith we include the most cited papers.

1. Imsland, AK, Foss, A, Conceição, LEC, Dinis, MT, Delbare, D, Schram, E, Kamstra, A, Rema, P, White, P. 2003. A review of the culture potential of *Solea solea* and *S. senegalensis*. Reviews in Fish Biology and Fisheries 13: 379-407. IF 2003/2007=1.386/2.531, n°C=33.
2. Aragão, C, Conceição, LEC, Martins, D, Rønnestad, I, Gomes, E, Dinis, MT. 2004. A balanced dietary amino acid profile improves amino acid retention in post-larval Senegalese sole (*Solea senegalensis*). Aquaculture 233: 293-304. IF 2004/2007=1.507/1.735, n°C=18.
3. Ozório, R, Van Ginneken, V, Van den Thillart, G, Verstegen, M, Verreth, J. 2005. Dietary carnitine maintains energy reserves and delays fatigue of exercised African catfish fed high fat diets. Scientia Agricola 62: 208-213. IF 2007=0.62, n°C=1.
4. Gouveia, L, Rema, P, Pereira, O, Empis, J. 2003. Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgal biomass. Aquaculture Nutrition 9(2): 123-129. IF 2003/2007=1.066/1.534, n°C=14.
5. Vaz-Pires, P, Seixas, P, Barbosa, A. 2004. Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): A review. Aquaculture 238(1-4): 221-238. IF 2004/2007=1.507/1.735, n°C=14.
6. Figueiredo-Silva, A, Rema, P, Bandarra, NM, Nunes, ML, Valente, LMP. 2005. Effects of dietary conjugated linoleic acid on growth, nutrient utilization, body composition and hepatic

lipogenesis in rainbow trout juveniles (*Oncorhynchus mykiss*). *Aquaculture* 248: 163-172. IF 2005/2007=1.374/1.735, n°C=12.

7. Rodrigues, MJ, Ho, P, López-Caballero, ME, Vaz-Pires, P, Nunes, ML. 2003. Characterization and identification of microflora from soaked cod and respective salted raw materials. *Food Microbiology* 20(4): 471-481. IF 2003/2007=1.049/2.039, n°C=9.

8. Figueiredo-Silva, A, Rocha, E, Dias, J, Silva, P, Rema, P, Gomes, E, Valente, LMP. 2005. Partial replacement of fish oil by soybean oil on lipid distribution and liver histology in European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) juveniles. *Aquaculture Nutrition* 11: 147-155. IF 2005/2007=1.441/1.534, n°C=10.

9. Cabrita, ARJ, Fonseca, AJM, Dewhurst, RJ, Gomes, E. 2003. Nitrogen supplementation of corn silages. 2. Assessing rumen function using fatty acid profiles of bovine milk. *Journal of Dairy Science* 86: 4020-4032. IF 2003/2007=2.139/2.361, n°C=10.

10. Ozório, R, Valente, LMP, Pousão-Ferreira, P, Oliva-Teles, A. 2006. Growth performance and body composition of white seabream (*Diplodus sargus*) juveniles fed diets with different protein and lipid levels. *Aquaculture Research* 37: 255-263. IF 2006/2007=1.051/1.067, n°C=9.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

LANUCE has published 6 full papers in national journals and 3 books/chapters. LANUCE has also participated with 11 oral communications/posters in national conferences. Besides the international papers we still think it is important to divulge our work at the national level to widespread our public.

Full papers

1. Martins da Costa, P, Nunes, T, Vaz-Pires, P, Bernardo, F. 2005. Influência do manejo alimentar no fenótipo de antibiorresistência de *Escherichia coli* e *Enterococcus* spp. isolados a partir da flora entérica de frangos. *Revista da Faculdade de Medicina de Lisboa, série III*, vol. 10, nº 7, Outubro de 2005, pp. 393-402.

2. Martins da Costa, P, Nunes, T, Vaz-Pires, P, Bernardo, F. 2006. Reprodutibilidade e especificidade dos métodos de detecção de *Escherichia coli* em águas e lamas colhidas em estações de tratamento de águas residuais. *Revista Portuguesa de Ciências Veterinárias*. Ano 105º, Vol. CI, Nº 559-560: 283-290.

3. Martins da Costa, P, Nunes, T, Vaz-Pires, P, Bernardo, F. 2006. Reprodutibilidade e especificidade dos métodos de detecção de *Enterococcus* spp. em águas e lamas colhidas em estações de tratamento de águas residuais. *Revista Portuguesa de Ciências Veterinárias*. Ano 105º, Vol. CI, Nº 559-560: 273-281.

4. Martins da Costa, P, Nunes, T, Vaz-Pires, P, Bernardo, F. 2006. Impacto da administração de uma associação antibiótica contendo furazolidona no nível de antibiorresistências em *Escherichia coli* e *Enterococcus* spp. em frangos. *Revista Portuguesa de Zootecnia* 13, Vol. I, 41-56.

5. Silva, P, Valente, LMP, Gomes, EFS. 2004. Pigmentação em peixes. Revista Portuguesa de Zootecnia, ANO XI-Nº1: 13-26.
6. Rema, P. 2007. Espécies Alternativas. Produção industrial de peixes de ornamento. Proceedings I Jornadas de Aquacultura da APEZ. Maio, 2007, UTAD, Vila Real, pp. 38-43.
7. Rema, P, Ozório, ROA, Puga, C, Pousão-Ferreira, P, Valente, LMP. 2005. Replacing fish oil with Soya-lecithine in diets of gilthead seabream (*Sparus aurata*). In XV Congresso de Zootecnia, 2-5 November, 2005, Vila Real, Portugal, pp. 77-82.

#### Books/chapters

8. Ozório, ROA. 2003. Estado da arte da piscicultura brasileira e mundial. In: Tópicos Especiais em Piscicultura Tropical De Agua Doce Tropical Intensiva. Castagnolli, N, Cyrino, JEP, Carrã, M (Eds). São Paulo, TecArt, 533 pp. ISBN 8590468917.
9. Vaz-Pires, P, Nunes, L, Batista, I. 2005. Terminologia de Produtos da Pesca e Aquicultura. Instituto Nacional de Investigação Agrária e das Pescas, colecção Publicações Avulsas do IPIMAR (Lisboa), nº 12, 87 p. ISSN 0872-914X.
10. Venâncio, C, Colaço, B, Rema, P, Ferreira, D, Lacilla, JV. 2005. Aspectos Gerais da Anatomia dos Peixes – Estudo da Truta arco-íris (*Oncorhynchus mykiss*). Série Didáctica. Ciências Aplicadas Nº 286. 28 p. ISBN: 972-669-719-0. Depósito Legal: 237529/06.

### **3. Other publications international (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)**

LANUCE has produced 9 full papers in international journals and/or conferences.

1. Costa, PM, Vaz-Pires, P, Bernardo, F. 2007. Antimicrobial resistance in *Escherichia coli* isolated in inflow, effluent and sludge from municipal wastewater treatment plants. Urban Water Journal 4(4): 275-281.
2. Olmedo, M, Linares, F, Álvarez-Blázquez, B, Valente, LMP. 2007. Crecimiento y composición corporal de juveniles de besugo, *Pagellus bogaraveo*, alimentados con diferentes niveles de proteína y carbohidratos. XI Congresso Nacional de Acuicultura, Setembro 2007, Vigo, Spain, pp. 1383-1386.
3. Ozório, ROA, Verreth, JAJ, Aragão, CR, Vermeulen, CJ, Schram, JW, Verstegen, MWA. 2003. Dietary carnitine supplements increased lipid metabolism and decreased protein oxidation in African catfish (*Clarias gariepinus*) juveniles fed high fat levels. Journal of Aquaculture in the Tropics, 3(18): 225-238.
4. Ozório, ROA, Cyrino, JEP, Turini, BGS, Moro, G, Oliveira, LST, Nascimento, A. 2005. Feeding dried yeast to Pacu (*Piaractus mesopotamicus*) as dietary fish meal replacement. World Aquaculture 36: 30-36.
5. Ozório, ROA, Iftoda, RM, Cyrino, JEP. 2003. Efeito de diferentes níveis dietéticos de levedura desidratada (*Saccharomyces cerevisiae*) sobre o desempenho e a composição corporal da Tilápia-

do-Nilo (*Oreochromis niloticus*) revertida sexualmente. In II Congreso Internacional Virtual de Acuicultura, <http://www.civa2003.org>, Spain, pp. 89-98.

6. Rema, P, Gouveia, L. 2005. Effect of various sources of carotenoids on survival and growth of goldfish (*Carassius auratus*) larvae and juveniles. *Journal of Animal and Vet. Advances* 4(7): 624-629.

7. Rema, P, Gouveia, A. 2005. Growth and survival of goldfish (*Carassius auratus*) larvae reared at different densities. *Journal of Animal and Vet. Advances* 4(2): 163-166.

8. Ribeiro, L, Couto, A, Olmedo, M, Álvarez-Blázquez, B, Linares, F, Valente, L. 2005. Digestive enzymes activity during black spot seabream larvae and post-larvae development. In: *Larvi' 05 - Fish & Shellfish Larviculture Symposium*. Hendry, CI, Van Stappen, G, Wille, M, Sorgeloos, P (Eds). European Aquaculture Society, Special Publication 36, 426-429. Oostende, Belgium.

9. Seixas, P, Rey-Mendes, M, Valente, L, Otero, A. 2007. Producción y enriquecimiento de *Artemia* sp. con distintas microalgas marinas como alimento para paralarvas de pulpo (*Octopus vulgaris*). XI Congreso Nacional de Acuicultura, Setembro 2007, Vigo, Spain, pp. 643-646.

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Martins, Dulce Alves. 2007. Role of dietary lipid in the Nutrition of Atlantic halibut (*Hippoglossus hippoglossus*). PhD in Aquatic Science, Instituto de Ciências Biomédicas Abel Salazar, University of Porto. Supervisor: LMP Valente (CIMAR), Co-supervisor: E Gomes (CIMAR) and Santosh P. Lall (National Research Council of Canada).

##### **MSc THESIS**

Moreira, Sandra. 2004. Variability of myostatin genes in rainbow trout strains exhibiting distinct hyperplastic growth. Master in Animal Production, University of Trás-os-Montes and Alto Douro. Supervisor: LMP Valente, Co-supervisor: Pierre-Ives Rescan (SCRIBE-INRA, Rennes, France).

Figueiredo-Silva, Amélia Cláudia. 2005. Effects of dietary conjugated linoleic acid in rainbow trout juveniles. Master in Aquatic Science, Instituto de Ciências Biomédicas Abel Salazar, University of Porto. Supervisor: LMP Valente (CIMAR), Co-supervisor: P Rema (University of Trás-os-Montes and Alto Douro).

Ramos, Maria Amélia. 2007. Time course deposition of conjugated linoleic acid in market size rainbow trout (*Oncorhynchus mykiss*) muscle. Master in Animal Production, Faculty of Veterinary Science of the University of Lisboa. Supervisor: LMP Valente (CIMAR), Co-supervisor: P Rema (University of Trás-os-Montes and Alto Douro).

Oliveira, Ana Paula. 2007. Validation of the food safety system of Diversumos. Master in Food Technology & Quality, Faculty of Sciences of University Nova de Lisboa. Co-supervisor: JF dos Santos Oliveira and P Vaz-Pires (CIMAR).



## **5. Patents/propotypes (2000 ca.)**

## **6. Organization of conferences (2000 ca.)**

Members of LANUCE have participated in the organization of several national and international conferences as local organizers or members of the scientific committee:

- II Jornadas Técnicas de Aquariorfilia. ORNIEX- EXPOZOO, 2001.
- I Jornadas de Aquacultura. UTAD, Vila Real, 2007 (Local organizer; 60 participants).
- Aquacultura e Novas Espécies. Funchal, Madeira, 12-15 September 2004 (Scientific committee, 100 participants).
- Workshop COST action 925 “The importance of prenatal events for postnatal muscle growth in relation to the quality of muscle based foods”, CIIMAR, Porto, October 2004 (60 participants from 16 countries; 32 oral presentations).

## **7. Industry contract research (2000 ca.)**

The Laboratory of Nutrition, Growth and Quality of Fish (CIIMAR) together with the Aquaculture research group (CCMAR) coordinate the OPTIDIETAS project in consortium with a feed company SORGAL, S.A. (Project IDEIA 70/00073, Support to investigation of applied research in companies). This project is focused on the optimization of dietary formulations and feeding practices for Senegalese sole and blackspot seabream, based on the specific nutritional requirements and feeding behaviour of each species.

- CIIMAR participated in the PULSEFISH project in consortium with a company SOTEXPRO and PROFAT and several other research institutes (INRA, CTC) (Project Craft). This project was focused on the technological improvement of the utilisation of pulse proteins and fish protein concentrate in fish feeds.
- A protocol was established with a fish farm, Isidro de la Cal, in order to improve dietary formulations and feeding practices for blackspot seabream.
- A protocol was established with a fish farm, A. Coelho e Castro, in order to establish a new bio filter to improve the effluents' water quality.
- A protocol was established with a private company for ornamental fish commercialization, Orniex, to give technical and scientific support.

## **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

LANUCE has participated in several meetings promoted by the Comissão de Coordenação para o Desenvolvimento da Região Norte (CCDR-N), contributing to the elaboration of regional priorities for the sustainable development of marine research in the North of Portugal and Galiza region that resulted in a final report “Regional Agenda for the Sea”.

A protocol was established with the National Veterinary Authority, Direcção Geral de Veterinária, in order to investigate the biological and economical consequences associated to the administration of nitrofurans to broilers during their rearing period. The results obtained were published in a portuguese journal:

Martins da Costa, P, Nunes, T, Vaz-Pires, P, Bernardo, F. 2006. Impacto da administração de uma associação antibiótica contendo furazolidona no nível de antibiorresistências em *Escherichia coli* e *Enterococcus* spp. em frangos. *Revista Portuguesa de Zootecnia* 13, Vol. I: 41-56.

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

LANUCE has long established collaborations with several international Institutes to develop research programs and co-supervision of PhD students.

Projects:

- Pulse-fish, Craft financed by the European Union. 3 Countries.
- SEACASE, financed by the EU, involving 5 Countries, coordinated by CCMAR.

Collaborative publication/ graduate training/Cooperation

- National Research Council of Canada, NRC, Canada. Co-supervision of a PhD thesis by Santosh Lall: 2 publications (+ 2 submitted).

Martins, DA, Valente, LMP, Lall, S. 2007. *Aquaculture* 263: 150-158.

Martins, DA, Afonso, LOB, Hosoya, S, Lewis-McCrea, LM, Valente, LMP, Lall, S. 2007. *Aquaculture* 272: 573-580.

- NIFES, Bergen, Norway. Co-supervision of a PhD thesis by Marit Espe: 1 publication.

Silva, JMG, Espe, M, Conceição, LEC, Dias, J, Costas, B, Valente, LMP. 2008. *Aquaculture Nutrition*. Submitted

- UMR, Nutrition Aquaculture Génomique, INRA, St-Pee-Sur Nivelles, France. Co-supervision of a PhD thesis by Geneviève Corraze: 1 publication. 1 COST action 825.

Figueiredo-Silva, AC, Corraze, G, Borges, P, Valente, LMP. 2008. *Aquaculture Nutrition*. Submitted.

- Bodo University, Norway. Co-supervision of a PhD thesis by Jorge Fernandes. 1 COST action 825, 1 proposal for a PhD thesis.

- Barcelona University, Spain (Joaquin Gutierrez). 1 publication. 1 COST action 825.

- Instituto Español de Oceanografía and Consellería de Pesca e Asuntos Marítimos (CPAM), Spain (T Peleteiro, M Olmedo, F Linares and JLR Villanueva). 1 Interreg project submitted and 1 Spanish project. 1 publication.

Valente, LMP, Olmedo, M, Álvarez-Blázquez, B, Borges, P, Soares, S, Gomes, EFS, Pazos, G, Linares, F. 2008.

- University of Santiago de Compostela (USC), Spain. Co-supervision of a PhD thesis by Manuel Rey Mèndez e Professora Ana Otero: 1 publication.

Seixas, P, Rey-Méndez, M., Valente, LMP, Otero, A. 2008. Aquaculture, In Press.

- SCRIBE-INRA, Rennes, France (Pierre-Ives Rescan). 1 publication. 1 COST action 825.

Valente, LMP, Moreira, S, Ralliére, C, Ramos, AM, Rescan, PY. 2006. Archives of Animal Breeding 49: 103-108.

- INPA, National Institute for Research in the Amazon (Manoel Pereira) and UFAC, Universidade Federal do Acre (Ricardo Amaral), Brazil. Co-supervision of a PhD thesis.

## 6e. Future research

### 1. Objectives (3000 ca.)

In the Division of Aquaculture & Marine Biotechnology of CIMAR-LA, there are several groups specifically dedicated to the research line Aquaculture. The objective of LANUCE is to contribute to the implementation and consolidation of Fish Nutrition through a more thigh collaboration with those groups. Until 2008, 5 collaborative projects have already been established, generating funds of 1.13 Million EUR. LANUCE and AQUAGROUP have currently joint PhD projects with the participation of international groups. These co-supervised projects were elaborated in order to optimize the expertise of each group and to guaranty the student the best institutional facilities to develop the proposed research line in the due time.

In specific terms, the central aim of LANUCE still is contributing to the sustainable development of the aquaculture industry. Plant protein ingredients will be considered for dietary incorporation. The effects of such diets on fish growth and on the final muscle quality will be evaluated. The optimization of feeding regimes and rearing conditions of Senegalese sole will be a priority in collaboration with Aquagroup from CCMAR. Large-scale experiments will be considered to validate our laboratory-scale results. These are applied research lines that we expect to develop further in collaboration with fish farmers from Portugal and North Spain.

Particular attention will be given to the development of new tools to assess muscle growth and final quality to combine with the classic morphometric approach already implemented in our laboratory. Understanding the molecular basis of environmental-induced phenotypic plasticity of muscle development and growth will be a main research line. Factors like rearing temperature and nutritional effects will be studied.

LANUCE will reinforce its activity towards the improvement of food quality and security of fisheries and aquaculture products. A new senior researcher has joint to our group by the end of 2007 (Paulo Costa). The predicted work includes the further development of sensory schemes based on Quality Index Method, the most recent sensory classification system for seafood items. In terms of physical methods, new methods for the evaluation of rigor mortis will be developed, as well as others that proved to be efficient and useful in quality and welfare evaluations, in

addition to the already used instrumental methods (Torrismeter and Freshmeter). In terms of microbiology, the first studies will be focused on the characterization of the microbiological environment of freshwater and seawater farms, including animals, water and surfaces, and eventual resistances to antibiotics (antibioresistances) shown by commensal (*Escherichia coli* and *Enterococcus* spp.) and pathogenic bacteria (*Salmonella* sp.); their ability to transmit those resistances to humans directly from the consumption of farmed products and also by the aquatic environment will also be considered.

**2. Funding, source, dates** (1500 ca.) (Indicate in full including amount of current and pending funding)

Several projects are still going on and 2 new projects were recently submitted, one coordinated by CIMAR involving 194.600 EUR. The plurianual funding of the 6 senior researchers will contribute to the most basic needs of LANUCE. The development of the main research topics will be dependent of funding from projects.

2005-2008: Optidietas: Feed formulation for blackspot seabream and sole. Project IDEIA 70/00073 in consort with a feed company, SORGAL, financed by AdI, Programme PRIME. (2 November 2005 to 30 June de 2008; total 380.343,48 EUR; CIIMAR 312.379,45 EUR). Coordinator: Luísa Valente. Partners: CCMAR and SORGAL.

2005-2008: Effects of hydrostatic pressure and other abiotic factors on growth of the blackspot seabream (*Pagellus bogaraveo*). Project POCTI/MAR/57022/2004, financed by FCT. Coordinator: João Coimbra.

2007-2009: SEACASE: Sustainable extensive and semi-intensive coastal aquaculture in Sothern Europe. Project FP6-2005-SSP5A, financed by European Union. Coordinator Maria Teresa Dinis. (25 Jan 2007 to 30 Dec 2009; total 2.391.401 EUR; CIMAR-LA 312.486 EUR, CIIMAR 49.970 EUR). Partners: LANUCE, IPIMAR and involving researchers of five European Countries (Portugal, Spain, France, Italy and Greece).

2009-2011: TEXBREAM - Post-mortem changes in gilthead sea bream muscle proteins: its implications to flesh texture. Project PTDC/MAR/70858/2006, financed by FCT. Approved Project in 2007, waiting for FCT contract to start.

2009-2010: SUPERLING - Sustainable development of Senegalese sole: Selection of reproducers, vaccination scheme implementation, probiotics and vegetable feeds. Submitted to INTERREG – Programa de Cooperação Transfronteiriça Espanha-Portugal: 194.600 EUR.

2009-2011: Conservation of trout (*Salmo trutta* L.) populations in the rivers of North Portugal: importance of the genetic preservation and ecological assessment. Submitted to EDP funding for Biodiversity: 92.000 EUR.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Ramos, A, Bandarra, NM, Rema, P, Vaz-Pires, P, Nunes, ML, Andrade, AM, Cordeiro, AR, Valente, LMP. 2008. Time course deposition of conjugated linoleic acid in market size rainbow trout (*Oncorhynchus mykiss*) muscle. *Aquaculture* 274: 366-374. IF 2007=1.735, n°C=0.

2. Seixas, P, Rey-Méndez, M, Valente, LMP, Otero, A. 2008. Producing juvenile *Artemia* as preys for *Octopus vulgaris* paralarvae with different microalgal species of controlled biochemical composition. *Aquaculture* 283: 83-91. IF 2007=1.735, n°C=0.
3. Ozório, ROA, Valente, LMP, Correia, S, Pousão-Ferreira, P, Damasceno-Oliveira, A, Escório, C, Oliva-Teles, A. (in press). Protein requirement for maintenance and maximum growth of two banded seabream (*Diplodus vulgaris*) juveniles. *Aquaculture Nutrition*, DOI: 10.1111/j.1365-2095.2008.00570.x. IF 2007=1.534.
4. Silva, P, Rowleron, AM, Valente, LMP, Olmedo, M, Monteiro, RAF, Rocha, E. (in press). Muscle differentiation and growth in blackspot seabream (*Pagellus bogaraveo*, Brunnich): histochemical and immunohistochemical study of the fibre types. *Tissue & Cell*, DOI: 10.1016/j.tice.2008.05.001. IF 2007=1.237.
5. Reis, PA, Valente, LMP, Almeida, CMR. 2008. A fast and simple methodology for determination of yttrium as an inert marker in digestibility studies. *Food Chemistry* 108: 1094-1098. IF 2007=3.052, n°C=0.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

- First of all it would be very important for us to have regular calls for national projects with a reasonable deadline to sign the respective contracts. The development of new research lines implies the possibility of finding funding to start those lines.
- The professional help of trained experts in appliances for EU funds would be desirable to guarantee the elaboration of competitive projects according the EU guidelines. This already exists in several EU countries.
- In order to develop new tools to assess muscle growth and final quality to combine with the classic morphometric approach already implemented in our laboratory it will be needed the collaboration of a researcher with large experience in the area. We expect to be able to recruit someone that can develop further our capacity to preview and control muscle growth in important species for aquaculture.
- The recruitment of a technician to the group would allow us to offer a more effective use of our equipment and eventually provide services to the exterior.
- New facilities for the rearing of the fish that can respect all security parameters are also required.

## **Fish Nutrition**

### **6a. Group description**

#### **1. Group name / denomination**

#### **Fish Nutrition**

#### **2. Principal investigator**

Aires Manuel Pereira Oliva Teles

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Nutrient requirement, Nutrient metabolism, Feedstuff utilization, Environmental sustainability

#### **5. Funding, source, dates (1000 ca.)**

Besides the Pluriannual funding, during this period research activities have been supported by EU projects (more than 100.000 EUR), FCT projects (283.000 EUR) and industry contract research (12.000 EUR).

### **6b. Group team**

#### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Aires Manuel Pereira Oliva Teles (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

002. António José Rogeiro Gouveia (Cat.: Professor Associado, Gr. Acad.: Agregação)

003. António Paulo Alves Ferreira de Carvalho (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

004. Maria Helena Tabuaço Rêgo Martins Peres (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

005. Rui Miguel Fonseca Neves de Sá (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

#### **2. Other Researchers in the group (Include here collaborators with PhD. only)**

001. Ana Maria Pimentel Rodrigues (Cat.: Professor Auxiliar, Gr. Acad.: Doutoramento)

#### **3. Other Researchers in the group (non PhD.)**

001. Ana Isabel Santos Couto (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

## 6c. Objectives & achievements

### 1. Objectives (2000 ca.)

The fish nutrition group directs its research to the study of basic and applied aspects of the nutrition and feeding of larvae and juveniles of fish species well established in marine aquaculture (sea bass, sea bream, turbot), candidate species for aquaculture (sargus, sole), and ornamental species (zebrafish, angelfish, kribensis, jewelfish).

Main research activities are directed towards the study of:

- 1) protein and amino acid requirements, metabolism and utilization efficiency;
- 2) utilization of conventional energy sources (lipids and carbohydrates);
- 3) phosphorus requirement and bioavailability;
- 4) utilization of feedstuffs alternative to fish meal and evaluation of processing treatments on the improvement of feedstuff utilization;
- 5) development of microparticulate diets for fish larvae and weaning strategies.

These studies aim contributing for a deep insight on the nutritional requirements and metabolic utilization of nutrients by fish, therefore providing basic information regarding the physiological aspects of comparative animal nutrition and contributing for a scientific based improvement of fish diets. From an applied perspective, studies aim maximization of fish growth and feed utilization while reducing the environmental impact due to feeding. This way it is expected to contribute for the sustainable development of aquaculture.

### 2. Main achievements (2000 ca.)

For the first time, protein requirement and energy utilization for white sea bream, a potential aquaculture species for the Mediterranean were evaluated. Protein requirement for a proximate species, two-banded sea bream was also evaluated. Nutritional requirements of sargus spp. were until now unknown, and this knowledge represents basic and fundamental data for a correct diet formulation for these species.

A comparative study of carbohydrate utilization by sea bass and sea bream was also undertaken, trying to elucidate the potential use of different concentrations and different sources of carbohydrates by these species and understand its use both at zootechnical and metabolic level.

Studies on protein and AA utilization and requirements were also prosecuted in sea bass and turbot. Lysine requirement of turbot juveniles was for the first times estimated. A deeper analysis of use of NPN sources was also undertaken.

Phosphorus requirement of sea bass was for the first time estimated as well as phosphorus availability in fish meals and inorganic phosphorus sources. This is fundamental data for the production of low pollution diets. It is also fundamental for a correct diet formulation when fish meal is not the main ingredient in diet formulation.

Use of alternative feedstuffs by sea bass and sea bream juveniles was also prosecuted.



Studies on utilization of microparticulate diets by larval fish were also continued aiming a precocious replacement of live feeding during larvae rearing.

Finally, effect of toxic agents and nutritional strategies to improve disease resistance were also analyzed.

Overall, during this period a total of 42 papers were published in peer-reviewed (ISI) journals, 3 book chapters, 7 papers in other journals, and 35 communications in scientific meetings.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Carvalho, AP, Oliva-Teles, A, Bergot, P. 2003. A preliminary study on the molecular weight profile of soluble protein nitrogen in live food organisms for fish larvae. *Aquaculture* 225: 445-449. IF 2003/2007=1.507/1.735, n°C=7.

2. Pereira, TG, Oliva-Teles, A. 2003. Evaluation of corn gluten meal as protein source in diets for gilthead sea bream (*Sparus aurata*) juveniles. *Aquaculture Research* 34: 1111-1117. IF 2003/2007=0.766/1.067, n°C=13.

3. Peres, H, Lim, C, Klesius, PH. 2003. Nutritional value of heat-treated soybean meal for channel catfish (*Ictalurus punctatus*). *Aquaculture* 225: 67-82. IF 2003/2007=1.507/1.735, n°C=12.

4. Carvalho, AP, Sá, R, Oliva-Teles, A, Bergot, P. 2004. Solubility and peptide profile affect the utilization of dietary protein by carp (*Cyprinus carpio* L.) during early larval stages. *Aquaculture* 234: 319-333. IF 2004/2007=1.627/1.735, n°C=11.

5. Oliva-Teles, A, Pimentel-Rodrigues, A. 2004. Phosphorus requirements of European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture Research* 35:636-642. IF 2004/2007=0.676/1.067, n°C=9.

6. Evans, JJ, Pasnik, DJ, Peres, H, Lim, C, Klesius, PH. 2005. No apparent differences in intestinal histology of channel catfish (*Ictalurus punctatus*) fed heat-treated and non-heat-treated raw soybean meal. *Aquaculture Nutrition* 11, 123-129. IF 2005/2007=1.441/1.534, n°C=5.

7. Enes, P, Panserat, S, Kaushik, S, Oliva-Teles, A. 2006. Effect of normal and waxy maize starch on growth, food utilization and hepatic glucose metabolism in European sea bass (*Dicentrarchus labrax*) juveniles. *Comparative Biochemistry and Physiology, Part A*, 143: 89-96. IF 2006/2007=1.553/1.863, n°C=12.

8. Sá, R, Pousão-Ferreira, P, Oliva-Teles, A. 2006. Effect of dietary protein and lipid levels on growth and feed utilization of white sea bream (*Diplodus sargus*) juveniles. *Aquaculture Nutrition* 12: 310-321. IF 2006/2007=1.642/1.534, n°C=7.

9. Santos, MM, Micael, J, Carvalho, AP, Morabito, R, Lamoree, M, Massanisso, P, Booy, P, Reis-Henriques, MA. 2006. Estrogens counteract the masculinizing effect of tributyltin in zebrafish. *Comparative Biochemistry and Physiology C - Toxicology and Pharmacology* 142: 151-155. IF 2006/2007= 1.991/2.345. n°C=9.

10. Peres, H, Oliva-Teles, A. 2007. Effect of dietary essential amino acid pattern on growth, feed utilization and nitrogen metabolism of European sea bass (*Dicentrarchus labrax*). *Aquaculture* 267: 119-128. IF 2007=1.735, n°C=4.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Oliva-Teles, A. 2007. Aspectos gerais da nutrição em peixes. *Aquacultura sustentada*. In: I Jornadas Aquacultura da APEZ – Situação Actual e Perspectivas Futuras, pp. 14-33.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Gomułka, P, Dąbrowski, K, Ostaszewska, T, Hliwa, P, Siennicki, M, Carvalho, AP. 2006. Wzrost, deformacje ciała i patologia przewodu pokarmowego larw karpia (*Cyprinus carpio*) żywionych pokarmem naturalnym, paszami komercyjnymi lub eksperymentalnymi. In: Rozród, podchów, profilaktyka ryb karpiojących i innych gatunków. Zakęś, Z, Demska-Zakęś, K, Wolnicki, J (Eds). Wyd, IRS, Poland, pp. 87-98.

2. Peres, H, Lim, C. 2008. Use of soybean products in non-salmonids marine finfish. In: *Alternative Protein Sources in Aquaculture Diets*. Li, SC, Lim, C, Webster, CD (Eds), The Haworth Press, Inc., New York.

3. Brown, PB, Kaushik, S, Peres, H. 2008. Protein Feedstuffs Originating from Soybeans. In: Li, SC, Lim, C, Webster, CD (Eds). *Alternative Protein Sources in Aquaculture Diets*. The Haworth Press, Inc., New York.

4. Gouveia, A. 2003. Aquaculture in the Mediterranean region: an overview. *Magazine of the European Aquaculture Society in World Aquaculture* 34(3): 3-7.

5. Davies, SJ, Gouveia, A. 2004. Cereal processing & improved carbohydrate digestibility in - Over-wintering diets for juveniles Gilthead Sea Bream. *Aquafeed International* 7(4): 18-23.

6. Rema, P, Gouveia, A. 2005. Growth and survival of goldfish (*Carassius auratus*) larvae reared at different densities. *Journal of Animal and Veterinary Advances*. Vol. 4(2): 274-275.

7. Sticklandm, NC, Koumoundouros, G, Sfakianakis, DG, Georgakopoulos, E, Papadakis, G, Divanach, P, Kentouri, M, Guedes, MJ, Carvalho, AP, Oliva Teles, A, Ashton, C, Weaden, J, Goldspink, G, Fauconneau, B, Kacem, A, Kranenbarg, S, Leeuwen, JL Van. 2005. Solving spinal deformity problems in Mediterranean sea bass. *Fish Farmer* 28: 22-24.

8. Laporte, J, Woodgate, ST, Davies, SJ, Serwata, R, Gouveia, A, Nates, SF. 2007. Biotechnological process & biological value of feather meal: evaluation of a novel protein source for the partial replacement of fish meal in aqua-feeds. *Aquafeed International* 10(4): 16-23.

9. Laporte, J, Woodgate, ST, Davies, SJ, Serwata, R, Gouveia, A. 2007. Poultry meat meal: a valuable source of protein for the partial replacement of fish meal in aquafeeds. *Aquafeed International* 10(2): 12-17.

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Sá, Rui Miguel. 2007. White sea bream juveniles nutrition. PhD in Biology, Faculty of Sciences, University of Porto. Supervisor: A Oliva Teles (CIIMAR).

##### **MSc THESIS**

Antão, Ana Maria. 2003. Estudo comparativo da comunidade de endohelmintas parasitas do tubo digestivo de diferentes populações de *Anguilla anguilla* (L.). Master in Applied Ecology, Faculty of Sciences, University of Porto. Supervisor: AP Carvalho (CIIMAR).

Araújo, Leonor. 2004. Effect of dietary vitamin A level on reproduction and larval performance of ciprinids. Master in Applied Ecology, Faculty of Sciences, University of Porto. Supervisor: AP Carvalho (CIIMAR).

#### **5. Patents/propotypes (2000 ca.)**

#### **6. Organization of conferences (2000 ca.)**

#### **7. Industry contract research (2000 ca.)**

Project: Poultry meat meal evaluation to sea bream. Funded by Fats and Protein Research Foundation, USA. Duration: 2005, Coordinator: A Gouveia (CIIMAR), Participating Institutions: CIIMAR and Plymouth University. Total funding: 3000 EUR.

Project: The digestibility study of Sea bream. Funded by Fats and Protein Research Foundation, USA. Duration: 2003, Coordinator: A Gouveia (CIIMAR), Participating Institutions: CIIMAR; Plymouth. Total funding: 3000 EUR.

Project: The digestibility study of Turbot. Funded by Fats and Protein Research Foundation, USA. Duration: 2003, Coordinator: A Gouveia (CIIMAR), Participating Institutions: CIIMAR; Plymouth. Total funding: 3000 EUR.

Project: The digestibility study of Sea bass. Funded by Fats and Protein Research Foundation, USA. Duration: 2003, Coordinator: A Gouveia (CIIMAR), Participating Institutions: CIIMAR; Plymouth. Total funding: 3000 EUR.

**8. Government/organization contract research** (2000 ca.) (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

Researchers of the laboratory of Nutrition established cooperation with other research groups both at national and international level.

Regarding international groups, research cooperation is well established with the groups of:

Prof. SJ Davies, Faculty of Sciences, Plymouth University;

Drs. SJ Kaushik and S Panserat, INRA, St. Pée sur Nivelle, France;

Prof. A E Morales, Faculty of Sciences, Granada University.

Recently, the following contacts have been established aiming the prosecution of works in new directions and integrating Pos-Doc and PhD students:

Prof. J Gutierrez, University of Barcelona;

Prof. S Zamora, University of Murcia;

Prof. A Krogdahl, Norwegian Science and Veterinary Science, Aquaculture Protein Center.

Besides these groups, research has also been established with the groups of:

Dr. K Dabrowski, School of Environment and Natural Resources, The Ohio State University, USA and University of Warmia and Mazury in Olsztyn, Poland;

Dr. C Lin, Aquatic Animal Health Research Laboratory of the United States Department of Agriculture, Agriculture Research Service in Auburn, Alabama, USA;

Prof. M Kenturi, IMBC, Greece;

Prof. NC Stickland, Royal Veterinary College, London;

Prof. G Goldspink, Royal F. Hospital, London;

Dr. B Fauconneau, INRA, Rennes, France.

Some of this international cooperation was funded by the following projects :

Project: Optimisation of rearing conditions in sea bass for eliminated lordosis and improved musculoskeletal growth. Funded by EU (Q5RS-2001-01233), Duration: 2001-2004, Coordinator: Aires Oliva Teles (CIIMAR). Participating Institutions: FCUP; CIIMAR; INRA- France; RVC and RFH, London University; IMBC, Greece. Participants of the Laboratory of Nutrition: AOliva Teles, AP Carvalho. Total funding: 89.000 EDUR.

Project: Species diversification and improvement of aquatic production in seaweeds purifying effluents from fish farms and from other waste sources. Funded by EU (Q5RS-2000-31334), Duration: 2001-2003, Coordinator: I Sousa Pinto (CIIMAR). Participating Institutions: FCUP; CIIMAR; UTAD. Participants of the Laboratory of Nutrition: A Gouveia.

## **6e. Future research**

### **1. Objectives (3000 ca.)**

Research within the next few years will continue to fall across the main research areas that were defined in our group as research priorities.

Research focused in commercial aquaculture species such as sea bass and sea bream juveniles will be focused on:

- deeper analysis on amino acid requirement and utilization;
- further understand the regulation of carbohydrate utilization;
- evaluate the antioxidant stress due to feedstuffs and additives and its effect on the innate defence mechanisms;
- evaluate feedstuff utilization and the effect of anti-nutrients on gut morphology and metabolism;
- use of probiotics and its effect on the immune system;
- further understand phosphorus utilization and uptake regulation.

In new species for aquaculture we will try to evaluate:

- the temperature effect on growth and nutrient utilization in sole;
- utilization of carbohydrate rich alternative feedstuffs in *Diplodus sargus*, which is a omnivorous species;
- establish macronutrient requirements of *Diplodus cervinus*.

Such studies will be carried out using conventional approaches already in use in our lab but we aim also to apply different approaches, namely nutrigenomics and proteomics that may give a new insight in the elucidation of such aspects. Also, aspects related to nutritional impact on fish health will also be prosecuted. All this implies a multidisciplinary approach, therefore a close collaboration with colleagues both from CIIMAR and external laboratories (national and international) will be necessary.

Nutrigenomics is a promising field in nutritional studies and a qualitative up-grade in CIIMAR skills would greatly benefice if an expert in this filed was contracted.

To adequately perform all the tasks proposed and in view of the new involvement in nutrigenomics, a few specific equipment specific for such studies will be necessary. Apart of that a multichannel plaque reader and a freeze-dryer will be required.

**2. Funding, source, dates** (1500 ca.) (Indicate in full including amount of current and pending funding)

Project: Utilisation of dietary carbohydrates by sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) juveniles. Funded by FCT (POCI / CVT/ 57695 / 2004), Duration: 2005-2008, Coordinator: A Oliva Teles (FCUP, CIIMAR)/ H Peres (CIIMAR). Participating Institutions: FCUP; CIIMAR. Participants of the Laboratory of Nutrition: A Oliva Teles, H Peres, P Enes. Total funding: 83.336,40 EUR.

Project: Effects of complex mixtures of antagonistic disrupting chemicals in two fish species with different life histories. Funded by FCT (POCI/MAR/60895/2004), Duration: 2005-2008, Coordinator: MM Santos (CIIMAR). Participating Institutions: CIIMAR. Participants of the Laboratory of Nutrition: AP Carvalho. Total funding: 100.000 EUR.

Project: OPTISOLE – Investigação e desenvolvimento na produção de juvenis de linguado: incremento da resistência a patologias e do crescimento. Funded by Quadro de Referência Estratégico Nacional 2007-2013 and ERDF - Sistema de Incentivos à I&DT, Duration: 2008-2011, Coordinator: A Oliva Teles. Participating Institutions: CIIMAR. Participants of the Laboratory of Nutrition: A Oliva Teles, H Peres, AP Carvalho.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Peres, H, Oliva-Teles, A. 2008. Lysine requirement and efficiency of lysine utilization in turbot (*Scophthalmus maximus*) juveniles. *Aquaculture* 275: 283-290. IF 2007=1.735, n°C=0.

2. Davies, SJ, Gouveia, A. (in press). Enhancing the nutritional value of pea seed meals (*Pisum sativum*) by either thermal treatment or specific isogenic selection for the African catfish, *Clarias gariepinus*. *Aquaculture*. IF 2007=1.735.

3. Enes, P, Panserat, S, Kaushik, S, Oliva-Teles, A. 2008. Rearing temperature enhances hepatic glucokinase but not glucose-6-phosphatase activities in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) juveniles fed with the same levels of glucose. *Comparative Biochemistry and Physiology, Part A* 150(3): 355-358. IF 2007=1.863, n°C=0.

4. Sá, R, Pousão-Ferreira, P, Oliva-Teles, A. 2008. Effect of dietary starch source (normal vs. waxy) and protein levels on performance of white sea bream (*Diplodus sargus*) juveniles. *Aquaculture Research* 39(10): 1069-1076. IF 2007=1.067, n°C=0.

5. Couto, A, Enes, P, Peres, H, Oliva-Teles, A. (in press). Effect of water temperature and dietary carbohydrate level on growth performance and metabolic utilization of diets in gilthead sea bream (*Sparus aurata*) juveniles. *Comparative Biochemistry and Physiology, Part A*. IF 2007=1.863.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

A technician for support of rearing systems and facilities would be highly appreciated. Concerning equipment, a ELISA reader would be required.

## **Biodiversity of Aquatic Ecosystems**

### **6a. Group description**

#### **1. Group name / denomination**

**Biodiversity of Aquatic Ecosystems**

#### **2. Principal investigator**

Isabel Maria Trigueiros Sousa Pinto Machado

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Marine Biodiversity, Ecosystem Functioning, Fish Behaviour, Marine Resources

#### **5. Funding, source, dates (1000 ca.)**

- Plurianual

- FCT

74.819 EUR; Distribution, use and conservation of the botanical heritage of the Northern Coast of Portugal: A sustainable development approach for a (future) "Natura 2000" site; 2001 to 2004.

- 5th Framework Program. EC

182.763 EUR; SEAPURA: species diversification and improvement of aquatic production in seaweeds purifying effluents from fish and other waste sources; 2001 to 2004.

260.020 EUR; BIOPLATFORM: European Platform for Biodiversity; 2001 to 2005.

97.200 EUR; SEAWEED AFRICA: Underpinning Sustainable Ecosystem Management of Seaweed Resources in Africa; 2001 to 2005.

171.199 EUR; SEABEE: A Mobile Lander for Autonomous Monitoring and Sampling; 2001 a 2005.

14.882 EUR; MARBENA: Creating a long term infrastructure for marine biodiversity research in the European Economic Area and the Newly Associated States; 2002 - 2005.

- Other

50k EUR; Reabilitação da lagoa das Furnas: estudo do impacto da biomanipulação através da redução da densidade dos ciprinídeos; 2003-2005.



2.5k EUR; Estudo Geoecológico das lagoas de sedimentação da região de Alvarães, Viana de Castelo; Projecto de investigação científica na pré-graduação; Reitoria da Universidade do Porto e Caixa Geral de Depósitos; 2006.

10k EUR; Estado actual da biodiversidade dos recurso natural Artemia, na área mediterrânea, Portugal; Acção integrada Luso-Espanhola; 2002-2004.

35k EUR; Caracterização hidrográfica e de qualidade da água nas ribeiras da costa entre o rio Minho e Barrinha de Esmoriz; CCDRN; 2005-2007.

14.963 EUR; Utilização de Macroalgas Marinhas em Cultura para Limpeza de Efluentes e Reciclagem de Nutrientes; FUP; 2001 to 2003.

27.433 EUR; POOC - Plano de Ordenamento da Orla Costeira - of S. Jorge and Terceira Islands, Azores: characterization of the communities of the coastal Zone; 2002-2003.

10.250 EUR, POOC of S. Miguel Island, Azores: characterization of the communities of the coastal Zone; 2003-2004.

15.600 EUR; Produção experimental da planta halófila Salicornia; AGRO; 2005-2006.

5000 EUR; Studies of integrated cultivation of red seaweeds for effluent cleaning, in aquaculture and in the laboratory. Financiado pelo programa de Investigação na pré-graduação – UP and Fundação Ilídio Pinho; 2004.

25.000 EUR; Caracterização do moliço da Ria de Aveiro e uma avaliação preliminar da biomassa disponível; Funding: Company MARTIFER; 2004-2005.

## 6b. Group team

### 1. Researchers in the group (Include only PhD. integrated in the LA)

001. Anake Kijjoa (Cat.: Professor Catedrático Gr. Acad.: Agregação)

002. João Paulo de Sousa Cabral (Cat.: Professor Associado Gr. Acad.: Doutoramento)

003. Maria Leonor Fidalgo (Cat.: Professor Associado Gr. Acad.: Agregação)

004. Isabel Maria Trigueiros Sousa Pinto Machado (Cat.: Professor Auxiliar Gr. Acad.: Doutoramento)

005. Maria Natividade Ribeiro Vieira (Cat.: Professor Auxiliar Gr. Acad.: Agregação)

006. Maria Teresa Martins Borges (Cat.: Professor Auxiliar Gr. Acad.: Doutoramento)

007. Nuno Miguel Pinto de Sousa Monteiro (Cat.: Professor Auxiliar Gr. Acad.: Doutoramento)

008. Paulo Jose Talhadas Santos (Cat.: Professor Auxiliar Gr. Acad.: Doutoramento)

009. Francisco Arenas Parra (Cat.: Investigador Auxiliar Gr. Acad.: Doutoramento)

010. Rawiwan Watanadilok (Cat.: Não aplicável (bolseiro) Gr. Acad.: Doutoramento)

011. Rui Pedro Goncalves Pereira (Cat.: Não aplicável (bolseiro) Gr. Acad.: Doutoramento)

012. Stefano Vaselli (Cat.: Não aplicável (bolseiro) Gr. Acad.: Doutoramento)

**2. Other researchers in the group (Include here collaborators with PhD. only)**

001. Abdelaziz Fassouane (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

**3. Other researchers in the group (non PhD.)**

001. Agnès Marhadour (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

002. Ana Cristina Lemos de Matos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

003. Andreia Filipa Domingues Braga Henriques (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

004. Carina Santos da Silva (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

005. Carla Alexandra Martins Peixe (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

006. Filipa Margarida Barroso Ferreira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

007. Hugo Manuel Silva Ribeiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

008. Isa Cristina Teixeira Santos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

009. Joana Costa Vilhena de Bessa Campos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

010. Mafalda Rangel Malheiro Dias de Oliveira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

011. Maria Helena Trindade de Abreu (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

012. Mariana Dias Almeida (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

013. Marisa Sárria Pereira de Passos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

014. Marta Tibaldo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

015. Rita Micaela dos Santos Fernandes de Araujo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)

016. Raquel Andreia Pinheiro Vieira (Cat.: Outra, Gr. Acad.: Licenciatura)

## 6c. Objectives & achievements

### 1. Objectives (2000 ca.)

The general research objectives of the group are the study of biodiversity of marine ecosystems, the promotion of their conservation and the development of sustainable use of their resources. Within this framework our specific objectives are to:

- determine the patterns of distribution and abundance of species on rocky shore communities and their variation at different spatial and temporal scales.
- develop and promote environmental monitoring schemes/long term data acquisition on biodiversity and habitats and build and populate databases to be able to detect long term biodiversity changes.
- determine the effects of different stressors on coastal biodiversity, detected from the level of individual physiological responses to the functional effects at the ecosystem level, and using different approaches: from observational studies to demographic modelling and experimental manipulations of assemblages. This includes a particular focus on the study of fish reproduction and behaviour, sexual selection and the evolution of mating systems, and the use of animal behaviour as a proxy for detecting xenobiotic contamination.
- develop Integrated Multitrophic Aquaculture systems (IMTAs), in collaboration with existing commercial fish farms, by integrating the cultivation of algae and bacteria, that have been previously isolated and characterized by the group, to produce economically valuable biomass and improve water quality.
- investigate the bioactive secondary metabolites produced by different organisms, including marine sponges, and to evaluate the in vitro anticancer and antifungal activities of these metabolites.
- develop new products from marine organisms, and increase the value of species used for improve water quality (IMTA systems) or discard organisms.

Besides these research objectives we also aim at:

- producing outreach programs and materials for the general public and school children.
- promoting research that is relevant to the conservation and sustainable use of marine biodiversity, and
- improve the effectiveness of communication and cooperation between researchers and policy makers to facilitate the design and implementation of knowledge based policies and action plans, at regional, National and European level.

### 2. Main achievements (2000 ca.)

1 - Regarding the study of biodiversity of marine ecosystems:

Implementation of an intensive sampling and fieldwork program, with observations, collection of species for reference and deployment of artificial substrates that resulted in:

New databases and checklists for Portuguese benthic species (21 new records of algae) and of changes in their distribution and abundance for the last 50 years

Detection of several invasive species, and evaluation of some for their invasiveness potential and impacts on benthic communities

Experimentally we were able to:

Demonstrate that spatial patterns are important in shaping the effects of diversity on productivity of macroalgal communities

Demonstrate significant and long lasting negative effects of human trampling on *Ascophyllum nodosum* communities.

Determine the main variables that cause or control sex-role reversal in different syngnathid species

Develop new methodologies to monitor natural pipefish populations to improve the study of sexual selection on these model organisms.

Determine the effect of some androgenic and estrogenic compounds in the life cycle and mating systems of fish species with contrasting life histories.

Much research was also done within Marbef and at an European scale (see Internationalisation)

2 - Regarding the sustainable use of marine resources we:

Developed and demonstrated the viability of a pilot IMTA system and optimised conditions for productivity and biofilter capacity for some native and economically valuable algal species, and clams.

Found that a dark treatment increased both yield and quality of carrageenan, making it a potential environmentally friendly alternative to the alkali treatment used by the industry.

Isolated and characterized a novel denitrifying bacteria, and evaluated its aerobic assimilatory and anoxic dissimilatory nitrate removal rates

Assessed the best substrate for fixed-film bioreactor development.

Isolated a number of novel compounds with interesting chemical structures from the marine sponges, fungal cultures and higher plant extracts.

Used NMR techniques to elucidate the structures of the new compounds and to understand the nature of some metabolites present in marine organisms.

Showed that some of these compounds have in vitro anticancer activity and elucidated some of these mechanisms.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Cardoso, JFMF, Langlet, D, Loff, JF, Martins, AR, Witte, JIJ, Santos, PT, van der Veer, HW. 2007. Spatial variability in growth and reproduction of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) along the west European coast. *Journal of Sea Research* 57(4): 303-315. IF 2007=2.204, n°C=3.
2. Silva, K, Vieira, MN, Almada, VC, Monteiro, NM. 2007 . The effect of temperature on mate preferences and female-female interactions in *Syngnathus abaster* . *Animal Behaviour* 74(5), pp. 1525-1533. IF 2007=2.752, n°C=1
3. Matos, J, Costa, S, Rodrigues, A, Pereira, R, Sousa Pinto, I. 2006. Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. *Aquaculture* 252(1): 31-42. IF 2006/2007=2.081/1.735, n°C=7.
4. Arenas, F, Sánchez, I, Hawkins, SJ, Jenkins, SR. 2006. The invasibility of marine algal assemblages: Role of functional diversity and identity. *Ecology* 87(11): 2851-2861. IF 2006/2007=4.782/4.822, n°C=6.
5. Pereira, R, Yarish, C, Sousa-Pinto, I. 2006. The influence of stocking density, light and temperature on the growth, production and nutrient removal capacity of *Porphyra dioica* (Bangiales, Rhodophyta). *Aquaculture* 252(1): 66-78. IF 2006/2007=2.081/1.735, n°C=5.
6. Francisco, SM, Vieira, MN, Almada, VC. 2006. Genetic structure and historical demography of the shanny *Lipophrys pholis* in the Portuguese coast based on mitochondrial DNA analysis. *Molecular Phylogenetics and Evolution* 39(1): 288-292. IF 2006/2007=3.528/3.994, n°C=0.
7. Cabral, JP, Natal Jorge, RM. 2007. Compressibility and shell failure in the European Atlantic *Patella* limpets. *Marine Biology* 150(4): 585-597. IF 2007=2.215, n°C=0.
8. Kijjoa, A, Wattanadilok, R, Campos, N, Nascimento, MSJ, Pinto, M, Herz, W. 2007. Anticancer Activity Evaluation of Kuanoniamines A and C isolated from the Marine Sponge *Oceanapia sagittaria*, Collected from the Gulf of Thailand. *Marine Drugs* 5: 6-22. IF 2007=1.103, n°C=1.
9. Vieira, LMM, Kijjoa, A. 2005. Naturally-occurring xanthenes: Recent developments. *Current Medicinal Chemistry* 12(21): 2413-2446. IF 2005/2007=4.904/ 4.944, n°C=9.
10. Castro, M, Santos, MM, Monteiro, NM, Vieira, N. 2004. Measuring lysosomal stability as an effective tool for marine coastal environmental monitoring. *Marine Environmental Research* 58(2-5): 741-745. IF 2005/2007=2.055/, n°C=10.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Kijjoo, A, Watanadilok, R, Sonchaeng, P, Puchakarn, S, Sawangwong, P, Herz, P. 2004. Bromotyrosine Derivatives from the Marine Sponge *Suberea* aff. *praetensa*. In: *Sponge Science in the Millennium*. Pansini, M, Pronzato, R, Bavestrello, G, Manconi, M (Eds.). *Bullettino del Musei e degli Istituti Biologici dell'Università di Génova*. Vol. 68: 391-397.
2. Sousa-Pinto, I, Muessner, R. 2004. EPBRS and the National Platforms for Biodiversity: Interactions, Synergies and the role of Bioplatform. In: *Romanian Biodiversity Research*. Mihailescu, S, Falca, M (Eds). Romanian Academy of Sciences, Bucharest, pp. 11-17.
3. Muessner, R, Sousa Pinto, I. 2005. How to halt biodiversity loss? The need for science policy interfaces. In: *Romanian Biodiversity Research*. Mihailescu, S, Falca, M (Eds). Romanian Academy of Sciences, Bucharest, Vol. II, pp. 11-16.
4. Sousa-Pinto, I, Araújo, R. 2006. Seaweed resources of Portugal. In *Seaweed Resources of the World*. Ohno & Critchley (Eds), JICA, Yokosuka.
5. Borges, M-T. 2006. Bacterial biofiltration studies and integrated system development for effluent treatment and water reuse in intensive aquaculture. *Proceedings 3rd International Water Conference, ISEP-IPP, Porto, Portugal*, pp. 505-511.
6. Yarish, C, Pereira, R. 2008. Mass Production of Marine Macroalgae. In: *Ecological Engineering*. Sven Erik Jørgensen and Brian D. Fath (Editor-in-Chief), Vol. [3] of *Encyclopedia of Ecology*, 5 vols, Oxford: Elsevier, pp. 2236-2247.
7. Young, J, Sousa Pinto, I, Hawkins, S, Serrão Santos, R, Watt, AD (Eds.). 2007. Life on the Blue Planet: Biodiversity research and the new European marine policies. Report of an e-conference.
8. Sousa-Pinto, I, Araújo, R. 2005. Seaweeds from Portugal: past and present description, research and use of this natural resource. CD rom (ed *Seaweed Africa*).
9. Anne, I, Fidalgo, ML, Thosthrup, L, Christoffersen, K. 2006. Influence of filtration and glucose amendment on bacterial growth rate at different tidal conditions in the river Minho estuary (North West of Portugal). *Limnetica* 25(3-4): 25-34.
10. Casimiro, S, Fidalgo, ML. 2007. Performance of the freshwater shrimp *Atyaephyra desmarestii* as indicator of stress imposed by textile effluents. *Web Ecol.* 7: 35-39.

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**4. Master and Ph.D. thesis completed** (3000 ca.)

**PhD THESIS**

Anne, I. 2003. Caracterização de duas Estações de Tratamento de Águas. Supervisors: L Fidalgo, W Tobias (RFA).

Wattanadilok, R. 2003. Bioactive Secondary Metabolites from Marine Sponges. Supervisors: A Kijjoa, P Sonchaeng (Burapha Univ., Thailand)

Pereira, R. 2004. Ecophysiology and Biochemistry of Porphyr. U. Porto. Supervisors: I Sousa Pinto and C Yarish (US)

Monteiro, N. 2005. Biologia de *Nerophis lumbriciformis* (Piscis, Syngnathidae): Uma perspectiva comparada. Supervisors: MN Vieira, V Almada (ISPA)

Jiamjit, O. 2007. Diversity and distribution of Caprophilous fungi from wildlife and domestic animals. Supervisors: A Kijjoa, L Manoch (Kasetsart Univ.)

Dethoup, T. 2007. Diversity and distribution of *Talaromyces* species and their potential uses as biological control. Supervisors: A Kijjoa, L Manoch (Kasetsart Univ.)

Silva, C. 2008. Reproductive ecology of the Mildly sex-role reversed pipefish, *Syngnathus abaster*. Supervisors: MN Vieira, NM Monteiro, V Almada (ISPA)

## **MSc THESIS**

Ferreiro, N. 2007. Caracterização da qualidade ecológica do Rio Tua. Supervisor: MN Vieira

Gonçalves, T. 2007. A barrinha de Esmoriz como recurso educativo. Supervisor: P Santos

Lansu, N. 2007. The relationship between native & invasive algae in rock pools. Co-supervisors: F Arenas, MP Berg (Vrije Universiteit Amsterdam)

Nunes, J. 2007. O Sítio da rede Natura «Valongo» como recurso educativo. Contributos para a formação de professores. Supervisor: P Santos

Rodrigues, C. 2007. Estudo da comunidade de diatomáceas de índices para a avaliação da qualidade da água de algumas ribeiras da ilha da Madeira. Supervisor: MN Vieira.

Silva, S. 2007. Bioremediação em águas residuais: remoção de fosfatos utilizando microalgas *Chlorella vulgaris* imobilizadas em meio de alginato de sódio. Supervisor: MN Vieira

Fonseca, H. 2006. In vitro studies of azo dyes cytotoxicity using *Tetrahymena pyriformis*. Co-supervisor: N Lima (Univ. Minho)

Coelho, C. 2005. Caracterização da ictiofauna do estuário do Rio Cávado, com particular incidência na fase juvenil. FCUP. Supervisor: P Santos

Santos, C. 2005. Efeitos Tóxicos da exposição de *Danio rerio* ao efluente bruto de uma ETAR. FCUP.

Valente, M. 2005. O conceito de Recurso Biológico nas orientações do ensino básico. Supervisor: P Santos

Andrade, R. 2004. Efeitos da exposição de peixe zebra, *Danio rerio*, a um efluente têxtil. FCUP.



Araújo, R. 2004. Estudo das comunidades de macroalgas bentónicas dos sistemas rochosos intertidais da região do Minho. Co-supervisors: Quintino V (CESAM), Barbara I (U. Corunha, Spain)

Costa, N. 2004. Influência de bactérias halófilas na dinâmica populacional de Artemia. Supervisor: MN Vieira

Pimentel, N. 2004. Avaliação do estado trófico da Albufeira de Crestuma-Lever. FCUP. Supervisor: MN Vieira

Rodrigues, A. 2004. Potencialidades de duas espécies de macroalgas marinhas para aquacultura integrada. Supervisor: I Sousa-Pinto

Santos, E. 2004. Avaliação da qualidade da água da zona costeira portuguesa: Estudo da resposta lisossomática em hemócitos de *Mytilus galloprovincialis*. Supervisor: MN Vieira

Sousa, A. 2004. Identification, characterization and evaluation of denitrifying capacity of marine bacteria isolated from a biological filter. Co-supervisor: P Castro (ESB-UCP)

Souza, P. 2004. Toxicidade do Efluente Final de uma Refinaria de Petróleo em *Danio rerio*. FCUP.

Silva, M. 2003. A evolução do estado trófico da Albufeira do Rio Sôrdo (Vila Real) - Indicadores Biológicos. Supervisor: MN Vieira.

## **5. Patents/propotypes (2000 ca.)**

We produced, developed and tested a prototype of a Integrated Multitrophic Aquaculture System (IMTA) system with the collaboration of the Aquaculture company Coelho e Castro and in the framework of two EC projects: SEAPURA and Raceways. This system produces fish, algae that are grown in the effluent of the fish tanks and take out excess nutrients and CO<sub>2</sub> and produce O<sub>2</sub>, and clams that filter the organic particles in suspension. These aquaculture may become systems without waste and environmentally friendly, reducing the impacts of aquaculture.

In the framework of the EC project SEABEE. we participated in the development and test of a prototype of an autonomous underwater vehicle (AUV) built to be used in the monitoring of coastal zone ecosystems and environment.

## **6. Organization of conferences (2000 ca.)**

Isabel Sousa Pinto has been member of the International Organising Committee of 10 of the Meetings of the European Platform for Biodiversity Research Strategy (EPBRS) that are organized with every Presidency of the EU since the Portuguese Presidency of 2000.

She was also the chair of the organizing Committee of the EPBRS meeting under the Portuguese Presidency of the EU in 2007 under the theme: “Life on the Blue Planet: Marine Biodiversity and the New EU Marine Policies” held in Porto, 6 to 9 of November 2007 with 107 participants from 28 countries, including representatives from the EEA, EC DGs Environment, Research and MARE, coordinators of all the relevant Networks of Excellence and ERA-NETS, and other projects as the IP Hermes and the Research Infrastructure Life Watch.

She also organised:

2005 - General meeting of the NoE Marbef: Marine Biodiversity and Ecosystem Functioning in Porto, with 105 participants from 27 countries.

2006 - Workshops “Best practice in managing biodiversity research and overcoming barriers to transnational cooperation” em Peterborough, UK with 28 participants of 16 countries and “Selecting thematic areas for collaboration in biodiversity research funding”, Porto, with 30 participants of 19 countries.

Was member of the Scientific Committee of the “1st European Congress of Conservation Biologists” in Eger, Hungary, 2006 with more that 1000 participants. She also co-organised and chaired of the Symposium “Conservation of Marine Biodiversity” and of the workshop – Managing Marine Biodiversity” in this congress. She is also member of the Scientific Committee of the “2nd European Congress of Conservation Biologists” to be held in Prague, September 2009.

Was a member of the Scientific Committee of the meeting “Neighbourhood for Sustainability: from Lisbon to Leipzig through research”, organised by the Federal Ministry of Education and Research of Germany, the Helmholtz Association, the Fraunhofer Gesellschaft, the Max Planck Society and the Leibniz Association” in the framework of the German Presidency of the EU held in Leipzig in 2007. She also organised and chaired a panel on the Sustainable Use of Marine Biodiversity.

Anake Kijjoa was a member of the organizing committee of the 5th European Conference on Marine Natural Products (V ECMNP) which took place in Ischia, Italy, from 16-21 September 2007.

## **7. Industry contract research (2000 ca.)**

## **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

- Santos, P. POOC S. Miguel Costa Sul. Participação na Caracterização e diagnóstico da zona costeira, CENARIZAÇÃO, regulamento, plano de intervenções. Quaternaire Portugal.
- Santos, P. PROT Açores. Participação na Caracterização, diagnóstico e CENARIZAÇÃO. Quaternaire Portugal.
- Santos, P. 2007. Plano de Gestão da Tapada Nacional de Mafra. Participação na Caracterização e diagnóstico, CENARIZAÇÃO e plano de intervenções. Quaternaire Portugal.
- Fidalgo, ML, Vieira, N, Valente, A, Martins, AS, Casimiro, S. Caracterização hidrográfica e de qualidade da água nas ribeiras da costa entre o Rio Minho e a Barrinha de Esmoriz. Qualidade ecológica. Relatório final do Projecto “Caracterização hidrográfica e de qualidade nas ribeiras da costa entre o Rio Minho e a Barrinha de Esmoriz” (Projecto financiado pela Comissão de Coordenação e Desenvolvimento da Região Norte).

- Reports from EPBRS meetings with recommendations for research topics and strategy (from 2000 to 2008) Bioplatform, Bistrat and EPBRS.
- Sousa Pinto, I. 2005. Report on the funding of Biodiversity Research in Portugal. For FCT and Biodiversa.
- Sousa Pinto, I, Marhadour, A. 2007. Report on Research of marine Biodiversity in Portugal. For Biostrat and EPBRS.
- Sousa Pinto, I, Marhadour, A, Pereira, R. 2007 Report on Research of marine Biodiversity in Europe. For Biostrat and EPBRS.
- Sousa Pinto, I. 2006. Report on the results of the 8th Conference of the Parties of the Convention for Biological Diversity in Curitiba, Brazil. For FCT and MCTES (Ministry of Sciences, Technology and Higher Education).
- Sousa Pinto, I. 2008. Report on the results of the 9th Conference of the Parties of the Convention for Biological Diversity in Bonn, Germany. For FCT and MCTES.

**9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

Our group has been working extensively with colleagues from Europe and around the world. In the last five years we have been funded by 9 EC projects, having coordinated one of them (Bioplatform) with 28 partners from 28 countries, and being part of the Steering , executive or management Committees of Biostrat, (32 countries), NoE Marbef with 56 partners and Era-Net Biodiversa,. We have also participate in ENCORA, an European coastal network, and in Naguisa (CoML) an international project, and several bi-lateral projects with Spain, Brazil and Thailand, that often include co-advisorship of students. We also participate in the Erasmus Mundus MSC in Marine Biodiversity and Conservation

So around 50% of the c 110 publications in peer review journals with IF produced by our group in the last 5 years have been in collaboration with colleagues from other countries.

Within the different European and International research projects and networks we: Participated in several European scale experiments: e-g- on effects of biodiversity on the functioning and stability of marine ecosystems, or to identify the intensity, timing, and temporal extent of production and settlement of pelagic propagules of benthic organisms at a Pan-European scale. Experiments are finished and data is now being assembled and analysed.

Developed an existing world database of seaweeds (ALGABASE)

Developed and tested a prototype of a new AUV for coastal monitoring

Participated in the work of Biodiversa: Era-Net on Biodiversity, helping to frame the research priorities of its first call (23 million Euros)

Furthermore I Sousa Pinto was/is member of the:

Editorial Board of the new Journal of Marine Biodiversity, Springer Verlag.

- Advisory Group from the EEA 's "State of the Environment Report 2005"
- Working group for the revision of "Biodiversity Action Plan – Fisheries" coordinated by DG Environment and DG Fisheries
- Study Group on Biodiversity Science (SG BioDiv) from ICES.
- Steering Committee of EPBRS (European Platform for Biodiversity Research Strategy) and of the International Program "Census of Marine Life (CoML)" and the "2020 Science Council" to plan the continuation of this program after 2010.
- Board of Directors of the "Society for Conservation Biology" Europe Executive Board of MARS – European Network of Marine Research Institutes and Stations.

## 6e. Future research

### 1. Objectives (3000 ca.)

The first objective is to increase the synergies between us and the six new researchers that are being hired to strengthen our research on biodiversity. The general research objectives of the group will continue to be the study of different aspects of marine biodiversity and its role on coastal aquatic ecosystems, and the promotion of the sustainable use of marine resources. We will further integrate our research with other groups at national and international level, to be able to do more interdisciplinary research. We will also increase our inputs for initiatives as international databases (Algalbase, GBIF, OBIS), international research programs (CoML and its successor 2020) and national initiatives as the network for Marine and Coastal Biodiversity that we founded in 2007.

The integration of two new members with modelling capabilities will allow developing and implementing hydrodynamic and biogeochemical models, to integrate knowledge on coastal ecosystems and develop decision support systems that can be used as management tools to answer questions as:

What are the net contributions of our coastal ecosystems to the carbon biogeochemical cycle and how will this change as a result of global warming?

What is the carrying capacity of selected shore areas for the polyculture of macroalgae and bivalves?

We also aim to contribute with know how to the implementation of different environmental and marine policies e.g. the EU Water Framework Directive (pending project) and the Habitats Directive for the marine environment, as well as participating in the development of methodologies for implementation of other EU Directives, as the Marine Strategy Directive and ICZM. Within this framework we will work further on:

Patterns of distribution and abundance of benthic species, focusing more on population genetics and subtidal assemblages.

Effects of different stressors, including sedimentation, habitat modification (coastal constructions) and xenobiotics (e.g. in a new sentinel species for the Atlantic Coast, *Lipophrys pholis*).

Invasive species to evaluate the need for management actions and start a survey of ports and marinas to detect introduced species and evaluate their potential for becoming invasive.

Fish ecology and behaviour, studying the influence of climate change on the expression of sexual selection pressure and alteration of fish mating systems.

Assessment of toxicity effects of urban and industrial wastewaters using different test organisms and developing methodologies to rehabilitate degraded water bodies

Development of new products from marine organisms and IMTA systems including:

New products from algae, e.g. biofuels, biomedical compounds (pending proposals with 3Bs group) and as ingredient for fish feed (ongoing project).

Development of tools for rapid determination of water quality in hyperintensive aquaculture and improve water quality rehabilitation units.

Isolation and structure elucidation of the secondary metabolites of soil fungi and higher plants, and research on bioactive secondary metabolites from marine fungi including endophytic fungi from marine sponges and from mangroves.

Taxonomic identification and mass culture of selected fungi, bio-assayed guided isolation of secondary metabolites from cultures, isolation, purification and structure elucidation of the bioactive compounds.

Evaluation of the isolated compounds for their in vitro anticancer and antifungal activities and study of the underlying mechanisms.

**2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

- Plurianual

- FCT

50.000 EUR; LIMITS: Dinâmica populacional, distribuição geográfica e diversidade genética de espécies de algas com limite de distribuição sul na costa Portuguesa; 2005-2008.

97.853 EUR; Caracterização e Avaliação da Actividade Antitumoral de Compostos Bioactivos de Esponjas do Mar.

100.000 EUR; Effects of complex mixtures of antagonistic endocrine disrupting chemicals in two fish species with different life histories.

100.000 EUR; Variações em latitude da biologia de espécies-chave estuarinas como indicadores para a previsão de efeitos das alterações climáticas.

100.000 EUR; TAILOREDALGAE: optimização de propriedades funcionais das algas integradas em aquacultura de peixes em Portugal. Pending

• 6th Framework Program EC

106.882 EUR; MARBEF: Marine Biodiversity and Ecosystem Functioning - Network of Excellence. 6th Framework Program; 2004 to 2010.

151.000 EUR; RACEWAYS: a hyperintensive fish farming concept for lasting competitiveness and superior production; 2006-2008.

203.440 EUR; BIODIVERSA: EraNet on Biodiversity; 2006-2010.

148.500 EUR; BIOSTRAT: Developing the EU Biodiversity Research Strategy; 2006-2009.

• Other

4.000 EUR; Ecophysiological response of *Fucus serratus* to thermal stress at its southern distributional limit: implications in a warming up scenario; AI Luso-Espanhola; 2008-2009.

35.000 EUR; MOBIDIC: Projecto de Monitorização Costeira com escolas secundárias; Ciência Viva; 2006-2008.

10.000 EUR; Efeito da carcinocultura na qualidade da água no sistema lagunar da Guraraira\_RN; Cnpq, UFRN Brasil; 2006-2008.

130.000 EUR; Avaliação de possíveis impactos no Ecossistema costeiro, da actividade de extracção de inertes na plataforma continental; 2007-2009.

404.400 EUR; Projects pending from QREN funds.

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Duarte, P, Labarta, U, Fernández-Reiriz, MJ. 2008. Modelling local food depletion effects in mussel rafts of Galician Rias. *Aquaculture* 274(2-4): 300-312. IF 2007=1.735.

2. Viejo, RM, Arenas, F, Fernández, C, Gómez, M. 2008. Mechanisms of succession along the emersion gradient in intertidal rocky shore assemblages. *Oikos* 117(3): 376-389. IF 2007=3.136.

3. Silva, K, Vieira, MN, Almada, VC, Monteiro, NM. 2008. Can the limited marsupium space be a limiting factor for *Syngnathus abaster* females? Insights from a population with size-assortative mating. *Journal of Animal Ecology* 77(2): 390-394. IF 2007= 3.747, n°C=0.

4. Borges, M-T, Sousa, A, De Marco, P, Matos, A, Höningová, P, Castro, PML. 2008. Aerobic and anoxic growth and nitrate removal capacity of a marine denitrifying bacterium isolated from a recirculation aquaculture system. *Microbial Ecology* 55(1): 107-118. IF 2007=2.558, n°C=0.

5. Araújo, R, Vaselli, S, Almeida, M, Serrão, E, Sousa-Pinto, I. (in press). Disturbance on marginal populations: effects of human trampling on *Ascomyllum nodosum* assemblages at its southern distribution limit. *Marine Ecology Progress Series*. IF 2007=2.546.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

We are in the process of hiring 6 new researchers assistant (post-doc level) to strengthen our group and to develop our research program. We should have 1 modeler (and another researcher that integrated our group this year), 2 geneticists, 2 ecologists and 1 chemist of natural products. Besides that, the other new resources we need

1. Laboratory technician(s) to help with some of the routine analysis as: nutrient, bioactive compounds, and that could be responsible for some of the equipment that was recently bought: e.g. an autoanalyser.
2. A laboratory unit for carrying out biological activity evaluation.
3. A laboratory unit for DNA extraction and PCR
4. Access to a boat for diving

We will also strengthen our capacity for computers and GIS programs in collaboration with other groups.



## **Oceanic and Coastal Dynamics**

### **6a. Group description**

#### **1. Group name / denomination**

**Oceanic and Coastal Dynamics**

#### **2. Principal investigator**

Maria Luisa Machado Cerqueira Bastos

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Coastal Monitoring, Oceanography, Satellite techniques, Sea level

#### **5. Funding, source, dates (1000 ca.)**

- Northwest Iberian Coastal Current – NICC (POCTI/CTA/49563/2002): Base study of the NICC system and its role in the dynamics of the NW Iberian shelf area. Funding FCT, total/CIIMAR budget: 80.000/20.444 EUR; 01/02/2005–31/07/2008.

- Estuarine Contributions to Inner Shelf Dynamics – ECOIS (POCTI/CTA/48461/2002): Variability of river runoff its effect on Douro and Minho plumes and coastal dynamics. Funding FCT; total/CIIMAR: 80.000/7.222 EUR; 01/08/2005–31/08/2008.

- Study the Portuguese Oceanic Coastal zone Using remote Sensing data – POCUS (PDCTE/CTA/50388/2003): Space/time characterization of ocean circulation patterns, temperature and colour with ground-truth and satellite image data. Funding FCT, total/CIIMAR: 60.000/4.480 EUR; 01/05/2005–31/10/2008.

- River Douro Estuarine Contributions to Inner Shelf Dynamics: Data collection in Douro and Minho for estuarine characterization. Funding APDL, total/CIIMAR: 77.000/77.000 EUR; 01/05/2005–31/07/2008.

- Global Loading and Deformation at Tidal Timescales (NE/C003438/1). Funding Co-I, NERC, total/CIIMAR: 161.200 EUR/travel and meeting costs; 2006–2009.

- Pluriannual 26.640 EUR

### **6b. Group team**

#### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Maria Luisa Machado Cerqueira Bastos (Cat.: Investigador Principal, Gr. Acad.: Doutoramento)

002. Ana Maria Ferreira Bio (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)
003. Isabel Gonçalves de Barbosa Araújo (Cat.: Ciência 2007, Gr. Acad.: Doutoramento)
004. Machiel Simon Bos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
005. Paulo Renato Enes Baganha Baptista (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
006. Rui Miguel Andrade Caldeira (Cat.: Ciência 2007, Gr. Acad.: Doutoramento)

## **2. Other researchers in the group** (Include here collaborators with PhD. only)

001. Helena Maria Leite Pato Granja (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

## **3. Other researchers in the group** (non PhD.)

001. João Paulo Ramalho Marreiros (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
002. Richard Deurloo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
003. Ana Isabel Gaspar Meirinho (Cat.: Outra, Gr. Acad.: Licenciatura)
004. Joana Sousa Rodrigues (Cat.: Outra, Gr. Acad.: Licenciatura)
005. Marlene Cristina da Silva Antunes (Cat.: Outra, Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives** (2000 ca.)

The group's main objectives have been concerned with monitoring coastal and estuarine dynamics, which is of utmost importance for model development in support of an integrated coastal zone management (ICZM). With the recent enlargement of the group, with experts in oceanography, these objectives broadened towards understanding of processes that occur at the continental platform.

The main objectives are: the study of estuarine and coastal processes, in particular on the Portuguese northwest coast, through regular monitoring and characterization of coastal dynamics, at a local and regional scale; assessment of river-ocean processes, in relation and interaction with coastal circulation, particularly the transfer processes between estuaries and the shelf, contributing for the design of model constraints and model output validations; and, morphodynamic characterization of sandy littorals. Technically the group focuses on the exploitation of different in-situ data acquisition methodologies, such as DGPS kinematic surveys and GPS/GSM equipped buoys (drifters) for the evaluation of estuarine and coastal dynamics. Resulting local data are integrated with satellite information, e.g. altimetry data, for the study of ocean dynamics.

Furthermore, the group cooperates with other CIMAR groups, working in estuarine, coastal and ocean systems, assessing the needs for oceanographic information within the LA and preparing oceanographic information and other data (e.g. processed remote sensing data). The group is

further involved in setting up LA-related databases, provides support to researchers and students in data-analysis and modelling issues, participates in MSc courses and in the (co )orientation of MSc and PhD students.

## **2. Main achievements (2000 ca.)**

Main achievements were:

- Construction of a reference frame for the estuarine and costal dynamics of the NW Iberian region, for a better understanding of exchange processes with the continental platform and for the parameterization and validation of circulation models; a proper understanding of a shelf process is a prerequisite of an operational system of coastal observations and forecast.
- Contributions to the clarification of the roles of buoyancy and wind in the North-West Iberian Coastal Current forcing, as well as identification of typical space and time scales.
- Application of DGPS monitoring techniques for the characterization of the morphodynamic evolution of coastal sectors. Particularly sensitive littoral sectors were selected as study areas, including the Douro river sand spit, the Aguda-Granja littoral beach and Costa-Nova to Mira beaches. Sedimentary analyses of samples were carried out to complement morphodynamical characterization.

These activities are relevant to assess the impact of natural and anthropic factors on coastal erosion, e.g. the impact of river run-off variations and of man made constructions in the study areas, and essential for the understanding of ongoing processes and for the design of new circulation models setting model constraints and allowing validation through in-situ measurements.

Other achievements include:

- Implementation of the computer program CARGA allowing state of the art ocean tide loading and atmospheric loading computations.
- Development of a new method to analyze temporal correlated time-series, such as GPS data, that is one order of magnitude faster than those previously available.
- Compilation of (mostly freely) available satellite oceanographic data, mainly Ocean Temperature and Ocean Colour, to support the characterisation of the Atlantic ocean surrounding the Iberian Peninsula. This database is intended to support several ongoing projects of the group (NICC, ECOIS, POCUS) as well as other CIMAR projects.
- Setting up of the «divMar–Rede de Biodiversidade Marinha e Costeira» expertise and publications database.
- Initiating the CIMAR-LA geographical metadata and database, to integrate information and data available at the LA in a system that is easy to assess and query.
- Development of drifter prototypes with GPS/GSM and data storage module.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Baptista, P, Bastos, L, Bernardes, C, Cunha, T, Dias, J. 2007. Monitoring sandy shores morphologies by DGPS – a practical tool to generate digital elevation models. *Journal of Coastal Research*. DOI: 10.2112/07-0861. IF 2007=0.883, n°C=0.

2. Fernandes, RMS, Miranda, JM, Meijninger, BML, Bos, MS, Noomen, R, Bastos, L, Ambrosius, BAC, Riva, REM. 2007. Surface velocity field of the Ibero-Maghrebian segment of the Eurasia-Nubia plate boundary. *Geophysical Journal International* 169(1): 315-324. IF 2007=2.112, n°C=1.

3. Combrink, AZA, Bos, MS, Fernandes, RMS, Combrinck, WL, Merry, CL. 2007. On the importance of proper noise modelling for long-term precipitable water vapour trend estimation. *South African Journal of Geology* 110: 211-218. IF 2007=0.602, n°C=0.

4. Soares de Carvalho, G, Granja, HM, Loureiro, E, Henriques, R. 2006. Late Pleistocene and Holocene environmental changes in the coastal zone of Northwestern Portugal. *Journal of Quaternary Science* 21(8): 859-877. IF 2006/2007=1.906/2.496, n°C=0.

5. Garcia-Amorena, I, Gómez Manzanque, F, Rubiales, JM, Granja, HM, Soares de Carvalho, G, Morla, C. 2007. The Late Quaternary coastal forests of western Iberia: A study of their macroremains. *Palaeogeography, Palaeoclimatology, Palaeoecology* 254(3-4): 448-461. IF 2007=2.162, n°C=0.

6. Fernandes, RMS, Bastos, L, Miranda, JM, Lourenço, N, Ambrosius, BAC, Noomen, R, Bastos, L, Noomen, R, Simons, W. 2006. Defining the Plate Boundaries in the Azores Region. *Journal of Volcanology and Geothermal Research* 156(1-2): 1-9. DOI:10.1016/j.jvolgeores.2006.03.019. IF 2006/2007=1.685/1.742, n°C=2.

7. Bos, MS, Baker, TF. 2005. An estimation of the errors in the gravity ocean tide loading computations. *Journal of Geodesy* 79: 50-63. DOI: 10.1007/s00190-005-0442-5. IF 2005/2007=1.205/1.636, n°C=4.

8. Fernandes, RMS, Ambrosius, BAC, Noomen, R, Bastos, L, Combrinck, L, Miranda, JM, Spakman, W. 2004. Angular velocities of Nubia and Somalia from continuous GPS data: implications on present-day relative kinematics. *Earth and Planetary Science Letters* 222: 197-208. DOI: 10.1016/j.epsl. IF 2004/2007=3.434/3.873, n°C=23.

9. Fernandes, RMS, Bastos, L, Ambrosius, BAC, Noomen, R, Matheussen, S, Baptista, P. 2004. Recent geodetic results in the Azores Triple Junction region. *Pure and Applied Geophysics (PAGEOPH)* 161(3): 683–699. DOI:10.1007/s00024–003–2469–y. IF 2004/2007=0.840/0.860, n°C=2.

10. Fernandes, RMS, Ambrosius, BAC, Noomen, R, Bastos, L, Wortel, MJR, Spakman, W, Govers, R. 2003. The relative motion between Africa and Eurasia as derived from ITRF2000 and GPS data. *Geophysical Research Letters* 30(16): 1828. DOI:10.1029/2003GL017089. IF 2003/2007=2.422/2.744, n°C=18.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Soares de Carvalho, G, Granja, HM. 2005. Dinâmica costeira e as praias entre o rio Minho e o rio Lima. In: 1as Jornadas de História, Ambiente e Urbanismo de Vila Praia de Âncora. Editado pela Junta de Freguesia de Vila Praia de Âncora, pp. 297-306. ISBN 972-95151-2-3.
2. Bos, MS, Lázaro, C, Bastos, L. 2007. Analysis of the gravity campaigns made in Faial, Azores. In: Acta da V Conferência Nacional de Cartografia e Geodesia. Casaca, J, Matos, J (Eds), Lidel, pp. 341-348. ISBN 978-972-757-478-0.
3. Ferreira, AR, Lázaro, C, Fernandes, MJ, Bio, A, Sobral, MC, Bastos, L, Gonçalves, JA. 2007. Base de Dados de Informação de Satélite para a Zona do Atlântico Nordeste. In: Acta da V Conferência Nacional de Cartografia e Geodesia. Casaca, J, Matos, J (Eds), Lidel, pp. 331-340. ISBN 978-972-757-478-0.
4. Baptista, P, Bastos, L, Bernardes, C, Veloso-Gomes, F. 2006. Evolução do litoral da Praia da Aguda (NW de Portugal) após a construção de um quebramar destacado. Acta do 5º Simpósio sobre a Margem Ibérica Atlântica, Aveiro, Portugal.
5. Bos, MS, Bastos, L, Fernandes, RMS. 2005. Atmospheric Loading at Portuguese GPS Stations. In: Acta da IV Conferência Nacional de Cartografia e Geodesia. Casaca, J, Matos, J. (Eds), Lidel, pp. 487-494. ISBN 978-972-757-478-0.
6. Baptista, P, Bastos, L, Veloso-Gomes, F. 2004. Impacte Morfodinâmico duma Obra de Protecção Costeira: O Quebra-mar da Aguda. Acta da 4ª Assembleia Luso-Espanhola de Geodesia e Geofísica, Figueira da Foz, Portugal, pp. 27-28.
7. Fernandes, RMS, Bastos, L, Miranda, JM, Ambrosius, BAC, Lourenço, N, Noomen, R, Baptista, P. 2004. Análise dos movimentos tectónicos no Arquipélago dos Açores usando observações GPS. In: Acta da III Conferência Nacional de Cartografia e Geodesia, Aveiro. Bastos, L, Matos, J (Eds), Lidel. ISBN 972-757-330-4.
8. Baptista, P, Bastos, L, Bernardes, C, Dias, A. 2004. Monitorização de litorais arenosos. In: Acta da III Conferência Nacional de Cartografia e Geodesia, Aveiro. Bastos L, Matos, J (Eds), Lidel. ISBN 972-757-330-4.
9. Bastos, L, Tomé, P, Cunha, T, Fernandes, MJ, Cunha, S. 2004. Avaliação do desempenho de um sistema GPS/INS para gravimetria aérea. In: Acta da III Conferência Nacional de Cartografia e Geodesia, Aveiro. Bastos, L, Matos, J (Eds), Lidel. ISBN 972-757-330-4.
10. Baptista, P, Bastos, L, Veloso-Gomes, F. 2004. Impacte Morfodinâmico de uma Obra de Protecção Costeira: O Quebra-mar da Aguda. 4ª Assembleia Luso-Espanhola de Geodesia e Geofísica. Figueira da Foz, Fevereiro 2004.

**3. Other publications international (3000 ca.)** (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Lomba, A, Vicente, J, Granja, HM, Santos, A, Fidalgo, F, Caldas, FB, Honrado, J. 2006. Towards an interdisciplinary methodology for the indication of stability conditions in coastal areas. Littoral 2006, Gdansk, Poland.
2. Baptista, P, Bastos, L, Granja, HM, Gama, C, Bernardes, C. 2007. Methodological approaches to shoreline assessment: short-term analysis of beach erosion rates. Proceedings of 8th International Symposium on GIS and Computer Mapping for Coastal Zone Management, Vol.2, pp. 187-188, Santander, Spain.
3. Ruiz, AM, Sousa, JJ, Hanssen, RF, Perski, Z, Bastos, L, Gil, AJ. 2007. Deformation in the Granada Basin (Southern Betic Cordillera) studied by PS-InSAR. Proceedings of the Envisat Symposium 2007, SP-636, N° 461440. 23-27 April 2007, Montreux, Switzerland.
4. Bos, MS, Fernandes, RMS, Bastos, L. 2006. (Mis)use of the BLQ-file for Atmospheric Loading modeling. Proceedings of the XIII Assembly of Wegener, Nice, France, 4-7 September 2006.
5. Fernandes, RMS, Miranda, JM, Bastos, L. 2006. Analysis of the surface velocity field derived from continuous GPS data in the Ibero-Maghreb region. Proceedings of the V Assembleia Luso Espanhola de Geodesia e Geofísica, Sevilha, Spain, January 2006.
6. Madeira, S, Gonçalves, JA, Bastos, L. 2006. Calibration and orientation problems in a Video-GPS-INS surveying system. Proceedings of the International Calibration and Orientation Workshop – EuroCOW, Castelldefels, Spain, 25-27 January 2006.
7. Marchenko, D, Meyer, U, Bastos, L. 2004. Airborne gravity disturbances in sequential multipole analysis for geoid determination and its test over the Azores. Bollettino di Geodesia e Scienze Affini, N° 2, June.
8. Cunha, S, Bastos, L, Tomé, P, Cunha, T. 2003. On the Integration of Inertial and GPS Data with an Odometer for Land Vehicles Navigation. Proceedings of ION GPS 2003, Portland, USA, September 2003.
9. Baptista, P, Bastos, L, Jesus, ME, Correia, E. 2003. Morphodynamic evolution of the sand-spit in the mouth of Douro river through DGPS monitorization. Proceedings of 3 IAHR Symposium on River, Coastal and Estuarine Morphodynamics (RCEM 2003), pp. 1060-1067, Barcelona, Spain, September 2003.
10. Baptista, P, Bastos, L, Cunha, T, Bernardes, C, Dias, J. 2003. Alongshore characterization of the intertidal zone using DGPS observations: the Aveiro lagoon case. IV Simposio sobre a Margem Ibérica Atlântica. Special Volume of Thalassas. An International Journal of Marine Sciences, pp. 139-140, Vigo, July 2003.

**4. Master and Ph.D. thesis completed (3000 ca.)**

**PhD THESIS**

Madeira, Sérgio dos Reis. 2007. Sistema Móvel Terrestre de Levantamento com Integração em SIG. PhD, Department of Applied Mathematics, Faculty of Science, University of Porto. Supervisor: José Alberto Gonçalves (FCUP/CICGEO), Co-supervisor: L Bastos (FCUP/CIIMAR).

Henriques, Renato Filipe. 2007. A monitorização da zona costeira tendo em vista a sua vulnerabilidade (aplicação à zona costeira noroeste de Portugal). PhD, School of Sciences, University of Minho. Supervisor: H Granja (UM).

Baptista, Paulo Renato. 2006. Utilização de técnicas espaciais para monitorização de litorais arenosos e modelação morfodinâmica. Department of Geosciences, University of Aveiro. Supervisor: C Bernardes (UA), Co-supervisor: L Bastos (FCUP/CIIMAR).

## **MSc THESIS**

Antunes, Marlene Cristina. 2005. Construção de um SIG da zona de maré com imagens de grande resolução obtidas a partir de avião. Master, Department of Applied Mathematics, Faculty of Science, University of Porto. Supervisor: JA Gonçalves (FCUP/CICGEO), Co-supervisor: L Bastos (FCUP/CIIMAR).

Meirinho, Ana Isabel. 2007. Distribuição de alcatraz (*Morus bassanus*) na costa continental portuguesa e sua relação com variáveis ambientais. Master, SPEA – Sociedade Portuguesa para o Estudo das Aves. Supervisor: A Bio (CIIMAR), Co-supervisor: JG Ferreira (FCT/UNL).

### **5. Patents/prototypes (2000 ca.)**

### **6. Organization of conferences (2000 ca.)**

Organization of the Datação e reconstrução de paleoambientes em zonas graníticas através de critérios paleontológicos, morfológicos e isotópicos, CEER (Centro de Estudos Eurorregionais Galiza/ Norte de Portugal) seminar, University of Minho, December 2004.

Organization of the seminar: Zona costeira – um olhar plural, University of Minho, supported by the Fulbright Commission and the Centro de Ciências da Terra, June 2006.

Organization of a workshop within the TIPGRAV project (FCT funded), at the Astronomical Observatory, Vila Nova de Gaia, 25-26 October 2007.

Organisation of the conference “Gravity, Geoid and Space Missions - GGSM 2004”, IAG International Symposium, Porto, Portugal. 30 August - 3 September 2004.

Memberships in the following Scientific Committees:

XI Encontro Nacional de Educação em Ciências, I Encontro de Educação para uma Nova Cultura da Água, Escola Superior de Educação, Instituto Politécnico do Porto, September 2005.

International Conference Iberian Coastal Holocene Paleoenvironmental Evolution, Coastal Hope 2005, Faculty of Sciences Ciências, University of Lisboa, July 2005.



International Conference on Nature restoration practices in European coastal habitats, Dunes and Estuaries 2005, Belgium; chair of the thematic workshop Shoreline Management.

International Conference Littoral 2006 – Coastal Innovation and Initiatives, September 2006, Gdansk, Poland.

XIIV Assembly of Wegener, Darmstadt, 15-18 September 2008.

6th Luso-Spanish Assembly for Geodesy and Geophysics, Tomar, Portugal, February 2008.

XIII Assembly of Wegener, Nice, France, September 2006.

5th Luso-Spanish Assembly for Geodesy and Geophysics, Seville, Spain, February 2006.

XII Assembly of Wegener, WEGENER 2004, Tangier, Morocco, September, 2004 (Member of the Scientific and Organizing Committees).

International Workshop Theory, Technology and Realities of Inertial / GPS Sensor Orientation, Barcelona, September 2003.

#### **7. Industry contract research (2000 ca.)**

A Protocol for Cooperation was signed between the APDL – Administração dos Portos do Douro e Leixões, S.A. and the CIMAR. This protocol established a cooperation to support data collection, i.e. observation campaigns in the Douro and Minho estuaries, for the characterization of estuarine dynamics. This protocol is related with the activities of the “River Douro Estuarine Contributions to Inner Shelf Dynamics” project, described in section E, which is co-financed by the APDL.

#### **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

NICC 1st Year Report. 2006. The observation system was organized, planned and installed, with GPS-equipped floaters. Observations next to the Douro and Minho estuary tide gages, to determine the hydrographical zero, took place.

NICC 2nd Year Report. 2007. Observations (from the NICC and ECOIS projects) were processed and related satellite data compiled. Hydrographical characterization of the study area and evaluation of the estuarine influence in coastal currents. Development of reference conditions for the study area. Collected data allow testing and validation of existing circulation models.

ECOIS 1st Year Report. 2006. Two observation campaigns (with collection of hydrological parameters, phytoplankton and sediments) were carried out for the characterization of the Minho and Douro estuaries in terms of hydrology, currents and sediments (including geo-chemistry).

ECOIS 2nd Year Report. 2007. A third and last observation campaign took place and estuaries were characterized in terms of their hydrology, currents, biology and sediments, involving all project partners. An additional sediment sampling was carried out and two surface floaters were launched. Laboratory analyses of samples. Interpretation of data. Development of a detailed

batimetry map for the Minho estuary and river up to Valença. Assessment of the sedimentary dynamics of both rivers, their interactions with each other and with the internal platform.

POCUS Report. 2007. A database of available remote sensing data was set up, for the Northern Atlantic Iberian region, with maps of sea level anomalies, absolute dynamic topography and geostrophic currents from satellite altimetry, SST and ocean colour. Marine survey (Jan. 2006) along the Envisat track 459. GPS raw data was collected onboard of NRP Auriga and inland GPS reference data acquired for Gaia, Cascais and Cabo Carvoeiro. Data were analysed and modelled.

Reports to the APDL 2006, 2007 described data and inferences about the dynamics of the Douro and Minho estuaries.

Machiel S Bos, Faial gravity campaign 14-20 July 2006. 2006. Description of a gravity survey campaign on Faial, Azores, during 14-20 July in 2006 in the scope of the TIPGRAV project (POCI/CTE-GIN/58494/2004), including sample data and conversion method from raw measurements to gravity values.

#### **9. Internationalization** (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

Editor

“Gravity, Geoid and Space Missions”. Proc. GGSM 2004. IAG Intern. Symp. Porto, Portugal. Series: Intern. Ass. of Geodesy Symp., Vol. 129. Jekeli, C, Bastos, L, Fernandes, J (Eds.). 2005. XVI, 368 p., 334 illus., ISBN: 3-540-26930-4.

Proceedings of the III National Conference of Cartography and Geodesy, Aveiro. Bastos, L and Matos, J (Eds.). 2004. Lidel, ISBN 972-757-330-4.

Referee

J. Geophysical Research - Solid Earth, AGU

IAG Symposia Series (conference proceedings), Springer

Physics and Chemistry of the Earth, Elsevier

J. Geodesy, Springer Verlag

J. Geodynamics, Elsevier

Pageoph, Birkhäuser

Computers & Geosciences, Elsevier

J. Applied Ecology, Blackwell

Invited speaker

Bastos, L, Fernandes, RMS, Bos, MS. 2006. Improvements in the determination of tectonic motions from GPS. International Geodetic Forum XiAn 2006, ChangAn University, Xian, China, 10-16 October 2006.

Scherneck, H-G, Bos, M S. 2006. Ocean loading tides - a review. General Assembly of the European Geophysical Union, Vienna, Austria, 2-7 April 2006.

Bastos, L. 2003. Exploring GPS/INS integration for airborne and terrestrial applications. Presented at Instituto Cartográfico da Catalunha. In: Cicle of International Conferences, Inst. Geomatics, Barcelona, Spain, March 2003.

#### Other

Invitation for participation in the COST Action ES0701: Improved Constraints on models of glacial isostatic adjustment (2008–2012).

Project partner of the project “Global Loading and Deformation at Tidal Timescales”. Principal Investigators: Dr. Peter Clarke, Dr. Nigel Penna and Dr. Matthew King of the University of Newcastle, United Kingdom (2006–2009).

“Development of aeolian sand transport events in a coastal system (Portugal)” and “Wave attenuation across a shore platform and wave reflection from a gravel beach”, 2005/2006 Fulbright Scholar in Coastal Geology framework, with Prof. Douglas Sherman, Texas A & M Univ.

“Efficient coastal morphodynamics monitoring” Proposal, approved by the LOICZ Scientific Steering Committee for affiliation in the IGBP, LOICZ. 2006.

#### Memberships

Am. Geophysical Union (AGU)

Inst. of Navigation (ION)

Int. Ass. of Geodesy (IAG) (associated member)

European Geophysical Society (EGS)

Sub-Comissão WEGENER of the Int. Geodesy Ass. (president).

COST 40 action, EOSS (European Sea Level Obs. Sys.)

## 6e. Future research

### 1. Objectives (3000 ca.)

The recently created Oceanic and Coastal Dynamics group originated from the Marine Technology group, following the broadening of the research areas and capabilities within the Associated Lab. The addition of researchers specialized in Oceanography (through the Ciência 2007 programme) will allow the group to analyse and model physical and biological oceanographic processes, using in-situ and remote sensing data.

The objectives of the marine technology group, described previously in Section 6c, will be continued within the scope of the current group. In addition, the group will focus on using GPS

measurements to study coastline morphology for management and impact assessments as well as using airborne systems to identify pollutants; assess marine sediments; evaluate water colour; and determine sea surface topography, which can be used to model surface currents. The data provided by these systems can complement data provided by Earth Observation satellites, which have lower spatial and spectral resolution but cover much larger areas.

Additional in-situ data will be available to the group as it is involved in the new Portuguese-Spanish ocean observatory. These data will be combined with remotely sensed data to study ocean meso-scale dynamics, e.g. study of wakes and spiral eddies (modelling results provide boundary conditions for the coastal ocean models); assimilation of sea-surface height dynamics (estimated from improved geoid and satellite altimetry data) into ocean models; use of the remote sensing technique such as SAR to complement in-situ and modelling data.

The coastal ocean dynamics will be modelled using a Regional Ocean Model System (ROMs). Special attention will be paid to turbulence phenomena which are important for mixing of the water. To assist the numerical modelling a powerful set of computers, a cluster, has been purchased. The estuaries will be modelled using Finite Volume Coastal Ocean Models

(FVCOM) that use unstructured grids. The advantage of this type of method is that it adapts very well to difficult geometries of estuaries and it allows that some areas fall dry during low sea-level.

The future research will also include projects in collaboration with local authorities and private companies. For example, together with APDL, a GPS station has been recently installed in the harbour of Leixões, Porto, to monitor its stability in the coming years. Furthermore, a project has been submitted with APDL to analyse its 100 years long tide gauge record. The results will contribute to a better understanding of long term sea-level changes in front of the Portuguese coast.

The group intends to engage in the training of young scientists and promote the development and exploitation of new marine technologies, and observational methodologies to support studies of ocean coastal dynamics in cooperation with other universities, research groups and companies.

The work is strongly interdisciplinary, involving cooperation within the CIMAR as well as with researchers from other national and international Centres.

## **2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

Projects ongoing in 2008 include: NICC, ECOIS and POCUS (see section 6a).

Other funding sources are:

- DEOSOM, Detection and Evaluation of Oil Spills by Optical Methods: Development a low cost, efficient system for the third level surveillance, which can be installed on watercraft and unmanned aerial vehicles and used for intensive surveillance of harbourages, rivers, channels, beaches, and coastal waters. Coordinator - INOV-INESC-INOVAÇÃO; Funding: AMPERA; total/CIMAR budget – 420.000/43.800 EUR; 01/09/2008–31/08/2011 (Approved December 2007).

- RAIA, Observatorio oceánico del margen ibérico: Implementation of an ocean observation network, for the consolidation of operational oceanography on the Spanish-Portuguese Iberian coast. Coordination: Consellería de Medio Ambiente e Desenvolvemento Sostible, Xunta de Galicia. Total budget of 4.2 M EUR. Funding: Interreg IV (75%), total/CIIMAR budget: 3.162.700/132.900 EUR; 01/01/2009–31/12/2011 (Approved September 2008).

- ICEMOS, Monitorização integrada da Eco-morfodinâmica Costeira (PTDC/AMB/69593/2006): Development and application of high-resolution, low-cost aerial and terrestrial for continued coastal eco-morphodynamical monitoring. IP: A Bio; Funding: FCT; total /CIIMAR budget – 196.560/43.920 EUR (Awaiting approval).

- AGUIA, Airborne Gravimetry with an Ultralight Independent Aircraft (PTDC/AMB/69593/2006): Development of a portable gravimetry system that can be installed in a small remotely controlled aircraft, allowing extension of gravimetry data collection overseas. IP: M Bos; Funding: FCT; total/CIIMAR budget – 188.245/81.780 EUR (Awaiting approval).

**3. Previous publications in the area (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)**

Future research will build on the research topics initiated in the 2003-2007 period and expand with the oceanographic topics exposed in section 6e-Objectives. The latter became possible after signing up Isabel Araújo and Rui Caldeira, two capable researchers specialised in SAR and meso-scale oceanography, respectively. Given that publications related to research topics of the 2003-2007 period are already cited in sections 6d, the following publications are related to the new oceanography-related research topics.

1. Marchenko, D, Meyer, U, Bastos, L. 2004. Airborne gravity disturbances in sequential multipole analysis for geoid determination and its test over the Azores. *Bollettino di Geodesia e Scienze Affini*, N° 2.

2. Baker, TF, Bos, MS. 2003. Validating Earth and ocean tide models using tidal gravity measurements. *Geophysical Journal International* 152: 468-485. IF 2003/2007=1.636/2.112, n°C=24.

3. Caldeira, RMA, Marchesiello, P, Nezlin, NP, DiGiacomo, PM, McWilliams, JC. 2005. Island wakes in the Southern California Bight. *Journal of Geophysical Research* 110: C11012. IF 2005/2007=2.784/2.953, n°C=3.

4. Araujo, IB, Pugh, DT. 2008. Sea levels at Newlyn 1915-2005: Analysis of trends for future flooding risks. *Journal of Coastal Research* 24: 203-212. IF 2007=0.383, n°C=0.

5. Carvalho, J, Soares, A, Bio, A. 2006. Improving satellite images classification using remote and ground data integration by means of stochastic simulation. *International Journal of Remote Sensing* 27: 3375–3386. IF 2006/2007=0.980/0.987, n°C=1.

**4. Special requirements (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)**

Oceanography

Software

Ocean buoy

Coastal buoy

Sensors to equip buoys (temperature, salinity, pressure)

Echo sounder

2 Post-doc

4 PhD students

2 MSc students

Coastal monitoring

Motor-quad (equipped with field computer, GPS and Video cameras)

Airborne sensors (digital Camera, Lidar)

1 Post-doc

2 PhD students

3 Msc

Other

Online database of meteorological and ocean products (LAS - Live access server)

Acquisition of satellite data (SAR, AVHRR, MODIS, MERIS, SeaWiFs etc.)

## Geology and Paleo-oceanography

### 6a. Group description

#### 1. Group name / denomination

#### Geology and Paleo-oceanography

#### 2. Principal investigator

João José Oliveira Dias Coimbra

#### 3. Location of group (Host institution)

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### 4. Keywords

Past, Climate Change, Environment, Coastal Dynamics

#### 5. Funding, source, dates (1000 ca.)

In terms of funding four sources have to be considered, PIDDAC, Externally funded projects, CIMAR, in the name of which we have some externally funded projects, and Other funds, which include special and small funds attributed to different members of the group by different funding agencies and for participation in meetings or short stays at foreign laboratories.

	2003	2004	2005	2006	2007
PIDDAC	18.0	30.0	0.0	114.3	0.0
EXT. PROJECTS	205.0	128.0	35.0	238.0	300.0
CIMAR	0.0	17.5	10.5	69.8	113.0
Other Funds	0.8	1.6	8.0	36.3	12.5
TOTAL	223.8	177.1	53.5	458.4	425.5

In terms of the type of expenses covered with the total amount, it is to note that for example in 2007, 25% went to personnel contracts and 21% to pay services, in particular analyses that have still to be performed in foreign laboratories, due to the lack of the necessary equipment in our own laboratory! Other expenses to consider are Project fellowships (2%), Consumables (9%), Bibliography (1%), Equipment Maintenance contracts (2%), Informatics Equipment and Software (10%), laboratory equipment (28%) and missions (2%)



## **6b. Group team**

### **1. Researchers in the group (Include only PhD. integrated in the LA)**

- 001. Channarayapattana Narasimhamurthy Prabhu (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)
- 002. Cristina Isabel Coelho Dias Lopes (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 003. Dr Antje Helga Luise Voelker (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 004. Isabel Martins Gil (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 005. Maria Emília Carvalho Salgueiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 006. Rui Manuel do Amaral Branco de Oliveira Quartau (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 007. Vitor Hugo da Silva Magalhães (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)
- 008. Lúcia de Abreu (Cat.: Não aplicável (bolseiro), Gr. Acad.: Doutoramento)

### **2. Other researchers in the group (Include here collaborators with PhD. only)**

### **3. Other researchers in the group (non PhD.)**

- 001. Andreia Seia Rebotim (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 002. Célia Teresa Neto dos Santos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 003. Luis Filipe Rodrigues Batista (Cat.: Outra, Gr. Acad.: Licenciatura)

## **6c. Objectives & achievements**

### **1. Objectives (2000 ca.)**

Investigação em Geologia Marinha - INGMAR is the main program of DGM-INETI, which objectives are to promote the geological research of the oceans and the coastal regions, the paleoceanography, and, the processes related to the occurrence of marine mineral resources of the marine areas under Portuguese jurisdiction, and the PALOP coastal and immersed territories. DGM's Research Plan is built around four main scientific areas: Paleoceanography / Paleoclimate and Environment, Geology and Resources, Natural Hazards, and Coastal Geology.

Research within Paleoceanography and Environment is centered in the reconstruction of climate and oceanographic conditions at scales that vary from annual to million years, on the basis of geologic records. A theme that is of major importance to assess future climate conditions, since the instrumental record is much too short to allow the separation between natural variability and the anthropogenic impact.

Research on Resources has been and will continue determined by the economic potential of resources in a near future. Basic work to characterize and mark the distribution of inerts (sand and gravel) on the shelf is complete but needs detailed studies at the regional level. The study of other resources such as phosphorites, polymetallic Ni and Co rich crusts and nodules, has been started by their basic characterization and mapping of their spatial distribution

Coastal Geology is centered in studies related to the understanding of the geologic evolution of coastal areas, including dune dynamics, and littoral processes.

Natural Hazards include research on themes of immediate social concern and of regional/local scale, such as risks associated to slope instability, earthquakes, tsunamis, and submarine mass movements. Any of these can cause extensive territory devastation and loss of human lives, as well as destruction of anthropogenic generated constructions and products what can cause first order economic problems.

To consolidate the international cooperation, to participate in an innovative educational program at the European level, to contribute for the transference of knowledge about the ocean, as well as its importance for the political and public question of how to be prepared to survive possible important climate changes in the near future, are other of the DGM objectives.

## **2. Main achievements (2000 ca.)**

- The existence of FeMn nodules and crusts on the Madeira seamounts and their high Co content makes them a potential mineral resource, and, the study of Pb, Sr and Nd radiogenic isotopes on their detritic fraction gave new information about the evolution of the NE Atlantic water masses;
- The presence of gas hydrates in the ocean bottom, revealed by the occurrence of mud volcanoes in the Cadiz Gulf, lead to on going investigation on the generating processes of both the carbonate chimneys and the methane;
- New seismic data allowed the modulation of the deep basins of the Portuguese margin, where natural gas and hydrocarbons can be formed and stored. This work and its publication have given a job position at Cardiff University and has already attracted a few foreign companies to do further studies in the area;
- The sulfur-rich mineral masses associated with the Azores hydrothermal fields sampled revealed them as the richest Zn-Cu-Ag-Au resources known;
- The high-resolution sedimentary sequences from the inner shelf allowed: the reconstruction of the climate of the last 2 kyr; the discovery of the offshore record left by the 1969 and 1755 tsunamis ; the history of deposition of antropogenic and pollutant materials in those regions; and, revealed high contents of Hg and Pb still present in the Tagus prodelta sediments;
- The detailed reconstruction of stages 11 and 1 and their comparison;
- The discovery of shelf instability associated to the cold phases of stages 4-2 millennial climate changes;
- Quantification and calibration of two microfossil groups that now allow quantitative reconstructions of the ocean surface temperature, primary productivity and nutrient availability

back in time, at least in regional terms. A result of great importance for regional climate modeling;

- Work on the Troia peninsula has revealed the evolution of both the dune field and coastline during the Quaternary. Luminescence dating point to a recent age (< 2 kyr) for the Troia Caldera;

- The ongoing study of the Sado delta evolution shows that the delta has lost something like 150 million m<sup>3</sup> of sediments in 72 yr (1930-2002).

One of the most important aspects of the group's activity is the organization / participation in scientific cruises. DGM organized 3 campaigns and our researchers participated in 19 other international cruises of which 2 IODP legs.

## 6d. Productivity

1. **Publications in peer review journals** (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)

1. Abrantes, F, 2003. A 340,000 year continental climate record from tropical Africa – news from opal phytoliths from the equatorial Atlantic. *Earth and Planetary Science Letters* 209: 165-179. IF 2003/2007=3.528/3.873, n°C=10.

2. Abreu, L, Shackleton, NJ, Schönfeld, J, Hall, M, Chapman, M. 2003. Millennial-scale oceanic climate variability off the Western Iberian margin during the last two glacial periods. *Marine Geology* 196: 1-20. IF 2003/2007=1.497/1.975, n°C=29.

3. Alves, T, Gawthorpe, R, Hunt, D, Monteiro, J. 2003. Cenozoic tectono-sedimentary evolution of the western Iberian margin. *Marine Geology* 195: 75-108. IF 2003/2007=1.497/1.975, n°C=10.

4. Tzedakis, PC, Roucoux, KH, Abreu, L, Shackleton, NJ. 2004. The Duration of Forest Stages in Southern Europe and Interglacial Climate Variability. *Science* 306: 2231-2235. IF 2004/2007: 31.853/26.372, n°C=18.

5. Abreu, L, Abrantes, FF, Shackleton, NJ, Tzedakis, PC, McManus, JF, Oppo, DW, Hall, MA. 2005. Ocean climate variability in the Eastern North Atlantic during interglacial MIS 11: A partial analogue to the Holocene? *Paleoceanography* 20: 1-15. IF 2005/2007=3.233/3.391, n°C=3.

6. Gil, IM, Abrantes, FFG, et al. 2006. The North Atlantic Oscillation forcing through the last 2000 years: Spatial variability as revealed by high-resolution marine diatom records from N and SW Europe. *Marine Micropaleontology* 60: 113-129. IF 2006/2007: 1.973/1.505, n°C=3.

7. Lopes, CA, Mix, C. 2006. Diatoms in northeast Pacific surface sediments as paleoceanographic proxies. *Marine Micropaleontology* 60: 45-65. IF 2006/2007=1.973/1.505, n°C=1.

8. Niemann, H, Duarte, J, et al., (V Magalhães). 2006. Microbial methane turnover at mud volcanoes of the Gulf of Cadiz. *Geochimica et Cosmochimica Acta* 70: 5336-5355. IF 2006/2007: 3.751/3.665, n°C=9.

9. Voelker, A, Lebreiro, SM, Schoenfeld, J, Cacho, I, Erlenkeuser, H, Abrantes, FFG. 2006. Mediterranean outflow strengthening during northern hemisphere coolings: A salt source for the glacial Atlantic? *Earth and Planetary Science Letters* 245: 39-55. IF 2006/2007: 3.887/3.873, n°C=11.

10. Matrat, B, Grimalt, J, Shackleton, N, de Abreu, L, Hutterli, M, Stocker, T. 2007. Four Climate Cycles of Recurring Deep and Surface Water Destabilizations on the Iberian Margin. *Science* 317(5837): 502-507. IF 2007=26.372, n°C=3.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Quartau, R, Teixeira, FC, Monteiro, JH, Cunha, T, Pinheiro, L, Cardigos, F, 2003. Sand resources on the continental shelf of Faial Island (Azores). 4º Simpósio sobre a Margem Ibérica Atlântica, Vigo, 7-10 Julho.

2. Rodrigues, T, Flores, J-A, Abrantes, F, Grimalt, J. 2004. Coccolithophore record in the Tagus Prodelta for the last 13.5 Kyr: A Comparison with the biomarker signal. 10th. Conference of the Internacional Nannoplankton Association, 28 August - 4 September 2004, Department of Geology, University of Lisboa, Portugal.

3. Alves, TM, Cunha, T, Bouriak, S, Volkonskaya, A, Monteiro, JH, Ivanov, M, TTR12-Leg 5 Scientific Party. 2005. Surveying the flanks of the Mid-Atlantic Ridge: Examples of Geosphere-Biosphere coupling in deep-sea areas of the North Atlantic. MoMAR (Monitoring the Mid-Atlantic Ridge) III Workshop, Lisboa, Portugal.

4. Rebêlo, LP, Brito, PO, Monteiro, HC. 2005. Evolução da Península de Tróia: as dunas frontais como indicadores de erosão e acreção. *Internacional Geosphere Biosphere Program - Portugal, MGNotícias*, nº 2, Outubro 2005.

5. Mil-Homens, M, Stevens, RL, Abrantes, F, Cato, I, Boer, W. 2005. Recent sediment deposition within three areas of the Portuguese Shelf: natural vs. anthropogenic components. Volume de resumos da "XIV Semana de Geoquímica dos Países de Língua Portuguesa". Aveiro.

6. Brito, P, Andrade, C, Terrinha, P, Rebelo, L, Monteiro, J, Freitas, MC. 2006. Variação da morfologia e volumetria do delta de vazante do estuário do Sado, livro de resumos do 5º Simpósio sobre a Margem Ibérica Atlântica, Aveiro, 33-34.

7. Magalhães, VH, Birguel, D, Peckmann, J, Pinheiro, LM, Vasconcelos, C, McKenzie, JA, Ivanov, MK. 2006. Biomarker and SEM evidences of microbial mediation in the formation of methane-derived authigenic carbonates from the Gulf of Cadiz. In: 5º Simposio sobre a Margem Ibérica Atlântica / 5º Simposio sobre el Margen Iberico Atlantico / 5th Symposium on the Iberian Atlantic Margin Aveiro, Portugal.

8. Salgueiro, E, Voelker, A, de Abreu, L, Abrantes, F, Meggers, H. 2006. Hydrographic and Productivity Variations off the Western Iberian Margin during the Last 150 ky. VII Congress National of Geology, Évora, Portugal.

9. Lopes, C, Mix, A. 2007. Ancient mega-floods in the Northeast Pacific. 1º Encontro Ciência em Portugal – Ciência 2007. Fundação C. Gulbenkian, Lisboa, 12-13 Abril.

10. Ferreira, PL, Dixon, JE, Murton, BJ, Boulter, C. 2007. Distribution of magma types along the Lucky Strike segment, 37°N on the Mid-Atlantic Ridge: the role of CO<sub>2</sub>. Abstract volume of the VI Congresso Ibérico de Geoquímica, 4 p., Vila Real, Portugal.

**3. Other publications international (3000 ca.)** (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Expedition 306 Scientific Party. 2005. Integrated Ocean Drilling Program Expedition 306 Preliminary Report: North Atlantic Climate 2. 2 March - 25 April 2005, DOI: 10.2204/IODP.PR.306.

2. Abrantes, F. 2006. Marine Diatoms. Encyclopedia of Quaternary Sciences, Elsevier.

3. Bartels-Jónsdóttir, HB, Knudsen, KL, Schoenfeld, J, Lebreiro, SN, Abrantes, F. 2006. Recent benthic foraminifera from the Tagus Prodelta and Estuary, Portugal: microhabitats, assemblage composition and stable isotopes. *Zitteliana* (A46): 91-104.

4. Channell, JET, Sato, T, Kanamatsu, T, Stein, R, Malone, M, Alvarez-Zarikian, C, IODP Expeditions 303 and 306 Scientists. 2006. IODP Expeditions 303 and 306 monitor Miocene - Quaternary climate in the North Atlantic. *Scientific Drilling*, 2: 4-10. DOI: 10.2204/iodp.sd.2.01.

5. Channell, JET, Kanamatsu, T, Sato, T, Stein, R, Alvarez Zarikian, CA, Malone, MJ, Expedition 303/306 Scientists. 2006. *Proc. IODP, 303/306: College Station TX (Integrated Ocean Drilling Program Management International, Inc.)*. DOI: 10.2204/iodp.proc.303306.

6. Roucoux, KH, Tzedakis, PC, de Abreu, L, Shackleton, NJ. 2006. Fine tuning the land-ocean correlation for the late Middle Pleistocene of southern Europe. *Deeklin Book*.

7. Stein, R, Kanamatsu, T, Alvarez Zarikian, CA, Higgins, S, Channell, JET, Aboud, E, Ohno, M, Acton, GD, Akimoto, K, Bailey, I, Bjørklund, KR, Evans, H, Nielsen, SHH, Fang, N, Ferretti, P, Gruetzner, J, Guyodo, YJB, Hagino, K, Harris, R, Hatakeda, K, Hefter, J, Judge, SA, Kulhanek, DK, Nanayama, F, Rashid, H, Sierro Sanchez, FJ, Voelker, A, Zhai, Q. 2006. North Atlantic paleoceanography: the last 5 million years. *EOS*, 87: 129-133.

8. Voelker, A, Abrantes, F. 2007. Circum-Iberia paleoceanography and paleoclimate. Workshop report in: *PAGES news*, vol. 15, no. 1, April, pp. 30-31.

9. Mil-Homens, M, Branco, V, Vale, C, Stevens, R, Boer, W, Lebreiro, S, Cato, I, Abrantes, F. in press. Historical trends in Hg, Pb and Zn sedimentation in the central shelf area of Portugal, *Journal Iberian Geology*.

**4. Master and Ph.D. thesis completed (3000 ca.)**

**PhD THESIS**

Bartels, Helga. 2005. Latest Holocene climate variability in the North Atlantic. Univ. Aarhus. Supervisor: K-L Knudsen, F Abrantes.

Nave, Sílvia. 2005. Oceanic Productivity Variations during Rapid Climatic Changes. Univ. Paris-Sud. Supervisor: L Labeyrie, F Abrantes.

Ferreira, Pedro. 2006. Melt Supply and Magmatic Evolution at a Large Central MOR Volcano, and its Relationship with Volcanic Style and Hydrothermal Activity. Univ. Southampton. Supervisor: B Murtion, C Inverno.

Gil, Isabel. 2006. Reconstructing Climate Variability in the Tagus Prodelta and in Skagerrak during the Last 2000 Years using High Resolution Marine Diatom Records. Univ. Bremen. Supervisor: D Hebbeln, F Abrantes.

Lopes, Cristina Isabel. 2006. Reconstruction of the Oceanic Paleoproductivity: Coastal Upwelling Comparison between Oregon and Portuguese Margins. Univ. Oregon. Supervisor: A Mix, F Abrantes.

Mil-Homens, Mário. 2006. Anthropogenic and Geological Influences Upon the Distribution of Heavy Metals and Other Elements on the Portuguese Shelf Near the Rivers Ave, Lis, Mira and Arade. Univ. Gotemburgo. Supervisor: R Stevens, F Abrantes.

Roque, Ana Cristina. 2006. Estrutura e Evolução Tectónica das Margens Continentais Sul e Sudoeste Portuguesas. Univ. Lisboa. Supervisor: P Terrinha.

Salgueiro, Maria Emília. 2006. Reconstructing of Productivity Conditions on Iberian Margin: a Foraminiferal Overview for the Last 130 Ky. Univ. Bremen. Supervisor: G Wefer, F Abrantes.

Magalhães, Vítor. 2006. Mud Volcanism and Gas Hydrates in the South Portuguese Margin and the Gulf of Cadiz. Univ. Aveiro. Supervisor: L Menezes, H Monteiro.

Quartau, Rui. 2006. Processos Sedimentares e Recursos em Areias e Cascalhos da Plataforma Continental das Ilhas do Faial, Pico e S. Miguel, Açores. Univ. Aveiro. Supervisor: L Menezes, P Terrinha.

Cunha, Tiago. 2007. Gravity Anomalies, Flexure, and Crustal Structure of the Iberia / Canada Conjugate Margin. Univ. Oxford. Supervisor: T Watts, L Menezes.

Freitas, Pedro. 2007. Stable Isotopes and Minor Element Records from Marine Bivalve Shells: Accurate Tracers of Environmental Conditions?. Univ. Wales/Bangor. Supervisor: H Kennedy, F Abrantes.

Teixeira, Francisco. 2007. Localização e Mapeamento Concorrentes em Veículos Submarinos Autónomos utilizando Dados de Magnetometria, Gravimetria e Sonar. Univ. Técnica Lisboa. Supervisor: A Pascoal, H Monteiro.

## **MSc THESIS**

Rodrigues, Teresa. 2004. Estudo de variações climáticas e inputs terrígenos no prodelta Tejo através da aplicação de n-alkanos e do índice UK37. University of Aveiro. Supervisor: F Abrantes, J Grimalt.

Gafeira, Joana. 2005. Cartografia morfo-estrutural da área da Falha Marquês de Pombal. University of Évora. Supervisor: P. Terrinha.

Muiños, Susana. 2005. Contribuição da análise multivariada para o estudo de crostas submarinas de ferro e manganês do Atlantico Nordeste. Univ. Nova Lisboa. Supervisor: Pereira HG, Monteiro H.

Vicente, Maris dos Santos. 2006. Registo antropogénico em sedimentos do Prodelta do Tejo nos últimos 200 anos. Univ. Nova Lisboa. Supervisor: A Rodrigues, F Abrantes, J Grimalt.

**5. Patents/propotypes (2000 ca.)**

**6. Organization of conferences (2000 ca.)**

**7. Industry contract research (2000 ca.)**

**8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

**9. Internationalization (2000 ca.)** (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

Convinced that reaching international level in Geological Oceanography research is dependent on the existence of qualified staff, DGM has been actively contributing to the education of a new generation of researchers, through the offering of PhD plans to be executed at DGM, at least partially, but supervised by internationally recognized researchers from excellent Institutions in the properly selected research area. This strategy shall increase and consolidate the cooperation with the international community and “generate” researchers capable to make important contributions in areas still non-existent in Portugal.

National and international Universities and Oceanographic and Marine Geology Research

Institutions, with which DGM has active collaboration are presented in Fig. 2 and include:

University of Aveiro, University Nova de Lisboa, University of Lisboa – Centro

CREMINER, Instituto Superior Técnico – Grupo de Robótica; Universities de Bremen (GE),

Barcelona (SP), Bergen (NO), Salamanca (SP), Cambridge (UK), Oregon (USA), Chicago (USA),

Aarhus (DE), Plymouth (UK), Southampton (UK), Cardiff (UK), Bangor (UK), Gothenburg (SE), or yet CNRS at GIF sur Yvette (FR), LAMONT (USA), WHOI-MIT (USA), etc

Besides inviting a few foreign colleagues to come and give short intensive courses at DGM, also our researchers have been invited to teach intensive courses, like it was the case of F. Abrantes



who went to the Instituto de Oceanografia da Universidade de São Paulo (Dec. 2007) to give a course on Introduction to Paleoceanography.

## **6e. Future research**

### **1. Objectives (3000 ca.)**

To become a reference group in Europe and the World in our area of activity is the major objective for the future. The research plan considers the continuation of the ongoing projects as well as new research projects in areas prioritized by the: IODP scientific plan, European Science Foundation Marine Board (MB-ESF), IMAGES- marine branch of IGBP /PAGES, EU blue book and 2007 IPCC report.

The paleo group will follow up the study of the five most important coastal upwelling regions since their high productivity plays a crucial role in modulating the flux of CO<sub>2</sub> between the ocean and the atmosphere and constitute one of: (1) the main mechanisms controlling atmospheric pCO<sub>2</sub> and regulating climate; (2) the climate reconstruction of the Holocene and preceding (warm) interglacial isotopic stages as an important source of information for future climate modeling; (3) the investigation of high-resolution records of the last 2 kyr and their calibration to instrumentally derived data sets; (4) the calibration and development of new proxies, necessary to better understand forcing processes and/or quantify past climate variability; (5) the drilling of the Portuguese margin, within the IODP GUCADRILL proposal, prepared with a significative contribution of our paleo group, and 1st in the line of new campaigns to be scheduled. Its main objective is to understand the importance of the Mediterranean Outflow Water in oceanic circulation and climate through time.

Research in the environment area will concentrate on the origin and temporal evolution of polluting elements on the Portuguese estuaries and shelf. That is, evaluate human-climate interactions since the explosion of the Neolithic population 9kyr ago.

In coastal geology, having in mind the most likely increase in storm frequency and intensity as well as sea level rise associated with global warming, studies will be related to the understanding the dynamics of coastal areas, to get the necessary knowledge for the pacification of a sustainable utilization of these heavily populated and environmentally sensitive regions. To contribute for the decision-making, in an effective way, an atlas containing the characterization of the coastal area as well as the vulnerability of each area to natural hazards is the main objective.

The study of generation/mineralization processes of Fe-Mn nodules and crusts on the Azores region Mid-Atlantic Ridge hydrothermal camps, will be one of the objectives of the resources group. Attention will also be centered in the Gulf of Cadiz Methane Seeps, both as a potential source of energy, as potential slope instability generators, or yet, as unique ecosystem environments, maybe similar to the first existing ones on earth.

For new areas of research, the group plans to contribute to the study of the deep biosphere origin and evolution and develop a new research line, dedicated to the microbial role in the processes involved in the hydrocarbons and methane anaerobic oxidation and the precipitation of Fe-Mn nodules and crusts. The potential discovery of unknown forms of life adapted to these extreme environments may have a great impact on management issues such as, potential deep-sea disposal

of wastes, water quality, bioremediation and pollution control as well as novel organisms and enzymes for biotechnology.

The effect of the acidification of the ocean on the organisms constitutes another of the new areas for which we plan to contribute through the study of the geologic record for past times of similar conditions.

**2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

FCT/ESF

PDCTM/2003/DIV/40017/99, 2005-2008, 275.000 EUR

PORTO-Variações nos padrões de circulação oceânica e paleoproductividade na margem portuguesa e oceano Atlântico Norte: ligações entre o hemisfério Norte e a Antártida entre 380.000 e os 800.000 anos, FCT, 2006-2009, 86.400 EUR - Managed by CIMAR

PDCTM/MAR/56963/2004, 2006-2009, 59.233 EUR - Managed by CIMAR

POCI/CTE-GEX/61007/2004, 2007-2009, 79.732 EUR

PDCT/MAR/56781/2004, 2007-2009, 79.732 EUR

PTDC/MAR/65197/2006, 2008-2010, 200.000 EUR

PTDC/ECM/70428/2006, 2008-2010, 50.924 EUR

PTDC/CTE-GEX771298/2006, 2007-2009, 11.054 EUR

POCI/MAR/61178/2004, 2007-2009, 32.460 EUR

PDCT/MAR/56823/2004, 2007-2009, 79.200 EUR

SNPC, 2006-2008, 55.707 EUR

Pending funding

Amocint project, FCT/ESF, 2008-2010, 182.043 EUR

PTDC/CLI/70772/2006, 2008-2010, 172.800 EUR - To be managed by CIMAR

Topomed project, FCT/ESF, 2008-2010, 196.320 EUR

Minho, Cooperação Transfronteiriça Portugal-Espanha, 2009-2011), 924.233 EUR (total) - To be managed by CIMAR

Geo-Seas, 7FP, 2009-2011, 176.000 EUR

PTDC/MAR/66022/2006, FCT, 2009-2011, 199.982 EUR

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Hensen, C, Nuzzo, M, Hornibrook, E, Pinheiro, LM, Bock, B, Magalhães, VH, Brückmann, W. in press. Sources of mud volcano fluids in the Gulf of Cadiz - indications for hydrothermally altered fluids. *Geochimica et Cosmochimica Acta*. DOI: 10.1016/j.gca.2006.11.022 2006.
2. Aranda da Silva, A, Gooday, AJ. 2007. Large organic-walled Protista (Gromia) in the Arabian Sea: density, diversity, distribution and ecology. *Deep-sea Research Part II*, 2007. IF=1.172, n°C=.
3. Duarte, H, Pinheiro, LM, Teixeira, FC, Monteiro, JH. 2007. High-resolution seismic imaging of gas accumulations and seepage in the sediments of the Ria de Aveiro barrier lagoon (Portugal). *Geo-Marine Letters* 27(2-4): 115-126. IF 2007=0.811, n°C=
4. Muiños, SB, Frank, M, Maden, C, Hein, JR, Labreiro, SM, Gaspar, L, Monteiro, JH, van de Flierdt, T, Halliday, AN. 2008. New constraints on the Pb and Nd isotopic evolution of NE Atlantic water masses. *Geochemistry Geophysics Geosystems*, 9, Q02007. DOI: 10.1029/2007GC001766. IF 2007=2.354.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

Access to sea going and laboratory equipment, is necessary to: (i) generate the capacity to produce scientific results at the level of our international partners; (ii) be competitive relatively to European/International funds; (iii) maintain and attract to Portugal the best quality researchers.

The group needs to have the possibility to do the basic necessary analytical work, normally done in foreign institutes, and the capability to organize oceanographic campaigns to respond to their own R & D questions, implement new projects, as well as test concepts and scientific hypothesis.

List of structural equipment and field-work funds that would allow increasing our competitiveness and broadening our research capacity.

Gravity Corer

Multi Scater Colour L

GEOSCAN II

Stable Isotopes Mass Spectrometer

ICP-MS

Multinet

CTD for rosette sampler

Tour Vehicle

Scuba Diving equipment

Flat bottom boat

Multybeam Eco-sounder

Marne Differential GPS unit

GPR (Ground Penetrating Radar)

Shallow water chirp sonar

## **Cellular and Molecular Studies (LECEM)**

### **6a. Group description**

#### **1. Group name / denomination**

**Cellular and Molecular Studies (LECEM)**

#### **2. Principal investigator**

Eduardo Jorge Sousa Rocha

#### **3. Location of group (Host institution)**

Centro Interdisciplinar de Investigação Marinha e Ambiental

#### **4. Keywords**

Fish, Molluscs, Biology, Toxicology

#### **5. Funding, source, dates (1000 ca.)**

69.000,00 EUR; Source: FCT (Project POCTI/BSE/46968/2002); Dates: 2003-2006; Title: Structural and metabolic regulation of fish peroxisomes by estrogenic compounds.

Funding: 32.830,00 EUR; Source: FCT (Project POCI/MAR/59462/2004); Dates: 2005-2008; Title: The molecular mechanisms of sexual differentiation in gastropods and the role of vertebrate-like steroid hormones.

Funding: ca. 60.000,00 EUR; Source: FCT (Pluriannual Basic Funding); Dates: 2003-2007.

### **6b. Group team**

#### **1. Researchers in the group (Include only PhD. integrated in the LA)**

001. Rogério Alves Ferreira Monteiro (Cat.: Professor Catedrático, Gr. Acad.: Agregação)

002. Alexandre Manuel Silva Lobo Cunha (Cat.: Professor Associado, Gr. Acad.: Agregação)

003. Eduardo Jorge Sousa Rocha (Cat.: Professor Associado, Gr. Acad.: Agregação)

004. Maria Joao Tome Costa Sousa Rocha (Cat.: Professor Associado, Gr. Acad.: Doutoramento)

005. Luís Filipe Costa Castro (Cat.: Investigador Auxiliar, Gr. Acad.: Doutoramento)

#### **2. Other Researchers in the group (Include here collaborators with PhD. only)**

### 3. Other Researchers in the group (non PhD.)

- 001. Albina Dolores Cardoso da Silva Castro Resende (Cat.: Assistente, Gr. Acad.: Mestrado)
- 002. Carla Batista de Carvalho Batista Pinto (Cat.: Assistente, Gr. Acad.: PAPCC)
- 003. João Soares Carrola (Cat.: Assistente, Gr. Acad.: Mestrado)
- 004. Ricardo Jorge Pereira Córdova Marcos (Cat.: Assistente, Gr. Acad.: PAPCC)
- 005. Paula Cristina Paulo Videira da Silva (Cat.: Assistente Convidado, Gr. Acad.: Mestrado)
- 006. Ana Margarida Pinto Henrique Machado (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 007. Claudia Maria Rosa Ribeiro (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 008. Fernanda Cristina Rodrigues Malhão Pereira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 009. Paulo César Nunes Pereira do Rêgo (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)
- 010. Pedro Alexandre Cardoso Matos (Cat.: Não aplicável (bolseiro), Gr. Acad.: Mestrado)
- 011. Tânia Vieira Madureira (Cat.: Não aplicável (bolseiro), Gr. Acad.: Licenciatura)

## 6c. Objectives & achievements

### 1. Objectives (2000 ca.)

- The primary strategic objectives set up in 2003 were to build a multidisciplinary core group and to establish a lab space in the CIIMAR “new” facilities in Porto. We aimed a team able to support multidisciplinary studies and keen to establish cooperation with other national and international groups. The research efforts at LECEM soon targeted both fundamental and applied studies, correlating structure with function in selected fish and molluscs, either in normal and pathological scenarios (namely in a toxicological context). Some emphasis was put in studying influences of steroid hormones and xenobiotics in unexplored targets, from the molecular (eg, molecular mechanisms of imposex and of PPARs involvement in peroxisomal changes) to the organ levels (eg, liver responses to subacute toxicity).
- As to non-experimental studies, the group’s main objective was to contribute for a better knowledge of the structure and function of the fish liver, and other related aspects of the digestive tract, considering the influences of gender and season. Recently, those studies also involved the kidney, establishing a comparative approach between peroxisomal events detected in liver and that are explored as a new thesis of endocrine (estrogenic) modulation of peroxisomal function. Comparative studies were also made in the digestive apparatus of molluscs, including hydrothermal-vent bivalves from the Azores. These approaches aimed to provide sound baseline data to support toxicopathological studies, which were initiated combining biomarkers with analytical chemistry targeting toxicants.
- As to experimental studies, they targeted two main biological problems: 1) how and to what extent fish peroxisomes are regulated and disrupted by estrogenic compounds; and 2) what are the

molecular mechanisms involved in the development of male penis and female imposex. Both themes derive from concerns about endocrine disruption in aquatic animals. In additional experiments, in cooperation with other research groups at CIMAR and from other institutions, our group intervened implementing histopathology know-how for diagnosis of toxicopathic lesions and also testing new quantitative approaches at tissue level for evaluating the intensity of effects of reference toxic compounds, like pesticides.

## **2. Main achievements (2000 ca.)**

- Scientific output in publications. We published 40 international works (38 articles in peer-reviewed indexed journals, and 2 peer-reviewed book chapters). The mean production rate was 1.8 peer-reviewed publications / per PhD / per year; taking into account that the team had only 4 PhDs up to 2005, and 5 PhDs 2006 onwards. Only from 2006 onwards one full time Auxiliary PhD Researcher was hired. Additionally, the team published 9 abstracts subjected to peer-review, in indexed journals, whereas attending national and international meetings, presenting over 60 works (poster and platform talks).
- Collaborations in scientific output. From our 40 international publications, we had 11 articles published in co-authorship with other Laboratories of the CIMAR LA (28% of our output). We had 24 international publications that included cooperation with colleague(s) outside CIMAR LA (60% of our output). A network of interactions was thus established.
- Support to advanced training. Our efforts to support both Master and PhD Students resulted in the conclusion of 2 MSc and 2 doctoral theses in the evaluation period. That effort continued, and in 2008 we already concluded 1 more MSc and 2 more doctoral theses. In 2007, 2 MSc theses just started and 1 was about to be finished, while 9 PhD students were starting/continuing works to end their thesis from 2008 onwards. We reinforced the group by opening, later in 2007, a position for Auxiliary Researcher (via an international call, won by a German researcher).
- International recognition by Publishing Houses and Editors. Team members have increasingly served as peer-reviewers. The PI is Editor-in-Chief of the journal *Comparative Hepatology* (BioMed Central - Springer). In addition to projects concluded up to 2007, team members were leading 1 book project (released early in 2008) and writing 1 book chapter: 1) Rocha MJ, Arukwe A, Kapoor BG. Fish Reproduction (Science Publishers); 2) Rocha E, Rocha MJ: Ovary: Development and Functions. In: Hormones and Reproduction in Vertebrates, Fishes (Academic Press).

## **6d. Productivity**

### **1. Publications in peer review journals (3000 ca.) (Up to a max of 10. Always indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language. DO NOT translate)**

1. Rocha, MJ, Rocha, E, Resende, AD, Lobo-da-Cunha, A. 2003. Measurement of peroxisomal enzyme activities in the liver of brown trout (*Salmo trutta*), using spectrophotometric methods. *BMC Biochemistry* 4: 2, 1-9. IF 2003/2007=NA/2.340, n°C=2.
2. Batista-Pinto, C, Rodrigues, P, Rocha, E, Lobo-da-Cunha, A. 2005. Identification and organ expression of peroxisome proliferator activated receptors in brown trout (*Salmo trutta* f. *fario*).



Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression 1731: 88-94. IF 2005/2007=2.506/1.704, n°C=6.

3. Kadar, E, Bettencourt, R, Costa, V, Santos, RS, Lobo-da-Cunha, A, Dando, P. 2005. Experimentally induced endosymbiont loss and re-acquirement in the hydrothermal vent bivalve *Bathymodiolus azoricus*. Journal of Experimental Marine Biology and Ecology 318: 99-110. IF 2005/2007=1.660/1.750, n°C=24.

4. Resende, AD, Rocha, E, Lobo-da-Cunha, A. 2005. Activity of purine catabolism enzymes during the reproductive cycle of male and female brown trout (*Salmo trutta*). Trends in Comparative Endocrinology and Neurobiology – Annals of the New York Academy of Sciences 1040: 1-4. IF 2005/2007=1.971/1.731, n°C=1.

5. Figueiredo-Fernandes, AM, Fontaínhas-Fernandes, A, Monteiro, RAF, Reis-Henriques, MA, Rocha, E. 2006. Temperature and gender influences on the hepatic stroma (and associated pancreatic acini) of Nile tilapia, *Oreochromis niloticus* (Teleostei, Cichlidae): A stereological analysis by light microscopy. Journal of Morphology 267: 221-230. IF 2006/2007=1.553/1.621, n°C=3.

6. Marcos, R, Monteiro, RAF, Rocha, E. 2006. Design-based estimation of hepatocyte number, by combining the smooth fractionator and immunocytochemistry with polyclonal antibodies for carcinoembryonic antigen. Liver International 26: 116-124. IF 2006/2007=2.344/2.559, n°C=6.

7. Castro, LFC, Lima, D, Machado, A, Melo, C, Hiromori, Y, Nishikawa, J, Nakanishi, T, Reis-Henriques, MA, Santos, MM. 2007. Imposex induction is mediated through the Retinoid X Receptor signalling pathway in the neogastropod *Nucella lapillus*. Aquatic Toxicology 85: 57-66. IF 2007=2.975, n°C=6.

8. Jordanova, M, Miteva, N, Rocha, E. 2007. A quantitative study of the hepatic eosinophilic granule cells and rodlet cells during the breeding cycle of Ohrid trout, *Salmo letnica* Kar. (Teleostei, Salmonidae). Fish and Shellfish Immunology 23: 473-478. IF 2007=3.160, n°C=2.

9. Lobo-da-Cunha, A, Kadar, E, Santos, RS. 2007. Histochemical and ultrastructural characterisation of mantle storage cells in the hydrothermal-vent bivalve *Bathymodiolus azoricus*. Marine Biology 150: 253-260. IF 2006/2007=1.756/2.215, n°C=2.

10. Matos, P, Fontaínhas-Fernandes, A, Peixoto, F, Carrola, J, Rocha, E. 2007. Biochemical and histological hepatic changes of Nile tilapia (*Oreochromis niloticus*) exposed to carbaryl. Pesticide Biochemistry and Physiology 89: 73-80. IF 2007=1.475, n°C=0.

**2. Other publications national** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

**3. Other publications international** (3000 ca.) (Include only Books, chapters or full papers published in conference proceedings up to max of 10. Give title and full citation in original language)

1. Rocha, E, Rocha, MJ, Monteiro, RAF. 2003. Seasonal Changes in Fish Hepato-cytes and Correlations with the Endocrine System. In: Fish Adaptations. Kapoor, BG, Val, AL (Eds). Science Publishers, Inc., Enfield, New Hampshire, USA, and Plymbridge Distributors Ltd., Plymouth, UK, pp. 383-403.
2. Rocha, MJ, Rocha, E. 2006. Morphofunctional Aspects of Reproduction from Synchronous to Asynchronous fishes – An Overview. In: Fish Endocrinology. Reinecke, M, Zaccane, G, Kapoor, BG (Eds). Science Publishers Inc., Enfield, New Hampshire, USA, pp. 571-624.

#### **4. Master and Ph.D. thesis completed (3000 ca.)**

##### **PhD THESIS**

Jordanova, Maja. 2004. The liver in female *Salmo letnica* Kar. (Teleostei, Salmonidae) during the reproductive cycle: A microscopic study of the natural population of Lake Ohrid. University of Sts. Cyril and Methodius, Skopje, Macedonia. Supervisors: E Rocha (CIMAR LA and ICBAS) and N Miteva (FYR Macedonia).

Batista Pinto, Carla. 2007. The peroxisomes of brown trout (*Salmo trutta fario*): Regulation by estrogenic compounds. ICBAS, University of Porto, Portugal. Supervisors: A Lobo da Cunha, E Rocha (CIMAR LA and ICBAS) and P Rodrigues (IBMC / INEB LA and ICBAS).

##### **MSc THESIS**

Carrola, João. 2003. Liver histopathology as a biomarker of pollution in aquatic systems - Douro and Ave watersheds. MSc at University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal. Supervisors: A Fontainhas-Fernandes (UTAD) and E Rocha (CIMAR LA and ICBAS, Univ. Porto).

Matos, Pedro Alexandre. 2003. Effect of carbaril in biochemical and oxidative stress parameters and in the liver histology of Nile tilapia (*Oreochromis niloticus*). MSc at the University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal. Supervisors: A Fontainhas-Fernandes (UTAD) and E Rocha (CIMAR LA and ICBAS).

#### **5. Patents/propotypes (2000 ca.)**

#### **6. Organization of conferences (2000 ca.)**

#### **7. Industry contract research (2000 ca.)**

#### **8. Government/organization contract research (2000 ca.)** (Include here work carried out by the group that resulted in a publication or report. Of particular importance are those involved in public policy advice)

## 9. Internationalization (2000 ca.) (Collaborative publication, Research, Graduate Training Networks or other forms of participation of the Research Group at the international level)

▪ Projects were initiated with groups in Macedonia (several partners), Japan (several partners), Norway (NTNU, Trondheim), UK (King's College and CEFAS), USA (Duke University), and Thailand (Kasetsart University, Bangkok). Joint articles start appearing in 2006 and continued in 2008, with eg:

1. Chatchavalvanich, K, Marcos, R, Poonpirom, J, et al. 2006. Histology of the digestive tract of the freshwater stingray *Himantura signifer* Compagno and Roberts, 1982 (Elasmobranchii, Dasyatidae). *Anat Embryol*: 211: 507-518. Partner: KAAsartsart University, Thailand.

2. Kortner, TM, Rocha, E, Silva, P, et al. 2008. Genomic approach in evaluating the role of androgens on the growth of Atlantic cod (*Gadus morhua*) previtellogenic oocytes. *Comp Biochem Physiol D: Genomics and Proteomics* 3: 205-218. Partner: NTNU, Norway.

3. Kortner, TM, Rocha, E, Arukwe, A. 2008. Androgenic modulation of early growth of Atlantic cod (*Gadus morhua* L.) previtellogenic oocytes and zona radiata-related genes. *J Toxicol Env Health* (in press). Partner: NTNU, Norway

4. Silva, P, Rowleron, AM, Valente, LMP et al. 2008. Muscle differentiation and growth in blackspot seabream (*Pagellus bogaraveo*, Brunnich): histochemical and immunohistochemical study of the fibre types. *Tissue & Cell* (in press). Partner: King's College, Univ. London, UK.

5. Jordanova, M, Miteva, N, Rocha, E. 2008. A qualitative and quantitative study of the hepatic pigmented macrophage aggregates during the breeding cycle of Ohrid trout, *Salmo letnica* Kar. (Teloestei, Salmonidae) *Microsc Res Techniq* (in press). Partner: Univ. Sts. Cyril & Methodius, Macedonia.

▪ The PI was invited to integrate his team in a European network project named Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM). Also, the PI is helping inserting CIMAR LA in AQUA-TNET (Socrates-Erasmus Thematic Network in Aquaculture, Fisheries and Aquatic Resource Management). This will allow networking the Master in Marine Sciences and Resources (UPorto), in which the team participates. The PI is a Deputy Director of the new inter-university PhD Program in Marine and Environmental Sciences, and is preparing a network with Institutions in Galicia (Spain). The PI's Group provides advanced training/classes in those Master and PhD programmes. The Group housed PhD students from Brazil (1) and Norway (1).

## 6e. Future research

### 1. Objectives (3000 ca.)

• The strategic objectives for the next 3 years are to: 1) increase in 10% the articles with international partners and the articles average impact; 2) increase in 10% the number of articles/PhD/year; 4) conclude at least 2 theses/year; 5) focus on 2 research lines; 6) run an annual course on cell and molecular biology techniques.

- Line 1) Fish peroxisomes and related nuclear receptors (PPARs, RXRs, RARs); interplay mechanisms, endocrine modulation, and metabolic consequences of disruption – We will continue to publish data derived from our latest efforts, proving that fish peroxisomes are modulated by sex steroids, particularly in liver and by estrogenic stimuli, apparently involving PPARs. Also, we will study interactions between peroxisomal / PPARs activating vs repressing (estrogens) ligands. Work will be conducted with the so-called peroxisome proliferators (PPs). Some of them have been detected worldwide, and concern about their use is growing due to their persistence, toxicity and bioaccumulation. We will explore the hypothesis that those exogenous PPARs ligands may disrupt lipid metabolism in fish, affecting health, survival, growth and, lipid/FA profiles. We think we will contribute with new insights to understand lipid and energy metabolism control in fish, with possible application on risk assessment and aquaculture grounds. In parallel, it will be also described the action of PPs on other genes not involved in lipid metabolism and possibly not under PPARs control, as the result of the use of advanced molecular tools. As it known that PPARs cross-talk with RXRs, we are co-responsible for a new project that involves a multiparametric approach, combining the study of variations at the gene and protein (enzymes) levels of key disruption prone targets, but also changes in sexual development and reproduction. We expect to identify relevant metabolic pathways prone to disruption by RARs, RXRs, PPARs agonists.

- Line 2) Toxicopathology, Toxicogenomics and Chemical Toxicology – We just launched a multidisciplinary project integrating transcriptomics, which will set grounds for an expansion and cooperative efforts into proteomics and metabolomics. The project background is the fact that toxicopathic injuries in estuarine fish are increasing. Histopathology often reveals liver lesions and intersex condition. These situations may translate either acute and/or chronic chemical stress. Field surveys targeting fish toxicopathic lesions are being made in Europe and USA, in parallel with the use of other biomarkers and chemical analyses. Pathology surveys are very scarce in Portugal and so we aim to lead them. The new project is unique in making a 1st systematic survey of toxicopathic lesions in Portuguese estuarine fish, whereas experimentally tackling effects of lesions at diverse biological levels. Some of the aims are to: 1) document toxicopathic fish lesions in estuaries in the north and centre; 2) clarify in what extent the lesion type and severity relates with pollution; 3) know better fish carcinogenesis and find ways to early detect related lesions; 4) clarify if/in what extent hepatic necrosis and neoplasia interferes with general liver function and detoxification, growth/survival; 5) conclude about gender susceptibility and implications of fish neoplasia; 6) study gene signatures of tumour liver progression in a fish model; 7) develop a focused cDNA array to analyse wildlife and experimental samples. We will generate field data and also mechanistic insights.

## **2. Funding, source, dates (1500 ca.) (Indicate in full including amount of current and pending funding)**

1. Lipid metabolism disruption in marine fish by xenobiotic ligands of peroxisome proliferator-activated receptors (PPARs). Source: FCT (Project PTDC/MAR/68885/2006). Starting date: July 2008. Funding: 184.929,00 EUR.

2. An integrative study on the toxicopathic lesions in Portuguese estuarine fishes - Assessing injury impact and toxicogenomic implications in experimental models. Source: FCT (Project PTDC/MAR/70436/2006). Starting date: October 2008. Funding: 187.028,00 EUR.

3. The modulation of retinoic acid signalling pathways by environmental pollutants in teleosts. Source: FCT (Project PTDC/MAR/68106/2006). Starting date: September 2008. Total Funding: 188.980,00 EUR. Project in cooperation with the Group Lab Ecotoxicology, Genomics and

Evolution and the Lab Environmental Toxicology of the CIMAR. Funding for our research Group (LECEM) = 71.164,00 EUR.

4. 2008 Pluriannual Funding for LECEM. Total Funding: 16.250,00 EUR (3.250 EUR / PhD).

**3. Previous publications in the area** (1500 ca.) (5 in the last 5 years. If available you must indicate at the end of the citation, impact factor of the journal (IF=) and number of citations (n° C=). Give title and full citation in original language)

1. Batista-Pinto, C, Rodrigues, P, Rocha, E, Lobo-da-Cunha, A. 2005. Identification and organ expression of peroxisome proliferator activated receptors in brown trout (*Salmo trutta* f. *fario*). *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression* 1731: 88-94. IF 2005/2007=2.506/1.704, n°C=5.

2. Castro, LFC, Lima, D, Machado, A, Melo, C, Hiromori, Y, Nishikawa, J, Nakanishi, T, Reis-Henriques, MA, Santos, MM. 2007. Imposed induction is mediated through the Retinoid X Receptor signalling pathway in the neogastropod *Nucella lapillus*. *Aquatic Toxicology* 85: 57-66. IF 2007=2.975, n°C=6.

3. Matos, P, Fontaínhas-Fernandes, A, Peixoto, F, Carrola, J, Rocha, E. (2007) Biochemical and histological hepatic changes of Nile tilapia (*Oreochromis niloticus*) exposed to carbaryl. *Pesticide Biochemistry and Physiology* 89: 73-80. IF 2007 = 1.475, n°C=0.

4. Ribeiro, C, Tiritan, ME, Rocha, E, Rocha, MJ. 2007. Development and validation of a HPLC-DAD method for determination of several endocrine disrupting compounds in estuarine water. *Journal of Liquid Chromatography & Related Technologies* 30: 2729-2746. IF 2007=0.977, n°C=2.

5. Kortner, TM, Rocha, E, Silva, P, Jørstad, TS, Castro, LFC, Arukwe, A. 2008. Genomic approach in evaluating the role of androgens on the growth of Atlantic cod (*Gadus morhua*) previtellogenic oocytes. *Comparative Biochemistry and Physiology Part D - Genomics and Proteomics* 3: 205-218. IF 2007=1.391, n°C=0.

**4. Special requirements** (1500 ca.) (equipment, facilities, staff or other special needs essential to carry out the future research program)

Equipment and facilities:

Within the CIMAR LA, the research group has access to virtually all that is needed to carry out the proposed research programme, both in terms of laboratory equipment and fish husbandry. The group has also direct access to other facilities and equipment at the ICBAS – University of Porto (eg, confocal microscope, transmission electron microscope, microdissector, histology equipment, etc.), where the PI and other senior researcher work as Professors; there are cooperation protocols signed between the CIMAR LA and the University of Porto. For accessing further equipment to support the genomic approaches, namely for building, reading and statistically analysing arrays, there is an undergoing official collaboration with the Department of Biology, of the Norwegian University of Science and Technology, Trondheim, Norway. The future projects include outsourcing specific tasks, such as customized gene array printing. The local husbandry facilities for the Group are being expanded at this exact moment.

Staff or other special needs:

The group has sufficient staff, and also ongoing established partners and cooperation, to pursue its research objectives. All the financed projects will be able to hire additional staff, namely via two research investigation grants. The group will also continue to host Master and PhD students, which will integrate the research efforts while receiving advance training. In 2008 the group was reinforced with: 1) one full time (German) auxiliary PhD researcher working; 2) one full time post-doc FCT grantee. At three to four recent PhD graduates may also join the efforts as team members and collaborators, despite they have teaching obligations as members of university staff.

## 7. OTHER LA ACTIVITIES

### 7a. Services & resources

1. **Internal services and resources** (3000 ca.) (Indicate here sharing of equipment and other resources both within the LA, between LAs or with other institutions of Higher Education nationally or internationally)

CIMAR LA occupies two distinct poles, one at the University of Porto (ca 3500 m<sup>2</sup>) and another one at the University of Algarve (ca 1800 m<sup>2</sup>). Each pole also includes facilities (1000 m<sup>2</sup>) with water closed circuits for animal maintenance with an overall capacity of 60000 liters of seawater and freshwater. CIMAR researchers have also access to an aquaculture experimental station located in the Algarve (ca 2 ha). Recently, CIMAR acquired two small vessels (12 and 17 meters) for coastal and estuarine campaigns that will be made available to researchers during 2008.

Since 2003, some significant equipment has been acquired mainly for analytical chemistry and molecular and genetic research. Most of this equipment (2 million EUR) was purchased through the national competitive program for the scientific re-equipment launched by FCT and includes:

- Integrated system for automating behaviour experiments and data collection
- Complete set-up of intra- and extra-cellular electrophysiological recording
- High Pressure Liquid Chromatography System with light scattering detector (HPLC)
- Mass detector for HPLC
- Isotope ratio mass spectrometer (IRMS)
- Gas chromatography with Mass Spectrometry
- Sequence detection system for Real Time PCR
- Automatic DNA sequencers
- Multipurpose liquid scintillation and luminescence counter
- Upgrade of Image Analysis System
- Phosphorimager and fluorimager
- Culture chambers with light, temperature and photoperiod controls
- Microplate Spectrofluorimeter
- Gas Chromatography coupled to Mass Spectrometry (GC-MS)
- Gamma Counter
- Ultracentrifuge, rotors and accessories



- Epi-fluorescence and phase contrast photomicroscope
- Sequence Detection System for Real-time Quantitative PCR
- Inverted Microscope for transmitted light, fluorescence, Nomarski DIC and Phase-contrast
- Cryostat
- Laminar flow cabinet
- Multianalysier FIA and Underwater autoanalyser
- Cell culture facility
- Microplate reader
- Atomic Absorption Spectrometer

This equipment is shared within CIMAR but is also available to other LAs and Higher Education Institutions. CIMAR can also access to equipment from other LA and Higher Education Institutions mainly for scanning and transmission electron microscopy. In the framework of the agreement between FCT and the Portuguese Hydrographic Institute, CIMAR researchers have also the possibility to hire ship time at low price.

Two services interface have been developed within the LA, for analytical chemistry and molecular and genetic research. Several services are installed in both centers for administrative and technical support, with a total of 23 staff members, including public and international relations, project management, finance, IT systems, image and communication, juridical, biotery, and maintenance. CIMAR has been stimulating the training of this technical staff (2 BSc and 4 MSc degrees have been accomplished during 2003-2007).

Through several protocol agreements with higher education institutions, CIMAR has been supporting pre and post graduation training by providing facilities and resources including the participation of non-academic staff in teaching activities.

**2. External services and resources (3000 ca.)** (Indicate here scientific or technical services provided both nationally or internationally to Governments/Institutions or the community)

CIMAR has been involved in a large number of activities to support public policies. Most relevant are:

- Member of European Science Foundation – Marine Board (final acceptance in 2008)
- Participation in the Report Europe of the Sea, a strategic evaluation of maritime activities from CRPM
- Detachment of two national experts to FP5 and FP6 (Environment Theme)
- Third party of FCT in the ERA-nets AMPERA and Biodiversa, FP6

- Detachment of a permanent staff element to integrate the Portuguese network of FP7 National Contacts Points, supported by the Minister of Science and Technology, to provide assistance to potential FP7 participants (Universities, Research Centers, SMEs, Industry) in all aspects regarding the “Environment, including Climate Change” Theme

- Founding member of the Institute for the Development of the Knowledge and the Economy of the Sea (IDCEM - Agency for the promotion of scientific knowledge, valorization of research and technology service agreements, and promotion of entrepreneurship)

- Leadership of the Ocean agenda for the territorial cooperation North of Portugal – Galicia

- Contribution to the implementation of the Water Framework Directive, National Institute for Water (INAG)

- Contribution to the Ocean Agenda of the North Region, North Regional Coordination and Development Commission (CCDR-N)

- Contribution to the Regional Spatial Planning of Algarve, CCDR-Algarve

- Coordination of the National Task Force regarding the environmental impact of the Prestige oil spill for the Intersectoral Oceanographic Commission

Moreover, CIMAR has been involved in several R&D projects aimed to give support to European Territorial cooperation (eg Interreg) and has been regularly requested by the government to give advice on legislation (eg. Environment and fishing) and bilateral agreements involving Portugal.

At the individual level, members of the Direction of CIMAR have also been involved in several activities:

- Member of the scientific council of FCT

- National delegate for FP5, FP6, FP7

- Director of IDCEM

- Member of the Strategic Commission for the Oceans (2003-2004)

- Member of the Council of the Task Group for Maritime Affairs (EMAM)

- Focal Point of the Minister of Science and Technology on the National Ocean Strategy

- Ambassador of the Commissioner Joe Borg (DG Mare) for the dissemination of the Green Book on the European Maritime Policy

In the last five years, CIMAR provided services for the industry in several areas including water quality (chemical, biological and toxicological; to 19 enterprises), fish sanitation and pathology (12 enterprises), diet formulation for aquaculture (2 enterprises), algal biomass monitoring and production (2 enterprises), DNA extraction and genotyping (2 enterprises and several national and European universities), physical oceanographic data collection (1 enterprises) and environmental consulting studies (3 enterprises). Recently, CIMAR has been qualified for providing R&D and innovation services to SMEs under the Portuguese National Strategic Reference Framework (QREN, 2007-2013).

## 7b. Networking actions

### 1. Networking actions (2000 ca.) (Indicate here both national and international activities)

CIMAR members have been involved in numerous network actions to build the National and European Research Areas. Most relevant:

EU Networks of Excellence - FP6

- MGE - Marine Genomics Europe, [www.marine-genomics-europe.org](http://www.marine-genomics-europe.org)
- MARBEF - Marine Biodiversity and Ecosystem Function, [www.marbef.org](http://www.marbef.org)

ERA Nets - FP6 (Third party of FCT)

- BiodivERsA, [www.eurobiodiversa.org](http://www.eurobiodiversa.org)
- AMPERA, [www.cid.csic.es/ampera](http://www.cid.csic.es/ampera)

Technological Platforms

- European Aquaculture Technology Platform, [www.eatpnet.eu](http://www.eatpnet.eu)
- ASEM Aquaculture Platform, [www.asemaquaculture.org](http://www.asemaquaculture.org)
- Water Supply and Sanitation Technology Platform, [www.wsstp.eu](http://www.wsstp.eu)

ESF Networking Programmes

- Functional Dynamics in Complex Chemical and Biological Systems, <http://funcdyn.org>
- Molecular Simulations in Biosystems and Material Science, [www.simbioma.cecarn.fr](http://www.simbioma.cecarn.fr)

COST Actions, [www.cost.esf.org](http://www.cost.esf.org)

- Welfare of fish in European aquaculture - n. 867
- Critical success factors for fish larval production in European Aquaculture: a multidisciplinary network - n. FA0801
- Oral facial development and regeneration - n. B23
- The importance of prenatal events for postnatal muscle growth in relation to the quality of muscle based foods - n. 925

International Thematic Networks

- European Network of Marine Institutes and Stations, [www.marsnetwork.org](http://www.marsnetwork.org)
- EPBRS, [www.epbrs.org](http://www.epbrs.org), and the supportive networks BioPlatform (CIMAR coordination, [www.bioplatform.info](http://www.bioplatform.info)) and Biostrat

- Global Network for the Hazard Management of Cyanobacterial Blooms and Toxins in Water Resources, UNESCO-IHP, [www.cyanonet.org](http://www.cyanonet.org)
- Historical Ecology of the Trans-Atlantic Marine Biota Network, [www.biology.duke.edu/corona](http://www.biology.duke.edu/corona)
- MedZoo Network, through CIESM program, <http://www.ciesm.org/marine/programs/zooplankton.htm>
- Red Iberoamericana de Toxicología y Seguridad Química, <http://ritsq.org>
- Rede Iberoamericana de Contaminação e Toxicologia Ambiental, SICTA

#### National Thematic Network

- DivMar - Rede de Biodiversidade Marinha e Costeira, [www.cimar.org/divmar](http://www.cimar.org/divmar)

#### Training Network

- European Erasmus Network in Aquaculture, Fisheries & Aquatic Resources Management, [www.aquatnet.com](http://www.aquatnet.com)

#### FP7 National Contact Points Network

- CIMAR has detached a permanent staff element to integrate the Portuguese network of NCP for FP7 (Environment including Climate Change theme) [www.gppq.mctes.pt](http://www.gppq.mctes.pt)

### 7c. Training

#### 1. Training activities (2000 ca.) (Indicate here activities of the LA as a whole)

Between 2003 and 2007, 142 MSc students and 87 PhD students have completed their degree under the (co)-supervision of CIMAR members. New theses are currently underway.

#### Post-graduate Studies

Doctoral School in Marine and Environmental Sciences (cooperation with CESAM, Porto and Aveiro Universities) [www.cimar.org](http://www.cimar.org)

#### Involvement in European Master programmes:

- Science in Marine Biodiversity and Conservation [www.embc.marbef.org](http://www.embc.marbef.org)
- Ecological Management of Catchments [www.ecocatch.eu](http://www.ecocatch.eu)
- Science in Fisheries and Aquaculture [www.maqfish.com](http://www.maqfish.com)

#### Agreement with ICBAS-UP for the Masters:

- Marine Sciences – Marine Resources

- Environmental Contamination and Toxicology

Organization of advanced training courses, Workshops and Internal Seminars Series. Most relevant:

- Experimental Design course. AJ Underwood & G Chapman (Univ. Sydney). Faro, 06/03 and 11-15/09/06.

- A field Guide to GenBank and NCBI Molecular biology resources. In collaboration with the National Centre for Biotechnology Information (USA). Faro, 04/04.

- PRIMER-E, Multivariate Statistics for Ecologists. Porto, 4-7/05/04.

- European Institute in Statistical Genetics. Bruce Weir, Bioinformatics Research Center, North Carolina State. Faro, 19-28/07/04.

- White and Black Scientific Illustration Techniques. Porto, 11/04, 05/05.

- Fish Vaccination. Porto, 3-4/02/05.

- II Molecular Evolution. D Swofford, G Naylor, M Holder (Florida State Univ, EUA), Faro, 2005.

- Techniques for Writing and Presenting a Scientific Paper. Porto, 25-28/10/05.

- Intensive Program AIDA (29274-IC-3-2004-1-AT-ERASMUS-IPUC-16). Faro, 21/04-13/05/06.

- Applied Aspects of Marine Parasitology, 21-24/05/06, Horta, Azores.

- ESF LESC Exploratory Workshops. Invasion of European shores by *Sargassum muticum*: research integration toward the future. Tavira, Portugal, 9-12/11/06.

- Advanced course in monitoring and management of pollution in coastal and estuarine areas. National Institute of Oceanography (NIO), Goa, India, 5-17/03/07.

- RNA extraction from different types of marine invertebrate organisms. In coll. with Max Plank Inst Berlin. Faro, 5-8/05/07.

- Technical training of human resources within Instituto do Emprego e Formação Profissional and FORPESCAS.

- Internal seminars in Faro and Porto on a fortnight basis.

## **7d. Outreach/science & society**

### **1. Outreach/science and society (4000 ca.) (Indicate here activities that the LA as a whole)**

CIMAR has become increasingly committed to outreach activities to foster the dialogue between scientists and society, to improve the public knowledge and perception of science and to increase

the interest of the young students towards scientific careers. Some of the most relevant outreach activities include:

Scientific management of public equipment

- CMIA's - Environmental Monitoring and Interpretation Centres

Through protocols with the municipalities of Matosinhos and Vila do Conde ([www.cmia-viladoconde.net](http://www.cmia-viladoconde.net)), CIMAR is directly managing two CMIA's, which opened in 2007 (7500 visitors in 6 months). Both centres have displayed several temporary exhibitions, organized conferences and professional courses and performed regular in house and field activities for public of different ages, with a particular emphasis on young students.

- Public aquariums and fisheries museums

Aguda Littoral Station - ELA ( [www.fundacao-ela.pt](http://www.fundacao-ela.pt), ca 50000 visitors/year) and River Minho AQUAMuseum ([www.cimsoft.pt/aquamuseu](http://www.cimsoft.pt/aquamuseu), ca 30000 visitors/year) have been created and managed by CIMAR researchers. Both stations have a special relevance in what concerns Environmental Education Programs for all age.

Participation at the International Polar Year Education & Outreach Programme

- LATITUDE60! (<http://latitude60.blogspot.com>)

The IPY has a strong education and outreach programme and the Portuguese educational project LATITUDE60! is considered as one of the most active and comprehensive projects internationally. More than 40 activities dealing with education and outreach were developed to explain polar research, involving more than 200 schools, 400 teachers and thousands of students. The top events include science and art competitions for schools culminating in an educational expedition of 7 high school students integrated in "Students on ice" to Antarctica; itinerant exhibitions and acting, workshops, lectures, pamphlets and books (also in Braille) and the Polar Science Weekend at Pavilhão do Conhecimento (6500 visitors).

Participation in the Ciência Viva Programme (<http://www.cienciaviva.pt>)

Several types of activities have been implemented at CIMAR, to contribute to the promotion of a scientific and technological culture among the Portuguese population and more specifically to encourage the use of experimental methods in science learning, involving both scientific and school communities. Most relevant activities include:

- Scientists go to school - CIMAR research groups, in collaboration with the Algarve Ciência Viva Centre, have prepared a number of scientific themes to present at elementary and high schools of Algarve; around 140 lectures were given in the last five years.
- Science on Holidays for Young People - CIMAR research groups have prepared more than 50 scientific activities (1-2 weeks each) aiming to offer high school students the opportunity for first-hand contact with the reality of scientific research work during their summer vacations (students from Spain have also been involved in 2007).

- Biology Activities in the Summer - CIMAR has prepared around 40 field activities (4-8 hours each) for general public of all ages, in particular family groups (e.g. Bird Watching, Ecology and Biology of the Littoral, etc.).
- Science and Technology Week - Since 2005, CIMAR has participated in the annual Science and Technology Week through the organization of several exhibitions, workshops and conferences for the general public. In 2007, both centers have decided to open their doors and promote a direct contact between researchers and public through guided tours.

#### Participation in other events and exhibitions

- Junior University (<http://universidadejunior.up.pt/>), within the School of Life and Health Sciences for high school students
- “Science, Education and Innovation” exhibition by the University of Porto, 2003-2007, through the presentation of the research developed at CIMAR to encourage high school students to engage in scientific careers
- Commemorations of the National Sea Day ([www.diadomar.mdn.gov.pt/actividades\\_continente.htm](http://www.diadomar.mdn.gov.pt/actividades_continente.htm))
- EXPOMAR exhibition, 2005-2007, by generating information regarding consumer concerns of aquaculture products to the general public
- Several exhibitions (e.g. ImaGene, 2007, within the Marine Genomic NoE)
- Several seminars to the general public and participation of volunteers within the scope of scientific projects (i.e. Biomares, FindKelp, Seacase)

#### CIMAR in the media

CIMAR activities have been also disseminated through the media: publications in non-scientific journals (i.e. National Geographic) and newspapers, interviews on radio station (i.e. TSF, Portugueses Excelentíssimos) and participation in TV programs (i.e. 4x ciência, Biosfera).

### 7e. International events

#### 1. Organization of international events (2000 ca.) (Indicate here events that are international in dimension and required the involvement of the LA for their implementation)

CIMAR has been involved in the organization of several international scientific congresses and symposia. Most relevant in the last five years included:

- 9th International Congress of the European Association for Veterinary Pharmacology & Toxicology. Lisbon, July 2003. 600 participants.
- 5th Iberian and 2nd Iberoamerican Congress of Environmental Contamination and Toxicology - Environmental Problems in an Iberoamerican Context. Porto, Sept 2003. 230 participants, 15 countries.



- 4th Iberian Limnology Conference. Porto, July 2004.
- 5th Congress of the Iberian Association of Comparative Endocrinology. Faro, 7-9 Sept 2005. 100 participants.
- 3 Symposia at the 23rd Conference of European Comparative Endocrinologists (Pheromone and other chemical communication, Endocrinology of calcium homeostasis, Comparative Thyroid Endocrinology). Manchester, 29 Aug - 3 Sept 2006. Editorial Material published in a special issue of General Comparative Endocrinology (vol 52, 2007, Elsevier).
- 17th Annual Meeting of SETAC Europe - Multiple stressors for the environment, present and future challenges and perspectives. Porto, 20-24 May 2007. 2000 participants, 65 countries.
- 10th Symposium of Aquatic Microbial Ecology - SAME. Faro, 2-7 Sept 2007. 200 participants.

The Director of CIMAR was co-chairman at EurOCEAN 2007, Aberdeen, June 2007. This event resulted in the Aberdeen Declaration, which synthesises the voice of marine scientists and express their priorities in the context of the development of the European Maritime Policy

<http://ec.europa.eu/maritimeaffairs/eurocean2007.html>

## PORTUGUESE PRESIDENCY

CIMAR organized two international events within the Portuguese Presidency to discuss the strategic research needed for the marine environment, particularly in the light of EU policies including the Marine Strategy Directive and EU Maritime Policy:

- The Role of Marine Sciences in Ocean Sustainability and Global Change. Lisbon, 8 October 2007. Target audience: Ministers of Science and Technology, scientists and policy makers and EurOCEAN Organising Committee. 150 participants.

[www.cimar.org/archive\\_meetings\\_ocean\\_sustainability2007.htm](http://www.cimar.org/archive_meetings_ocean_sustainability2007.htm)

- Life in the Blue Planet - Biodiversity Research and the New European Marine Policies. EPBRs meeting, Porto, 6-9 September 2007. 100 participants.

[www.cimar.org/epbrs](http://www.cimar.org/epbrs)

## 7f. Summary

### 1. Final summary comparison between original objectives and achievements (2000 ca.)

In the research contract with FCT, the following four horizontal programs were consigned:

1. Develop links with the institutions and companies using the results of the lab activities in the form of products and/or services, contributing to the socio-economic development and stimulating innovation;

2. Promote the development of the Scientific Culture through actions with schools and the general public, the preparation of information and didactic material, and collaboration with the specific programmes of the Science and Technology Ministry;
3. Promote and take part in platforms and other national and international thematic networks;
4. Promote the training of scientific and technical human resources through the support to post-graduation courses, including Masters and Doctoral courses.

A total of 29 missions were also indicated as example of actions to undertake under these programs.

The first program was the most difficult to implement for two main reasons: a) low level of technology utilization by the majority of enterprises in the marine and maritime sector; b) difficulties on the implementation of a technology transfer system inside the LA. To surpass these difficulties, CIMAR decided to maintain a maximum level of direct services available to enterprises and, simultaneously, create with other partners the Institute for the Development of the Knowledge and the Economy of the Sea (IDCEM). This institution was created in 2006 as an agency for the promotion of scientific knowledge, valorization of research and technology service agreements, and promotion of entrepreneurship. CIMAR has been progressively more requested by governmental sectors to support public policies.

Concerning the other three programs, CIMAR has overcome the planned objectives. Main achievements included:

- Large programme in outreach science and society, including the Scientific management of public equipments (ca 100.000 visitors/year), and the participation at the International Polar Year Education Programme and in the Ciência Viva Programme
- Participation in 2 Networks of Excellence and 2 Era-nets (FP6), 3 Technological Platforms, 6 ESF Networking Programmes and cost actions, and several national and international thematic networks
- Creation of a Doctoral School in Marine and Environmental Sciences in cooperation with CESAM and Univ. of Porto and Aveiro (open to international extension), and collaboration in several national and European Master programmes

## 8. INTERNAL EVALUATIONS

### 8.1 Reports (Include here pdf copies of internal evaluations and the application of the recommendations)

- Report FCT (See [Annex 2](#))
- Report Prof. S.J. Hawkins (See [Annex 3](#))
- Report Prof. Edward Donaldson (See [Annex 4](#))
- Notes Prof. S.J. Hawkins (See [Annex 5](#))
- Report Prof. S.J. Hawkins\_10\_2008 (See [Annex 6](#))

### 8.2 Implementation of the recommendations (2000 ca.)

By difficulties of agenda, it was not possible to gather all the members of the Advisory Board in 2006. Only Prof. Steve Hawkins and Prof. Edward Donaldson made their reports. Previously (2004) we had a visit of an Advisory Group from FCT, which produced a report that is also annexed. We attached also the critical notes produced by Prof. Steve Hawkins at the meeting with CIMAR Board of Directors (2006) and a recent report relative to his visit to CIMAR-Porto. Globally, the reports noted an improvement of CIMAR organisation and results in the different areas of activities.

The most relevant recommendations are relative to:

1. The need for a better cross-relationship between research groups;
2. Development of the Horizontal Programmes, including the commitment of research groups on common activities (i.e. monitoring);
3. The need for an integrated training programme;
4. The need for recruitment of young scientists in some key areas (i.e. physical oceanography, biogeochemistry, environmental economics, ecosystem modelling and social sciences).
5. The need to proceed with networking with other institutions (national and international), particularly with state laboratories (IPIMAR).

CIMAR has dealt with these recommendations in the following manner:

1. The increase collaboration between research groups resulted in the reduction from 38 (2003) to 24 (2008) groups (in these way three groups of Porto and one of Algarve have merged in one, more coherent, group of Aquaculture). Even among unaltered groups, the number of articles shared by researchers of different groups has significantly increased.
2. The Horizontal Programmes were strongly reinforced (see section 7). A monitoring programme involving CIMAR, CESAM, ISPA and another LA (ISR) was launched after

an agreement in the framework of the DIVMAR network. Financial difficulties are still postponing its full implementation.

3. CIMAR launched the Doctoral School with CESAM and 2 Universities.
4. The recruitment of researchers in most of these areas was already done. Recruitment on missing areas is foreseen for the next years (Ciência 2008 and FCT contract – see section 9)
5. Networking at National and European level has strongly increased. Relationships with State Laboratories are foreseen by the consortium OCEANO to be launched in 2009.

### **8.3 Future internal evaluation (2000 ca.)**

The end of 2008 or the beginning of 2009 is the time foreseen for the next evaluation by the External Advisory Board. The implementation of the recommendations will be more visible by then. We hope to join all the members of the board and we intend to show them the facilities in the three centres (including ISPA-Lisbon) and to offer them the possibility of a better contact with researchers.

Visits are expected from advisors of each centre as it has been done in the past (e.g. William Wiebe, almost once a year in the period 2003-2006 and Peter Chapman - 2005).

It is also foreseen to complete the Advisory Board with one more member in the field of Physical Oceanography.

## 9. FUTURE OBJECTIVES

### 9.1 Summary of proposed future objectives (2000 ca.)

Future objectives of the LA are aimed at consolidating existing lines of research, synergistic interactions of research teams, and integrating new approaches that may contribute to a holistic view of marine ecosystems, responsiveness to changes of natural or anthropogenic origin and contribution to social, educational and economic sectors.

Specific objectives are:

- 1) Develop and contribute to integrate marine biodiversity initiatives and databases at a national and European level through research networks.
- 2) Develop genomics and post-genomics capabilities to understand biogeochemical processes, short and long-term adaptation including species invasions, extreme environments, analysis of historical samples and potential biotechnological applications such as marine biofuels.
- 3) Develop physical oceanography and modelling competences which can be associated with existing remote sensing and biology expertise to understand ecosystem dynamics, dispersion of pollutants and long term climatic variations .
- 4) Establish risk management procedures for natural toxins and xenobiotics by integrating information from multiples levels of biological organization from molecules to ecosystem.
- 5) Integrate social sciences in the evaluation of ecosystem services including fisheries.
- 6) Develop analytical chemistry and bioscience platforms (“omics”, imaging) to support the LA and provide external services.
- 7) Stimulate technology transfer, setting up of spin offs, startups and partnerships with industry, in particular aquaculture.
- 8) Develop an action plan to improve and stimulate cooperation between different research groups within research lines and across research lines with the aim of reducing fragmentation and stimulating cooperation.
- 9) To promote public understanding of sciences for the general public educational programmes targeting schools at all levels.
- 10) Strive to improve cost-effectiveness of research and reduce waste and carbon footprint.

### 9.2 Future vision of the LA (2000 ca.)

It is envisioned that CIMAR LA will acquire critical mass and dimension in key areas of Marine and Environmental Sciences and progressively become player at European level. As part of this process the Eco-Ethology Unit (ISPA, Lisbon) will join CCMAR and close ties will be maintained with the Marine Geology Department of INETI. Strong links are also maintained with CSAM (Univ. Aveiro) in training and research.

CIMAR LA has been having and will continue to have a leadership role in promoting national networking and clustering to face the challenges of sharing of infrastructures, international dimension, creating visibility and promoting technology transfer to industry.

Internal organization will have to be adapted to the growing demands and will be developed in consultancy with the advisory council and relevant partners. Annual meetings and more specialized initiatives, such as workshops, have an important cohesive function and facilitate the development of collaborations in poles geographically separated and across fields of research.

To increase visibility of the LA will be an objective. This will involve 1) establishing a coordinated communication plan between LA centres, 2) establishing a position for a research-industry relationship officer with previous experience in science and/or industry sectors and 3) widen outreach activities with schools and the general public.

To increase the offer of post graduate courses, targeting partnerships with institutions in Portuguese speaking countries.

Finally, a strategy for infrastructure and technical platform expansion in coordination with national and international initiatives will be developed.

### 9.3 Human resources (Proposed hiring of new researchers in 2008-2011)

	2008	2009	2010	2011	Total
Nr of researchers from previous contract	0	0	0	23	23
Nr of researchers to be hired	0	2	6	4	12
<b>Total</b>	0	2	6	27	35

### 9.4 Justification for human resources (2000 ca.) (Justify here individually each new researcher position)

These positions are aimed at reinforcing research groups and reflect also areas of development within CIMAR. Slight adjustments may be made in the topic at the time of recruitment.

1 scientist to implement ecosystem based management strategies for fisheries incorporating the role of top predators, multispecies modeling (predator-prey interactions etc.)

1 scientist to study essential fish habitat, habitat mapping and implementation of protected areas

1 scientist for incorporation of socio-economic aspects in fisheries management

1 scientist to develop bioinformatics capacities for an increasing number of genomics and proteomics projects

1 scientist to study plant and animal adaptation to climate change and species invasions, including impacts on fisheries

1 scientist to develop systems for bio-fuel production from microalgae.

1 scientist for marine bio-economics to study the economy of marine conservation and resource management in relation with marine ecosystem services.

2 scientists to reinforce the ecological modeling capabilities of the LA, which is essential to match the needs for the study of ecosystem functioning and risk management.

1 scientist to study energy exchanges along the trofic chain, also needed to increase the LA capacity for knowledge of ecosystem functioning.

1 scientist specialist in Bio-Geo-Chemistry to study element cycles (filling the gap indicated by external advisors) which will also reinforce the capacity on ecosystem functioning and global changes.

1 scientist in the area of Physical Oceanography to give support to ecologists on genetic connectivity issues, making the bridge between the physical and the biological domains.

As previously indicated, CIMAR needs to double the number of technicians (from 10 to 20) to support the increasing needs for laboratory and field work.

## **9.5 Training (1000 ca.) (Participation in training programs)**

CIMAR will consolidate the Doctoral School in Marine and Environment Sciences in cooperation with the other partners and will enlarge the supporting network to include European institutions - negotiations have already started with Bangor University, University of Bergen and NIO. (The Netherlands).

Cooperation at other post-graduate levels with 3 universities of Galicia (Spain) have also started in 2007.

The reinforcement of CIMAR collaboration with Portuguese Universities at the level of M.Sc. is also expected to increase.

Particularly attention will be given to international M.Sc. courses, namely the ERAMUS MUNDUS M.Sc. programme. The agreement with CESAM includes in a second stage (after the creation of the Doctoral School) a programme of capacity building oriented to the Developing Countries, particularly to the Portuguese speaking countries and territories. This programme of training-through research is expected to be launched in 2009 profiting from the intense scientific collaboration already existing with some of those countries.



