

Centro de Ciências do Mar do Algarve  
Algarve Centre of Marine Sciences

2002

Publications List.....	1
Division Aquaculture and Biotecnology .....	1
Articles in international refereed journals (listed in SCI) .....	1
Articles in other refereed journals (national and international).....	2
Papers in refereed conference proceedings and published abstracts .....	2
Conferences papers (not in proceedings).....	3
Project and consultancy final reports .....	6
Prizes and Honours.....	6
Division of Living Resources.....	6
Books, edited books, chapters of books .....	6
Articles in international refereed journals (listed in SCI) .....	6
Articles in other refereed journals (national and international).....	7
Papers in refereed conference proceedings and published abstracts .....	7
Conferences papers (not in proceedings).....	7
Project and consultancy final reports .....	9
Prizes and Honours.....	9
Division of Aquaculture and Biotecnology .....	10
Theses PhD .....	10
Theses Master of Science.....	11
Graduation Honours thesis (Estágio de licenciatura).....	11
Division of Living Resources.....	12
Theses PhD .....	12
Theses Master of Science.....	13
Graduation Honours thesis (Estágio de licenciatura).....	14
Description of the research activities .....	15
Division of Aquaculture and Biotecnology.....	15
Group Molecular Biology of Marine Organisms .....	15
Research team.....	15
Summary of activities and progress during 2002 .....	15
Biotecnology and Molecular Biology of Microalgae Group .....	17
Summary of activities and progress during 2002 .....	17
Plan for 2003.....	18
Group of Comparative and Molecular Endocrinology .....	18
Research team.....	18
Summary of activities and progress during 2002 .....	19
Plan for 2003.....	23
Group of Physiopathology .....	24
Research team.....	24
Summary of activities and progress during 2002 .....	24
Plan for 2003.....	27
Biophysics Group.....	28
Summary of activities and progress during 2002 .....	28
Plan for 2003.....	28
Group Aquaculture.....	28
Research team.....	28
Summary of activities and progress during 2002 .....	28
Plan for 2003.....	32
Optimization of sole ongrowing .....	32
Division of Living Resources.....	33
Group: Ecology and Evolution of Marine Organisms.....	33
Research team.....	33

Summary of activities and progress during 2002 .....	33
Plan for 2003 .....	34
Group of Marine Plant Ecology .....	35
Research team .....	35
Summary of activities and progress during 2002 .....	35
Global-related changes in Portuguese marine flora over a long time scale.....	37
Group crustacean biology and fisheries .....	39
Research team .....	39
Summary of activities and progress during 2002 .....	39
Plan for 2003 .....	39
Group Fisheries Biology and Hydrobiology .....	40
Research team .....	40
Summary of activities and progress during 2002 .....	40
Plan for 2003 .....	40
Coastal Fisheries Research Group.....	40
Research Team.....	40
Summary of activities and progress during 2002 .....	40
Plan for 2003 .....	40
Group Biodiversity and Biology in Fisheries (BIOPESCAS) .....	41
Research team .....	41
Summary of activities and progress during 2002 .....	41
Plan for 2003 .....	41
Fish Parasitology and Reproduction .....	42
Research team .....	42
Summary of activities and progress during 2002 .....	42
Group Coastal Ecology .....	44
Summary of activities and progress in 2002 .....	44
Plans for 2003 .....	44
Externally funded Projects .....	45
Division of Aquaculture and Biotechnology.....	45
New and ongoing beyond 2002 .....	45
Completed 2002 .....	55
Division of Living Resources.....	58
New and Ongoing beyond 2002 .....	58
Completed in 2002.....	61
International and Interinstitutional Cooperations.....	65
Division of Aquaculture and Biotechnology.....	65
Division of Living Resources.....	66
CCMAR Seminar programme .....	66
Seminars given by CCMAR members in other institutions .....	67
Dissemination of scientific culture .....	67
Visiting scientists.....	67
Organization of Conferences, workshops, courses .....	68
Financial Report.....	69

## Publications List

### Division Aquaculture and Biotechnology

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- Aureliano, M., Joaquim, N., Sousa, A., Martins, H., and Coucelo, J. M. (2002). Oxidative stress in toadfish (*Halobatrachus didactylus*) cardiac muscle - Acute exposure to vanadate oligomers. *J. Inorg. Biochem.* **90**, 159-165.
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Ortiz-Delgado, J.B. Prize François-Vincent Raspail (1794-1878) awarded by the Royal Academy of Medicine and Surgery of Cadiz (Spain) for the work entitled "Haematopoietic organs in fishes (spleen and kidney). Blood cells in fishes". 2002

### **Division of Living Resources**

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- Pearson, G., Serrão, E., Dring, M., Schmid, R. Blue and green light signals for gamete release in the intertidal brown alga, *Silvetia compressa*. *37th European Marine Biology Symposium*, Aug 5-8, 2002, Reikjavik, Iceland.
- Sá, R.; Bexiga, C.; Vieira, L.; Veiga, P. and Erzini, K. The diet of the soles (*Solea vulgaris* and *Solea senegalensis*) in the lower estuary of the Guadiana River (Algarve, Southern Portugal. XII Simposio Ibérico de Estudios del Bentos Marino (22 - 25 October, 2002). Poster presentation.
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- Silva, J and Santos, R. Light Effects on the Circadian Evolution of Photosynthesis in *Zostera noltii* and *Cymodocea nodosa* Along a Vertical Gradient in Ria Formosa (South Portugal) . 5th International Seagrass Biology Workshop, Ensenada, Mexico, October 2002.
- Vieira, L.; Bexiga, C.; Sá, R.; Veiga, P. and Erzini, K. Population dynamics of *Carcinus maenas* (L., 1758) in the Guadiana estuary: preliminary results. XII Simposio Ibérico de Estudios del Bentos Marino (22 - 25 October, 2002). Poster presentation.
- Vieira, L.; Bexiga, C.; Sá, R.; Veiga, P. and Erzini, K. Abundance and diversity of the fish fauna of the Guadiana river estuary and the Castro Marim salt marsh (SE Portugal). 6th International Conference LITTORAL 2002 (22 – 26 September, 2002). Poster presentation.

#### **Project and consultancy final reports**

- Borges, T.C., co-ord. (2002) Managing by-catch and discards: a multidisciplinary approach (BYDISCARD). *Final Report to the European Commission, D.G. Fisheries*, 422pp.
- Chicharo, L.; Gaspar, M.B.; Falcão, M. Vafidis, D.; Marin, M.G.; da Ros, L. ; Pellizato, M. , Labarta, U.; Reiriz, M-J.; Cravo, A., Chicharo, M.A. (2002). Impact of bivalve fisheries in the Ria Formosa lagoon (Portugal), Venice lagoon (Italy) and Aegean sea (Greece), and on the juvenile flatfish in the south coast of Portugal. Project DGXIV 062/99 Final Report. Vol I + II, 627 p.
- Chicharo, L.; Chicharo, M. A. F. Alves & J. Regala (2002). Evaluation and improvement of shellfish dredge design and fishing in relation to technical conservation measures and environmental impact. Final Report - University of Algarve- of the Project ECODREDGE EC FAIR-CT98-4465. 82 p
- Erzini, K.; Bentes, L.; Coelho, R.; Correia, C.; Lino, P.G.; Monteiro, P.; Ribeiro, J. and Gonçalves, J.M.S. 2002. Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (Southern Portugal). Final Report, DG XIV/C/1, Ref. 99/061, Universidade do Algarve, Faro, 182pp + annexes.
- Erzini, K., Bentes, L., Coelho, R., Correia, C., Lino, P., Monteiro, P., Ribeiro, J. e Gonçalves, J.M.S. 2002. "A study to identify, quantify and ameliorate the impacts of static gear lost at sea". Relatório final. FANTARED II, UE-FAIR-PL98-4338. 300pp.

#### **Prizes and Honours**

- International Seagrass Biology Workshop Prize for best student presentation: Silva, J and Santos, R. Light Effects on the Circadian Evolution of Photosynthesis in *Zostera noltii* and *Cymodocea nodosa* Along a Vertical Gradient in Ria Formosa (South Portugal)

## Division of Aquaculture and Biotechnology

### Theses PhD

#### **Completed**

- Conceição, Natércia Maria da Silva (2002) Regulation of Matrix Gla protein gene expression: comparative studies in fish and amphibians. Universidade do Algarve. 9 de Dezembro de 2002 (supervisors Leonor Cancela e Roland Schule, University of Freiburg, Germany).
- Faustino Manuel Almeida dos Ramos (2002). Developmental osteology of the gilthead seabream (*Sparus aurata*, L.). Universidade do Algarve, 28th January 2002 (Supervisor Deborah Power)
- Guerreiro P.M. Physiological function of calcium and PTHrP in sea bream (*Sparus aurata*). University of Nijmegen, Holland (supervisors Deborah Power and Gerit Flik).
- Natércia Joaquim. *Non invasive characterization of Halobatrachus didactylus heart morphology and function: application of echocardiography*. (supervisor: Josefina Coucelo).

#### **Ongoing**

Isabel Morgado

Marco Campinho

- Almeida, Manuela Roxo. Study of the parasite *Perkinsus atlanticus* (Protista, Apicomplexa) endemic infection of clams, *Ruditapes decussatus*, in Portugal. Development of an *in vitro* and *in vivo* infestation system to study the host/parasite interaction and improve production management. Universidade do Algarve (supervisors Leonor Cancela and Maria Teresa Dinis). Completion expected in 2003.
- Aragão, Cláudia. Determinação dos requisitos em aminoácidos na dieta das larvas e pós-larvas do linguado (*Solea senegalensis*) e da dourada (*Sparus aurata*). Universidade do Algarve (supervisors Maria Teresa Dinis and Luis E.C. Conceição). Completion expected in 2003.
- Brinca, L. Regulação por neuropeptídeos da secreção e síntese da hormona de crescimento e da prolactina em pituitárias de dourada (*Sparus aurata* L.). Universidade do Algarve (supervisor Deborah Power). Completion expected in 2004.
- Cardoso, J.C.R. Estudo da estrutura e função genética dos receptores acoplados a proteínas-G no peixe-balão. Universidade do Cambridge, UK (supervisor Deborah Power and Melody Clark). Completion expected in 2004.
- Carvalho, Suzana. Papel das Comunidades de Macrofauna Bentónica na Gestão de Tanques para Piscicultura (supervisors Maria Teresa Dinis and Luis Fonseca). Completion expected in 2003
- Coesel, Sacha. Regulation of the carotenoid biosynthetic pathway in the halotolerant alga *Dunaliella salina*. Universidade do Algarve. (supervisor João Varela). Completion expected in 2005.
- Fagundes, Teresa Ecologia comportamental do blenídeo *Salaria pavo* na Ria Formosa: tácticas alternativas de reprodução e inversão de papéis sexuais (supervisors Rui Oliveira and Adelino Canário). Completion expected in 2005
- Frade, Pedro Alexandre. Chemical identification and function of pheromones in the reproduction of tilapia, *Oreochromis mossambicus* (Pisces: Cichlidae). Universidade do Algarve (supervisors Eduardo N. Barata, Adelino V.M. Canário and Peter C. Hubbard). Completion expected in 2005.
- Gavaia Paulo J. Functional analysis of osteocalcin (Bone Gla protein, BGP) from bony fish during skeletal development. Universidade do Algarve (Supervisors Leonor Cancela e Carmen Sarasquete, CSIC Cadiz, Espanha). Completion expected in 2004.
- Gisela Borges *Endothelium dysfunction in microvascular diseases*. (supervisor: Josefina Coucelo). Completion expected in 2005.
- Henriques, Nuno. Regulation of the gene expression associated with the carotenoid biosynthesis in the microalga *Dunaliella salina*. Universidade do Algarve. (supervisors Leonor Cancela and João Varela). Completion expected in 2003.
- Modesto, Teresa Hormonal control of the reproductive cycle of the Lusitanian toadfish, *Halobatrachus didactylus* Universidade do Algarve (supervisor Adelino V M Canario). Completion expected in 2003.
- Moncaut, Natália Universidade do Porto
- Morais, Sofia. The role of nutrition in the larval quality of flatfishes. (supervisors Maria Teresa Dinis and Ivar Ronestad). Completion expected in 2005.

- Pinto, Patricia Isabel Silvestre - Diversidade, expressão e mecanismo de acção do receptor de estrogénio na dourada, *Sparus aurata*. Universidade do Algarve (supervisor Adelino V.M. Canário). Completion expected in 2005.
- Ribeiro, Maria Laura. Ontogenic development of *Solea senegalensis*: digestive system and nutritional aspects. Universidade do Algarve (supervisor Maria Teresa Dinis). Completion expected in 2003.
- Serrano, Rui Manuel. Pheromones in the reproduction of *Salaria pavo* and *S. fluviatilis* (Pisces: Blenniidae): a comparative study. Universidade do Évora (supervisors Eduardo N. Barata, Adelino V.M. Canário and Peter C. Hubbard). Completion expected in 2005.
- Simes, Dina Cristina Costa. Purification, biochemical characterization and localization at single cell resolution of Matrix Gla protein (MGP) and Bone Gla protein (BGP) in the teleost fish *Argyrosomus regius* Universidade do Algarve. To be completed in January 2003 (supervisor Leonor Cancela).

### **Theses Master of Science**

#### **Completed**

#### **Ongoing**

- Diogo, Maria de Lurdes. Qual o papel da interregional na diferenciação sexual induzida pela temperatura na tilapia *Oreochromis mossambicus*. MSc Aquaculture, Universidade do Algarve (supervisor Adelino V M Canario). Completion expected in 2003.
- Martins, Rute. Does the interrenal influence sex differentiation in sea bass, *Dicentrarchus labrax*? MSc Aquaculture, Universidade do Algarve (supervisor Adelino V M Canario). Completion expected in 2003.
- Vieira, Nuno. Contribuição para o conhecimento da estrutura do gene da POMC na dourada (*Sparus aurata*). MSc Aquaculture, Universidade do Algarve (supervisor Deborah M Power). Completion expected in 2003.
- Pais, Susana Isabel da Silva A regulamentação portuguesa de segurança biológica como um instrumento de gestão ambiental Mestrado em Gestão e Conservação da Natureza, Univerisdade do Algarve (completion expected in 2003) (supervisor from CCMAR- Leonor Cancela)
- Pombinho, António Ribeiro – Effect of extracellular calcium on MGP gene expression. Mestrado em Biotecnologia, Universidade do Algarve (completion expected in 2004). Supervisors Leonor Cancela and Vincent Laize.
- Violante, Ana. Estudo da doença de inverno em dourada (*Sparus aurata* L.) de cultivo, a sul de Portugal. Universidade do Algarve (supervisor Maria Teresa Dinis). Completion expected in 2003.

### **Graduation Honours thesis (Estágio de licenciatura)**

#### **Completed**

- Cabaço, S. "Population dynamics of *Zostera noltii* along a nutrient gradient". Universidade do Algarve (supervisor, Rui Santos).
- Cunha, Manuela (2002) Determinação da influência de dois imunoestimulantes (B-glutano e FMI) na ontogenia do sistema imunitário de larvas e juvenis de *Solea senegalensis*. (Supervisores: Maria Teresa Dinis, Pavlos Makridis and Florbela Soares)
- Fernandes, Tânia (2002) Infection with *Trypanoplasma borreli* (Laveran and Mesnil, 1901) in *Clarias gariepinus* (Burchel, 1822) and parasite sensibility to fish serum in vitro. (Supervisor: Maria Teresa Dinis)
- Ferreira, Joao David Farinha (2002) Efeito dos oligómeros de vanadato na polimerização e expressão da actina no músculo esquelético do *Halobatrachus didactylus*. Univeridade do Algarve. Supervisors Leonor Cancela e Manuel Aureliano, (FCT/UALG)
- Fidalgo João P.M. (2002) Caracterização de uma cultura celular derivada de osso de *Xenopus laevis*. Implicações na regulação hormonal da expressão do gene da proteína Gla da matriz (MGP). Universidade do Algarve. Supervisor Leonor Cancela.
- Gomes, L. (2002). Efeitos do estímulo químico da glândula anal de machos *Salaria pavo* (Pisces: Blenniidae) na escolha sexual das fêmeas (supervisors Eduardo N. Barata, Peter C. Hubbard and Adelino V.M. Canário).

- Guerreiro, Miguel (2002) Monitorização da actividade bioelectroquímica da bomba de cálcio de retículo sarcoplasmático Universidade do Algarve. Supervisors Manuel Aureliano (FCT/UALG), Leonor Cancela and Henrique L.Gomes (FCT/UALG).
- Inês Figueiredo (2002). [*Liver and kidney oxidative stress induced by vanadate oligomers in Halobacterichus didactylus.*]. Universidade do Algarve. Orientadores: M. Aureliano Alves, Natércia Joaquim and Josefina Coucelo
- Machás, R. "The role of *Zostera noltii* on the food web of Ria Formosa" (supervisor, Rui Santos).
- Martins, Silvia (2002) Isolamento, caracterização e identificação de bactérias isoladas de *Solea senegalensis* e *Tetraselmis chui* (FERN, Ualg) (Supervisores: Pavlos Makridis and Lidia Dionisio)
- Molha, Duarte (2002) Estudo sobre a regulação da mineralização in vitro de duas linhas celulares derivadas de vértebra de um peixe teleosteo marinho, *Sparus aurata*. Universidade do Algarve. Supervisors Leonor Cancela e Vincent Laize.
- Pestano, Rita (2002) Infecção experimental em juvenis de *Diplodus sargus* (Linnaeus, 1758) com *Photobacterium damsela* subsp. *piscicida* e efeito de uma vacina comercial (Supervisor: Maria Teresa Dinis)
- Rocha, Rui (2002) Nitrogen removal in recirculating aquaculture systems. (Supervisor: Maria Teresa Dinis)

## Division of Living Resources

### Theses PhD

#### **Completed**

- Borges, Patrícia Ribeiro Abreu Gonçalves Simões 2002- Aspectos da biologia e da pesca do espadarte (*Xiphias gladius*, L. 1758) no Arquipélago dos Açores. Doutoramento em Ciências do Mar, Universidade do Algarve (Supervisor: J P Andrade)..
- Palma, Jorge Afonso Martins da 2002 – Morphological variation in eight Sparidae species of the Eastern Atlantic and Mediterranean Sea. Doutoramento em Ciências do Mar, Universidade do Algarve (Supervisor: J P Andrade).

#### **Ongoing**

- Alberto, Filipe. Univ. Algarve (supervisor E. Serrão, C. Duarte). Completion expected in 2003.
- Amaral, A. "Ecofisiologia de Ruditapes decussatus na Ria Formosa" (Supervisor Luís Chícharo Co-orientação com o Doutor Uxio Labarta (CSIC – Vigo) (Espanha).
- Anibal, Jaime. Effects of the seasonal dynamics of green algae of the nutrient flux in the sediments of the Ria Formosa lagoon. Universidade do Algarve (supervisor Martin Sprung). Completion expected in 2004
- Cabaço, S. Population dynamics of *Zostera noltii* along a nutrient gradient. Universidade do Algarve (supervisor, Rui Santos)
- Candeias, A. "The Processes Of Feeding In The Physiological Energetics Of Coastal Meroplankton" (Supervisor Alexandra Chícharo em co-orientação com Doutor Andrew Bruce Yule - School Of Ocean Sciences, University of Wales)
- Costa, M.E. By-catch e rejeições da pesca comercial de arrasto na costa Sul de Portugal. Universidade do Algarve (supervisor Teresa Cerveira Borges) Completion expected in 2003.
- Coelho, R. Biologia, dinâmica espacio-temporal, gestão e conservação de tubarões de profundidade. (Supervisor: Karim Erzini).
- Dias, Ana Natália. Ecology of the saltmarsh fauna in the Ria Formosa lagoon. Universidade do Algarve (supervisor Martin Sprung). Completion expected in 2004
- Elena Diaz-Almela. Univ. Algarve e Univ. Illes Balears (supervisor E. Serrão, C. Duarte). Completion expected in 2005.
- Esteves, Eduardo Bruno Oliveira. Recrutamento e condição larvar de savelha, *Alosa fallax fallax*, nos rios Mira e Guadiana. (Supervisor: J. Pedro Andrade)
- Godinho, C. "The impact of anti-fouling paints on seagrass populations of *Zostera noltii* in the Ria Formosa Lagoon". University of Plymouth (supervisors, Rui Santos, M. João Bebianno and Murray Brown).
- Hazim, H. Influência das variáveis oceanográficas na dinâmica populacional do espadarte, *Xiphias gladius*, no Oceano Atlântico. (Supervisor: Karim Erzini).
- Lago, Asuncion. Univ. Algarve (supervisor G. Pearson, E. Serrão). Completion expected in 2006.
- Machás, R. Fluxos de matéria orgânica na Ria Formosa", Universidade do Algarve (orientador, Rui Santos)

- Marques, Alexandra. Population dynamics of dominant copepod species in the Ria Formosa lagoon. University of Bangor (supervisors Andy Yule and Martin Sprung). Completion expected in 2004
- Mendes, J.C “Long-Term Time Series of Continuous Plankton Recorder Survey off Portuguese Coast” (Supervisor Alexandra Chicharo em co-Orientação com o Doutor Miguel Santos do IPIMAR e com o Doutor Chris Reid, do SAPHOS (Plymouth))
- Morais, P. - “*Engraulis encrasicolus* (Linnaeus, 1758) population dynamics in the Guadiana estuary and adjacent coastal area” (Supervisores Alexandra Chicharo e Luis Chicharo)
- Pais, MC. Use and selection of habitats by non-breeding Bonelli's eagles in southern Portugal. (supervisors Pedro Beja and Leonor Cancela). Completion expected in 2006.
- Ribeiro, J. Ecologia e dinâmica da ictiofauna da Ria Formosa. Universidade do Algarve (supervisor Karim Erzini). Completion expected in 2006.
- Santana, J. - “Comparação bioeconómica das pescas no rio Tocains Amazônia-Brasil”, (Supervisor Luis Chicharo em co-orientação com o Doutor Miguel Petrere da Universidade de Pernambuco (Brasil)).
- Serafim, Maria Paula. Universidade do Algarve (supervisor Margarida Castro). Completion expected in 2006.
- Silva, J. Carbon acquisition and nitrogen uptake in the seagrasses of Ria Formosa. Universidade do Algarve (supervisor, Rui Santos)
- Teodósio, J. “Dinâmica populacional e caracterização do estado fisiológico e bioquímico da ameijoia asiática *Corbicula fluminea* na bacia hidrográfica do rio Guadiana”. (Supervisores Alexandra Chicharo e Luis Chicharo)
- Moschino, V. - “Impact of fishing activity on the morphology, physiology and biochemistry of the bivalves *Chamelea gallina* and *Tapes philippinarum* from coastal and lagoon areas of the Northern Adriatic Sea (Italy)” (Supervisor Luis Chicharo em co-orientação com a Dra. Maria Gabriella Marin da Universidade de Padova (Itália)).

### Theses Master of Science

#### **Completed**

- Alves, F. (2002) “Análise sazonal do impacte a curto prazo na estrutura das comunidades meio e macrobentónicas resultantes da pesca com ganchorra”. Tese apresentada na Universidade de Coimbra, 68 p. (Supervisores Luis Chicharo e António Nogueira – Univ Aveiro)
- Coelho, S. (2002). Alimentação de *Sardina pilchardus* (Walbaum, 1792) ao largo da costa continental portuguesa e implicações da condição nutricional das fêmeas na qualidade dos oócitos. Tese de mestrado em Ciências do Mar- Recursos Marinhos no Instituto de Ciências Biomédicas de Abel Salazar da U. do Porto, 156 p. (Supervisor Alexandra chicharo e Yorgos Stratoudakis)
- Constança Maria de Sousa Bexiga (2002). Comunidade ictiológica estuarina do Guadiana e sapal de Castro Marim. 79 p. + anexos. EMAC - University do Algarve. (Supervisor: Karim Erzini)
- Dias, A.N.: Dinâmica populacional e produção do isópode *Tylos ponticus* (Oniscidea: Tylidae) num sapal da Ria Formosa (Sul de Portugal). – EMAC - University do Algarve (supervisor: Martin Sprung)
- Massei, Karina (2002). A alimentação de otárias-do-Cabo, *Arctocephalus pusillus pusillus* (Schreber, 1776) num parque zoológico: aspectos calóricos, digestivos e comportamentais. Curso de Mestrado em Estudos Marinhos e Costeiros, Universidade do Algarve. (Supervisor: J. Pedro Andrade)
- Mata, L. (2002). Cultivo de macroalgas em efluentes de piscicultura. Mestrado de Aquacultura, FCMA, Universidade do Algarve (supervisor, Rui Santos).
- Regala, J. (2002) – “Contribution to the knowledge of forager aggregative behaviour at clam dredged grounds off southern Portugal”. Tese apresentada na Universidade de Liverpool, 106 p. (Supervisores Luis Chicharo e Doutor Andrew Brand – University of Liverpool)
- Sykes, António de Vilhena Andrade Ferreira (2002). On the use of live grass shrimp (*Palaemonetes varians*) as the only prey for cuttlefish (*Sepia officinalis*, Linnaeus, 1758) culture throughout the life cycle. Mestrado em Ciências do Mar, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto. (supervisor: J. Pedro Andrade)

#### **Ongoing**

- Alcazar, R. *Biodiversidade e intensificação agrícola: modelação ecológica e orientações para um planeamento ambiental*. Mestrado em Ordenamento do Território e Planeamento Ambiental, Universidade Nova de Lisboa, Faculdade de Ciências e Tecnologias. Completion expected in 2002.



- Alexandre, A. "Biologia da reprodução de *Zostera noltii*", (supervisor, Rui Santos).
- Barata, F. *A avifauna aquática das zonas húmidas da costa leste da Ilha Terceira – um contributo para a sua conservação*. Mestrado em Gestão e Conservação da Natureza, Universidade dos Açores, Departamento de Ciências Agrárias. Completion expected in 2002.
- Bentes, L. Supervisor: Karim Erzini.
- Ferreira, R. Influência de parâmetros oceanográficos e pesqueiros na captura accidental de tartarugas marinhas pela pesca de espadarte nos Açores. Completion expected in 2003. Supervisor: Karim Erzini.
- Gonçalves, I.A. Caracterização morfológica e histoquímica do tracto digestivo de paralarvas de *Octopus vulgaris*. (supervisor Teresa Cerveira Borges) Completion expected in 2003.
- Malaquias, A.A.E. Contribuição para o conhecimento da sistemática e da ecologia do género Haminoea Turton and Kingston, 1830 (Mollusca: Gastropoda: Cephalasidea). Mestrado em Ecologia, Faculdade de Ciências e Tecnologia da Universidade de Coimbra. Completion expected in 2003
- Olim, S. Biologia e morfometria de várias espécies da família Triglidae na costa Sul de Portugal. (supervisor Teresa Cerveira Borges) Completion expected in 2003.
- Oliveira, M. Contribuição para o estudo da pesca desportiva (costeira e de alto mar) e caça submarina no norte de Portugal. MSc Thesis on Marine Science – Marine Resources, Instituto de Ciências Biomédicas de Abel Salazar - IPIMAR (supervisors Karim Erzini – CCMAR – Universidade do Algarve). Completion expected in 2002.

### **Graduation Honours thesis (Estágio de licenciatura)**

#### **Completed**

- Alexandre, A. Nitrate reductase activity in two intertidal morphotypes of the seagrass *Zostera noltii*, (supervisors, Rui Santos and João Silva)
- Branco, Francisco Alexandre Ferreira (2002) – Memória táctil em *Octopus vulgaris* (Cuvier, 1797): aspectos comportamentais e fisiológicos. Universidade do Algarve. Orientador: J. Pedro Andrade, em co-orientação com o Doutor Pedro Lima.
- Costa, Clélia Regina Lopes (2002) – Aspectos do comportamento e da ecologia alimentar do Perna-longa, *Himantopus himantopus* (Linnaeus, 1758) no Parque Natural da Ria Formosa. Universidade do Algarve. Orientador: J. Pedro Andrade.
- Dinis, Ana. Alguns aspectos da biologia do polvo cabeçudo – *Eledone moschata* e *E. cirrhosa* – na região Algarvia. Estágio de Licenciatura em BMP, Universidade do Algarve. 47 pp + anexo. (supervisor Teresa Cerveira Borges)
- Ferreira, M<sup>a</sup>. (2002). Hábitos alimentares das tartarugas verdes *Chelonia mydas* em Ra's Al Hadd, Mar Árábico (Omã). Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve. Supervisor: Karim Erzini and Jorge Gonçalves.
- Lima, A. (2002). "Formação da concha durante as fases embrionárias e larvares de *Anodonta cygnea*" Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve.
- Rosa, A. (2002) Idade e crescimento da raia-lenga, *Raja clavata* (Linnaeus, 1758), no Arquipélago dos Açores. Tese de Licenciatura. Faculdade de Ciências do Mar e do Ambiente da Universidade do Algarve. 60 pp. + Anexos. Co-supervisor: Karim Erzini
- Soares, J.
- Rocha, M. (2002)- Estudo de Idade e Crescimento de Sardinha (*Sardina pilchardus* Walbum) capturada na costa do Algarve. ). Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve.
- Silva, D. (2002). Organização das comunidades ictiológicas) - Determinação da idade e crescimento de duas espécies de peixes demersais dos canais principais e secundários da Ria Formosa. Tese de Licenciatura. Faculdade de Ciências do Mararquipélago dos Açores, Boca-negra (*Helicolenus dactylopterus* DelaRoche, 1809) e do Ambiente da Universidade do Algarve. 69 pp + annexes. Superviso: Karim Erzini and Jorge Gonçalves
- Teixeira, Rita Alexandra Claro (2002) – Determinação do efeito de um inibidor específico dos receptores NMDA, o AP5, num processo de aprendizagem associativa discriminatória em *Octopus vulgaris*. Universidade do Algarve. Orientador: J. Pedro Andrade, em co-orientação com o Doutor Pedro Lima.
- Wise, L. (2002) Estudo da colonização de armadilhas de pesca (covos) perdidas no mar. Tese de Licenciatura. Faculdade de Ciências do Mar e do Ambiente, Universidade do Algarve. 60pp. + annexes. Supervision: Karim Erzini and Jorge Gonçalves

## **Description of the research activities**

### ***Division of Aquaculture and Biotechnology***

#### **Group Molecular Biology of Marine Organisms**

##### **Research team**

Leader: Leonor Cancela

Researchers and post docs: Vincent Laizé, Laurence M. Elandalloussi, Jorge P. Pinto, Juan B. OrtizDelgado, Sandra P. Marques, Pedro M. Rodrigues

PhD students: Natércia Conceição, Dina Simes, Paulo Gavaia, Nuno Henriques, Sara Mira Silva, Patricia Cabrita,

Technicians/ research assistants: Carla Viegas, Catarina Canas, António Pombinho, Ricardo Leite, Ricardo Afonso, Carla Cruz

Undergraduate students: João Fidalgo, Duarte Molha, João David Ferreira, Miguel Guerreiro, Brian Schaff, Susana Domingues, Bruno Pardelha

##### **Summary of activities and progress during 2002**

###### **General description of the major objectives:**

The major objective of the group is the study of molecular determinants of tissue mineralization and abnormal calcification, both during development and in adulthood. Specific genes currently being used as molecular markers for bone and cartilage have been cloned from all model organisms (non-mammalian) presently in study in our laboratory and their sites of gene expression and protein accumulation identified. Functional analysis of promoter regions through construction of specific deletion mutants, site-directed mutagenesis and electrophoretic mobility has led to the identification of previously undetected DNA regulatory regions in selected genes of interest. These results will further permit the identification of the nuclear factors involved in specific gene regulation. Following the recent development of fish bone and cartilage-derived cell lines in our laboratory, major emphasis is presently directed towards understanding mechanisms involved in bone and cartilage cell differentiation, role of Gla protein in extracellular matrix mineralization and response to environmental parameters through the use of integrated genomic/proteomic approaches.

A different project was initiated more recently on host-parasite interaction using as model organism the parasite *Perkinsus atlanticus* and its natural host the clam *Ruditapes decussatus*. A pure cell culture of *Perkinsus atlanticus* was developed and good progress was made towards initiating studies on the biology of the parasite and host-parasite interactions using an integrated approach involving histological, molecular and cellular biology techniques. Molecular diagnostic tools were developed to detect parasite infection in host tissues and environmental factors affecting parasite growth and development.

Members of the group are also involved in work related with the obtention of molecular markers for studies on genetic variability, paternity and sexing, in particular for endangered species. Two projects are currently on going, concerning 1) the development of molecular markers for studies on molecular sexing, identification and genetic variability of the Bonelli's eagle Portuguese population, and 2) the use of molecular markers for measuring genetic variability and estimating the effective size population of the coastal otter in South-western Portugal.

## **Detailed description of the research objectives, major achievements in 2002 and plan for 2003**

### **1. Molecular determinants of extracellular matrix calcification**

#### Main purposes:

During 2002 research focussed primarily on i) understanding molecular pathways of tissue mineralization and its regulation in adult life and during development, using as model organisms fish (gilthead seabream and zebrafish) and amphibian (Xenopus), and ii) the role of vitamin K dependent proteins (matrix Gla and Bone Gla proteins) in this process. Additional goals include iii) studies on the effect of genetic or environmental factors on alterations of the normal pattern of tissue calcification and its relation with altered expression of vitamin K-dependent proteins and iv) molecular adaptations of mechanisms that control extracellular matrix mineralization throughout evolution. In order to perform studies on bone- and cartilage-related protein function, gene expression/regulation and functional promoter analysis, it was imperative to obtain bone and cartilage derived cell lines from fish and amphibian, not available at present. For this purpose the major goals were 1) To develop and optimize conditions for obtaining primary cell cultures/cell lines derived from marine organisms and amphibians. And 2) To develop transfection conditions for primary cells cultures and cell lines suitable to direct specific gene expression in vitro.

#### Achieved in 2002:

Major results included 1) identification of sites of BGP/MGP gene expression and protein accumulation in adult teleost fishes and during larval development., 2) Functional analysis of MGP gene promoter from *Xenopus laevis*. 3) Studies towards elucidation of 3D structure of BGP from lower vertebrates. Comparative studies between fish and mammalian BGPs. 4) Identification of signal transduction pathways involved in the mechanisms of regulation of expression of BGP/MGP genes 5). Development and characterization of bone and cartilage derived cell lines from fish and amphibian. 6) Studies on environmental factors affecting bone biology

#### Plan for 2003:

Major expected goals include integrated multidisciplinary approaches to perform in vivo/ in vitro functional analysis of bone/cartilage specific genes and promoter organization; Further characterization of cell lines developed in our laboratory. Evolutionary studies on specific gene function. Effect of environmental parameters on bone biology.

### **2. Studies on the characterization of infection levels of mollusk bivalves by the parasite *Perkinsus atlanticus*. Studies on the biology of the parasite *Perkinsus atlanticus*.**

#### Main purposes:

In this project, our initial objectives were to follow bivalve infection patterns and levels by *Perkinsus* sp along the Portuguese and Galicia coast as part of an epidemiology study and to analyse in vivo and in vitro parasite-host interactions. In order to fulfill this last objective, it was necessary to develop a cell culture of the parasite *Perkinsus atlanticus* and characterize it. The availability of this culture will permit to initiate studies on the molecular characterization of specific parasite genes involved in host infection.

#### Achieved in 2002:

Major results included the development, within a collaborative study, of a pure culture of *P. atlanticus* derived from infected clams from Ria Formosa, an integrated two year survey study along the Portuguese coast and Galicia using both histological and molecular techniques to detect parasite infection, the development of a molecular diagnostic assay for the parasite capable of differentiating between different species of *Perkinsus*.

#### Plan for 2003

Identification of specific genes involved in host-parasite interaction and studies on its regulation of expression through a genome/proteome approach. Identification of environmental parameters capable of modulating parasite growth in vivo and in vitro. Validation of the molecular diagnostic assay developed in 2002. Identification of similarities and differences with other Apicomplexa.

### **3. Population conservation studies for the Bonelli's eagle, *Hieraetus fasciatus*, and the European otter *Lutra lutra*.**

#### Main purposes

Major objectives have been to develop suitable molecular markers for genetic variability studies in order to improve our knowledge on Portuguese populations of two endangered species, the Bonelli's eagle and the coastal otter. The absence of sexual dimorphisms in juvenile eagles makes sexing quite difficult based on morphological parameters only, therefore molecular markers for sexing were also developed. Since eagles are protected species and all handling is very difficult and stressful for the animal, coupled with the inaccessibility of the nests, a method for extracting DNA from feathers was recently developed with success. The otters, on the other hand, pose different problems. Being a nocturnal species and very difficult to catch, methods for purifying DNA from faeces are currently being optimized in our laboratory, in order to obtain the required biological material to perform genetic analysis.

#### Achieved in 2002

For the Bonelli's eagle: microsatellites were successfully developed and are currently being used to determine degree of polymorphism and initiate genotyping of the population. Method for DNA extraction from feathers is currently being used to increase our population sampling. As an associated project, microsatellites were successfully developed for another bird of prey, *Gyps fulvus* and their degree of polymorphism determined for a restricted sample. For otters, DNA from faeces was successfully purified and proven to be from otter using an additional marker from mitochondrial DNA, cytochrome b. DNA was also extracted from tissues collected from dead animals kept frozen or currently in captivity in order to optimize DNA amplification for microsatellite detection and compare it with results obtained from faeces. A monitoring of individuals resulting from faeces collection in the wild is currently being initiated.

#### Plan for 2003

Obtention and characterization of additional microsatellites for the Bonelli's eagle. Studies on genetic variability and paternity on the Bonelli's eagle population from Southern Portugal. Comparison with populations from different sites in Europe and analysis of older populations previously collected and in storage. Studies on otters will measure genetic variability and estimate effective population size in coastal area of Southern Portugal.

## **Biotechnology and Molecular Biology of Microalgae Group**

Leader - João Varela

PhD students: Nuno Henriques, Sacha Coesel

### Summary of activities and progress during 2002

#### **Biotechnology of marine microalgae**

The research carried out in 2002 focused on finding out whether genes coding for enzymes involved in the carotenoid biosynthetic pathway are indeed key players in the regulation of stress-induced carotenogenesis in the microalga *Dunaliella salina*. In order to address this question, northern analysis of cells exposed to different stresses, namely nutrient, high light, osmotic or heat shock, and combinations thereof, showed that there is a lack of correlation between high levels of *Pds* and *Psy* transcripts and accumulation of carotenoids. Because *Psy* and *Pds* encode the first two enzymes of this pathway, these results suggest that carotenogenesis may be regulated by other genes.

Recently, our group was able to clone two additional genes of this pathway, namely the *Lcy* gene, coding for lycopene cyclase, and a member of the *IspH / LytB* gene family. As far as we know we are the first research group to clone these genes in algae. Interestingly, the *IspH / LytB* gene has been characterized as a key player in the carotenogenesis in cyanobacteria. As both genes were

found in a subtractive cDNA library of carotenising cells, it is likely these genes will further elucidate how the accumulation of carotenoids in microalgae is regulated.

The characterization of the subtractive cDNA library mentioned above suggests that several classes of genes are induced during the stress-induced carotenogenic process, namely terpenoid and carotenoid biosynthetic enzymes, heat-shock proteins (HSPs), oxidative stress-related gene products (e.g. glutathione S-transferase) and membrane transporters. Concerning the latter, it is interesting to notice that genes involved with nutrient influx and Na<sup>+</sup> efflux were isolated, suggesting that the accumulation of carotenoids is just one of the metabolic changes taking place in stressed algae.

Because of the approval of the OVERCAROTEN project, another line of research was introduced: the isolation of carotenoid-overproducing strains by chemical mutagenesis. Using EMS as a mutagenic agent we have defined a expedite protocol for screening carotenoid-overproducing mutants. This mutant screen is carried out in 96-well microtiter plates containing DPA (diphenylamine), a known carotenoid biosynthesis inhibitor in algae. So far, we were able to select 44 putative overproducing strains. These strains will be further characterized by our partners, namely the SME Necton, INETI and ESB-UC.

In alternative to the generation of mutants, our research group is developing ways of genetically transform microalgae such as *Dunaliella salina*. Early attempts to obtain stable transformants were not successful. However, we are presently constructing new transformation vectors with homologous genes, instead of relying on existing plant transformation vectors. Although *Chlorophyta* algae resemble higher plants in terms of physiology and gene structure, it seems that factors such as codon bias and the presence of homologous introns are essential for expression of resistance markers in microalgae.

#### **Plan for 2003**

In the year 2003 we will have fully characterized the *lspH* / *LytB* and *ClpC* genes, the latter a member of the HSP100 heat-shock protein family. This characterization will provide an important insight as to how carotenogenesis is regulated in microalgae and, presumably, in higher plants. We will also develop macro- and microarrays containing 500 cDNAs from the subtractive cDNA library obtained from carotenising cells. These DNA chips will allow us to understand how these genes are regulated when cells are exposed to the several stressful conditions able to induce carotenogenesis in microalgae.

Concurrently, we will begin the characterization of carotenoid-overproducing strains by studying their physiology as well as their metabolic and protein profile. The most interesting mutants will be further characterized by means of gene-specific northern blot and microarray analyses.

In collaboration with Dr. Chris Bowler, we will use novel transformation vectors and DNA-coated microprojectiles to transform *Dunaliella salina*. The transformants will be characterized concerning their stability as well as the levels of homologous and heterologous gene expression.

## **Group of Comparative and Molecular Endocrinology**

#### **Research team**

Leader(s): Adelino V. M. Canário and Deborah Power  
Visiting scientist: Pratap Singh

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### **Summary of activities and progress during 2002**

The main topics of the group are the molecular mechanisms underlying hormone action and the physiological response of the whole animal. The processes that are the focus of attention are growth and development (with particular emphasis on cartilage, bone and muscle metabolism), reproduction (with recent emphasis on sex determination), endocrine disruption in the wild and the stress response to normal physiological challenges (with emphasis on ion regulation). An integrated approach is being taken and genomics, biochemistry, cell biology and whole animal physiology are deployed in order to give an overview of hormone function. The approach encompasses studies of gene regulation, gene expression, post-translational and post-secretory processing, receptor binding, signal transduction and finally the response at a cellular level and also the whole animal response. In the last two years we have initiated a new area of research in collaboration with other European teams, the object of which is to generate a map of the sea bream (*Sparus auratus*) genome. This project will give scope for comparative studies of genome evolution and gene family evolution, promoter analysis and related functional studies and an integrated map will open the way for QTL analysis and selection programs in collaboration with industry.

### ***Parathyroid hormone-related protein and calcium homeostasis***

Mechanisms of ionic calcium homeostasis in fish are different from those in terrestrial vertebrates because there is an almost constant supply of calcium ions from the surrounding water, whilst terrestrial vertebrates rely on dietary sources of calcium, which are very variable and intermittent. In higher vertebrates there are three hypercalcaemic hormones involved in calcium homeostasis, vitamin D, calcitonin and parathyroid hormone (PTH). In fish, the hormones regulating calcium homeostasis are poorly studied and the hypocalcaemic hormone stanniocalcin is assumed to be the principal regulatory factor. In fact PTH has not been identified in fish and the parathyroid gland which produces the hormone in terrestrial vertebrates is absent.

The program of work aims to characterise hypercalcaemic hormones in fish and to establish at the whole organism and cellular level the mechanisms that regulate calcium homeostasis. The organisms studied are a marine teleost, the sea bream (*Sparus aurata*), and a euryhaline species tilapia (*Oreochromis mossambicus*). The first phase of the work was to determine if hypercalcaemic factors occur in fish. Initially the existence of parathyroid hormone-related protein (PTHrP) in fish was inferred from immunocytochemical and biochemical studies using heterologous antisera in the sea bream. Subsequently the gene was characterised in *Fugu* and the cDNA in the sea bream confirming the existence of this hormone in fish. The function of this hormone in fish has yet to be characterised but our studies with the well-conserved N-terminal fish peptide of PTHrP have shown a function for this domain in calcium metabolism in sea bream larvae. It remains to be determined if this hormone is principally a hypercalcaemic factor or if it has other functions such as the regulation of normal chondrogenesis and bone development. This year saw a substantial advance in this area and a group of PTH-like genes have been identified and are

currently under intense study in order to characterise their structure and function. Several new radioimmunoassays/ELISA are under development. The way in which cells producing hyper- and hypocalcaemic hormones are able to sense extracellular calcium is unclear but may be by the mediation of a calcium sensing receptor. The calcitonin gene, a complex transcriptional unit that codes for two bioactive peptides, calcitonin and calcitonin gene related peptide (CGRP), has also recently been cloned and will be utilised in coordinate regulation by all the factors identified to date. In the context of studying calcium balance/regulation a bone and cartilage cDNA library has been made and has been partially arrayed. Additionally using subtractive hybridisation a microarray has been generated to identify genes up and down regulated in chondrogenesis and bone development. Additional hormone/peptides and their receptors have been characterised and will contribute to an integrative analysis of this process.

The interaction between cortisol and PTHrP in sea bream has been further investigated *in vitro* and *in vivo*. For this purpose a homologous radioimmunoassay (RIA) for sea bream PTHrP was developed. A number of antagonist/agonists and truncated forms of PTH/PTHrP have been produced and their potency in the cortisol/PTHrP axis characterised. The receptors and signalling pathways utilised by PTH/PTHrP in different tissues and cell types have been partially characterised. Such studies have provided further confirmation that PTHrP can act as a classical hormone and paracrine factor.

### ***Hormonal control of development and growth of fish eggs and larvae***

In order to understand the development of the musculo-skeletal system of the sea bream (*Sparus aurata*) it has been necessary to generate the tools for these studies. Moreover, in the absence of background studies of skeletal development it was necessary to first, carry out basic studies characterizing its normal development in sea bream and this work was recently concluded with the characterization of the viscerocranial skeleton. The importance of the muscle mass to aquaculture has meant that several studies of the morphology of this tissue exist in the literature making it unnecessary to replicate this work and attention has been focused on isolating muscle specific genes and initiating studies of their developmental expression in control larvae. The next phase will be to characterize the developmental expression of skeletal and muscle specific genes and determine which genes are involved in body patterning and the regulation of cartilage/bone formation. The way in which developmental ontogeny of skeleton and muscle may be altered in abnormal larvae also represents an important applied aspect of the work and has wide implications for a range of aquaculture species.

1. Egg and larvae are frequently exposed to changing external conditions and the way in which this affects development and the route by which it occurs is fundamental to the understanding of development. A range of experiments have been conducted in which culture temperature has been manipulated and the effect of this on the skeleton and expression of muscle specific genes determined. The way in which different endocrine axis (such as the pituitary gland, thyroid gland etc) are also been affected and how these may interact and affect development is also being determined.
2. The normal thyroid hormone (TH) balance at different life stages in the sea bream has been characterized and demonstrates a clear annual cycle that appears to vary according to the age and reproductive stage. As an essential part of the study the receptor for the hormone was also cloned ; two transcripts were identified and classified as alpha and beta on the basis of similarity with previously isolated receptors. The relative importance of the two forms in the various biological activities identified for TH remains to be determined. In the context of understanding the hormonal control of metamorphosis in round fish and flatfish, in collaboration with a European consortium a project was initiated which aims to characterize biochemical and molecular mechanisms controlling normal and abnormal metamorphosis.
3. Studies are continuing to characterize more fully the structure of genes of interest, such as prolactin (PRL) and its receptor, parathyroid hormone related protein (PTHrP) and a range of G-protein coupled receptors in order to identify regulatory sequences (eg. promoters) and develop assays to identify factors which influence promoter activity.

4. Good progress has been made in the development of methodologies for gene expression. Several expression vector constructs have been generated using different vectors and strategies and they are currently being tested *in vitro* and *in vivo*. Methodologies are now available in the group permitting the application of this technique to a range of genes currently under investigation.
5. The hardware for most of the physiological studies planned in the area of osmoregulation, reproduction and calcium regulation are now in place. Studies are now ongoing to evaluate the affect on these processes of a range of different hormones.

### ***Sea bream genome mapping***

The sea bream has been selected as a model organism for the perciformes and in particular for the Sparidae a commercially important group of fish. The sea bream has a genome (0.8pg/haploid nuclei) which is only slightly larger than that of Fugu. This year the objective of the Comparative and Molecular evolution group will be to obtain between 500-1000 expressed sequence tags (ESTs) and map them on a sea bream radiation hybrid. The methodology for this process should be fully developed by the end of the current year. In addition, 100-200 microsatellites will be developed and also mapped on the radiation panel. Work will be initiated to identify polymorphic microsatellites which will be utilised in the future for QTL analysis.

### ***Steroid receptor expression and function***

The estrogen receptor (ER) is a transcription factor of the nuclear receptor family with a wide range of functions in vertebrates. The objective of the research is to study the function of estrogen receptor in relation to reproduction in sea bream. We have previously isolated three ERs ( $\alpha$  and  $\beta 1$  and  $\beta 2$ ) in sea bream and analysed their pattern of expression in different tissues and in relation to reproductive stage. These various ERs show tissue specific patterns of expression, but in addition there is evidence that each ER homologue will produce more than one transcript according to tissue or developmental stage. Several transcripts have now been isolated and their expression is being analysed in control and estrogen primed tissues. The potential role of estrogen in testicular function is being analysed and a several estrogen responsive genes have been isolated using a subtractive hybridization approach. These genes have been macroarrayed and their expression patterns according to developmental stage of the testis is under study.

### ***Control of sexual determination and differentiation***

Environmental conditions in fish farms have a strong influence on the differentiation of sex in seabass. Recent evidence indicates that temperature during early development is the main environmental factor driving phenotypic sex. The objective of the work at this point is to clone sex determining genes and steroidogenic enzymes, putatively involved in the control of sex differentiation in sea bass and related the expression of these to the environment. A series of genes have been now isolated including several steroidogenic enzymes (CYP11, CYP17), known mammalian sex-determining genes (DMRT-1, DAX-1, SF-1) which are being analysed for expression in individual larvae taken from different environmental situations and of different ages. This work is being carried out as part as an European consortium which have isolated many other genes and carried out a series of experiments which altogether should provide a comprehensive view of gene expression during early development and of the factors that influence sex differentiation in sea bass fish farms. Experiments to test the effect of rearing density on sex differentiation have been carried out and results are being analysed.

### ***Development of biomarkers of endocrine disruption***

The primary objective of the research is to monitor the presence of endocrine disrupters and their effects in Portuguese coastal waters and rivers, including "pristine" reference waters and those subject to industrial or urban pollution. We have been developing *in vitro systems* using yeast for screening of estrogenic substances as well as an ELISA assay for monitoring blood vitellogenin as an *in vivo* biomarker of estrogenic effects. Fish, water and sediment samples have been taken



from the main portuguese estuaries and are being analysed for contamination. Results are expected in 2003.

### ***Fish Chemical Senses: identification of active compounds and modes of action***

The understanding of olfaction in fish is fundamental to investigations into chemical communication, reproduction, ion-homeostasis and feeding. Although the importance of pheromones in fish reproduction is well recognised, given the phylogenic diversity and the wide range of habitats and reproductive strategies used by teleosts, very few species have been studied in detail, the model species being the goldfish. The main impediment in this respect is the lack of knowledge of the chemical identity of the active compounds involved. This is particularly true of the cichlids and blennids, especially considering their highly developed mating systems and use of parental care. In addition, many exploited fish species are marine (e.g. *Sparus aurata*, *Solea senegalensis*), but the limited studies by other laboratories have chiefly focused on freshwater species. In such marine species, very little is known about the potential role of pheromones in reproduction, and more detailed knowledge would facilitate informed and efficient management of broodstock. Both marine and freshwater species have been object of research by the research team; the peacock blenny (*Salaria pavo*), the gilthead seabream (*Sparus aurata*), the goldfish (*Carassius auratus*), the Mozambique tilapia (*Oreochromis mossambicus*) and the tench (*Tinca tinca*).

During the breeding season, *S. pavo* males develop an androgen-dependent anal gland (AG) from the first two rays of the anal fin. Behavioural experiments have shown that a putative pheromone from the AG promotes female attraction to nesting sites and influences female mate choice, thereby affecting male reproductive success. However, this putative pheromone is not involved in sex recognition, since male visual cues are sufficient to trigger female courtship behaviour. This is the first demonstration of an external structure specialised in the production/release of sex pheromones in teleosts. Research work has been initiated in collaboration with Prof. John Pickett from Rothamsted Research (U.K.) aiming at the identification of the putative sex pheromone. Knowledge of the pheromonal system(s) in *S. pavo* is also important for management of its wild populations. The only habitat of this species in Portuguese waters, classified as vulnerable in the Red List of Portuguese vertebrates is the Natural Park of Ria Formosa (southern Portugal). The research project, Reproductive Biology of the Blenny *Salaria pavo* from Ria Formosa: Knowledge for Population Management and Conservation – (PNAT/1999/BIA/15090; 01/3/2001 – 01/2/2003) has continued. Although, it has not been possible to establish a procedure for rearing of *S. pavo* from egg to adult in the laboratory, environmental and social factors that influence reproductive strategies of *S. pavo* have been identified. The results will be described in the progress report (2<sup>nd</sup> year) of this project.

*O. mossambicus* is a maternal, mouth-brooding cichlid; territorial males group in "leks", and are actively sought out by reproductive females. After the female has chosen her mate, spawning takes place and the female picks up the fertilized eggs in her mouth and incubates them, until they hatch, in solitude. We have obtained strong evidence for chemical communication between the sexes during reproduction. Females showed strong olfactory responses to the water of males, as well as to their urine, faeces and bile fluid. Furthermore, the urination rate of males is highly dependent on social context, being markedly increased in the presence of pre-ovulatory females. In addition, it was shown that males have the ability to discriminate via olfaction between pre- and post-ovulatory females. This suggests that both sexes are transmitting chemical information about their reproductive status. Our aim is to identify these chemicals, and to establish their biological roles. Progress has been made in such direction, as unique HPLC fractions of urine from territorial males were shown to activate olfactory receptor neurones in females. These fractions were isolated in collaboration with Prof. Peter Sorensen (Univ. Minesota, USA).

Although the goldfish has been used as a model for ground-breaking studies on the role(s) of pheromones in fish reproduction, the possibility that it uses pheromones in other aspects of its biology has received much less attention. One such possibility is the alarm response. Given the rapid and large increase in circulating catecholamines that occur in stressed fish (the goldfish included), we hypothesised that these, and/or their metabolites may also have some sort of communicative function between individuals, as well as their much better understood physiological

roles. To test this hypothesis, we recorded the olfactory responses of goldfish to the catecholamines (adrenaline, noradrenalin and dopamine) and their common metabolites (8). The olfactory system proved to be highly sensitive to the catecholamines, particularly adrenaline and dopamine, and their 3-methoxy metabolites, metadrenaline and 3-methoxy-tyramine. Cross adaptation studies, and use of  $\alpha$ - and  $\beta$ -adrenoreceptor and dopamine antagonists, suggest that the receptors involved in this process are distinct, both functionally and pharmacologically, from "conventional" adrenoreceptors and dopamine receptors involved in neurotransmission *etc.* The results have been accepted for publication in *Chemical Senses*. In 2003, the physiological and/or behavioural consequences of exposure to these compounds will be investigated.

A potentially important aspect of fish chemical senses is its ability to monitor levels of physiologically important ions in the environment, mainly calcium. We have presented evidence that the olfactory system of the gilthead seabream (*Sparus aurata*) is highly sensitive to reductions in environmental  $[Ca^{2+}]$  and suggest that this sensitivity is mediated by an extracellular  $Ca^{2+}$ -sensing receptor. This phenomenon is not restricted to species that normally experience large fluctuations in external ion concentrations (*e.g.* moving from sea-water to freshwater), since the olfactory system of the stenohaline freshwater cyprinid, *C. auratus*, is also sensitive to fluctuations in environmental  $[Ca^{2+}]$ . Furthermore, the teleost calcium-sensing receptor (Ca-SR) is highly expressed in a sub-population of olfactory receptor neurones. Thus, the olfactory sensitivity to calcium, likely to be mediated by the Ca-SR, is probably a widespread phenomenon in teleosts, and may have an input into the physiological mechanisms regulating internal calcium homeostasis. Now we plan to identify the olfactory neurones that carry this information and to which centres of brain are they passing it. This will form the first step in understanding how the olfactory system of teleosts may influence the neuroendocrine pathways regulating calcium homeostasis and, as such, suggests a role for the olfactory system of fish that has not yet been investigated. To this end, we have established a collaboration with Professor Richard Balment (University of Manchester, U.K.) to investigate this phenomenon in the flounder (*Platichthys flesus*), an estuarine species which migrates between seawater and freshwater on almost a daily basis. Preliminary evidence (funded by the Treaty of Windsor programme) strongly suggests that the flounder has high sensitivity to environmental calcium. It is, therefore, an ideal model in which to study both the neural and endocrine pathways by which this olfactory input may affect calcium homeostasis and the changes imposed on the olfactory transduction process when moving from an ion-rich to an ion-poor environment.

### Plan for 2003

#### **Molecular evolution of hormones and receptors**

The genome of the model species *Fugu rubripes*, a teleost with a remarkably small genome (400Mb), was fully sequenced in 2001. This resource has opened the door for comparative studies of hormones and receptors in *Fugu* and other teleost fishes. Several models of genome evolution exist, the most popular suggests that 2 rounds of whole genome duplication occurred before the emergence of the jawless fish and that subsequently in the teleost lineage a further round of duplication occurred. The persistence of duplicated hormone and receptor genes in the genome of the *Fugu* will be studied in the secretin family of G-protein coupled receptors in order to establish mutation rates and the reason that duplicate genes have persisted. In parallel the corresponding cDNA for these receptors will be isolated in the sea bream to establish transcript number and the existence of splice variants. This will entail completion of work already initiated in *Fugu* to fully characterise the genes in the secretin family of G-protein coupled receptors. Extensive analysis *in silico* of the genes from *Fugu* and available sequences from representatives of other taxa will be carried out. Functional studies to establish the affinity of ligands for duplicate receptors will be performed. The approach taken will form the basis of future studies to establish the cross talk which occurs between gene evolution and function of other hormone and receptor genes.

#### **Parathyroid hormone-related protein and calcium homeostasis**

A range of physiological and molecular studies are being carried out to elucidate the role of PTH-like molecules in reproduction, skeletal development, calcium balance and immune response in sea bream and other teleosts. The approaches include 1) the mechanisms through which PTHrP regulates calcium balance in fish using Ussing chambers and cell culture (enterocyte and gill mitochondria rich cells); 2) receptor characterization and PTHrP responsive genes using subtractive hybridization and microarrays.

#### ***Steroid receptors and development of biomarkers of endocrine disruption***

A main target tissue for analysis of ER function is the testes, where there are high levels of expression of ER $\beta$ , one of the less know ERs. The effect of and antiestrogen on testicular function and on gene expression will be studied by a subtractive hybridization, macroarray and quantitative PCR approach. The estrogen-responsive genes which have been isolated are now being characterized and some will be chosen for detailed anaylsis. During 2002 the analysis of samples to screen for signs of endocrine disruption in Portuguese estuaries should be finalized.

#### ***Control of sexual determination and differentiation***

From the sea bass isolated putative sex determining genes a PCR analysis of the experiments carried on the influence of environmental factors will be carried out. One or two of the genes will be selected for in depth study which will include promoter isolation and analysis to confirm the existence of putative regulatory elements that can be under environmental influence. In addition, studies for the confirmation of the role of these genes in sex differentiation and reproductive physiology will be initiated.

#### ***Fish Chemical Senses: identification of active compounds and modes of action***

- 1) Chemical identification of the putative pheromone in the urine of male *O. mossambicus* and study the mode(s) of action of identified compounds at behavioural and physiological levels; chemical identification of putative pheromone released by female *O. mossambicus*
- 2) Chemical identification of the putative pheromone from the anal gland of male *S. pavo* and investigate the mode(s) of action of identified compounds at behavioural and physiological levels.
- 3) Investigate if and how olfactory detection of calcium and sodium in the environment influences the neural and neuroendocrine pathways regulating ion homeostasis.
- 4) Initiate investigations aiming at the identification of attractants associated with natural sources of food of the Senegal sole (*Solea senegalensis*).
- 5) Initiate investigations into the olfactory transduction process of marine teleosts, particularly where and how it differs from freshwater fish with respect to the use of external ions.

### **Group of Physiopathology**

#### **Research team**

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Post-doc: Natércia Joaquim  
PhD students: Gisela Borges; Sandra Soares  
Undergraduate students: Ricardo Gândara; Isabel Costa

#### **Summary of activities and progress during 2002**

##### ***1- Regulation mechanisms of vertebrate cardiac function – strategies of environmental adaptation***

Our main research goal is to contribute to clarify mechanisms of vertebrate cardiac function regulation, with special attention to strategies of species adaptation to specific habitat conditions and way of life. During 2002, our progresses regarding this objective were:

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### **Non-invasive study of heart morphology and function of aquatic turtles** (collaboration with Zoomarine and UIC)

This work aims to contribute to clarify the mechanisms that regulate cardiac function in marine turtles, in particular the dynamics of blood flows during cardiac cycle in relation with the respiratory frequency. During 2002 we did several echocardiographic exams along the year in order to evaluate seasonal variation of cardiovascular function in *Caretta caretta*. This work will proceed in the following years. Studies in development also deal with non invasive hemodynamic quantification of filling and ejection flow velocity and determination of functional indices such as cardiac output.

### **Non-invasive hemodynamic assessment of systolic and diastolic function of the toadfish heart.** (collaboration with UIC)

The hemodynamic study of cardiac flows allows for the evaluation of important parameters of cardiac performance. In lower vertebrates, there are a very few studies which used a non-invasive hemodynamic approach. For example, in fish, most available data on the hemodynamics of ventricular ejection flow come from isolated perfused heart preparations, and *in vivo* studies are limited to Doppler or Transonic® (electromagnetic) flow probes fitted around to the ventral aorta. Therefore, the aim of this study was to use Doppler echocardiography to perform a non-invasive study of the systolic and diastolic function of the Lusitanian toadfish heart. Doppler echocardiography velocity spectral records of ejection ventricular flow presented a typical spectrum, characterised by two phases: rapid acceleration and slow deceleration. Peak flow velocity varied between 0.13 and 0.25 m/s, and the pressure gradient between the ventricle and the bulbus, ranged between 0.07 and 0.25 mmHg. Using the described approach, stroke volume and cardiac output were calculated independent of ventricular shape, and averaged of  $0.245 \pm 0.066$  ml/kg and  $9.9 \pm 2.0$  ml/min/kg, respectively. These values for the for *H. didactylus* heart, are in the range of values reported using invasive methods for other fish species with 100 % trabecular myocardium and low ventricular mass. Doppler velocity records of ventricle filling showed a typical biphasic pattern, characterized by two waves: an E-wave (early filling), with peak velocities of 4.8-25.0 cm/s, and an A-wave (atrial contraction), with peak velocities that ranged from 4.7-33.9 cm/s. The E/A ratio presented two patterns, depending on HR ( $p < 0.05$ ): for individuals with high values ( $> 40$  bpm), E/A ratio was  $< 1$ , while individuals with low HR values ( $< 20$  bpm), presented an inverse profile, with an E/A ratio  $> 1$ . Thus, the present study shows that ventricular relaxation contributes significantly to ventricular filling in *H. didactylus*, and that ventricular filling is highly dependant on HR (especially on duration of isovolumic relaxation and early filling).

### **Cardiac Function and Critical Swimming Speed of the Winter Flounder (*Pseudopleuronectes americanus*) at Two Temperatures** (collaboration with the Oceans Science Centre – Memorial University de Newfoundland)

Using Transonic® flow probes and a uniquely designed swimming flume, we directly measured cardiac output (Q) before and during a critical swim speed ( $U_{crit}$ ) test at 4 and 10 °C. Resting Q, stroke volume ( $S_v$ ) and heart rate ( $f_H$ ) averaged  $9.8 \text{ ml min}^{-1} \text{ kg}^{-1}$ ,  $0.5 \text{ ml kg}^{-1}$  ( $1.0 \text{ ml g ventricle}^{-1}$ ) and 21 beats  $\text{min}^{-1}$  at 4°C and  $15.5 \text{ min}^{-1} \text{ kg}^{-1}$ ,  $0.5 \text{ ml kg}^{-1}$  ( $0.95 \text{ ml g ventricle}^{-1}$ ) and 34 beats  $\text{min}^{-1}$  at 10°C. Although Q,  $S_v$  and  $f_H$  increased by approx. 270, 160 and 175% at both temperatures during the  $U_{crit}$  test, all cardiac parameters reached near maximal levels almost immediately upon swimming and remained at these levels until  $U_{crit}$  ( $0.63 \pm 0.06 \text{ bl s}^{-1}$  at 4°C and  $0.73 \pm 0.07 \text{ bl s}^{-1}$  at 10 °C). Although we confirm that SV (per  $\text{g}^{-1}$  ventricle) is high in the flounder and that "lift-off"/slow swimming is energetically expensive, our results strongly suggest that Q and  $U_{crit}$  have been significantly overestimated in flatfishes.

### **2- Physiopathological responses to toxic metals intoxication**

We have been studying the effects of toxic metals, in several tissues of our experimental model, the toadfish. A combination of biochemical, cellular biology and physiological studies were performed in order to clarify the toxicological effects of cadmium and vanadium as well as the possible role of vanadium in muscle contraction.

During the last year, several important progresses were obtained, in order to determine effects of these metals:

#### **Effects of vanadate oligomers on lipid peroxidation and antioxidant enzymes in the lusitanian toadfish kidney and liver: Short-term exposure**

The objective of this study was to evaluate antioxidant defence system responses induced by an acute exposure to a sub-lethal concentration (5mM) of “meta” and “decavanadate”, on the kidney and liver of *Halobatrachus didactylus*. Different effects for both vanadate solutions in liver and kidney, were observed. In the kidney, antioxidant enzymes activities and lipid peroxidation increased both in Meta V and Deca V groups. Major alterations occurred in Deca V CAT cytosolic, after 8 days, SOD mitochondrial, after 24 hours and Se-GPx activities. Also, there was a significant increase in lipid peroxidation on Deca V group, which indicates an ineffective response of the cellular defence mechanisms against oxidative stress caused by this metal. In the liver, CAT and SOD activities were in general stimulated in both groups. Deca V group, after 24 hours, has shown the highest difference in comparison to CTRL group (139.4%). There were no significant alterations in lipid degradation products. These results indicate that, in the liver, the antioxidant enzymes play an important role against oxidative stress. The antioxidant enzymes activities in kidney had the same pattern, except in CAT activity, that decreases after 24 hours and an increase after 7 days. All oligomeric species of vanadate studied induced oxidative stress in both tissues, but have also shown to affect differently antioxidant enzymes activities and lipid peroxidation. Apparently, “decavanadate” induces stronger antioxidant responses than “metavanadate” and stronger effects, as well as lipid peroxidation, in the kidney.

#### **Effects of vanadate oligomers and cadmium on *Halobatrachus didactylus* sarcoplasmatic reticulum calcium pump – dependence on incubation and pH**

The Ca<sup>2+</sup>-ATPase of sarcoplasmatic reticulum (SR) membrane is a transmembranar protein that catalyses both the hydrolysis and synthesis of ATP playing an essential role in relaxation and contraction of the muscle. The activity of Ca<sup>2+</sup>-ATPase is normally described by a cycle postulating the existence of two protein conformations, E<sub>1</sub> and E<sub>2</sub>, with high and low affinity for Ca<sup>2+</sup> and ATP, respectively. The alternation between the two distinct conformations of Ca<sup>2+</sup>-ATPase during the transport cycle seems to affect the interactions with different oligomeric species of vanadate (e.g., decameric and tetrameric) that affects the activity of the calcium pump. Such as, vanadium, cadmium is well known by its toxic effects in live organisms, being an inhibitor of the calcium pump even at very low concentrations. In this work we compared the effects of cadmium and vanadium on the ATP hydrolysis by the calcium pump at different pH values and incubation times from skeletal muscle of *Halobatrachus didactylus*. It was concluded that, at pH 6.0, 7.0 and 8.0, cadmium inhibits strongly calcium pump ATP hydrolysis and the cadmium incubation with the protein favours enzymatic inhibition. “Decavanadate” affects more strongly the ATP hydrolysis by Ca<sup>2+</sup>-ATPase than “metavanadate”, being the relative order of inhibition affected by pH as followed: pH 6.0>8.0>7.0. It is suggested that oligomeric species of vanadate inhibition of ATP hydrolysis is favoured by the E<sub>2</sub> conformation (pH 6.0).

#### **Effect of vanadate oligomers on the Lusitanian toadfish heart (*Halobatrachus didactylus*): Functional noninvasive analysis**

Previous studies have identified several physiopathological effects of vanadate on the cardiovascular system, such as negative inotropy and essential hypertension. Recently, studies developed in our lab suggest that different vanadate species, namely monomeric and decameric species, may contribute differently to oxidative stress and to histopathologic changes on cardiac tissue, which may be related to cardiac dysfunction. In order to explore this hypothesis, systolic and diastolic functions of Lusitanian toadfish heart were analysed, using echocardiography, after acute *in vivo* exposure to “metavanadate” or “decavanadate” solutions.

It was observed that “metavanadate” significantly reduced the systolic flow peak velocity ( $p < 0.05$ ), after both 1 and 7 days. However, “decavanadate” only reduced cardiac function after 1 day. The pressure gradient between the ventricle and bulbus and stroke volume varied in accordance with ventricular systolic peak velocity. The maximum velocities of ventricular filling were also changed in

the presence of vanadium: E and A waves velocities diminished significantly after 1 day in both groups, and after 7 days in just the Meta group. These results indicate a reduced ventricle filling, which may be related to decreased stroke volume. On other hand, the reduction of both E and A waves indicates a deficient ventricular relaxation and atrial contraction. Although the vanadium species used affected flow velocities, there was no evidence that systolic or diastolic time intervals or heart rate were affected by vanadium exposure. In conclusion, vanadium significantly affects toadfish cardiac performance. The results obtained suggest that different vanadate species present in vanadium (V) solutions, may contribute to vanadium toxicity by decreasing ventricular filling flow and inducing diastolic ventricle dysfunction. Both ventricle relaxation and atrial contraction are depressed in the presence of vanadate compounds.

### **3 - Endothelial dysfunction and cardiovascular diseases**

The objective of this work is to study oxidative stress and endothelium dysfunction mechanisms in hypertensive and diabetic patients, and in experimental models (mouse and fish). During 2002, 19 diabetic (type II) patients and 30 controls were referred and blood samples were collected in order to determine: antioxidant enzymes activities, lipid peroxidation products and endothelial dysfunction markers. These analyses are in progress and future work will deal with isolated endothelial cells studies, related mainly with reactive oxygen species and nitric oxide.

### **Plan for 2003**

#### **1- Regulation mechanisms of vertebrate cardiac function – strategies of environmental adaptation**

It is our propose to continue the work developed in this area during the past years, specifically:

##### **Cardiovascular function of aquatic turtles**

- 1) Non invasive hemodynamic quantification of filling and ejection flow velocity and determination of functional indices of *Caretta caretta*.
- 2) Heart morphology and blood flow characterization of *Chelonia mydas*.
- 3) Natural seasonal temperature variations effects on cardiovascular function in *Caretta caretta*.
- 4) Respiratory behaviour of *Caretta caretta* individual under restrained conditions.
- 5) Haematologic parameters in several species with different water dependencies.

##### **Cardiovascular physiology of North Atlantic flatfishes**

- 1) Temperature effects on exercise capacity of Atlantic Halibut
- 2) Respirometry experiments with several North Atlantic benthic species: winter flounder, witch flounder, halibut, lumpfish, wolfish and sculpin.

#### **2-Metal ions induced oxidative stress in cardiac muscle**

This project aims to continue studying acute and chronic effects of toxic metals intoxication to heart tissue, specially its role on cardiac muscle function and toxicity mechanism.

##### **Toxic metals effects on antioxidant protection systems** (collaboration with FCT, UAIG)

- 1) In vivo studies: several sub-lethal concentrations of cadmium or vanadium compounds will be administrated through intravenous injections in order to study metals concentration effects upon acute exposure.
- 2) In vitro studies will be performed to clarify the contribution of metal-protein interactions to their toxic effects.

##### **Cadmium and vanadium compounds interactions with sarcoplasmic reticulum calcium pump** (collaboration with FCT, UAIG)

- 1) Comparative effects of cadmium and vanadium compounds on SR calcium pump activities from rabbit and toadfish: dependence on pH, incubation, temperature and natural ligands
- 2) Interaction of cadmium and vanadium with the calcium pump: atomic absorption spectroscopy UV/vis, NMR and EPR spectroscopy studies

### **3 - Endothelial dysfunction and cardiovascular diseases**

- 1) Antioxidant enzymes activities, lipid peroxidation products and endothelial dysfunction markers and vanadium concentration analysis in blood samples from diabetic (type II) patients and controls.
- 2) Hypertensive patients' reference and blood collection.

## **Biophysics Group**

Leader - Leonor Cruzeiro-Hansson  
 Paulo Silva, PhD Student

### Summary of activities and progress during 2002

From a theoretical point of view computational simulations of 4 proteins (myosin S1, ubiquitin, calmodulin and prion) showed that it is possible that they can adopt more than one tertiary structure. It was developed the idea that the formation of protein structure includes first a kinetic step, which is determinant of the final structure, followed by a second thermodynamic step (energy minimization) which is determinant of the time the structure takes to set itself (folding rate). In collaboration with prof. Chris Eilbeck a study was carried out of the type of states formed by electron pairs and their dynamic and thermodynamic stability.

### Plan for 2003

New studies of protein structure will be carried from the multi-funnel point of view, i.e., the notion that proteins can assume different structures. More general research on non-linear networks will be carried out in collaboration with Prof. Chris Eilbeck.

## **Group Aquaculture**

### Research team

Leader: Maria Teresa Dinis  
 Post-docs: Luis Conceição, Florbela Soares, Pavlos Makridis  
 PhD students: Laura Ribeiro, Cláudia Aragão, Sofia Morais  
 Technicians/ research assistants: Sofia Engrola, Pedro Cação, Ana Clara Marques, Marc Lacuisse, Silvia Martins, Cristina Pita, Isabel Noronha, François Hubert  
 Undergraduate students: Rita Colen, Joana de Pina, Manuela Castro e Cunha  
 Aquaculture technician students: Helena Damásio, Rodolfo Isidoro, Helena Teixeira

### Summary of activities and progress during 2002

The research was focused on sole broodstock (*Solea senegalensis*), and also on the quality of the first feeding preys for larval rearing. Within this context a close cooperation with a private enterprise for the study of a concentrated phytoplankton live cells was done. The cooperation with the aquaculture industry was also achieved through cooperation agreements between Spanish and Portuguese companies, in order to transfer and scale up the achievements of sole cultivation.

### **Broodstock management**

Two new groups of sole broodstock were established. All fish were tagged and sexes were determined. From the existing four groups of fish, three were maintained in an open system at the Experimental station of Ramalhete, and the fourth in a closed system at the University campus. At Ramalhete one group was under temperature and photoperiod control, and the two remaining groups had been used for hormonal induction using GnRH $\alpha$  slow-releasing devices provided by the University of Maryland, Center of Marine Biotechnology. Feeding was the same in all groups and consisted of squid, worms and mussel. The objectives were not only egg production, but to identify possible parameters responsible for sole reproduction, one important bottleneck in sole cultivation. Only a few spawnings of bad quality eggs were obtained. No positive results were achieved with hormonal induction, probably due to a very early induction.

### **Effect of dietary amino acid profiles on sole (*Solea senegalensis*) post-larvae on metabolism and growth**

Methionine has been suggested as the limiting amino acid in the growth of early stages of marine fish. The effect of a low-methionine diet on growth and metabolism of *Solea senegalensis* post-larvae was evaluated. Sole post-larvae ( $15.1 \pm 6.4$  mg dry weight) were distributed by six 4L trays. Each tray had 70 fish. Two experimental diets were tested in triplicate. One of the diets was fishmeal-based (control diet) and the other based on soy protein concentrate (low-methionine diet). The control diet had 56.0% DM crude protein, 19.1% DM lipids, 3.9% DM starch and 3.9 g methionine/100g IAA. The low-methionine diet had 57.2% DM crude protein, 15.8% DM lipids and 13.0% DM starch and 1.3 g methionine/100g IAA. The experiment lasted 19 days (from 62 to 81 days after hatching). Survival and growth were monitored. At the end of the experiment oxygen consumption and ammonia excretion were determined. Survival was high in both treatments (average value of 88.6% at the end of experiment). Condition factor and dry weight were also similar for both treatments. Relative growth rate had an average value of 5.5%/day, and feed conversion ratio was around 3.8. Oxygen consumption did not vary significantly, neither between treatments nor between fast and fed larvae in the same treatment. The average value found was  $74.7 \mu\text{mol O}_2 \cdot \text{g}^{-1} \text{DW} \cdot \text{h}^{-1}$ , with a variation coefficient around 13%. This might indicate that fed larvae reduce the costs of maintenance to accommodate the costs of growth. No significant differences were found in ammonia excretion rates between treatments, due to the high variability of the values, in particular in the low-methionine treatment. In this treatment no significant differences were found between fast and fed fish (average value  $14.4 \pm 6.99 \mu\text{mol NH}_4^+ \cdot \text{g}^{-1} \text{DW} \cdot \text{h}^{-1}$ ). In the control treatment fasted larvae had a significant higher ammonia excretion than fed larvae ( $15.0 \pm 3.4 \mu\text{mol NH}_4^+ \cdot \text{g}^{-1} \text{DW} \cdot \text{h}^{-1}$  and  $9.8 \pm 1.1 \mu\text{mol NH}_4^+ \cdot \text{g}^{-1} \text{DW} \cdot \text{h}^{-1}$ , respectively). This suggests that sole eating the control diet catabolise more protein for energy purposes when starved than after feeding, when they probably catabolise more non-protein energy and thereby spare amino acids for growth. The high variability in ammonia excretion in the low-methionine treatment may imply that some fish are more sensitive to the methionine deficiency in the diet than others, suggesting different capacities to adapt to this dietary deficiency.

### **Weaning of sole (*Solea senegalensis*) into artificial diets**

Weaning is one of the traditional bottlenecks in sole culture. In continuation of studies from the previous years in CCMAR, additional weaning strategies for sole were tested in this study. The commercial inert diet Aglonorse, (SSF, Bergen, Norway) was used again based on previous good results. Sole were weaned at 20, 30, and 40 days after hatching (DAH) using a "sudden weaning" strategy. Sudden weaning before 40 DAH led to high mortalities in all cases (over 50%). It seems to be advisable to use a period of co-feeding with *Artemia* if inert diets are to be used for sole before 40 DAH.

### **Growth of sole (*Solea senegalensis*) juveniles reared under different feeding frequencies**



Feeding frequency influences growth and growth dispersion in different species. Sole differ from most other culture species in having a passive feeding behaviour in the bottom. A study was carried out to determine the effect of four feeding frequency regimes on juveniles of sole, *Solea senegalensis*. Fish 187 days old ( $14.81 \pm 8.75$ g) were randomly distributed in four treatments: Night (fed 10pm to 7am), Day (10am to 7pm), Control (10am to 5am) and Pulse (fed at 10am, 2pm, and 6pm), in triplicates (34 fish per tank). A commercial inert diet was used (ProAqua 1.5mm) and supplied by automatic feeders. Throughout the experiment it was attempted to feed fish close to satiation, based on predicted maximum growth and daily adjustments. Temperature and salinity were maintained, respectively, at  $21.0 \pm 1.0^\circ\text{C}$  and  $32.0 \pm 1.0\text{‰}$ . Dissolved oxygen in water was around  $85 \pm 10\%$  saturation. A photoperiod of 12h light: 12h dark cycle was used, with an indirect dim light being provided by fluorescent tubes. Average fish weight about doubled during the experiment with small differences between treatments. The only significant difference ( $P < 0.05$ ) was found between the Night ( $27.02 \pm 9.87$ g) and Day ( $31.31 \pm 12.65$ g) treatments. The fish that were in the Control treatment had the lowest coefficient of variation (CV, 30.56%), and the highest CV was found in the Day treatment (CV= 40.40%). The condition factor at the end of the experiment had no significant differences between treatments. Sole eats well both with light and in the dark, but seems to eat slightly better with light. However growth dispersion seems to be reduced if sole is fed during a longer period (19 hours compared to 9 hours).

#### ***Effect of dietary carbohydrate to lipid ratios on growth, nutrient utilisation and tissue lipid deposition in juvenile Senegal sole (Solea senegalensis).***

[Study in cooperation with researchers from CIIMAR (University of Porto, Portugal) and Laboratory of Aquaculture & Artemia Reference Center (Ghent University, Belgium)]

A study was undertaken to determine the effect of various dietary carbohydrate to lipid ratios on growth performance, whole-body composition and tissues lipid content in Senegal sole (*Solea senegalensis*) juveniles. Four isoproteic (crude protein: 52% DM) diets were formulated to contain one of two lipid levels (11 and 21% DM). Furthermore, within each dietary lipid level the digestible carbohydrate content was varied by the incorporation of extruded or crude peas meal. The dietary digestible carbohydrate to digestible lipid ratios ranged from 0.2 to 1.6. Triplicate groups of 50 sole (mean initial body weight:  $23.6 \pm 1.2$  g) were grown in recirculated seawater (temperature:  $20^\circ\text{C}$ ; salinity: 35 ppt) over 67 days. During the growth trial, each diet was fed to satiation by means of automatic feeds, with daily adjustments when needed. Feed intake was quantified throughout the experimental period. At the end of the study, whole body, liver, viscera and muscle samples were withdrawn for analyses. During the experimental period the mean fish weight about doubled in all treatments. Despite a tendency to an overall improvement of growth performance (weight gain, feed efficiency and protein utilisation) of fish fed diets with low dietary lipid levels, statistical differences did not prove significant at  $P = 0.05$ . High fat diets increased whole-body fat content. Similarly, daily fat gain ranged from 0.54 to 0.78 g/kg/day and highest values were found in fish fed high-lipid diets. Dietary treatments were also found to affect tissue lipid content (liver, viscera and muscle), with highest values being generally observed in fish fed high digestible carbohydrate diets. On the overall, growth performance of Senegal sole juveniles was highest under low dietary lipid levels. The nature of dietary carbohydrates has little influence on performance criteria, but seems to condition tissue lipid deposition.

#### ***Pigmentation and skeletal abnormalities of sole (Solea senegalensis) larvae and post-larvae***

The effect of live food dietary in the development of pigmentation and in skeletal abnormalities in *Solea senegalensis* larvae was studied. Histological and histochemical techniques were used to characterize the pigmentation development. Different patterns of malpigmentation were identified. Histochemistry characterization of the skin of white, brown and albino juveniles of Senegal sole were done. Morphological characterization of the pigmentation during larval ontogeny was described. In larvae of Senegal sole (*Solea senegalensis*), pigment cells occur symmetrically on both sides of the body, while in adult fish, melanophores develop predominantly on the ocular side of the body. The first skin pigment cells identified on sole are the larval chromatoblasts which

includes larval melanophores and xanthophores. Iridophores (another type of chromatophores) appear only after metamorphosis giving a white or silvery appearance, due to scattering of light by guanine inclusions. Melanophores are the more abundant pigment cells in the first life stages of the larvae. Xanthophores produce yellow to orange pigments based on carotenoid compounds and were also observed on the first day after hatching. The adult melanophores begin to differentiate from stem chromatoblasts during metamorphosis, but larval melanophores are still present.

### **Ongrowing of sole (*Solea senegalensis*) juveniles**

The growth and growth dispersion of sole were evaluated in two sub-experiments made simultaneously. Fish with 10g were tested in four treatments, with three replicates each. The first experiment consisted in test the same density but with different number of fish per tank (Big (n=22, 1.2Kg/m<sup>2</sup>), Mix (n=31, 1.2Kg/m<sup>2</sup>), Small High (n=40, 1.2Kg/m<sup>2</sup>)). In the second experiment it was tested the performance of fish in two different densities: Small Low (n=20, 0.62Kg/m<sup>2</sup>) and Small High (n=40, 1.2Kg/m<sup>2</sup>) density. The experiment lasted 10 weeks. A commercial inert diet was used (ProAqua 2.0 mm) and supplied by automatic feeders every hour during 18 hours a day. A photoperiod of 12h light: 12h dark cycle was used, with an indirect dim light being provided by fluorescent tubes. Samples were taken to evaluate weight, length, RGR, CV and K. There larvae were significantly bigger (p>0.05) and with a higher condition factor in treatment "Big" when compared with the larvae from treatment "Mix". The larvae from "Small Low" were significantly bigger (p>0.05) and with a higher condition factor when compared with "Small High". The sole grew better in the treatment that had less fish in the treatment "Big". The juveniles grew better in the less density and crowded treatment. In addition to this controlled experiment, three groups of weaned sole were followed in practical conditions with the cooperation of 3 fish farms. Growth, as well as shape, and pigmentation abnormalities were measured.

### **Microbiology of fish larvae**

A group of candidate probiotic bacteria were isolated from juvenile sole fed natural diet (*Nereis* sp.). A second group of candidate probiotics were isolated from *Tetraselmis* sp. Feeding with a natural diet increased the number of strains present in the fish gut, showing that the diversity of microflora of the gut oncreased. A phenotypic characterisation of the collected bacteria was attempted using a large number of tests. The invitro inhibition of two pathogenic strains (*Vibrio anguillarum* and *Photobacterium damsela*) by the candidate probiotic bacteria strains was examined using the double layer technique. A high percentage of the bacterial strains (>40%) found in fish fed a natural diet shows in vitro inhibition of three pathogenic strains. The ontogeny of the immune system in sole during the first 120 days was examined using histological techniques. The effect of two immunostimulants ( $\beta$ -glucan and mannuronic acid) was examined in the same rearing experiment. Two in vivo tests were performed to test the effect of the candidate probiotic bacteria on marine fish larvae. In a small scale experiment a large number of strains were tested in two different concentration to see how they influence the survival of starved sea bream larvae. Whereas in a first feeding experiment several strains were examined for their effect on Senegalese sole larvae. The cultures added probiotic bacteria showed a survival around 80%, while the controls tanks showed a survival around 50%.

### **Feed for aquatic animals that contains cultivated marine microorganisms as alternatives for fish oil**

The main purpose of our work in this project was to perform experiments with enrichment emulsions for rotifers and Artemia, testing different percentages of Single Cell Oil (produced by heterotrophic growth of microalgae (*Cryptocodinium cohnii*) incorporated in a microalgae biomass, using commercial oils as reference. The trials were conducted according with the protocols of the commercial enrichments for each case (DHA Protein Selco® for rotifers and DC Super Selco for Artemia) in order to provide comparison with commercial available products. These experiments have a final objective to purpose a protocol for the enrichments emulsions based on microalgae pastes + Single Cell Oil. The experiments consisted in feeding trials with rotifers and Artemia enriched with *Cryptocodinium cohnii* incorporated at different amounts in a Nannochloropsis oculata based paste were done Biochemical composition (protein, total lipids and

fatty acids) of both enriched rotifers and Artemia were also determined. An enrichment protocol for rotifers was proposed based on the best results for the different enrichment times. Regarding Artemia, no protocol was produced due the considerably low levels of DHA found, and because no evidence of a positive performance of the paste was obtained. The main conclusion of the experiments was that none of the pastes used provide better DHA levels than the standard commercial emulsions used as a control.

### **Plan for 2003**

The research during 2003 will be focus on broodstock management and aspects of larval and juvenile rearing of sole (*Solea senegalensis*), seabream (*Sparus aurata*), red porgy (*Pagrus pagrus*) and grouper (*Epinephelus marginatus*), as well on methodologies for enrichment of rotifers and Artemia, as part of the research projects where the group is involved.

#### **Broodstock management**

- a) Identify the principal environmental and zootechnical parameters impinging on reproduction success and quality of eggs and larvae of sole.
- b) Reinforce, in collaboration with fisherman, the existing grouper and red porgy broodstocks. Adapt new fish to captivity.
- c) Attempt hormonal induction of spawning in sole and grouper using GnRH $\alpha$  slow-releasing devices and/or boosting injections.

#### **Nutritional and zootechnical aspects of larval rearing**

- a) Study the effect of diets with unbalanced amino acid profiles on the growth and metabolism of sole and seabream fish larvae. Growth trials and tube-feeding assays will be used, together with measurements of respirometry, activities of enzymes of intermediary metabolism and tracer studies.
- b) Determine the influence of dietary natural zooplankton, larval density and rearing volume in the growth performance, survival rates and fish quality for grouper and red porgy. Fish quality will be assessed based on morphological (e.g., skeleton malformations, pigmentation, histology) and biochemical (e.g., RNA/DNA ratios, free amino acid contents, enzyme activities) criteria.
- c) Optimise weaning zootechnical conditions and feeding regimes for sole. This will be based on zootechnical trials, with evaluation of growth performance and survival rates.

#### **Microbiological aspects of larval rearing**

The effect of probiotics on larval and juvenile survival, growth and viability will be further investigated, as well as the mechanisms involved in these processes. DNA analysis by use of 16S rRNA PCR will be applied for the identification of the bacterial strains.

The ontogenetic development of the immune system in sole and the influence of immunostimulants on the non-specific and specific immune defenses will also be investigated.

#### **Optimization of sole ongrowing**

- a) Development and testing of methods for ongrowing of Senegal sole in earthen ponds. Adapt techniques developed for ongrowing seabass and seabream in earthen ponds to Senegal sole, and verify its feasibility both in monoculture and in polyculture with these species.
- b) Determine the effect of grading and density on social behaviour, feeding behaviour and growth of sole.

#### **Pigmentation abnormalities in sole**

- a) Set up an assay to measure  $\alpha$ -melanophore-stimulating hormone ( $\alpha$ -MSH), a major hormone involved in colour change and background adaptation in fish.
- b) Investigation of background adaptation in sole involving the rearing of fish in white, green and black tanks. Adaptation in normal and malpigmented sole will be compared by measuring pituitary

and plasma  $\alpha$ -MSH levels, plasma cortisol, tyrosine kinase activity and histological examination of the skin melanophores.

c) Development of *in vitro* tests to determine the chemical signals involved in the aggregation/dispersion of melanin within the melanophores.

d) *In vitro* superfusion studies to compare the release of  $\alpha$ -MSH from the pituitaries of normal and malpigmented sole adapted to different backgrounds.

e) Morphological and histological characterisation of the epithelium skin (pigmentation) , in normal and malpigmented fishes results for the different rearing conditions. The use of histological and histochemistry techniques, will allow the identification of the main changes at the tissue level.ill also be taken.

f) Immunohistochemistry studies of the development of the melanotropes can be studied in normal and malpigmented fish.

#### ***Methodologies for enrichment of rotifers and Artemia***

a) Trials to evaluate proposed protocol for rotifers enrichment with DHA rich biomass will be conducted with seabream larvae, checking larval growth, survival and biochemical composition, mainly fatty acids

b) New type of enrichments will be tested with incorporation in microalgae paste of EPA rich biomass both in rotifers and *Artemia*

## ***Division of Living Resources***

### **Group: Ecology and Evolution of Marine Organisms**

#### **Research team**

Leaders: Ester Serrao, Gareth Pearson

Post-docs: Sophie Arnaud, Martin Billingham, Claire Daguin, Carolyn Engel, Lydia Ladah

PhD students: Filipe Alberto

Technicians/ research assistants: Sara Teixeira, Carla Viegas, Vera Fonseca, Luis Correia, João Ferreira, Marta Valente, Asuncion Lago, Tiago Simões, Rafael Bermudez, Carla Monteiro, Claudia Faustino, Sofia Caetano.

#### **Summary of activities and progress during 2002**

#### ***Genetic Structure of Populations***

##### Main purpose:

To analyse the genetic variability and population structure in a variety of species, either inhabiting marine environments or coastal regions, as well as endangered/protected species, in order to understand effects of various factors that affect populations, such as different reproductive strategies, population fragmentation, commercial exploitation, environmental threats.

##### Achieved in 2002:

1. Development of, and optimisation on an automated sequencer of microsatellite markers for *Posidonia oceanica*, *Cymodocea nodosa*, *Fucus vesiculosus*, *Fucus spiralis*.
2. Sampling of the relevant populations for each species and genotyping of individuals, to select the best combination of primers to resolve clonal diversity.
3. Investigation of the best statistical analysis to describe the patterns of spatial and clonal diversity.

#### ***Gene expression associated with stress in macroalgae***

##### Main purpose:

To identify genes that are differentially expressed under different physical stresses and to compare their regulation in populations and species living under contrasting stress conditions.

Achieved in 2002:

1. New subtractive cDNA libraries for identification of genes associated to desiccation stress.
2. Isolation of stress-responsive full-length clones in *Fucus*.

***Sequencing and expression of chloroplast genes in intertidal macroalgae***

Main Purpose:

To obtain gene sequences from macroalgal chloroplasts (plastids) in order to study the regulation and control of expression in photosynthetic organisms living in marine intertidal environments.

Achieved in 2002:

1. Sequencing of the chloroplast genome in target species (nearly complete).
2. Gene expression analysis of various photosynthesis-related genes in response to simulated low and high tide conditions.
3. Use of gene array techniques to allow large scale plastome gene expression analysis.

***Molecular evolution, phylogeny and phylogeography***

Main purpose:

To use molecular data to infer the evolutionary history of taxonomic groups from a range of organisms and to relate this with their biogeographic distribution and the evolution of other characters (e.g., physiological, reproductive, etc).

Achieved in 2002:

1. Screening of several *Fucus* sp. chloroplast spacer sequences as markers to solve taxonomic questions in the genus *Fucus* and to infer the evolution and phylogeography of fucoid algae.

***Reproductive strategies in algae and seagrasses***

Main purpose:

To investigate the investment into, and the success of, sexual reproduction in populations of algae seagrasses with different reproductive modes and/or under contrasting regimes of disturbance.

Achieved in 2002:

1. Estimation of the investment in sexual reproduction by seagrass species.
2. Estimation of the timing of reproduction and dispersal distances by algal species with hermaphroditic and dioecious reproductive modes.

***Ecophysiology and local adaptation of marine populations***

Main purpose:

To assess local adaptation of disjunct populations.

Achieved in 2002:

1. Study of a natural population of *P. oceanica* showing a high rate of mortality attributed to the proximity of aquaculture installations, and genetic analyses, to try to infer the potential impact of mortality on the genetic diversity of seagrasses.

**Plan for 2003**

**Genetic Structure of Populations**

1. Concluding the sampling of the relevant populations for each species and very intense genotyping of individuals in an automated sequencer.
2. Statistical analyses to evaluate genetic divergence between populations and genetic structure within populations of several species.

**Gene expression associated with stress in macroalgae**

1. New subtractive cDNA libraries for identification of genes associated to desiccation stress.

2. Isolation of stress-responsive full-length clones in *Fucus*.

Sequencing and expression of chloroplast genes in intertidal macroalgae

1. Conclusion of the sequencing of the chloroplast genome in a *Fucus* sp.
2. Gene expression analysis of new photosynthesis-related genes in response to simulated low and high tide conditions, manipulating light and desiccation levels.
3. Use of gene array techniques to allow large scale plastome gene expression analysis for new genes.

Molecular evolution, phylogeny and phylogeography

1. Screening of several *Fucus* sp. chloroplast, nuclear and mitochondrial DNA sequences as markers to infer the phylogeographic history of furoid algae and the species *Fucus vesiculosus*

Reproductive strategies in algae and seagrasses

1. Estimation of the investment in sexual reproduction by seagrass species in colonizing and fragmented versus stable and continuous seagrass meadows
2. Estimation of demographic parameters of seagrass meadows as a basis to understand the population genetic structure

## **Group of Marine Plant Ecology**

Research team

Leader: Rui O. P. Santos

Post-docs: Raquel Carmona, Alexandra Cunha

PhD students: João Silva, Raquel Machás, Susana Cabaço, Cecile Godinho

Master's students: Ana Alexandre

Research Assistants: Aschwin Engelen, Leonardo Mata, Andreas Schuenhoff, Estibaliz Berecibar

Technicians: Catarina Alves, Luís Dias, Rui Candeias.

Summary of activities and progress during 2002

### ***The role of plants in coastal ecosystem functioning***

Main purpose:

- 1 - To assess the role of vegetation in the carbon and nitrogen cycles of coastal ecosystems such as the Ria Formosa lagoon and the Guadiana estuary.
- 2 - To investigate the ecophysiology of the carbon and nitrogen uptake in seagrasses and their adaptation to varying environments.
- 3 - To assess the role of primary producers in the trophic dynamics of coastal systems
- 4 - To assess the effects of anthropogenic disturbances on plant communities
- 5 - To map the benthic communities of Ria Formosa using Geographic Information Systems tools
- 6 - To model the production and outwelling (export/import dynamics) of dissolved organic carbon, particulate matter and plant detritus in Ria Formosa.

Achieved in 2002:

- 1 - The seasonal variation of productivity and population dynamics were described for two lower/saltmarsh species, *Spartina densiflora* and *Spartina maritima*, along their distribution range in the Guadiana estuary.
- 2 - The seasonal productivity of two morphotypes of *Spartina maritima* in Ria Formosa was determined.

- 3 - The circadian evolution of seagrass photosynthesis along an intertidal vertical gradient was established (Silva, J and R Santos, accepted for publication in Marine Ecology Progress Series)
- 4 - The nitrate reductase activity of the seagrass *Zostera noltii* (Hornem.) was found to be dependent on the vertical location of the plants (Alexandre, A, J Silva and R Santos, submitted to Journal of Experimental Marine Biology and Ecology).
- 5 - The Interaction between the use of light and inorganic carbon acquisition in *Zostera noltii* was established. It was depend on the vertical location of the plants. (Mercado, J M, Niell, FX, Silva, J and Santos, R, accepted for publication in Journal of Experimental Marine Biology and Ecology).
- 6 - The flow of organic matter from primary producers to filter feeders in Ria Formosa lagoon was traced (R. Machás, R. Santos and B. Peterson, in press, Estuaries).
- 7 –The flow of organic matter from primary producers to consumers along the Guadiana estuary was revealed.
- 8 – The seasonal production and population dynamics of the seagrass *Zostera noltii* was established along an urban effluent gradient.
- 9 – The seasonal and spatial variation of *Zostera noltii* reproduction was described. The effects of clam digging on the reproductive potential of *Zostera noltii* was determined.
- 10 - The effects of the inlet natural migration and inlet human-relocation on the patch dynamics of *Zostera noltii* in Ria Formosa were determined.
- 11 – The benthic communities of the western sector of Ria Formosa were mapped using GIS tools. The total seagrass and lower saltmarsh production (dissolved and particulate) were calculated.
- 12 – The exchange of dissolved organic carbon, particulate matter and plant detritus through the western inlet of Ria Formosa was studied. An hydrodynamic model of Ria Formosa was used to estimate the transport and total annual fluxes through the inlet.

### **Population ecology of seaweeds**

#### Main purpose:

- 1- To investigate the ecophysiological/population dynamic traits that determine the genetic structure (diploid dominance) of the natural populations of the commercial agarophyte *Gelidium sesquipedale*.
- 2- To develop population matrix models to determine the sensitivity of the genetic structure to the different vital rates.
- 3- To reveal the population dynamics of an invasive seaweed species, *Sargassum muticum*, in its southern distribution limit (southwest coast of Portugal).
- 4– To develop population matrix models to determine the invasive potential of *Sargassum muticum* in southwestern Portugal.

#### Achieved in 2002:

- 1- Ecophysiological traits of *Gelidium sesquipedale* diploids and haploids (males and females), such as photosynthetic capacity, nutrient uptake, enzymatic activity of carbon and nitrogen uptake, were measured.
- 2- Population dynamic traits related to sexual, spore and vegetative recruitment were investigated in *Gelidium sesquipedale* diploids and haploids (males and females).
- 3- The population dynamics of *Sargassum muticum* was followed monthly in sites of the southwest coast. Vital rates such as growth, breakage, reproduction and recruitment are being monitored.
- 4- A conceptual model for the demography of *Sargassum muticum* in the southwest coast of Portugal was developed. A simpler model was constructed to apply to four European sites along the geographical distribution of the species in Europe so that the latitude variability of the species demography can be analysed.

### **Integrated aquaculture of seaweeds in fish farm effluents**

#### Main purpose:

To develop an integrated culture system in which seaweeds are grown in the nutrient/rich effluents of a fish farm to both biofiltrate them, reducing the environmental impact of fish farms, and to produce commercially valuable biomass.

Achieved in 2002:

A successful integrated cultivation system was developed in a fish farm of Ria Formosa. The species selected has higher biofiltration capacity than any other described in the literature. Moreover, the species produces halogenated compounds that have a strong and wide spectrum of antibiotic activity. It is under consideration to patent the whole process of seaweed cultivation, extraction of compounds and application of extracts.

**Plan for 2003**

To develop further the above research lines and to initiate the following ones.

***Metabolism of Ria Formosa lagoon***

Ecosystem metabolism is intimately linked to carbon and nutrient cycles. The metabolism of coastal systems such as Ria Formosa lagoon includes primary production, the process by which communities consume CO<sub>2</sub> and nutrients to produce organic matter materials and respiration, the opposite process by which communities oxidize organic to inorganic matter releasing CO<sub>2</sub> and nutrients. Autotrophic ecosystems are CO<sub>2</sub> and nutrient sinks, whereby organic matter and nutrients are either accumulated or exported from the ecosystem. Heterotrophic ecosystems, where depend on inputs of organic matter from adjacent autotrophic ecosystems. Therefore, the ecosystem metabolism of autotrophic and heterotrophic system are often energetically related (eg watersheds, estuaries/lagoons, coastal zone). Ecosystem metabolism determines, therefore, the role of biota in gas (e.g. O<sub>2</sub> and CO<sub>2</sub>) fluxes in the ecosystem, linking ecosystem function to important processes, such as the regulation of atmospheric CO<sub>2</sub> concentrations and to global change scenarios.

**Global-related changes in Portuguese marine flora over a long time scale**

Financial support was obtained from FCT to develop this project.

Abstract. It is generally accepted that climatic changes influence marine species distributions through species-specific physiological thresholds of temperature. Most studies on the responses of marine communities are speculative and offer predictions of poleward shifts in species' ranges in response to the global warming observed over the past century (Fields et al., 1993; Lubtchenko et al., 1993; Paine, 1993; Walther et al., 2002). With climate changes, non-native species from adjacent areas, or long-distance introductions mediated by human activities, may become new elements of the biota. The Portuguese continental coast has been recognized as particularly relevant in biogeographical terms (Lusitania Province), as it represents a boundary between the southern warmer communities and the northern colder communities, and hence has a high biodiversity. Ardré (1971) reported that more than 40 northern species and more than 20 southern species had their distribution limits in Portugal. The main aim of this proposal is to describe the long-term changes in the benthic marine flora of the continental coast of Portugal by comparing the actual situation with the only available description of the Portuguese marine flora, which was done in the 1960's by Ardré (1970, 1971). Range shifts of species, variations in their abundance or new introductions will be analysed to test the hypotheses that the richness and abundance of warm-water species has increased, as opposed to cold-water species. Furthermore, it is hypothesized that the species distribution range has shifted northwards. The same intertidal zones of the twenty-three sites considered in this study will be visited in the summer and winter. Sampling will be extended into the shallow sublittoral to provide the first general description of the subtidal flora of the Portuguese continental coast. This project will provide an updated database on the benthic marine communities of the Portuguese coast. 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 are very scarce.

## **Group crustacean biology and fisheries**

### **Research team**

Researchers: Margarida Castro, Margarida Cristo and Margarida Machado.

PhD students: Paula Serafim.

Research assistant: Jordi Sala.

Technician: Artur Araújo.

### **Summary of activities and progress during 2002**

#### ***Non-cladoceran branchipods in temporary ponds***

##### **Main purpose:**

To do an inventory of species present in ponds from the South of Portugal. To study the population dynamics of the most important species. Proposal of management plans for this sensitive areas.

##### **Achieved in 2002:**

1. Identification of species present in 19 ponds.
2. Registration of 1 new species for Portugal.
3. Registration of one new species worldwide.
4. Following of the biological cycles of 4 species in 7 ponds.
5. Study of the accompanying fauna, mainly amphibians.

#### ***Feeding ecology of decapods***

##### **Main purpose:**

To assess diets (seasonal and sexual variation) for the deep water shrimp *Plesiopenaeus edwardsianus* from the Southwest coast of Portugal.

##### **Achieved in 2002:**

1. Preliminary analysis of fall samples.

#### ***Survival of invertebrate non-target species of crustacean trawling, discarded on board***

##### **Main Purpose:**

To obtain estimates of survival rates of discarded invertebrates. To assess stress and damage cause by capture and release.

##### **Achieved in 2002:**

1. Testing of field methodologies.
2. Choice of species for captivity studies.

#### ***Spiny lobster biology and management***

##### **Main purpose:**

To update the information of the biological cycle of *Panulirus elephas*.

Characterization of the fishing activity of the artisanal fleet in the port of Sagres.

Set up of lobster larvae collectors for estimation of recruitment.

##### **Achieved in 2002:**

1. Construction and testing of larvae collectors.
2. On board sampling of the catches, by-catch, gear characteristics and fishing practices.

### **Plan for 2003**

- Continuation of the ecological studies in temporary ponds. A sample of the previously studied ponds will be observed for understanding year-to-year variation. Submission of the newly recorded species to international zoological boards.

- Analysis of winter, spring and summer samples of *Plesiopenaeus edwardsianus* for studying the feeding ecology of this species.
- Field and laboratory studies to quantify survival of discarded invertebrate species.
- Setting of the lobster larvae collectors in the southwest coast for identification of settling areas for *Panulirus elephas*.

## **Group Fisheries Biology and Hydrobiology**

### **Research team**

Leader: José Pedro Andrade

Principal researchers and post docs: Pedro Domingues, Jorge Palma

PhD students: Eduardo Esteves,

Technicians/ research assistants: Rita Sá, António Sykes

### **Summary of activities and progress during 2002**

Ongoing projects:

Trophic interactions of the cuttlefish, *Sepia officinalis* (Cephalopoda, Sepiidae) in the Ria Formosa, Sado Estuary and Ria the Aveiro: a toll for cephalopod resource management (ref<sup>a</sup> PDCTM/C/MAR/15259/1999).

Breeding and Foraging ecology of Little Terns to provide ecological indicators in estuarine environments. (ref<sup>a</sup> POCTI/BSE/37385/2001)

### **Plan for 2003**

Activities will be focussed on the feeding ecology and culture of cuttlefish, *Sepia officinalis*. Efforts made during 2001 to establish cooperative research with private enterprises, which can be interested in the culture of cuttlefish in order to extend pilot-scale experiments were successful and a project on cuttlefish culture will start in 2003. This project will be funded by the Agência de Inovação, S.A.

Breeding and foraging ecology of Little Terns, *Sterna albifrons* will also be addressed under the frame of a research project funded by the Fundação para a Ciência e a Tecnologia.

## **Coastal Fisheries Research Group**

### **Research Team**

Leader: Karim Erzini

Post Doc: Jorge M.S. Gonçalves

Technicians/ research assistants: Luis Bentes, Joaquim Ribeiro, Pedro Monteiro and Rui Coelho

### **Summary of activities and progress during 2002**

Ongoing Projects:

- Experimental By-catch Reducing Devices (BRD) in the demersal purse-seine fishery and evaluation of survivorship. (Project Ref.: FCT - POCTI/BSE/43113/2001)

### **Plan for 2003**

The group will focus on testing by-catch reducing devices (BRDs) for the demersal purse seine in order to reduce the by-catch and discarding of under-sized and/or non-commercial species. In particular, we will evaluate the use of both larger mesh sizes and square pannels. These modifications should increase the escapement of juveniles and non-commercial species.

Quantification of the effectiveness of BRDs will be carried out by means of experimental fishing trials using small-mesh covers over the BRDs sections. In addition, the condition of fish that have escaped will be evaluated and post-escapement survivorship monitored in tanks. The relationships between condition (scale loss and other signs of external damage), stress (monitored by cortisol radioimmunoassay) and long-term survivorship will be evaluated experimentally. The results of these experiments will be transmitted to the fishing community.

Another line of research will be the biological impact assessment of coastal dredging, the recruitment of commercially important species in the Arade estuary (Algarve) and the ichthyofauna monitorization in the Guadiana estuary.

## **Group Biodiversity and Biology in Fisheries (BIOPECAS)**

### **Research team**

Leader: Teresa Cerveira Borges

PhD students: Maria Esmeralda Costa

MSc students: Inês Gonçalves, Sónia Olim

Technicians/ research assistants: João Sendão

### **Summary of activities and progress during 2002**

During this period 1 research project was completed, 1 continued and 1 Concerted Action was started (October 2002). The research project completed was BYDISCARD "By-catch and discards: a multidisciplinary approach" (Ref: 99/00). The research area of this project was on the quantification and identification of the by-catch and discards on the commercial fisheries, the reasons of it, the ways of avoiding discards using BRDs, an ecological modeling of the area and a bio-economical study on this problem in Algarve. The project to continue was CORRAM "Cephalopod octopodid: relation between de resource and the marine environment". Project ID: 2/2.1/MAR/1707/95. This project had to be extended and transferred to the programme POCTI of the FCT due to the late start. The main focus of this project is the fishery biology of octopods. The Concerted Action, which started at the end of 2002, aims to review current knowledge and issues in cephalopod fisheries science, to assemble, organise, analyse and synthesise data from ongoing national projects, previous and new EC-funded R&D projects, and to recommend future actions for scientists and cephalopod fishery managers in European waters. During this period, a Phd thesis was completed. The project directed to education (Observation of an aquatic ecosystem), starting at the end of 2001, with the participation of a primary school and financed by the Programme "Ciência Viva", continued. The construction of a lake and 2 trips to different small rivers gave the opportunity to build an aquatic ecosystem in which the children are able to make several observations and experiments, which degree of difficulty depends on their age.

### **Plan for 2003**

One of the main objectives for next year is to analyse and publish most of the data compiled during several of the research projects done for the last years, especially on cephalopods. It is also planned to finish the research project CORRAM and to continue the work began in the different tasks for the Concerted Action. The 2 MSc students will finish their thesis in 2003 as well as the PhD student.

## Fish Parasitology and Reproduction

### Research team

Post-doc: Isabel Afonso-Dias (under Manuel Afonso-Dias of Faculdade de Ciências do Mar e do Ambiente)

### Summary of activities and progress during 2002

#### **“Anglerfish (*Lophius piscatorius* and *L. budegassa*) Parasites: Study to assess the possibility of using anglerfish parasites as biological tags”**

The identification of parasites that can be used to tag anglerfish is the main objective of this study (see Afonso-Dias & MacKenzie, 2002). The use of parasites as biological tags has been applied to many fish population studies (Williams *et al.*, 1992). Although it has limitations, like any other study, it also has advantages, especially for deep-water species such as the anglerfish. In this case parasites are better tags than mechanical tags for host population studies (Mackenzie & Abauza, 1998). According to these authors, one of the most important features of using parasites as tags is the fact that fish from commercial catches and research vessel cruises can be used for biological tag studies as well as for other biological studies. This makes the use of parasite tags less expensive than mark, release and recapture experiments.

During 2002 the sample collection progressed as planned. However, the number of samples available was not the same as intended. Samples of *L. piscatorius* were only collected from the region off the west coast of Scotland, while samples of *L. budegassa* were collected off the west and south coast of Portugal. Although it would be desirable to collect more samples of the two species of anglerfish from other geographical areas, this will not be possible with the time available to conclude this study (February, 2004). Some attempts were made to get samples of both species from the North Sea and of *L. piscatorius* from the west coast of Portugal. However, there were logistic problems that could not be overcome. The occurrence of *L. piscatorius* off the Portuguese coast is somewhat erratic. The specimens collected for GENASSESS (the project that supported the sample collection) were sent elsewhere for other studies (included in the GENASSESS project) and were thus not available for this project. The same happened with specimens of *L. budegassa* from different Northern European areas.

Regarding the laboratory work, the host samples (anglerfish viscera) are still being examined to recover the parasite specimens. This is a very time-consuming process for one person, since the anglerfish guts can be very large. All specimens of *L. budegassa* were examined, but there are still samples of *L. piscatorius* to examine. Digenetic trematodes are among the most abundant parasites found in anglerfish. Although many digeneans were identified to species level during one stay in Aberdeen, in December 2001, there are still a large number of these (and other parasite groups) to be identified to species level. Since some of these specimens had to be prepared (dehydrated in a graded series of ethanol, stained and mounted) to allow identification to species level, the identification took more time than expected to be completed.

The identification at species level within some groups required collaboration with specialists. In the case of Myxosporidia, the collaboration with Prof. Kalavati was very productive. Three new species of myxosporidia were identified from the gall bladder of *Lophius* spp: *Alatospora* n. sp. (from *L. budegassa*), *Pseudalatospora* n. sp. (from *L. piscatorius*), and *Ceratomyxa* n. sp. (from *L. piscatorius*). The *Ceratomyxa* sp. is quite different from *Ceratomyxa appendiculata* previously described from *Lophius budegassa*.

Major difficulties were found in the identification of the parasites and in the collation of dispersed papers. The literature available in the University of Algarve library is insufficient to correctly identify genera and species of parasites. Collection of host-parasite records began with the references traced from the host-parasite catalogue at the British Museum (Natural History) in London. This checklist is based largely on original papers, which appeared in many journals and in different languages. I am also collating information about marine parasites on the Internet, namely photographs and taxonomic characters, useful for the identification of the parasites recovered from *Lophius* spp. Literature searches are time-consuming and are not always successful or complete.

During this year the first results of this pilot project were presented in the form of a poster **“Gastrointestinal parasites of the NE Atlantic anglerfish as biological tags: a pilot study”** at the 10<sup>th</sup> International Congress of Parasitology, held in Vancouver in September 2002. My participation in this Congress was made possible due to the financial support provided by FCT (Fundação para a Ciência e Tecnologia) and CCMar (Centro de Ciências do Mar), which is gratefully acknowledged.

**“Validation of the macroscopic maturity scale of Sardine (*Sardina pilchardus*, (Walbaum 1792) currently in use ”**

Consultancy and data analysis, which include: routine histological examinations, including preparation of resin-embedded tissue (sardine's gonads), cutting of sections, staining and interpretation. These activities are integrated in different projects carried out at Instituto de Investigação das Pescas e do Mar (IPIMAR) to describe temporal and regional variation in sardine maturation patterns.

**“Reproductive aspects of the bastard sole *Microchirus theophila* (Risso, 1810) (Pisces: Soleidae) from the South coast of Portugal”**

During this year a paper was written with material collected and processed two years ago. This study presents new information on the reproductive biology of *M. theophila* in the South coast of Portugal and submitted in a shape of a scientific paper to Fisheries Research.

*Plan for 2003*

**“Anglerfish (*Lophius piscatorius* and *L. budegassa*) Parasites: Study to assess the possibility of using anglerfish parasites as biological tags”**

At this stage it is possible to say that the amount of work involved in the collection of host samples, recovery and identification of parasites was underestimated. Additional time required for literature collation and research is also very time-consuming for just one person. However, with all the information collected it is possible to perform two different types of studies: one is to assess the use of gastrointestinal parasites as tags in *L. budegassa* from at least two different adjacent areas – the west and south coast of Portugal – and to compare the parasite fauna of the two sympatric host species.

At this stage of the study it is necessary to continue the laboratory procedures to recover and identify parasites. One collaborative study is currently in progress: to publish the descriptions of the myxosporean parasites identified in the gall bladder of both species of *Lophius*, with Professor C. Kalavati (Andhra University, India) and Doctor K. MacKenzie (University of Aberdeen); In addition, a checklist of parasite species recorded from the two *Lophius* spp. is being prepared for publication.

The activities planned in this field of research for the coming year include two disparate tasks: to recover and identify the last lot of parasites and to publish the results obtained during this study.

**“Validation of the macroscopic maturity scale of Sardine (*Sardina pilchardus*, (Walbaum 1792) currently in use ”**

The activities planned in this field of research for the coming year include the preparation of written material for publication.

**“Testicular infestation of *Sardina pilchardus* (Walb.) by the coccidia *Eimeria sardinae* (Thélohan)”**

Male gonads collected during an annual sampling experiment (during 2002) that took place in the University of Algarve were found to be infected with *E. sardinae* (Thélohan, 1890) Reichenov, 1921. This study was firstly intended to follow the maturation pattern of the sardine in the Algarve. However, the prevalence of *E. sardinae* in the testes created a different question. Of 49 testes processed, 73% were infested with small alveoli that were later identified as testicular coccidiosis,

caused by *Eimeria sardinae*. If this so, how serious in terms of male reproductive success? During the 2003, if possible, some work will be carried out to attempt answering this question.

## **Group Coastal Ecology**

Team leader: Martin Sprung

PhD students: Jaime Aníbal, Natália Dias, Alexandra Marques

Research assistants: Telma Conduto, Carlos Manuel Afonso

### **Summary of activities and progress in 2002**

Additional macrobenthos samples have been taken in sequence of the project EVODRAG. The project came to an end in 2001, however changes continued also during the second year in the channels of the Ria Formosa at the sites affected by dredging. The samples have been evaluated and the results are compiled in species list (including previous published records) with an elaboration of an identification key for the Ria Formosa macrobenthos species.

The estimation of nutrient content in the pore water of sediments with and without green algal cover has been finished and the results are about to be integrated into a model.

Zooplankton has been analyzed monthly in a gradient from the outer to the inner Ria Formosa at incoming and outgoing tide. The identification of the copepod species and its developmental stages is currently under progress.

### **Plans for 2003**

The identification key for macrobenthos species is about to be completed. A final sampling campaign for the third year after dredging is planned for summer 2003 also to test the keys developed. Additional directed samples will be taken for special animal groups (ascidians, bryozoans). The model nutrient flux in the sediment at the presence of green algae is developed further. The last zooplankton samples will be evaluated and the results compiled. Further sites affected by sand extraction in front of Albufeira will be monitored for its macrobenthos (project RENSUB).

## Externally funded Projects

### *Division of Aquaculture and Biotechnology*

#### **New and ongoing beyond 2002**

**Title:** Hormones and life-history trade-offs and plasticity: a study on alternative reproductive tactics in blenniid fish

**Reference and funding entity:** Fundação para a Ciência e a Tecnologia POCTI/BSE/38395/2001

**Summary and Objectives:** Diversas espécies apresentam histórias vitais alternativas. Contudo, os mecanismos causais subjacentes à plasticidade das histórias vitais e aos "trade-offs" (e.g. investimento na reprodução presente vs reprodução futura) só recentemente têm sido tema de investigação.

Numa população de *Salaria pavo* (Blenniidae) da Ria Formosa (Portugal) ocorrem dois tipos de histórias vitais alternativas. De entre os machos recrutados no próprio ano, os mais pequenos reproduzem-se como "sneakers", enquanto os maiores continuam a crescer, apenas se reproduzindo na segunda época de reprodução, no ano seguinte, como "nest-holders". Assim, nesta população existem dois tipos de machos sexualmente activos: machos mais velhos e maiores que defendem ninhos e cuidam dos ovos (CT »14 cm; idade <sup>3</sup> 2 anos) e machos mais pequenos e mais jovens (CT » 10 cm; idade < 1 ano) que imitam o comportamento e a coloração nupcial das fêmeas na tentativa de se aproximarem dos machos que defendem ninhos e fertilizam parte dos ovos (Gonçalves et al 1996). Os machos que se comportaram como "sneakers" durante a sua primeira época de reprodução podem tornar-se "nest-holders" em épocas de reprodução subsequentes. Deste modo, o comportamento de "sneaking" parece ser uma estratégia condicional nesta espécie.

Neste projecto tencionamos investigar os mecanismos causais que permitem a expressão das histórias vitais alternativas acima mencionadas e dos "trade-offs" envolvidos. Os principais objectivos são os seguintes: 1) caracterizar as vias ontogenéticas alternativas e a plasticidade do comportamento de acasalamento dos machos jovens (i.e. classe etária 0+/1), através de um programa de marcação e recaptura intensivo e de longa duração (3 anos), que recorrerá à implantação de marcas magnéticas; 2) caracterizar os perfis hormonais dos diferentes tipos de machos recém recrutados (i.e. "sneakers" vs. machos não reprodutores), assim como dos "nest-holders" que servirão de referência para os parâmetros de reprodução. Proceder-se-á à quantificação das seguintes hormonas: esteróides sexuais em circulação (e.g. testosterona, 11-Cetotestosterona); ii) neuropéptidos da família da prolactina (e.g. hormona do crescimento, somatolactina, prolactina); iii) gonadotrofinas (GtH I e II); e iv) GnRH e AVT na área pré-óptica; 3) testar experimentalmente os efeitos do tamanho relativo, da densidade e do estatuto social nas decisões da história vital dos jovens machos, manipulando estas variáveis em grupos mantidos em cativeiro e avaliando o impacto da manipulação experimental nos parâmetros referidos em 1); 4) testar de que forma a presença de machos grandes, defensores de ninhos afectam a direcção da via ontogenética dos machos jovens, avaliando o efeito da exposição dos machos recém recrutados aos machos defensores de ninhos.

O conhecimento da biologia reprodutora constitui um excelente modelo para o estudo integrado dos mecanismos causais da plasticidade sexual e das histórias vitais dos teleósteos.

**Duration:** 2002-2004

**Research team:** Rui Oliveira (Ispa- coordinator), A Canário, D. Power

**Total budget:** 103.080 Euro; **Funding for CCMAR:** Euro

**Title:** Bridging genomes: an integrated genomic approach toward genetic improvement of aquacultured fish species

**Reference and funding entity:** EC Q5RS-CT-2001-01797

**Summary and Objectives:** The project will use a far reaching but parsimonious approach to:

- transfer genetic information from model organisms to commercial species;



- transfer technology and know-how from leading laboratories in genome analysis and mapping to more classical fish genetics laboratories, as well as knowledge from an interesting biological model, the sea bream, in the opposite direction;
- bridge the gaps in maps by merging physicals and linkage maps;
- bridge the distance between research and industry;
- integrate evolutionary theory and modern technology to generate an applied endpoint;
- integrate genome maps of various teleosts with data from higher vertebrates, thanks to the high potential of comparative mapping.

The project workplan is centered around the application of modern biotechnological methods to aquaculture. It is subdivided into 7 main workpackages, each of which is co-ordinated by the partner with the relevant expertise, and the completion of which will contribute to the attainment of the project objectives.

The workpackages articulate with each other and can be subdivided into 3 main groups according to the nature of the methods they use:

- i) molecular biotechnology, which includes methodologies for generating mapping panels for the linkage map (WP1) and HAPPY mapping (WP2), a highly automatable new method for physical mapping, genotyping the linkage map (WP3) and HAPPY map (WPS) by use of high throughput automated methods, and isolating STS markers for HAPPY mapping (WP4);
- ii) conventional genetic; the tools generated in WPI-5 will be used to screen sea bream (*Sparus aurata*) generated in a breeding program on a SME fish farm. This will result in the transfer of molecular biotechnological methodologies to conventional genetics and the implementation of technology transfer from science to industry, and;
- iii) the final workpackage which will run simultaneously with the other project tasks is the analysis of the extensive data which will be generated by the various workplans of the project. Bioinformatics will be essential for the handling and interpretation of the data and for its successful dissemination, in order that the project can have a maximum impact in the fields of aquaculture research, fish genetics, and comparative mapping.

**Duration:** 1/11/2001 – 31/10/2005

**Research team:** D M Power, A Canário.

**Total budget:** Euro; **Funding for CCMAR:** 211 906 Euro

**Title:** ARRESTED DEVELOPMENT: The molecular and Endocrine Basis of Flatfish

**Reference and funding entity:** Q5RS-CT-2002-01192

**Summary and Objectives:** Flatfish species form a major focus of the diversification of European marine aquaculture industry. However, production has been severely hampered by biological problems in larval rearing. This project focuses on solving the major problem of arrested metamorphosis, in order to reliably control the resulting juvenile quality and production quantity. This will be achieved by addressing key questions regarding the morphological, endocrine and molecular basis of metamorphosis in the Atlantic halibut as a model species. The ultimate goal of the project is therefore to strengthen European aquaculture of marine flatfish species, an important emerging industry in many rural areas, by facilitating the production of fully functional juveniles.

#### Objectives

The high incidence of metamorphic abnormalities represents a serious impediment to the successful aquaculture of marine flatfish by limiting the cost-effectiveness of the juvenile production industry. The objectives of this project are to determine the biological bases for abnormalities arising during metamorphosis of a model cultured marine flatfish, the Atlantic halibut. This will be achieved by comparing normally and abnormally metamorphosing larvae in terms of differential gene expression, endocrine regulation, and biochemical and morphological transformations. This will help establish improved, cost-effective rearing techniques for the production of marine flatfish juveniles, ultimately strengthening European aquaculture of marine flatfish species, an important emerging industry in many rural, coastal areas.

#### Description of the work

1) The events of metamorphosis in halibut will be analysed by monitoring biochemical and morphological markers of metamorphosis. These markers are skeletal development, erythrocyte morphology, expression of troponin-T isoforms, production of gut enzymes, and expression of immunoglobulins.

2) Differential gene expression will be analysed by screening of a gene microarray based on genes induced during metamorphosis. This will allow high throughput analysis of an extensive panel of genes involved in metamorphosis.

3) Endocrine regulation of metamorphosis will be analysed in terms of hormone levels and hormone responsiveness. Tissue and plasma levels of thyroid hormones, cortisol, growth hormone and IGF-1 during metamorphosis will be determined by RIA. Prolactin expression will be monitored by assaying its mRNA. The genes encoding receptors for prolactin, cortisol and growth hormone will be cloned. The temporal and spatial expression of these genes, along with the already-cloned thyroid hormone receptor will be analysed during metamorphosis by competitive RT-PCR and in situ hybridisation. The role of individual hormones during metamorphosis will be further investigated through hormone treatment experiments on pre-metamorphic and metamorphosing larvae.

4) Abnormally metamorphosing larvae will be collected, grouped according to type of abnormality (arrest of metamorphosis, pigmentation defect, inappropriate eye migration etc). These larvae will be compared with normally metamorphosing larvae from the same culture in terms of expression of biochemical and morphological markers of metamorphosis, differential gene expression, expression of hormone receptors and hormone content. This will indicate the underlying molecular and/or endocrine bases of the various abnormalities.

5) In vivo treatments with hormones and nutritional supplements will be carried out to establish functional relationships.

#### Milestones

A staging scheme for metamorphosis, based on biochemical, morphological endocrine and genetic markers. A "metamorphic" microarray of 700 genes to identify endocrine and molecular basis of metamorphic arrest. Larval treatments with hormones, iodine and selenium to establish functional relationships between nutritional factors, endocrine axes and metamorphosis, establishing "functional feeds" and scientific tools for effective metamorphic larval aquaculture

**Duration:** 1/10/2002 – 31/09/2005

**Research team:** D M Power

**Total budget:** Euro; **Funding for CCMAR:** 217 536 Euro

#### **Title:** The Role of Olfaction in the Feeding Behaviour of *Solea senegalensis* – SOLFACTO

**Summary and Objectives:** Food consumption is the primary determinant of growth in fish. Many fish species, in particular those with nocturnal activity and/or in habitats with frequent high water turbidity, rely mostly on chemo-sensory mechanisms for food detection and location. In different species, feeding behaviour is triggered by different chemical substances, some of which may act as attractants via olfaction, and others may act as promoters or enhancers of food consumption via both olfaction and gustation. In general, free amino acids, nucleotides, nucleosides and quaternary ammonium bases have been identified as feeding stimulants in some species.

The Senegal sole (*Solea senegalensis*) is a good model species to investigate olfactory mechanisms underlying feeding behaviour, due to its feeding strategy, and a well-developed olfactory system accessible to electrophysiological recordings. The central aim of this project is to identify substances released by natural food sources that act as olfactory cues involved in food-search behaviour, and ingestion of food. This objective will be achieved by an integration of electrophysiological, behavioural and food ingestion measurements in response to candidate olfactory stimuli.

A method for the electrophysiological recording from the olfactory system of juvenile sole will be employed, based on electro-olfactogram and/or multi-unit olfactory nerve recordings. This will give quantitative measurements to define relative olfactory sensitivities to various chemical stimuli. Stimuli to be tested include water containing live natural food items (e.g. polychaetes), crude filtered macerates of food items, chromatographic fractions of water containing the food items and

of its macerate, and a range of amino acids, nucleosides and nucleotides. The most potent olfactory stimuli identified will be used in behavioural assays.

Two types of behavioural assays will quantify how a given chemical stimuli affect the patterns of food-search behaviour in juvenile sole. The first will employ Y-maze aquaria to quantify the end result of food-search behaviour. The second assay will quantify specific behavioural acts during food-search behaviour observed in single fish. In both assays, sole behaviour will be videotaped for a fixed period of time and subsequently analysed for pre-defined behavioural responses. The strength of these responses will be related to stimulus quality and intensity.

Food consumption measurements and growth trials will be conducted to test the effect of olfactory stimuli shown to improve food-search behaviour on the ingestion of inert food pellets by juvenile sole. Measurement of ingestion will be obtained by a method employing X-radiography.

Finally, we will examine whether olfactory stimuli that affect feeding behaviour of juvenile sole also act in stimulating behavioural elements associated with feeding in early juveniles (after metamorphosis) during the weaning period.

Ultimately, the project will identify the chemical cues that are important in the initiation of food-search behaviour, food ingestion, and therefore growth. This may be the ground for future technological development of new inert food diets that enhance the farming of this commercially important species.

**Reference and funding entity:** POCTI/CVT/38831/2001, FCT

**Research team:** Eduardo N. Barata (Coordinator), Peter C. Hubbard, Adelino V.M. Canário, Luis Conceição, Pavlos Makridis, Maria T. Dinis.

**Total budget:** 100.000 Euro; **Funding for CCMAR:** 100.000 Euro

**Title:** Chemical Identification and Functional Roles of Reproductive Pheromones in the Tilapia, *Oreochromis mossambicus*.

**Summary and Objectives:** That pheromones play important roles in many aspects of fish biology is beyond doubt. However, the most complete evidence to date has been obtained from a few well-studied species, most notably the goldfish. Given the phylogenetic diversity and wide range of lifestyles and habitats of fishes, to achieve a fuller understanding of pheromonal systems in fish, a comparative approach must be taken. Despite their distinctive reproductive strategies, which include pair-bonding and parental care, the cichlids have received surprisingly little attention in this respect. Thus, the aim of this project is to identify putative pheromones in the Mozambique tilapia (*Oreochromis mossambicus*; a maternal mouth-brooding cichlid) and to investigate their possible biological functions, focusing initially on reproduction. Considerable preliminary data have been accrued that suggest that female tilapia have acute olfactory sensitivity to conspecific males, and that this sensitivity is strongly correlated with sexual status. By a combination of electro-olfactogram (EOG) recording and chromatographic techniques, the aim is to identify the substances released to the water by males that evoke the strongest olfactory responses in the female. This will then allow the testing of putative biological functions of these compounds by behavioral assays ("releaser" effects) and physiological assays ("primer" effects). Although it is strongly expected that the candidate pheromones are likely to be sex hormones, or their metabolites, this strength of this approach is that it makes no prior assumptions as to their exact chemical nature. Firstly, crude chemical fractions of male body fluids (male water, urine, faeces and bile) will be tested for olfactory potency in females by EOG recording. The fractions giving the strongest responses will then be further fractionated by high performance liquid chromatography (HPLC). Once single "peaks" have been identified by HPLC, these peaks will then be collected, concentrated and again tested for olfactory sensitivity by EOG. Once those HPLC peaks giving the largest EOG responses in females have been identified, some conclusions as to their chemical identity may be drawn. This will be tested by use of pure chemical standards in the HPLC system, to see if they co-elute with the peaks obtained from the biological samples. Final chemical identification will be by gas-chromatography linked to mass spectroscopy. If available commercially, olfactory sensitivity to pure compounds will be confirmed by EOG. If not, some compounds may be synthesised in the laboratory. Secondly, the effects of these compounds on the behaviour of females will be assessed initially by Y-maze experiments, and the effects on female physiology will be assessed by endocrine assays. If appropriate, assays can be utilized or

developed to measure the release rates of these putative pheromones to establish whether this is correlated with socio/sexual status. Clearly, this will depend on their chemical identity, and whether assays for these compounds are already in existence. Thus, this project forms the first stage in establishing the tilapia as an alternative model species for pheromonal studies. Not only does it have a distinct reproductive strategy, and is therefore worthy of study in its own right, but it also is reproductively active all-year-round and sexual status can be determined by external morphology, making it an ideal subject for such studies.

**Reference and funding entity:** POCTI/BSE/38815/2001, FCT

**Research team:** Peter C. Hubbard (Coordinator), Eduardo N. Barata, Adelino V.M. Canário, Pedro A. Frade (PhD student).

**Total budget:** 83.000 Euro; **Funding for CCMAR:** 83.000 Euro

**Web site:**

**Title:** Isolation of carotenoid-overproducing *Dunaliella salina* strains. OVERCAROTEN.

**Summary and Objectives:** To isolate novel strains of microalgae, namely *D. salina* and *H. pluvialis* able to accumulate higher levels of carotenoids at early stages of growth. This will be accomplished by several strategies: 1) generation and screening of mutants by chemical mutagenesis and carotenoid biosynthesis inhibitors; and 2) metabolic engineering. The latter strategy will be implemented by the development of genetic transformation procedures and expression of homologous and heterologous genes associated with the carotenogenesis in these microalgae.

**Reference and funding entity:** FCT, PDCTM / MAR / 15237 / 99

**Duration:** February 2002 – February 2005

**Research team:** CCMAR: João Varela, Vanessa Duarte, Sacha Coesel, Nuno Henriques. **INETI**, Rui Mendes e Luísa Gouveia; **NECTON**, João Navalho e Vítor Duarte; **ESB-CU**: Rui Morais.

**Total budget:** 175000 Euros. **Funding for CCMAR:** 77935 Euros.

**Title:** Feed for aquatic animals that contains cultivated marine microorganisms as alternatives for fish oil **PUFAFeed**

**Reference and funding entity :** European Community RTD Project QLRT- 1999-30271

**Duration:** Jan 2000 – Dez 2003

**Summary and Objectives:** The main objective of the current project is the development of alternative feed resources to fish meal and fish oil employing heterotrophic and mixotrophic microorganisms in order to supply the aquaculture industry with feed of constant and good quality that are free of toxins or genetically modified materials. In addition, feed based on a combination of heterotrophic and autotrophic produced microorganisms will be developed. PUFAfeed aims at the evaluation of Single Cell Oils (SCO), obtained from micro-organisms which include microalgae and diatoms, that are rich in the polyunsaturated fatty acids docosahexaenoic acid (DHA) and / or eicosapentaenoic acid (EPA) as alternative / complementary feed ingredients for fish oils. Within the project fundamental aspects of lipid accumulation in algae will be elucidated and the technologies to produce microbial biomass and novel feeds, based on this biomass, will be developed. Furthermore the performance of this feed and the economic feasibility of the integrated process will be established in order to provide a cost-effective alternative or complementary solution for fish oils use in feeds for aquaculture.

**Research team:** CCMAR: Maria Teresa Dinis, Florbela Soares, Pedro Cação

**Total budget:** 48.000 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** Determination of the ideal dietary amino acid profile for larval and post-larval marine fish

**Summary and Objectives:** Optimisation of fish growth is closely linked to the understanding of protein and amino acid (AA) metabolism in order to supply dietary protein with an appropriate AA composition in the right quantities. The dietary AA profile that will allow for optimal growth depends

on the efficiency of absorption of each AA, on the AA profile of proteins being synthesised as well as on the use of individual AA for energy or other purposes.

The central objective of this project is to determine the ideal dietary AA profile in different developmental stages of early life stages of fish and evaluate the effects of eventual imbalances in the dietary AA profiles on fish metabolism and growth. Larval and post-larval Senegal sole (*Solea senegalensis*) and the Gilthead sea bream (*Sparus aurata*) will be used as models.

In this study, the larval and post-larval AA profiles will be determined during ontogeny, and compared to the dietary AA profiles. Whole fish AA profiles of other species change during larval ontogeny, and are different from adult profiles. Small changes in the AA profile may have important implications in terms of AA requirements. This comparison of fish and dietary AA profiles will provide a rough indication of the dietary AA imbalances in the different stages. A precise knowledge of the ideal dietary AA profile implies the calculation of the relative bioavailabilities, i.e., quantification of the rates of absorption and catabolism, for the individual AA. This will be done with a novel method using high resolution <sup>13</sup>C-NMR spectroscopy. This method enables the simultaneous study of 16 AA, in comparison to the few AA that could be studied using single labelled AA or dose-response methods.

Next, the consequences of imbalances in the dietary AA profiles on overall metabolism and fish growth will be evaluated. As larvae can only store AA in the form of proteins, imbalances between dietary and larval AA profiles will tend to bring an unavoidable AA loss. Different methodologies will be used to study the effects of imbalanced AA profiles: biochemical composition as a parameter of growth quality; food intake as the primary determinant of growth; protein turnover, as a sensitive indicator of dietary AA imbalances; oxygen consumption, for the calculation of the costs of maintenance and growth as measures of growth efficiency; ammonia excretion, for estimation of AA catabolism and indication of protein utilisation; and the activities of the enzymes of intermediary metabolism, to ascertain to what extent do larvae regulate AA metabolism, and which metabolic pathways are more active as result of the AA imbalances.

Ultimately, this project expects to contribute to a better understanding of the metabolic processes impinging on AA requirements for optimal growth in fish and other animals, in particular in the definition of ideal dietary AA profiles, and their importance.

**Reference and funding entity:** Fundação para a Ciência e Tecnologia (POCTI)

**Duration:** Out 2000- Out 2002

**Research team:** CCMAR: Maria Teresa Dinis, Luis Conceição, Claudia Aragão. ICETA: Emidio Gomes, Jorge Dias.

**Total budget:** 104.650 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** Aquaculture and coastal economic and social sustainability **AQCESS**

**Summary and Objectives:** The overall aim of the proposal is a multidisciplinary study of the interactions between environment, fisheries and aquaculture taking the socio-economic and biological areas as the prime focus. The key objective is to define the sustainability of fisheries and aquaculture in biological and socio-economic terms. The proposal will assess the effects of interactions between environment, fisheries and aquaculture. It will focus on the effects of aquaculture on local economies and employment, fishery performance, abundance of fish species and coastal biodiversity. The proposal will suggest tools and methods to assess the contribution of aquaculture and fisheries to the economic development of coastal areas and their socio-economic interactions with other available economic activities. The proposal will review options and strategies for integrated utilisation of renewable marine resources in different rural regions. It will propose methods to obtain participation of local actors in rural development processes and strategies, and tools for the transfer of experience, innovation and knowledge where they are needed

**Reference and funding entity:** European Community C RTD Project QLRT- 1999-31151

**Duration:** Jan 2000 – Dez 2003

**Research team:** CCMAR: Maria Teresa Dinis, Karim Erzini, Luis Conceição, Jorge Gonçalves

**Total budget:** 116.192 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** Fish restocking associated to the Algarve artificial reefs: environmental mitigation, biodiversity and fisheries management - **RESTOCKING**

**Summary and Objectives:** The project objectives are related to: (i) evaluate the efficiency of restocking of finfish species associated with artificial reefs, using native species where the artificial reproduction and juvenile production are standard procedures, and (ii) develop the methodology necessary for the production in captivity of juveniles of other native with interest for restocking of artificial reefs, and whose populations are depleted as a result of a long and intense fishing effort. Different methodologies will be developed in order to optimise tagging and releasing techniques. Initially, this will be done for available juveniles of *Sparus aurata* (gilt head seabream and *Diplodus sargus* (white seabream). In a later stage, this will be done for juveniles of species which production methods will be developed during the project, (grouper *Epinephelus* sp. and the red porgy *Pagrus pagrus*). It should be noted that these are species with 1) ecology interest in relation to artificial reefs; 2) economical interest in terms of fisheries; and 3) interest for the revitalisation of over-exploited marine resources. The evaluation of restocking of fish in artificial reefs areas will be done in function of the size of the specimens, the season and the types of reefs. Classic methodologies will be used in this project, based on analysis of catch (recapture) and direct observation (underwater surveys) carried out in a regular basis. This restocking initiative will be advertised to the general public, in particular fishermen and anglers, to maximise the recapture records. Standard procedures for juvenile production in captivity of species that are not produced in Portugal and of interest for restocking (i.e., grouper and red porgy) will be developed. In particular, methodologies for induction of spawning in captivity and adequate feeding regimes and zootechnical conditions will be studied. This will be done using the methodologies available for other species as reference. This project expects to contribute for an integrated coastal management, promoting biodiversity, biological production and environmental mitigation. It will also contribute to the management of the local fisheries in order to promote their sustainability, with the subsequent socio-economic effects.

**Reference and funding entity:** Fundação para a Ciência e Tecnologia, POCTI 35608/99

**Duration:** Jul 2001- Jun 2004

**Research team:** CCMAR: Maria Teresa Dinis, Luis Conceição, Marc Lacuisse, Florbela Soares; IPIMAR: Carlos Costa Monteiro, Miguel Neves dos Santos, Miguel Gaspar, Pedro Pousão-Ferreira.

**Total budget:** 149.639 Euro; **Funding for CCMAR:** 74.221 Euro

**Title:** Design and development of commercial scale farming technologies for sole - **SOLEMATES**

**Summary and Objectives:** The overall objective of this project is to design and develop technologies for commercial scale farming of the sole species *Solea solea* and *Solea senegalensis*. The project will include the development of a commercial scale fingerling production system, namely optimisation of weaning zoo technical conditions and feeding regimes. It will obtain data on feeding and growth of both species of sole, including a comparison of growth potential in different conditions between the two, and development of an optimised feed for sole. This project will also work in the development of ongrowing and feeding systems suited for different areas within Europe, more specifically: earth ponds, shallow raceways, circular tanks and terraces.

**Reference and funding entity:** European Community - CRAFT Project Q5CR-2002-71039

**Duration:** Nov 2002 – Out 2004

**Research team:** CCMAR: Maria Teresa Dinis, Luis Conceição, Florbela Soares, Sofia Engrola; CIIMAR: Emidio Gomes, Paulo Rema, Luisa Valente; RIVO (NL): Andries Kamstra, Edward Schram; Akvapna Niva (NO): Patrick White, Albert Imsland; DVZ-CLO (BE): Daan Delbare.

**Total Budget:** 1.801.857 Euros **Funding for CCMAR:** 165.480 Euros

**Title:** Nutritional requirements and feeding of blackspot seabream (*Pagellus bogaraveo*), a new species for aquaculture – GORAZ

**Summary and Objectives:** In order to ensure a sustainable growth of the Portuguese mariculture, it is necessary to diversify the offer of cultivated species to avoid market saturation, competition among producers and to increase the efficacy of production facilities, namely hatcheries. The marine teleost, blackspot seabream (*Pagellus bogaraveo*), has a high market price and is considered as a strong candidate species for intensive aquaculture in Atlantic coasts. Up to now, studies with blackspot seabream under captivity are extremely scarce and have dealt mainly with the control of reproduction, larvae and juveniles cultivation techniques. To our knowledge, blackspot seabream has been fed with diets developed for other marine fish, namely gilthead seabream, and no available literature data exists on the specific nutritional requirements of this species. Therefore, the overall objective of this project is to contribute towards a better knowledge of the nutritional requirements and feeding strategies of the larvae and juveniles of blackspot seabream. Concerning the larvae, studies will cover: 1) optimisation of feeding strategies with live preys; 2) evaluation of precocious feeding strategies with micro-particulate diets; 3) optimisation of the dietary composition of micro-particulate feeds (i.e. dietary lipid, protein and energy level and source, adequate dietary PUFA level and DHA/EPA ratio). Regarding the juveniles, studies will evaluate: 1) the dietary protein requirements; 2) the relative potential of proteins, fats and carbohydrates as energy donors; 3) the optimal dietary DP/DE ratio. Given the economic importance and ecological implications of man-made feeds and feeding in aquaculture, the development of nutritionally balanced and environmental friendly diets is of utmost importance for a future establishment of blackspot seabream as a consolidated species in intensive aquaculture.

**Reference and funding entity:** Fundação para a Ciência e Tecnologia, POCTI 39239/2001.

**Duration:** Out 2002 – Set 2005

**Research team:** CCMAR: Maria Teresa Dinis, Luis Conceição, Florbela Soares, Laura Ribeiro; CIIMAR: Emidio Gomes, Paulo Rema, Luisa Valente; DAM-SRP da Madeira: Carlos Andrade, Nuno Gouveia

**Total budget:** 100.000 Euros **Funding for CCMAR:** 15.081 Euros

**Title:** Probiotics and immunomodulation in marine fish larvae and juveniles - **PROBIMU**

**Reference and funding entity:** FCT Project nº 38781/BSE/2001

**Duration:** Mar 2002 – Feb 2005

**Summary and Objectives:** Most bacteria causing disease in marine fish are opportunistic pathogens that are present as part of the normal seawater microflora. Environmental stress may weaken the immune system of the larvae and allow opportunistic pathogenic bacteria to invade the fish tissues, leading to disease.

Fish larvae drink water soon after hatching, and ingest bacteria associated with the water. Members of the "pioneer" microflora established in the larval gut may acquire a competitive advantage and become part of a persistent flora at the juvenile stage. Therefore, the early colonisation with non-pathogenic bacteria seems to be essential and has been shown to reduce mortalities after infection with pathogenic bacteria. Beneficial effects of probiotics in growth promotion and disease prophylaxis have also been reported. During the early life stages, fish have a very limited specific immune system, making difficult the use of vaccines. Successful use of immunostimulants in marine fish larviculture has been reported in recent years. Use of immunostimulants may aid the fish larvae to overcome stressful situations and critical stages during the early phase of rearing. Immunostimulants may act, either by stimulation of the non-specific mechanisms, or by acceleration of the development of the specific immune system.

This project aims at improving growth and survival of larval and juvenile marine fish using both probiotics and immunostimulants, as well as to find possible interactions between the two approaches. The Senegal sole (*Solea senegalensis* Kaup) and gilthead sea bream (*Sparus aurata* L.) will be used as models. In particular, it is intended to verify to what extent the immune system can be influenced by potential immunostimulants, and whether growth and survival can be improved by manipulation of the species composition of the gut microflora. The project also intends

to study the effect of exposure of fish larvae and juveniles to virulent pathogens after immune stimulation and/or modulation of the water microflora.

Ontogeny of the immune system organogenesis, non-specific cellular immunity and humoral immunity will be characterised in sole. Candidate probiotic strains will be isolated from the larval gut microflora based mainly on their inhibitory activity on virulent bacteria. The relative virulence of different pathogenic bacterial strains will be determined through challenge tests.

The effects of immunostimulants and selected probiotics on the development of the immune system, on the immune response, and on fish growth and survival will be assessed. This will be carried out by using standard microbiological, immunological and physiological techniques, both in normal conditions and when exposed to virulent pathogens.

**Research team:** CCMAR: Maria Teresa Dinis, Luis Conceição, Pavlos Makridis; IBMC :Pedro Rodrigues.

**Total budget:** 71.500 Euro; **Funding for CCMAR:** Euro

**Title:** Development of Virtual Learning Environment in Environmental Science, with Online Re-usable Interactive modules for remote users in marine pollution and ecology, with self learning languages packages in English, Greek, Portuguese and Swedish - **ORION**

**Summary and Objectives:** ORION will develop a state-of-the-art Virtual Learning Environment, housing a Resource Repository for a 4-language glossary, self-instruction language modules in English, Greek, Portuguese and Swedish, with environmental science course materials (marine pollution studies, ecology, marine biodiversity, etc.) suitable for the vocational and tertiary sectors at various levels, prepared by university departments and research organisations in Greece, Ireland, Portugal and Sweden. The major goal of ORION is the development of a powerful state-of-the-art ICT learning/teaching tool, to be used within a Virtual Learning Environment and delivered by means of a Distributed Network, in the subject area of the marine environment (pollution studies, ecology, biodiversity, aquaculture, etc.) relevant to both the vocational and tertiary sectors at various levels. This is an area of great importance, subject to increasing legislative and regulatory demands affecting a range of coastal zone users and decision-makers. These multi-disciplinary materials, prepared by top-level trainers in native English, Swedish, Portuguese and Greek, will be developed as innovative re-usable learning objects which can be shared and re-used by teachers and target group users situated in remote areas of Europe: Stockholm in the North, Cork in the north-west, Portugal in the south-west and Greece in the south.

To underpin the training in language which will be needed to maximise the benefits from this innovative methodology, an online marine environmental glossary in English, Greek, Portuguese and Swedish will be created, to form a major resource freely available to partners only, with accompanying modules in ESP (environmental science) and basic Greek, Portuguese and Swedish language modules, to reflect the needs of the target users.

**Reference and funding entity:** European Community Programme LEONARDO DA VINCI, Community Vocational Training Programme Project n° EL 2001 BP LA 114443,

**Duration:** April 2002 – April 2005

**Total Funding:** 404.672 Euros

**Partners:** FEAP (Federation of European Aquaculture Producers), IMBC, Crete, Greece, EKTHE (National Centre for Marine Research, Athens), TEREUS, SA, Athens, Department of Zoology, University of Cork, Ireland, AQUALEX Multimedia Consortium Ltd Ireland, Centre for Marine Sciences, University of Algarve, Portugal, Department of Systems Ecology, University of Stockholm, Sweden

**Title:** Characterization of the endemic infection of the clam *Ruditapes decussatus* by the parasite *Perkinsus atlanticus* (protista, apicomplexa) in the Portuguese coast.

**Summary and Objectives:**

To evaluate the status of bivalves towards infection by *Perkinsus* sp. in the Portuguese coast. To initiate an epidemiological study ..



**Reference and funding entity:** PDCTM/P/mar/15308/1999

**Duration:** October 2000-2003

**Research team:** L. Cancela:Project Coordinator;. C. Azevedo,(CIMAR-ICBAS- Univ Porto)

CCMAR team:M. T. Dinis, ; R. Leite, Ricardo Afonso, Laurence Elandalloussi. -, -.

**Total budget:** 125.000 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** "Desenvolvimento de um modelo de infestação in vitro adaptado ao estudo das interações hospedeiro-parasita entre as espécies *Ruditapes decussatus* e *Perkinsus atlanticus*. Obtenção de informação directamente aplicável em moluscicultura (CLAM).

**Funding Institution:** PRAXIS/C/BIO/12143/98

**Duration:** Setembro 2001-Agosto 2003

**Objective:** Development of an in vitro model to study the infection of Clams by the parasite *Perkinsus atlanticus* through analysis of host-parasite relationship using in vitro cell culture model systems.

**Research team:** L. Cancela: project coordinator. M.Teresa Dinis (UALG\_CCMAR), Carlos Azevedo (ICBAS-Univ Porto) CCMAR team: L. Elandalloussi, R. Leite, R. Afonso, P.Cabrita, P.M. Rodrigues

**Funding:** 98,000 Euro **Funding for CCMAR:** Euro

**Web site:**

**Title:** Biomarkers for environmental endocrine disruptors in Portuguese waters

**Summary and Objectives:** The primary objective of this proposal is to monitor the presence of endocrine disruptors and their effects in Portuguese coastal waters and rivers, including "pristine" reference waters and those subject to industrial or urban pollution. Locations to be included are Ria Formosa, Ria de Aveiro, estuaries of Sado, Guadiana, Tejo, Mondego, and Douro, and specific locations in rivers where industrial activities take place.

In vitro systems for screening of estrogenic, androgenic and thyroidogenic substances will be developed and standardized in the laboratory to be used for screening of natural waters. Specific enzyme linked immunoassays (ELISA) will be developed for monitoring blood vitellogenin in plasma of male fish. Evidence for endocrine disruption in aquatic populations will be sought using incidence of abnormal gonads and tumours evidenced by histological methods, blood vitellogenin in male fish as bioindicators of disruption.

An autochthonous fish species, preferably colonizing all the surveyed habitats, will be used as an in vivo test for endocrine disruption.

**Reference and funding entity:** FCT

**Duration:** 1/2001-12/2003

**Research team:** Adelino Canário, Deborah Power, Ana Lúcia Passos, José Eduardo Cavaco, Natália Moura.

**Total budget:** 219.470 Euro; **Funding for CCMAR:** Euro

**Web site:** [www.ualg.pt/ccmar/mar-endo](http://www.ualg.pt/ccmar/mar-endo)

**Title:** Improving production efficiency of sea bass farming by developing methodologies to eliminate environmental androgenesis

**Summary and Objectives:** The overall objective of the project is to understand the mechanisms regulating sex differentiation in cultured sea bass, in order to develop methodologies to minimize the proportion of males in cultured stocks.

**Reference and funding entity:** European Commission

**Duration:** 1/2001-12/2003

**Research team:** Coordinator: Silvia Zanuy, Consejo Superior de Investigaciones Científicas – Spain; Costadinos Mylonas, IBMC, Greece; Francesc Piferrer, CSIC, Barcelona, Spain; Glen Sweeney, Univ. Wales; Abigale Elizur, Elat, Israel; UK; CCMAR: Adelino Canário, Rute Martins, João Condeça.

**Total budget:** 239.832 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** Calcium, the backbone of fish culture: importance in skeletal formation, reproduction and normal physiology - Fishcal

**Summary and Objectives:** Egg and larval viability in sea bream culture is still low, as a consequence of mortalities and a high incidence of skeletal deformities (dystrophies). Dystrophies are not always immediately apparent, leading to wasteful use of food, energy, space and human resources. Abnormal cartilage growth and calcification are key features of skeletal deformities. Parathyroid hormone-related protein (PTHrP), recently identified as a hypercalcaemic hormone in fish, appears to mediate ossification. Calcium is also essential in many other physiological processes, such as reproduction and growth. The project will: i) establish the relative contribution of the diet and the environment to calcium balance; ii) determine the part played by PTHrP in larval development, growth and vitellogenesis; iii) identify genes regulated by PTHrP and iv) generate guidelines for the use of calcium in sea bream husbandry.

**Reference and funding entity:** European commission

**Duration:** 11/2001-6/2004

**Research team:**

**Total budget:** Euro; **Funding for CCMAR:** Euro

**Web site:**

### Completed 2002

**Title:** Reproductive biology of the blenny fish *Salaria pavo* from Ria Formosa: implications for population management and conservation strategies.

**Summary and Objectives:** The project investigates different aspects involved in conservation of *S. pavo* in Ria Formosa. The main goals are: a) Acquisition of knowledge for a future captive breeding program if necessary, b) the identification of ecological factors that influence reproductive strategies, and c) to propose guidelines for the management of *S. pavo* population in Ria Formosa.

**Reference and funding entity:** FCT/ICN

**Duration:** 24 months

**Research team:** Coordinator: Rui F. Oliveira, Instituto Superior de Psicologia Aplicada; CCMAR: Eduardo Barata, Rui Serrano, Adelino Canário

**Total budget:** 74.820 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** Molecular responses to water stress in highly salt- and desiccation- tolerant algae. ALGSTRESS.

**Summary and Objectives:** To identify genes and / or gene products involved in the process of tolerance to salt and /or desiccation in water stress-tolerant macro- and microalgae. In order to identify these genes two subtractive cDNA libraries will be constructed. These libraries will enable us to identify and isolate cDNAs whose expression is upregulated upon water stress conditions. Characterization of the positive cDNA clones will include DNA sequencing and Northern analyses. Priority will be given to clones homologous to genes known to be involved in stress tolerance in higher plants and other eukaryotes. However, other interesting clones, such as those possibly involved in signal transduction, will also be investigated.

**Reference and funding entity:** FCT. Praxis /P/ BIO / 12203 / 1998

**Duration:** May 2000- May 2002

**Research team:** CCMAR: João Varela, Nuno Henriques, Sacha Coesel, Ester Serrão and Gareth Pearson.

**Total budget:** 100.000 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** New applications for concentrated microalgae - **NOVALGAS L0032**

**Summary and Objectives:** The main objective is to find new applications for concentrated microalgae with respect to the aquaculture and poultry feed industries. During the last year, the consortium has developed two new products, one of them called Phytobloom. As far as BMBM is concerned, the final goal of this research is to provide Necton with novel procedures and / or strains able to accumulate higher amounts of carotenoids or synthesize novel compounds that otherwise the microalgae do not produce. These carotenoids would give our industrial partner an important place in the valuable world market of carotenoids (over 200,000,000 keuro / year).

**Reference and funding entity:** Agência de Inovação, S.A.

**Duration:** July 1999-July 2001

**Research team:** CCMAR: João Varela, Nuno Henriques, and Sacha Coesel; Maria T. Dinis e Pedro Cação. ESB – Catholic University: Rui Morais, Maria de Fátima Poças e Maria João Monteiro; IBET – João Paulo Crespo; STF – Faculdade de Farmácia do Porto: Maria Fernanda Bahia. NECTON, S.A. – João Navalho, Inácio Oom do Valle.

**Total budget:** 310.000 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** Immune competence and immune resistance in *Solea senegalensis* (Pisces, Soleidae) larvar and post-larvae - **IMUNO**

**Reference and funding entity:** FCT 7 POCTI Project nº 34115/99

**Duration:** Jan 2000 – Dez 2001

**Summary and Objectives:** Most bacteria causing disease in marine fish are opportunistic pathogens that are present as part of the normal sea water microflora. Alterations in the environmental conditions may weaken the larval defences and allow bacteria to invade tissues, leading to disease.

Fish larvae drink water soon after hatching, and ingest the associated bacteria. These bacteria may be of immunological importance by presenting antigen determinants. This primary microflora to become established in the larval gut tends to acquire a competitive advantage and develop into a persistent flora at the juvenile stage. Therefore the early colonisation with non-pathogenic bacteria seems to be essential, and has been shown to reduce mortalities after infection with pathogenic bacteria. The beneficial effects of probiotics in growth promotion and disease prophylaxis is also well documented. The early life stages of fish have a very limited immune defence system, making impossible the use of vaccines. Promotion of the non-specific immune defence of fish larvae through the use of immune stimulants has had some success.

This project aims at improving the understanding of the interactions between the environmental conditions and the immune competence and immune resistance of larval and post-larval fish, using the Senegal sole (*Solea senegalensis*, Pisces) as a model. In particular it is intended to verify to what extent immune stimulation and manipulation of the water microflora composition can affect immune competence and immune resistance, both in standard conditions and when fish are exposed to virulent pathogens and temperature and/or salinity stress.

Ontogeny of the non-specific cellular immunity (phagocytosis, oxygen burst), humoral immunity (polyclonal antibody anti-IgM) and the immune system organogenesis (histology) will be characterised. Candidate Probiotics will be isolated from the larval gut microflora based on its inhibitory activity and polyclonal antibodies tests. The relative virulence of different pathogenic bacterial strains and tolerance to temperature and salinity fluctuations will be determined through challenge tests.

The effects of immune stimulation and selected probiotics on the immune response, the development of the immune system, the bacterial populations of the larval gut, the microflora of rearing environment, and the fish physiological condition will be determined both in standard conditions and when exposed to virulent pathogens and temperature/salinity stress. The immunological methods already mentioned will be used. Bacterial populations will be characterised by cultivation of samples in selective media and phenotypic characterisation of randomly selected colonies. The effects on fish physiological condition will be studied through: (1) food consumption and protein turnover rates ( $^{15}\text{N}$  used as tracer); (2) RNA/DNA and Protein/RNA ratios (standard spectrophotometric methods); (3) and the energetic costs of maintenance and growth (respirometry)

**Research team:** CCMAR: Maria Teresa Dinis, Luis Conceição, Pavlos Makridis; IBMC :Pedro Rodrigues.  
**Total budget:** 15.000 Euro; **Funding for CCMAR:** Euro  
**Web site:**

## **Division of Living Resources**

### **New and Ongoing beyond 2002**

**Title:** Study of the environmental conditions of the Guadina River estuary and adjacent areas

**Summary and Objectives:** The study was initiated from the necessity of the portuguese and spanish administrations to characterize the environment at the lower reaches of the Guadiana, in particular the estuary and the coastal area, defining desirable scenarios of environmental quality for the area. It should identify management strategies for the dams being built that will lead to ecosystem sustainability.

**Reference and funding entity:** Funded by Instituto da Água

**Duration:** 05/2003 (delayed by INAG)

**Research team:** Karim Erzini (Ictiofauna e Cadeia Trófica), José Calvário e Martin Sprung (Macrofauna Bentónica), Maria Alexandra Chícharo e Luís Chícharo (Produtividade Primária – Fitoplâncton e Zooplâncton), Rui O. Santos (Cadeia Trófica e Produtividade Primária - Spartina)

**Total budget:** 402 280 Euro; **Funding for CCMAR:** 402 280 Euro

**Web site:**

**Title:** Contribution to the knowledge of the phylogeny of flat fishes (Pleuronectiformes, Teleostei)

**Summary and Objectives:** The goal of the present study is to establish the phylogenetic relationships among species of Atlantic and Mediterranean flatfishes. We propose to approach the phylogenetic relationships among species from the six existing families of Pleuronectiformes (Citaridae, Scophthalmidae, Bothidae, Pleuronectidae, Soleidae and Cynoglossidae) by molecular means. This work will be done on the basis of nucleotide sequence of different mitochondrial regions - cytochrome b, 12S RNA and 16S RNA.

**Reference and funding entity:** POCTI / 1999 / BSE / 34891 FCT

**Duration:** 05/2003

**Research team:** Rita Castilho, Regina Cunha

**Total budget:** 37400 Euro; **Funding for CCMAR:** 37400 Euro

**Web site:** <http://w3.uaig.pt/~rcastil/Flat.htm>

**Title:** "The Portuguese coast as a biogeographic boundary: consequences for reproductive success, local adaptation and genetic structure of populations living at their distributional limits (BIOPORT)"

**Funding institution:** FCT (PDCTM / P / MAR / 5292 / 1999).

**Duration:** Feb 2001-Jan 2004

**Objectes/Summary:** To compare the reproductive success and ecophysiological differentiation of species at their southern distributional limits, and their genetic consequences.

**Research team:** CCMAR-UAig: E Serrao, G Pearson, C Daguin, L Ladah, C Engel, et al.

**Funding:** 225 000 Euro

**Title:** "Resilience and genetic diversity of seagrasses affected by anthropogenic perturbations in the Natural Park of Ria Formosa". (SEAGRASSRIA)

**Funding institution:** FCT (PNAT/1999/BIA/15003/C)

**Duration:** Apr 2001-Mar 2004.

**Objectives/Summary:**

Evaluation of the genetic structure and reproductive ability of the seagrasses in the Natural Park of Ria Formosa.

**Research team:** CCMAR-UAig: E Serrao, R Santos, M Billingham, F Alberto, et al.

**Funding:** 75 000 Euro

**Title:** "Local adaptation and population genetic structure in intertidal algae" (GENFUCUS)

**Funding institution:** FCT (POCTI/BSE/35045/99.)

**Duration:** Dec 2001-Nov 2004

**Objectes/Summary:**

Populations of two closely related algal species with contrasting fragmented distributions are investigated concerning their population genetic structure and divergence.

**Research team:** CCMAR-UA|g: E Serrao, G Pearson, C Daguin, C Engel, et al.

**Funding:** 150 000 Euro

**Title:** Monitorização de lontras costeiras no Sudoestes de Portugal [Monitoring coastal otters in southern Portugal].

**Summary and Objectives:** This project aims at monitoring the population of otters inhabiting the coastline of southwestern Portugal. For this, the project will use DNA markers recovered from faeces to identify individual otters occurring along the coast. This will allow for an estimate of population numbers and habitat preferences. The project will also optimize techniques for the extraction and analysis of DNA in otter faeces, with potential applications to other species of endangered mammals.

**Reference and funding entity:** Transgás Atlântico, SA

**Duration:** January 2001 –December 2004

**Research team:** Pedro Beja, Leonor Cancela, Sara Mira, Catarina Canas

**Total budget:** 47386 Euro; **Funding for CCMAR:** 47386 Euro

**Web site:**

**Title:** Formosa dune: dinâmica espaço-temporal da vegetação de ilhas barreira da Ria Formosa.

**Summary and Objectives:** Evaluation of the ecological succession of vegetation of barrier islands in the Natural Park of Ria Formosa.

**Reference and funding entity:**

**Duration:** .2 years

**Research team:** R Santos (CCMAR) J. Fernandes

**Total budget:** 35000 Euro; **Funding for CCMAR:** 35000 Euro

**Web site:**

**Title:** SEAPURA. Species diversification and improvement of aquatic production in seaweeds purifying effluents from integrated fish farms,

**Summary and Objectives:** Use of seaweeds of economic value to biofilter fish farm effluents.

**Reference and funding entity:** QLRT - 1999 - 31334

**Duration:** .3 years

**Research team:** R Santos. L. Mata and A. Schuenhoff

**Total budget:** **Funding for CCMAR:** 150 000 Euro

**Web site:** <http://www5.ulpgc.es/servidores/algologia/seapura.html>

**Title:** ALIENS - ALgal Introductions to EuropeaN Shores.

**Summary and Objectives:** 1.- To explain the underlying ecological causes of the introduction, establishment and development of seaweed invasions on European shores.

2.- To generate a baseline dataset on the present status of seaweed introductions to European shores, and of future susceptibility to further introductions/invasions

3: To elucidate the genetic structure of various populations of selected invasive seaweeds in Atlantic and Mediterranean Europe, with a view to determining whether there have been multiple cryptic European introductions

4.- To evaluate the economic impact of existing seaweed invasions on a European scale, comparing losses with costs associated with prevention and eradication

5: To carry out risk assessment and propose a screening protocol for invasive macroalgae to be used in coastal zone management

**Reference and funding entity:** EVK3-2001-0008

**Duration:** .3 years

**Research team:** Rui Santos (coordinator), Ester Serrao, Gareth Pearson, Aswin Engelen

**Total budget:** Funding for CCMAR: 177 000 Euro  
**Web site:**

**Title:** " EPICAH - Ecological and population impact of commercial agarophyte harvesting".

**Summary and Objectives:**

- To assess the ecophysiological differences between life cycle phases that can explain the genetic structure of natural populations, *i.e.*, the relative abundance of the haploid and diploid life history phases.
- To assess the effect of the commercial harvest on the genetic structure of the populations and its demographic consequences;
  - To develop structured population models, including both life history phases to simulate the observed harvest impacts.
- To assess the impact of the *G. sesquipedale* commercial harvest on the associated fauna of the fronds, and its consequent effects along the trophic web, with particular attention to the local commercial fish species.

**Reference and funding entity:** PDCT/P/Mar/15299/1999.

**Duration:** 3 years

**Research team:** R Santos, R. Carmona

**Total budget:** Funding for CCMAR: 120 000 Euro

**Title:** Experimental By-catch Reducing Devices (BRD) in the demersal purse-seine fishery and evaluation of survivorship

**Summary and Objectives:** Preliminary studies have shown that mean discard rates in Algarve (southern Portugal) purse seine fisheries vary between 0.20 and 0.30; with between 5,000 and 10,000 mt of discards per year (Borges et al., 1997, 2000, Erzini et al., 2001). The objective of the proposed project is to test simple by-catch reducing devices (BRDs) for the demersal purse seine in order to reduce the by-catch and discarding of under-sized and/or non-commercial species. In particular, we will evaluate the use of both larger mesh sizes and square meshes in part of the net. These modifications should increase the escapement of juveniles and non-commercial species. Quantification of the effectiveness of BRDs will be carried out by means of experimental fishing trials using small-mesh covers over the BRD sections. In addition, the condition of fish that have escaped will be evaluated and post-escapement survivorship monitored in tanks. The relationships between condition (scale loss and other signs of external damage), stress (monitored by cortisol radioimmunoassay) and long-term survivorship will be evaluated experimentally. The results of these experiments will be transmitted to the fishing community.

**Reference and funding entity:** FCT/POCTI/BSE/43113/2001

**Duration:** 2002-2004

**Research team:** CCMAR: Jorge M.S. Gonçalves, Karim Erzini and Adelino Canário

**Total budget:** 73000 Euro; **Funding for CCMAR:** 73000 Euro

**Web site:**

**Title:** Effects of river flow changes on the fish communities of the Douro, Tejo and Guadiana estuaries and adjoining coastal areas. Ecological and socio-economic predictions (ERIC).

**Summary and Objectives:** Characterisation of the fish community structure (species composition, abundance and length distributions, biomass, diversity, evenness, nursery function) and their relation with environmental factors. Determination of the optimal values and the tolerance limits to some environmental factors for the most important fish species. Determination of the carrying capacity of the environment for the most important fish species, based on the knowledge of the major limiting resources and the relations with some environmental factors. Diagnostic model to support an integrative and rational management of these estuarine and coastal areas. Simulation of different scenarios according to river flow.

**Reference and funding entity:** FCT/MAR/15263/99

**Duration:** December 2000- December 2003

**Research team:** CCMAR: Maria Alexandra Chícharo and Luis Chícharo; - Instituto de Oceanografia, Universidade de Lisboa, Maria José Costa

**Total budget:** 523.738 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** CORRAM - Cephalopod octopodid: relation between de resource and the marine environment). Project ID: 2/2.1/MAR/1707/95

**Summary and Objectives:** The main objective of this project is to know the basic bio-ecological parameters of the species *Octopus vulgaris* in Portuguese waters, like the population structure, age and growth, reproduction and the distribution and abundance in different phases of the life cycle and their trophic relations. Therefore, complementary studies will be performed in the areas of eco-physiology, ethnology, biochemistry, nutrition and fishing technologies. There will also be done a social-economic impact study as well as attempts of stock assessment. A on board programme will be study to perform all direct studies. Samples will be bought at the first sell market to perform the necessary laboratory studies. Specimens will be caught and kept in captivity for all the studies on nutrition, eco-physiology ethnology, etc.

**Duration:** 01.02.1999 to 30.06.2003

**Funded by:** FCT, PRAXIS XXI, passou para POCTI

**Research team:** Teresa Cerveira Borges (coordinator); José Pedro Andrade, João Sendão, António Seykes; Other participating institutions: Fundação da Faculdade de Ciências da Universidade de Lisboa, Universidade dos Açores, Direcção de Serviços de Estudos e Investigação das Pescas da Madeira, Universidade Aberta

**Total budget:** 42.160 euro **Funding for CCMAR:** 10.500 euro

**Web site:**

**Title:** CEPHSTOCK "Cephalopod stocks in European waters: Review, Analysis, Assessment and Sustainable Management" (QOL-2001-5.1.2)

**Summary and Objectives:** This project aims to review current knowledge and issues in cephalopod fisheries science, to assemble, organise, analyse and synthesise data from ongoing national projects, previous and new EC-funded R&D projects, and to recommend future actions for scientists and cephalopod fishery managers in European waters. This will be achieved through (a) co-ordinated reviews and (b) development of common databases and associated GIS analysis capability, leading to (c) a series of co-ordination meetings and workshops at which the status of fished European cephalopod stocks will be reviewed, stock assessments carried out and management recommendations proposed.

**Duration:** 01-10-2002 to 30-09-2005

**Funded by:** EC- DGXII

**Research team:** University of Aberdeen (Coordinator) and 20 institutions from all Europe; from CCMAR/UAlg Teresa Cerveira Borges (coordinator) and Pedro Andrade.

**Total budget:** **Funding for CCMAR:** 27 600 Euro

**Web site:**

## Completed in 2002

**Title:** Assessing the impact of bivalve fisheries on the benthic ecosystems of the Ria Formosa lagoon (Portugal), Venice lagoon (Italy), Aegean sea (Kavala-Greece)

**Summary and Objectives:** This project aimed to study the impact of traditional clam harvesting in Ria Formosa, and also to analyse similar impacts on the Venice lagoon and Aegean Sea.

**Reference and funding entity:** ECDGXIV

**Duration:** March 2000- march 2002

**Research team:** CCMAR: Luis Chícharo; Maria Alexandra Chícharo and Pedro Andrade - The project is coordinated by CCMAR but have other partners CSIC and IEO (Spain), University of Padova, CNR and Agriteco (Italy), FRI (Greece) and IPIMAR (Portugal).



**Total budget:** 569.976 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** Fisheries & population structure of *Scomber* spp. in the Mediterranean and S. Iberian Atlantic waters

**Reference and funding entity:** Studies 99/034

**Duration:** 02/2002

**Research team:** Rita Castilho, Sofia Caetano

**Total budget:** 545 872 Euro; **Funding for CCMAR:** 86 880 Euro

**Web site:** <http://w3.ualg.pt/~rcastil/Scomber.htm>

**Title:** ICTIORIA Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (southern Portugal)

**Summary and Objectives:** This project focused on the early life history stages of commercially important species, especially those of the sea bream family (Sparidae). A total of 35 sampling sites representing the range of natural habitats within the Ria were sampled on a monthly basis using a 25 m beach seine. In addition, beam trawl, push nets and lift nets were used to sample specific habitats and a 50 m beach seine was used to provide data for a comparative study. This project permitted the evaluation of possible changes in the fish community of the Ria Formosa by comparing the species composition and abundance in 2000-2001 with data from 12 to 15 years ago. Tagging studies were carried out in order to study spatial and temporal dynamics. This project was the basis of a PhD and four undergraduate theses.

**Reference and funding entity:** European Commission Directorate General XIV Fisheries

**Duration:** April 2000-March 2002

**Research team:** CCMAR: K. Erzini

**Total budget:** 92.682 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** FANTARED II A study to identify, quantify and ameliorate the impacts of static gear lost at sea.

**Summary and Objectives:** The high level objective of this project was to identify, quantify and ameliorate the impact of static gear lost at sea in European waters. There were nine lower level objectives, which derived from that: 1) To investigate gear losses in representative static gear fisheries in all European waters including some Mediterranean and Norwegian métiers; 2) To establish the main causes of gear loss in fisheries not covered by the previous study; 3) To identify métier descriptors which can be used to indicate those métiers most likely to suffer significant gear loss; 4) To establish the extent of gear loss in representative métiers by a range of methods including terrestrial and marine surveys; 5) To determine the physical evolution of gears lost under a range of conditions; 6) To quantify as far as necessary the ecosystem impacts of lost gears particularly with respect to stocks of commercially important marine species; 7) To review a range of mitigating measures with potential to reduce the extent and/or impact of lost gears; 8) To transfer research methods between new research partners and 9) To maximise the involvement of national and sectoral industry groups in the programme and the transfer of information between all interested parties.

This project deal predominantly with the gillnet sector (using that term in its generic sense to include gill, trammel and tangle nets) but it included a limited amount of work on fish and shellfish traps. These were included because fish trapping is gaining in popularity in northern waters and because a previous study of shellfish traps covered only the waters around the British Isles.

This research extended the work undertaken during contract no. 94/095, which covered methodology and shallow water areas only. This new study extended the information on lost gears to a wider range of métiers prosecuted in European waters as well as a comprehensive range of seabed and hydrographic conditions. The partnership and the specific tasks allocated to each partner were set up with these requirements as a priority. Previous work has shown the extent to which the evolution and impacts of lost gear are métier and site-specific. To have any credibility in

the context of European fisheries management, this kind of research must be undertaken on commercially fished grounds. The integrity of the work was further enhanced by the involvement of specially convened liaison groups comprising fishermen from each participating nation.

**Reference and funding entity:** UE-FAIR-PL98-4338

**Duration:**

**Research team:** CCMAR: K. Erzini

**Total budget:** ; **Funding for CCMAR:** Euro

**Web site:**

**Title:** "Managing by-catch and discards: a multidisciplinary approach" (Ref: 99/058)

**Summary and Objectives:**

The aim of this proposal is to approach the problem of by-catch and discards on a multidisciplinary basis in the Algarve, the Portuguese region most dependent on fisheries, where the fisheries have an important role and a significant source of employment. To achieve this, the present project aims to look at the by-catch and discards in the south coast of Portugal (Algarve) with the following objectives:

1. To implement a database system with all information from previous projects;
2. To continue to identify and quantify the by-catch and discards from the main fishing métiers off the Algarve coast;
3. To continue to study biology aspects of species with low or no commercial value;
4. To describe the energy and biomass flow among the different fish guilds, and to investigate the impact of fishing on the different levels of the foodweb;
5. To evaluate the possible use of by-catch reducing devices (BRDs) in trawls to reduce the by-catch and discards, studying the positive and negative aspects from technical, economic and fishermen point of views.

To achieve the above mentioned objectives, the present project aims to look at the by-catch and discards in the south Portugal during a period of 24 months, in two fishing gears: trawl and purse seine. Data collection methodology will be with observers on board commercial fishing boats.

For the BRDs experimentation in the trawl fishery, the priority will be to test simple and inexpensive techniques, which can be accepted by the fishermen. It is expected to test two different systems on board commercial fishing trawls (crustacean): square mesh panels and sorting grids. Accepted statistical methods for evaluating the effects of tested techniques will be applied. In addition, underwater video cameras will document the results of the experiments.

Economic studies will accompany the entire project to quantify economic costs and benefits of the use of BRDs, to model the fishermen's behaviour responding to the use of BRDS and to new management measures necessary to implement (technical measures, enforcement procedures and types of control).

**Reference and funding entity:** EU, DG Fisheries

**Duration:** Feb 2000-Jan 2002

**Research team:** CCMAR — T.C.Borges (co-ordinator), K. Erzini & Grupo Biopescas; IPIMAR — Aida Campos e Paulo Fonseca; University of Tromsø, Norway — Jorge Santos, Roger Larsen & Arne Eide. **Consultant:** Broadhurst, M. - University Federal Rural of Pernambuco, Brasil

**Total budget:** 274.352 Euro; **Funding for CCMAR:** Euro

**Web site:**

**Title:** Identificação dos elementos de conservação (Fauna de crustáceos filópodes e anfíbios) nos charcos temporários do Parque Natural do vale do Guadiana e áreas limítrofes

**Summary and Objectives:** Temporary ponds in the Mediterranean region are among the most fragile aquatic environments, but are extremely important in terms of biodiversity. For this reason were classified by the EC as priority habitats (DH 92/43/EEC).

In order to define conservation measures in the PNVG area, basic knowledge has to be collect, in order to define conservation strategies (monitorization or active measures). The main objectives of the study are: faunistic lists, mainly of filopod crustaceans and amphibians; characterization of the habitat to explain the presence and abundance of different groups and their relations; identification

of the elements to conserve and their limiting factors; to propose managing strategies of these systems

**Reference and funding entity:** ICN/PROA

**Duration:** November/2001 – October/2002

**Research team:** Margarica Cristo, Margarida Machado, Jordi Sala

**Total budget:** 21947Euro; **Funding for CCMAR:** Euro

**Web site:**

## International and Interinstitutional Cooperations

### **Division of Aquaculture and Biotechnology**

Acção integrada Luso Britânica nº B25/02. Investigation of regulation of caudal neurosecretory system function by sensory input in a marine teleost (*Sparus aurata*). Peter Hubbard (CCMAR) and Prof. Richard Balmonte, University of Manchester.

**Title:** Identification of Sex Pheromones from the Anal Gland of Male Blennies, *Salaria pavo* and *S. fluviatilis* (Pisces: Blenniidae).

**Summary and Objectives:** *Salaria pavo* is small bottom living fish in the shallow littoral zone of the Mediterranean and adjacent Atlantic coast. The closely related freshwater species, *S. fluviatilis*, inhabits rivers and lakes in the vicinity of the Mediterranean. In both species, the mating system is promiscuous. Males occupy holes or crevices in the hard substrate where females come to spawn and the males subsequently guard the eggs. These are a good model fish species to investigate specialization in sex pheromone production. The males develop anal glands from the epidermis of the first two rays of the anal fin concurrent with development of the gonads. The Portuguese team has shown that the anal gland of *S. pavo* is a source of substances that attract pre-ovulatory females which promotes male reproductive success. This suggests that male blennies are “active signallers” in contrast with known pheromone systems in teleosts, where receivers are “chemical spies” detecting gonadal steroids or prostaglandins passively excreted by females into the water via the urine or gills.

The proposed work aims to identify the chemical structures of putative sex pheromones in the two species. Although strictly a freshwater species, the marine origin of *S. fluviatilis* is well established and, as with *S. pavo*, it is plausible that the anal gland has a pheromonal function in female attraction. If so, one would expect the pheromonal components of the two species to have similar or closely related chemical structures. In addition, the inclusion of *S. fluviatilis* in the project will simplify the chemical identification, since recording of electro-olfactograms is technically easier in freshwater, and can be combined with chromatographic separation of anal gland-produced substances.

**Reference and funding entity:** British Council/CRUP (Acções Integradas Luso-Britânicas)

**Duration:** January 2003 - December 2003

**Research team:** CCMAR: Eduardo N. Barata, Peter C. Hubbard, Ana Freitas, Rui Serrano, Adelino V.M. Canário. Rothamsted Research – Biological & Ecological Chemistry Division, UK: Mike Birkett, Lester Wadhams & John Pickett.

**Total budget:** 3.000 Euro; **Funding for CCMAR:** 1.500 Euro

**Title:** Thermal stability of electron pairs in the Hubbard-Davydov model.

**Summary and Objectives:** The model of Hubbard-Davydov is a non-linear model, which represents the interactions between quantic particles (electrons, excitations, etc) with the vibrations of a net. It is known that some non-linear systems can sustain localized excitations localized as the solitons and the discrete breathers. While the studies done until now do not include the influence of thermal energy, the objective of the project is to simulate the model of Hubbard-Davydov as finite temperatures.

**Reference and funding entity:** British Council

**Duration:** June 2001-May 2002

**Research team:** CCMAR: Leonor Cruzeiro-Hansson; Heriot-Watt University, Edinburgh, UK: Professor J.C. Eilbeck and Professor F.M. Russell.

**Total budget:** 3.000 Euro; **Funding for CCMAR:** 3.000 Euro

**Title:** Histomorphology, citohistology and ultrastructural study of *Solea senegalensis* pigmentation under different rearing conditions

**Summary and Objectives:** The main objective of the project is to optimise larval cultivation of sole (*Solea senegalensis*), a species with high potential for marine fish farming. As a consequence

of an ongoing project some bottlenecks for sole cultivation were identified. These included control of reproduction, weaning and pigmentation abnormalities (total or partial albinism). This project focus on this last problem. A significant proportion of pigmentation abnormalities may occur in hatchery produced sole, and are a problem for marketing of this species. Pigmentation abnormalities in sole larvae and post-larvae will be studied using histochemical and immunohistochemical techniques. It will be investigated whether zootechnical parameters (density, temperature, salinity, photoperiod, light intensity, type of substrate) or nutritional factors are related to pigmentation abnormalities.

**Reference and funding entity:** Convénio ICCTI/ CSIC

**Duration:** Dez 2000-Dez 2004

**Research team:** CCMAR: Maria Teresa Dinis, Florbela Soares, Laura Ribeiro, Pedro Cação; Instituto de Ciencias Marinas de Andalucia, Spain - Maria del Carmen Sarasquete, Emilio Pascual, Manolo Yúfera.

**Total budget:** 3.500 Euro; **Funding for CCMAR:** Euro

**Web site:**

### **Division of Living Resources**

Asmus, Harald, - Alfred- Wegener-Institut List/Sylt, Germany: Epifauna of seagrass beds. Financial support: Acção integrada CRUP/DAAD (contact person in CCMAR: Martin Sprung)

**Title:** "Monitoring and Management of European Seagrass Beds (M&MS)".

**Funding institution:** EU- EnvSD (EVK3-CT-2000-00044).

**Duration:** Feb 2001-Aug 2005.

**Objectives/Summary:** To evaluate the status of European seagrass beds, particularly for CCMAR to evaluate their capacity for recovery via sexual reproduction and their genetic diversity and structure.

**Research team:** Coordinator: FBL (Denmark): J. Borum et al. Other main partners: CCMAR-UAIG (Portugal): E. Serrao et al, CSIC (Spain): C. Duarte et al., Univ. Wales (UK): H. Kennedy et al. Associated partners: PNRF (Portugal), DGP (Spain), DMU (Denmark)

**Funding for CCMAR:** 330 000 Euro

**Title:** SEMAPP "Science, Education and Marine Archaeology Programme in Portugal "

**Summary and Objectives:** Co-operation to study the marine ecosystem at selected locations in Portuguese territorial waters. The aim of this programme is to develop the knowledge and research of oceanography related to fisheries, an important and desirable area of research in Portugal.

The University of Connecticut has access to, and expertise in utilising underwater systems and technologies that allow for detailed *in-situ* observations and research in water depths up to 2,000 meters. The initial proposed study site is Portimão Submarine Canyon and adjacent Continental Slope and Shelf region, off Portugal's Algarve coast. The plan is to spend the first 2 years conducting detailed bathymetric surveys and sub bottom profiling in order to map the ocean floor in those regions. In 2004, it is planned using manned submersibles and robot technology to assist with studies in Fisheries Biology, Benthic Ecology and Environmental Sciences.

**Duration:** 2000 ...

**Funded by:** OTF, USA & others

**Research team:** University of Connecticut: Prof. Richard Cooper (co-ordinator of programme); CCMAR: Teresa Cerveira Borges (Marine Biology); Autonomous University: Dr. Adolfo Silveira Martins (Marine Archaeology); Hidrographic Institute.

**Total budget:** variable

**Funding for CCMAR:**

**Web site:**

### **CCMAR Seminar programme**

Erzini, K. (2002). Dynamics of mean size: the influence of life history parameters, recruitment and exploitation patterns. (May 20, 2002).

## Seminars given by CCMAR members in other institutions

- Barata, E.N. 2002 "Comunicação química na reprodução de peixes: Dos espiões de informação aos emissores de sinais". Conferência do âmbito do ciclo de palestras "Biologia ConVida", organizado pelo Departamento de Biologia da Universidade de Évora, 19 de Junho de 2002, Universidade de Évora.
- Dinis, M. T. Técnicas de cultivos de peixes marinhos. Licenciatura em veterinária. Universidade de Évora, 2002.
- Dinis, M. T. Situação actual dos cultivos de peixes em Portugal, entro de Investigaciones Marinas de - CIMA, Xunta de Galicia, 2002.
- Soares, F. Histopatologia em peixes marinhos. DRPM, Funchal, Março de 2002.
- Lydia B. Ladah: "The Interdisciplinary Coastal Ecology Team" Institución: *Woods Hole Oceanographic Institute, MIT, Biocomplexity Project Workshop* 23-24 Nov 2002
- Lydia B. Ladah: "*Evolutionary advantage of some brown algal life histories*" University of Evora, Evora, Portugal, May 30, 2002
- Lydia B. Ladah: "*Life histories as survival strategies in brown algae*" American University of Beirut, Beirut, Lebanon, May 21, 2002

## Dissemination of scientific culture

- Castro, Margarida "Exploração e conservação dos ecossistemas. É possível a conciliação?" Centro de Ciência Viva do Algarve, 23 Novembro de 2002.
- Cruzeiro-Hansson, L. Os Ingredientes da Vida, no Centro de Ciência Viva, Faro, Portugal, 7 de Julho.
- Cruzeiro-Hansson, L. Os Ingredientes da Vida, no Centro de Ciência Viva, Faro, Portugal, 24 de Novembro.
- Participation in the "NW Seamounts 2002 (Cape Verde) Expedition" in close cooperation with the Atlantic Wildlife Association. This activity included a scientific dissemination program through newspaper articles, television documentaries and multimedia exposition. (Jorge Gonçalves, Rita Castilho, Pedro Monteiro, Daniel Ribeiro, Regina Cunha, Carlos Afonso, António Malaquias).
- Project "Observatório de um Ecossistema" (Observation of an Ecosystem). Projecto Ciência Viva Ref. (PV-0356)
- Financed by: Programa Operacional Ciência, Tecnologia, Inovação (POCTI), (670 500\$00)
- Participants: Escola EB1 de Alto Rodes e F.C.M.A, University of Algarve.
- Responsible: Teresa Cerveira Borges
- Duration: (20 months) 2001-2003

## Visiting scientists

- Bishop, Tina & Tuddenham, Peter – College of Exploration, USA. Objective: Planning and discussions with UAlg and other institutions, like Zoomarine and Ciência Viva, for planning future cooperation on education aspects of Programme SEMMAP (August 2002) (contact person in CCMAR: T.C.Borges)
- Cooper, Richard & Cooper, Christopher – Ocean Technology Foundation and University of Connecticut, USA. Objectives: Campaign 2002 off Portimão for diving in specific sites identified from before. Planning and discussions with UAlg and other institutions, like Zoomarine and Ciência Viva, for planning future cooperation on education aspects of Programme SEMMAP (August 2002) (contact person in CCMAR: T.C.Borges)
- Cruz, Raul, 1-30 September 2002 –University of Havana, Cuba. Collaboration with Dr. M. Castro to construct and set lobster larvae collectors. During his stay Dr. Cruz supervised the construction and testing of a prototype adapted from a model used with the same objective in tropical areas.
- Harris, Robert - 1 July to 31 August 2002 –, University of Leicester, UK. Collaboration with Dr. M. Castro with the objective of studying the impact of trawling on non-target invertebrate species. Field and laboratory experiments were conducted during this period.
- Roland Schule. University of Friburg. Germany. Research collaboration within a bilateral cooperation project with L. Cancela. FCT-BCC. December 2002
- Sarasquete, M.Carmen CSIC.Cadiz/ICMAN, Spain. Research collaboration within a bilateral cooperation with L. Cancela, project CSIC/ICCTI. April e December 2002.
- Santos, Jorge – Norwegian Fisheries College (NFH), University of Tromsøe, Norway. Visit for 3 months to work data for project Bydiscard. (contact person in CCMAR: T.C.Borges)

## **Organization of Conferences, workshops, courses**

XIII Congresso Nacional de Bioquímica. 5 –7 December 2002, Lisboa (L.Cancela member of the Scientific Committee and organizer of Symposium S11 on Gene Expression and Cell Differentiation)

## Financial Report