Centro de Ciências do Mar do Algarve
Algarve Centre of Marine Sciences
Annual Report
2001

Relatório sumário

O Centro de Ciências do Mar continuou a crescer do recrutamento de pós-doutorados e da finalização de doutoramentos. Muitos dos investigadores pós-doutorados são de nacionalidade estrangeira, o que é um reconhecimento da posição do CCMAR na ciência internacional. O número total de membros, incluindo estudantes pós-graduados e técnicos de laboratório, é agora 145.

A produtividade continuou a subir sendo agora de aproximadamente 1 artigo SCI por ano por investigador doutorado. No entanto, o espaço de laboratório e de gabinetes está agora para além do estado crítico contribuindo para um clima de frustração e de que o trabalho feito não e reconhecido pela instituição de acolhimento.

A despesa em investigação atingiu o valor recorde de 1 670 000 euro dos quais 390 000 euro foram gastos em equipamento e a maior parte do restante em consumíveis. Quarenta por cento dos gastos foram de financiamento europeu, um pequeno decréscimo em proporção relativamente a anos recentes reflectindo maior competição internacional, sendo possivelmente uma causa de preocupação sobre o 6º Programa Quadro que se aproxima..

Tal como em anos anteriores o CCMAR despendeu a maior parte do Financiamento Plurianual na participação em conferências, aquisição de pequenos equipamentos e consumíveis. O Financiamento Estratégico foi gasto para aquisição de equipamento maior e em suporte administrativo.

Summary report

The Center of Marine Sciences continued to grow from recruitment of post doctoral researchers and PhD completions. Many of the post doctoral researchers are of nationalities other than Portuguese, recognition of the position of CCMAR in international science. The total number of members, including postgraduate students and technicians is now 145.

Productivity has continued to rise now at 1 SCI article per year per researcher, but laboratory and office space is now beyond the critical stage and is a large contributor to general frustration and feeling that the work being done is not recognized by the hosting institution.

Expenditure reached an all time record of 1 670 000 euro of which 390 000 euro was spent on equipment and most of the remaining on consumables. Forty percent of the expenditure was of European funding, a small decrease in proportion from recent years that reflects stronger competition internationally and is perhaps a cause for concern about the approaching 6th Framework Programme.

As in previous years CCMAR uses most of the Financiamento Plurianual for travel, purchasing of small equipment and consumables. The Strategic funding was channeled for larger equipment, and salaries for administrative support and dissemination of science in schools and to the wider public.

Publications List

Division Aquaculture and Biotecnology

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Division of Living Resources

Books, edited books, chapters of books

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Ladah L.B., Bermudez R., Serrão E.A., Pearson G.A. (2001) The effect of water motion on reproductive success of marine species with external fertilization: monoecious reproduction as an adaptation to high wave energy in *Fucus*? *Phycologia* 40(4): 124-125.

Pearson G, Viegas C, Serrão E, Cancela L. (2001) Plastid genes and gene expression in the intertidal alga *Fucus vesiculosus* L (Heterokontophyta, Phaeophyceae). *European Journal of Biochemistry*, 268, suppl 1, 216.
Pearson G, Viegas C, Serrão E, Cancela L. (2001) Plastid genes and gene expression in the intertidal alga *Fucus vesiculosus* L. *Phycologia* 40(4): 93-94.
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Alberto, F., C. Billot, L. Correia, E. Serrão. Isolation and characterisation of new microsatellite loci for three seagrass species (*Enhalus acoroides, Cymodocea nodosa* and *Posidonia oceanica*). *"Workshop on Population Genetics and Evolution of Seagrasses"*, Stazione Zoologica 'A.Dohrn', Ischia, Naples, Italy.
Ávila, S. & Malaquias, M. A. E:. Moluscos do pico Ormonde (Banco Gorringe) – Check-list e relações biogeográficas. - I Colóquio Nacional de Malacologia, Lisbon, 26-27 January 2001
Beja, P 2001. Ecology and conservation of temporary ponds in Mediterranean farmland: a case study in Southwestern Portugal. *European Temporary Ponds 2001, 23 February, Birmingham, UK.* (Invited lecture)
Billingham, M., F. Alberto, J. Ferreira, E. Serrão. Genetic structure within and among populations of *Zostera marina* in the Ria Formosa coastal lagoon, Portugal. *"Workshop on Population Genetics and Evolution of Seagrasses"*, Stazione Zoologica 'A.Dohrn', Ischia, Naples, Italy.
Castro, A. Araújo, P. Monteiro, A. Madeira and W. Silvert (2001). The Efficacy of Releasing Caught Nephrops as a Management Measure. Life Histories, Assessment and Management of Crustacean Fisheries. La Coruña (oral presentation)
Costa, M.E.; Olim, S.; Pais, C. & Borges, T.C. (2001). The importance of by-catch from commercial trawl fisheries off the south coast of Portugal. Havana, Cuba, 12-14 September.
Dias, N. & Sprung, M.: Population dynamics and production of the isopod Tylos ponticus in a Ria Formosa salt marsh. - 5th Symposium on the Biology of Terrestrial Isopods, Irakleio, Crete, 19 - 23 May 2001
Domingues, P., T. Kingston, A. Sykes & J. P. Andrade. Growth of young cuttlefish *Sepia officinalis* (Linnaeus, 1758) at the upper end of the biological distribution temperature range. (Comunicação escrita). World Aquaculture Society annual meeting (Orlando, Estados Unidos da América).
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Erzini, K.; Bentes, L.; Lino, P.G.; Ribeiro, J.; Coelho, R.; Monteiro, P.; Correia, C. and J.M.S.Gonçalves (2001). Trammel nets selectivity studies in the Algarve (southern Portugal): II. Size selectivity. ICES 2001 Annual Science Conference, Oslo, Norway, 26-30 September 2001 (Poster).
Erzini, K.; Bentes, L.; Lino, P.G.; Ribeiro, J.; Coelho, R.; Monteiro, P.; Correia, C. and J.M.S. Gonçalves (2001). Age and growth of seven sparid species of the South coast of Portugal. Tenth European Congress on Ichthyology ECI X, Prague, Czech Republic, 3-7 September 2001 (Poster).
Erzini, K.; Bentes, L.; Lino, P.G.; Ribeiro, J.; Coelho, R.; Monteiro, P.; Correia, C. and J.M.S. Gonçalves (2001) .Reproductive aspects of seven sparid fishes from the southern coast of Portugal (Algarve).Tenth European Congress on Ichthyology ECI X, Prague, Czech Republic, 3-7 September 2001 (Poster).
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Malaquias, M. A. E. & Borges, T.C. (2001). Impacto da pesca de arrasto sobre as comunidades malacológicas da costa do Algarve (Impact of the trawl fisheries on malacological communities off the Algarve coast). I Colóquio Nacional de Malacologia. Lisboa, 26-27 January (oral com.)
Malaquias, M. A. E.,K. Erzini, L. Bentes e Teresa. C. Borges (2001). Impact of commercial fisheries on the malacological communities inhabiting the continental shelf and slope of the Algarve (Southern Portugal): An assessment of the effect of two different métiers. World Congress of Malacology. University of Vienna, Austria, 19-25 Agosto (poster).

Malaquias, M. A. E.: Impacto da pesca de arrasto sobre as comunidades malacológicas da costa do Algarve. - I Colóquio Nacional de Malacologia, Lisboa, 26-27 de Janeiro 2001

Malaquias, M. A. E.: Moluscos opistobrânquios do arquipélago da Madeira. - I Colóquio Nacional de Malacologia, Lisboa, 26-27 de Janeiro 2001Costa, M.E.; Pais, C.; Olim, S. & Borges, T.C. (2001). By-catch Biodiversity from the commercial crustacean trawl fisheries in the Algarve coast. 1st International Congress on Marine Science and Technology – "Oceans III Millenium" – Oceanology and Human Development between the coastline and the continental Margin. Pontevedra, Spain, 24-29 April.

Malaquias, M.A.E., Machás, R., Condinho, S., Santos, R. & Sprung, M.: Diet assessment of Haminoea orbygniana (Férussac, 1822) (Mollusca: Opisthobranchia: Cephalaspidea): a comparative approach by direct observation, gut content and stable isotope analysis. - World Congress of Malacology, Vienna, 19 - 25 August 2001

Mata, L and Santos, R. Cultivation of *Ulva rotundata* (Ulvales, Chlorophyta) in raceways, using semi-intensive fishpond effluents: yield and biofiltration. International Seaweed Symposium, Cape Town, South Africa.

Morgado, R., Delgado, A., Gordinho, L., Reino, L., Pereira, P., Borralho, R., Beja, P. & Moreira, F. 2001. Factores ambientais que influenciam a distribiuição de calhandra-comum *Melanocorypha calandra* em pousios do Baixo Alentejo. *III Congresso de Ornitologia da Sociedade Portuguesa para o Estudo das Aves*, Castelo Branco, 1-4 Novembro de 2001.

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Santos M.N., M. B.Gaspar, C.C. Monteiro, R. Sá, L. Bentes, J.M.S. Gonçalves, P.G. Lino, J. Ribeiro and K. Erzini (2001). Comparison of long-line and monofilament gill net selectivity for hake (Merluccius merluccius) in the Algarve (southern Portugal). NAFO Symposium on Deep Sea Fisheries. Varadero, Cuba, 10-14 September 2001 (oral com.)

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Silva, P. & Beja, P. 2001. Breeding habitat of the Black-shouldered Kites (*Elanus* caeruleus) in a rural area of southern Portugal. *4th Eurasian Conference on Raptors, 25- 29 September, Sevilla, Spain* (Poster)

Sprung, M., Afonso, C.M., Coelho, T., Machado, M., Malaquias, M.A. & Morenito, P.: Succession in macrobenthic communities affected by dredging activities - a study in the Ria Formosa lagoon (S. Portugal). - 36th European Marine Biology Symposium, Maó, 17 - 22 September 2001

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Project and consultancy final reports

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Castro, M., Araújo, A. And Monteiro, P (2001). University of Algarve. Project Coordinator: F. Andrade, University of Lisbon. Impacto das rejeições ao mar na abundância dos recursos demersais de crustáceos. Relatório da participação da Universidade do Algarve. PRAXIS/2/2.1/MAR/1734/95.

Castro, M., Araújo, A., Monteiro, P. And Silvert, W. (2001) . The efficacy of releasing caught Nephrops as a management measure. CE-DG XIV/C/1/98-081.

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Santos, R. F-ECTS: feedbacks of estuarine circulation and transport of sediments on phytobenthos, MAS3-CT97-0145 (DG12 – VOMA). Final report of CCMAR participation.

Santos, R., Sprung, M., Machás, R., Aníbal, J., Dias, N., Mata, L., Vieira, V., Piedade, F., Pérez-lloréns, L., Hernández, I, Vergara, J., Peralta, G., 2001. Produção bentónica e fluxos de matéria orgânica na Ria Formosa, Algarve, Portugal, INTERREG II, 105 pp.

Sprung, M. (2001). Impacte de variação das características fisicoquímicas do sedimento na ecofisiologia de Ruditapes decussatus na Ria Formosa (ECORUDI). PRAXIS/C/BIA/14220/1998. Project financed by the Fundação para a Ciência e a Tecnologia. – October 2001

Prizes

G.F. Pappenfuss prize from the International Phycological Society, for "best presentation in ecology and physiology of algae": Pearson G, Viegas C, Serrão E, Cancela L. (2001) Plastid genes and gene expression in the intertidal alga *Fucus vesiculosus.* (International Phycological Congress, Thessaloniki, Greece).

List of thesis supervised by members of the research unit

Division of Aquaculture and Biotechnology

Theses PhD

Completed

Pinto, Idílio Jorge Matias Pereira: "Cloning of the Bone Gla protein gene from the teleost fish Sparus aurata (gilthead seabream). Molecular organization, developmental appearance and evolutionary implications". 27 July 2001. Universidade do Algarve. (supervisor Leonor Cancela).

Socorro, S. Cloning and characterization of seabream (*Sparus aurata*) estrogen receptors. Faculdade de Ciências do Mar e do Ambiente. Faro, Universidade do Algarve**:** (supervisor Adelino Canário and Deborah Power).

Freitas, Ana Maria M. S. Characterization of oils rich in essential fatty acids and preliminary assay of its potential application in fish culture. *Faculdade de Ciências do Mar e do Ambiente*, Faro: Universidade do Algarve.

Ongoing

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.

Brinca, L. Regulação por neuropeptideos 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 genetica dos receptores acoplados a proteínas-G no peixe balão.Universidade do Cambridge, UK (supervisor Deborah Power and Melody Clark). Completion expected in 2004.

Carneiro, Luis Alberto Pinto Eusebio. Androgéneos e AVT na modelação dos comportamentos sociais e reprodutivos de tilapia. . Universidade do Algarve (supervisor Adelino V.M. Canário e Rui Oliveira). Completion expected in 2004.

Coesel, Sacha. Regulation of the carotenoid biosynthetic pathway in the halotolerant alga *Dunaliella salina.* Universidade do Algarve. (supervisor João Varela). 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.

Gisela Borges *Endothelium dysfunction in microvascular diseases.* (supervisor: Josefina Coucelo). Completation expected in 2005.

Guerreiro P.M. Physiological function of calcium and PTHrP in sea bream (*Sparus aurata*). University of Nijmegan, Holland (supervisors Deborah Power and Gerit Flik). Completion expected in 2002.

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.

Natércia Joaquim. *Non invasive characterization of Halobatrachus didactylus heart morphology and function: application of echocardiography.* (supervisor: Josefina Coucelo). Completion expected in 2002.

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.

Serrano, Rui Manuel. Pheromones in the reproduction of *Salaria pavo* and *S. fluviatilis* (Pisces: Blenniidae): a comparative study. Universidade do Algarve (supervisors Eduardo N. Barata, Adelino V.M. Canário and Peter C. Hubbard). Completion expected in 2005.

Theses Master of Science

Completed

Graduation Honours thesis (Estágio de licenciatura)

Completed

Frade, P. (2001). Detecção olfactiva de conspecíficos em Tilápia, *Oreochromis mossambicus*. Universidade de Évora e Universidade do Algarve. Supervisores: Eduardo N. Barata, Peter C. Hubbard e Adelino V.M. Canário.

Freitas, Paulo (2001) *Echocardiographic characterization of heart morphology and function in marine turtles*. Supervisors: Josefina Coucelo and Natércia Joaquim in collaboration with Bernardo Nascimento, Zoomarine).

Martins, Patrícia (2001) *Angiotensin Converting Enzyme in Halobatrachus didactylus– reproductive cycle study* (supervisor: Josefina Coucelo and Natércia Joaquim).

Mendonça, Paula (2001) *Histological study of vanadate effects on cardiac tissue in Halobatrachus didactylus*. Supervisors: Josefina Coucelo, Natércia Joaquim, José Coucelo (UIC) and Manuel Aureliano (FCT, UAlg).

Soares, Sandra (2001) *Cadmium and vanadium effects on metahaemoglobin redutate activity.* supervisors: Josefina Coucelo, Natércia Joaquim and Manuel Aureliano (FCT, UAlg).

Division of Living Resources

Theses PhD

Completed

Cristo. M.L.J.B. (2001). Ecologia alimentar do lagostim. Universidade do Algarve, Faro.

Ongoing

Cabaço, S. Dinâmica populacional de Zostera noltii e sua relação com os nutrientes, na Ria Formosa"., Universidade do Algarve (supervisor, Rui Santos)

Machás, R. Fluxos de matéria orgânica na Ria Formosa", Universidade do Algarve (orientador, Rui Santos)

Padinha, C. Cycling of metals in *Spartina maritima* populations within Ria Formosa tidal lagoon Universidade do Algarve (supervisor, Rui Santos)

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.

Serafim, Maria Paula. Universidade do Algarve (supervisor Margarida Castro). Completion expected in 2006.

Silva, J. Fotobiologia dos macrófitos e algas da Ria Formosa. Universidade do Algarve (supervisor, Rui Santos)

Theses Master of Science

Completed

Monteiro, Pedro (2001). Destino das rejeições do arrasto de crustáceos da costa Algarvia. Metodologias de estudo. EMAC – University of Algarve (supervisors Margarida Castro and Karim Erzini).

Sousa, Carlos Ferreira do Carmo (2001). Ajustamento de distribuições a dados de captura de pescado. Mestrado em matemática Aplicada, Uviversidade de Évora (Supervisors C. Brauman and M. Castro)

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.

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.

Mata, L. Cultivo de macroalgas em efluentes de piscicultura. Mestrado de Aquacultura, FCMA, Universidade do Algarve.

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.

Quaresma, A. Caracterização dos gradientes de matéria orgânica e de nutrientes ao longo do canal de descarga da ETAR Faro Noroeste da Ria Formosa. Mestrado de Gestão e Conservação do Ambiente, FCMA, Universidade do Algarve.

Graduation Honours thesis (Estágio de licenciatura)

Completed

Andrade, A.C. (2001). Avaliação do efeito da marcação com marcas em T ("T-Anchor") na sobrevivência e no crescimento de juvenis de Diplodus vulgaris (Geoffrey, 1817) e avaliação da retenção da marca. Estágio de Licenciatura em Biologia Marinha e Pescas, Univ. do Algarve, Faro, 34p + anexos.

Carvalho, L. 2001. Avaliação de erros potenciais em estudos sobre a dieta de aves de rapina: os casos da águia de Bonelli (Hieraaetus fasciatus) e águia-calçada (*Hieraaetus pennatus*). Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve.

Constâncio, B. 2001. Comunidades de gastrópodes da ribeira de Quarteira: composição, estrutura e condicionantes ambientais. Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve.

Costa, M. 2001. *Efeitos da remoção da galeria ripícola na abundância, estrutura dimensional e dieta do verdemã, Cobitis paludica, numa ribeira mediterrânica*. Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve.

Cunha, Maria Regina Oliveira Lopes da (2001). Contribuição para o conhecimento da filogenia dos Pleuronectiformes. *Estágio de Licenciatura em Biologia Marinha e Pescas*, Univ. do Algarve, Faro. Orientação Rita Castilho.

Rodrigues, D. e Henriques, N. Estudo da evolução espacio-temporal de uma sucessão ecológica dunar na Península do Ancão e Ilha da Barreta (Ria Formosa). FCMA, Universidade do Algarve.

Description of the research activities

Division of Aquaculture and Biotechnology

Group Molecular Biology of Marine Organisms

Research team

Leader: Leonor Cancela

Principal researchers and post docs: Pedro M. Rodrigues, Vincent Laizé, J. P. Pinto, Laurence Elandalloussi

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

Technicians/ research assistants: Juan Bosco, Catarina Canas, António Pombinho, Ricardo Leite, Ricardo Afonso

Summary of activities and progress during 2001

Control of Gene expression and mineralization of the extracellular matrix

Main purposes:

I- to understand the pathway of tissue mineralization and its regulation during vertebrate development, in particular in fish and amphibians and the role of vitamin K dependent proteins (matrix Gla and Bone Gla proteins) in this process.
II- To study 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.
III- To understand the molecular adaptations of the mechanisms that control extracellular matrix mineralization throughout evolution.

Achieved in 2001:

1. Analysis of BGP/MGP gene expression and regulation in fish and amphibians. Comparative analysis.
2. Studies towards elucidation of 3D structure of BGP-MGP from lower vertebrates.
3. Studies towards elucidation of pathways of regulation of BGP/MGP.
4. Studies on the regulation of sea bream and sole BGP gene in adults and during larval development.

Studies towards the establishment of cell cultures from marine organisms

Main purposes:

I. To develop and optimize conditions for obtaining primary cell cultures derived from marine organisms.
II. To develop transfection conditions for the primary cells cultures suitable to direct specific gene expression in vitro.
III. To study the possibility of immortalizing these cells and obtain cell lines suitable to study control of fish and bivalve gene expression using homologous systems.

Achieved in 2000:

Invertebrates derived cells:

1. A pure culture of *Perkinsus atlanticus,* to be deposited to ATCC in 2002.

Vertebrate-derived cells:

1. Development of bone and cartilage derived cell lines from fish and amphibian and initiation of cell characterization.
2. Studies on the expression of BGP/MGP in newly developed fish and amphibian cell lines

Studies on infection of mollusk bivalves by the parasite* Perkinsus atlanticus*.

Main purposes:

I. To develop pure cultures of Perkinsus atlanticus.
II. To analyse Parasite-host interactions
III. To analyse the infection patterns and levels of bivalves by perkinsus sp along the Portuguese and Galicia coast. As part of an epidemiology study

Achieved in 2000:

1- Survey study for the first year of the project along the Portuguese coast and galicia.
2- Development of a pure culture of P. atlanticus derived from clams from Ria Formosa
3- Development of specific molecular markers suitable to differentiate infection by P. atlanticus or P. marinus.

Plan for 2002

Biotechnology and Molecular Biology of Microalgae Group

Leader - João Varela
PhD students: Nuno Henriques, Sacha Coesel

Summary of activities and progress during 2001

Biotechnology of marine microalgae

During 2001, research focused on two major questions: 1) how microalgae such as *Dunaliella salina*, regulate the carotenogenic process at the level of gene expression; and 2) what gene products are directly involved in the biosynthesis and accumulation of carotenoids in this and other algae. These two scientific questions stem from the fact that *D. salina* is able to produce high levels of carotenoids (up to 13% of its dry weight) in a short period of time.

To answer these questions we have conducted a series of experiments concerning the regulation of gene expression directly involved in the biosynthetic pathway of carotenoids in *D. salina.* Previously, we have been able to clone the genes *Psy* and *Pds* encoding phytoene synthase and phytoene desaturase, respectively. In addition, we have isolated one cDNA coding for Cbr, a gene product thought to bind to carotenoids in the chloroplast of *D. salina.* By means of Northern analysis, we have shown that the microalga *D. salina* has apparently two different responses to the usual carotenogenic triggers, osmotic stress and nutrient stress: 1) a short-term response, in which the most important factor for a rapid accumulation of carotenoids in this alga; and 2) a long-term response, in which the most important factor seems to be the combined action of osmotic and nutrient stress for a massive accumulation of carotenoids in *D. salina* cells. During the short-term response (1-5 days upon stress onset), *Psy* and *Cbr* transcripts are induced in cells exposed to osmotic stress, but not in cells exposed to nutrient stress (lack of nitrates). In contrast, *Pds* messenger RNA (mRNA) seems not to fluctuate significantly during this period upon any of the imposed stresses. However, the higher levels of *Pds* and *Cbr* transcripts upon osmotic stress do not translate in a higher accumulation rate of carotenoids in this alga, as cells under nutrient stress are those showing the highest levels in carotenoids. This discrepancy disappears if the cells are allowed to grow under osmotic and / or nutrient stress for a longer time (5-21 days). During this long-term response, cells exposed to osmotic stress seem to show higher levels of carotenoids than cell submitted to nutrient stress, and thus mirroring the observed gene expression patterns. These results suggest that the induction at the level of mRNA levels during the short-term response is only expressed phenotipically during the long-term response. This delay between gene expression induction and phenotype may be caused by the adaptation process that *D. salina* cells must undergo when exposed to hyperosmotic shocks and / or lack of nutrients, such as nitrate. It is likely that cells establish priorities, in which the response to stress (osmoregulation, membrane, protein and DNA repair) must take place before carotenogenesis is able to begin.

Although the previous work already sheds light as to which cellular components participate in the carotenogenic process in the microalga *D. salina*, other gene products are surely involved in the biosynthesis and accumulation of these compounds. With this in mind, a subtractive cDNA library was constructed and is currently being analyzed. As a first step, 100 independent clones were tested by differential dot blot analysis. This analysis has shown that around 50% of these cDNA clones seem, indeed, to be induced during the carotenogenic process. This half set of positive clones was subjected to DNA sequencing analysis at a later stage. Preliminary results demonstrated that most clones show no significant homology with known genes. However, there were a few clones that are homologous with genes involved in the stress response and / or the import of proteins into the chloroplast (e.g heat shock proteins / chaperones and ubiquitine-conjugating enzymes). These results are most significant, as most gene products involved in the carotenogenic seem to be encoded in the nucleus and imported into the chloroplast upon translation in the cytoplasm. Another clone, possessing domains consistent with a FAD-dependent oxireductase, has shown a high degree of conservation from algae to man. Interestingly, several enzymes involved in the carotenoid biosynthetic pathway of high plants and algae belong to this class of proteins.

Plan for 2002

During 2002, our group will focus on developing vectors and methods for DNA transformation of *D. salina.* A year ago, we were able to transform *Chlamydomonas reinhardtii*, a microalga belonging to the same order (*Volvocalles*) as *D. salina*. Recently, we have found a herbicide able to kill *D. salina*, so that it can be used in a selection procedure for *D. salina* transformants. In collaboration with Dr. Chris Bowler, an international authority in DNA transformation of algae, we will study different methods and test different clones for the DNA transformation of this microalga. The development of this methodology will enable us to identify the metabolic bottlenecks regulating the biosynthesis and accumulation of carotenoids by (anti-sense or sense) suppression or gene overexpression.
Concomitantly, we will continue the characterization of the subtractive cDNA library clones. A high priority will be given to a cDNA showing strong homology with a heat-shock protein involved in the import of proteins to the chloroplast in higher plants. Another priority will be the putative, highly conserved FAD-dependent oxireductase. Both cDNAs will be further characterized by the isolation of the full-length cDNA and expression analysis. Eventually, their cellular location will be determined by gene product-tagging and confocal microscopy, so that their function can be ascertained.
A third line of research will begin during this year due to the approval of a new project (OVERCAROTEN). In this project we will develop new strains producing higher levels of carotenoids by means of classic mutagenesis and mutant screening. In collaboration with INETI, Lisbon, the School of Biotechnology of Catholic University, Porto, and NECTON, S.A., Olhão, these mutants will be characterized concerning their carotenoid extractability (INETI), carotenoid profile (School of Biotechonology) and growth parameters and scale-up procedures (NECTON).
All these lines of research have been further enhanced due to the approval of yet another project (ALGINET). ALGINET is a thematic network of European-based labs interested in surveying and developing new applications for microalgae that may be benefic SMEs established in the EU. Because of our strong ties with a Portuguese SME (NECTON, S.A.), we believe that our group is well positioned for the formation of new links between our research center (CCMAR) and European-based SMEs.

Group of Comparative and Molecular Endocrinology

Research team

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Summary of activities and progress during 2001

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) and the stress response to normal physiological challenges. Recently studies on the molecular evolution of hormones and related molecules have been initiated. 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. Last year we initiated a new area of research with the objective of establishing methods to analyze endocrine disruption in the wild. Molecular tools previously developed for physiological studies form the basis of this work.

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 present 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. 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. Recently a CaSR cDNA was isolated from a sea bream kidney library and comparative analysis of the gene isolated, with that from species phylogenetically distant demonstrated that the sequence of the CaSR receptor has been highly conserved throughout evolution. This is in line with a long phylogeny of biological function for ionic calcium and mechanisms for sensing and responding to concentration variations in the extracellular environment which is essential for survival. 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 experiments to study the coordinate regulation of calcium by all the factors identified to date.

A possible interaction between cortisol and PTHrP in sea bream has been investigated in vitro and in vivo. For that purpose a homologous radioimmunoassay for sea bream PTHrP was developed. PTHrP stimulated the release of cortisol from interrenal glands isolated from control sea bream in a concentration dependent fashion. A highly negative correlation between plasma cortisol and PTHrP levels in fish submitted to various forms of stress has been found suggesting that in teleost fish PTHrP may act as a classical hormone, in addition to its paracrine functions.

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 several endocrine axis (such as the pituitary gland, thyroid gland etc) have 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 and studies of the importance of TH during embryonic development and metamorphosis are also being initiated.
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 planed 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.

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 two ERs (α and β) in sea bream and analysed their pattern of expression in different tissues and in relation to reproductive stage. During 2001 we have isolated a second ERβ (denominated ERβ2) and are proceeding with its characterization. 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. Studies to identify alternative transcripts have been initiated using standard PCR techniques and comparative analysis of gene organization.

Control of sexual determination and differentiation

Environmental conditions in fish farms have a strong influence on the differentiation of sex in seabass. However, the nature and relative importance of the factors involved are not known. Since steroid hormones (androgens and estrogens) are the ultimate mediators of phenotypic sex, environmental factors most likely will directly or indirectly influence activity and / or expression of

steroidogenic enzymes. Indirect effects would act via genes involved in the sex differentiation cascade, some of which, like steroidogenic factor-1 are known to interact with steroidogenic enzymes. The objective of the work is to clone sex determining genes and steroidogenic enzymes, putatively involved in the control of sex differentiation in sea bass. The choice of sex determining genes targeted was on the basis of their putative roles in other vertebrates. Most genes that were targeted were isolated by polymerase chain reaction (PCR). These included a fragment of 309 bp of steroidogenic factor 1, a fragment of ~500 bp of DMRT-1 and two fragments of 791 and 892 bp of 11β-hydroxylase (CYP11B). Screening of a testis library has yielded positives using the 892 bp clone of 11β-hydroxylase which are being characterized. Screening of testis and ovary libraries using the PCR products as probes is ongoing. Primers have been designed and some PCR experiments have started for DAX-1 and 17-hydroxylase (CYP17). The products are being sequenced.
The possibility that other as yet unknown genes have a role in sex differentiation in fish is being examined by a subtractive hybridisation strategy. This strategy may also yield sex markers that may be useful to determine sex at least in juveniles. A subtractive library for testis and ovary is under construction to be used to screen differentially expressed genes in the two tissues.

With the isolation of the targeted genes and eventually novel genes by subtractive hybridisation we aim to be able to establish the sex differentiation cascade of gene expression in individual fish and hopefully find a sexually dimorphic pattern of gene expression, corresponding to male and female sex differentiation. We also hope to relate the pattern of gene expression to environmental changes, namely temperature and stocking density. With this information we aim at pinpointing which genes are being affected by the environment and eventually use that information to provide methodologies for the fish farmer to control androgenesis.

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. During 2001 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. Starting from the sea bream ERβ a vector has been constructed and inserted into yeast and its expression has been confirmed. The sea bream vitellogenin and its antiserum were produced in collaboration with Dr Per-Erik Olsson from Umea University (Sweden). The ELISA for sea bream has been under development. Isolation of mullet vitellogenin has been carried out by Dr Pratap Singh who has been visiting for 6 months. The objective is to have an assay that can be applied worldwide in marine waters.

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. Knowledge of the pheromonal system(s) in this species 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). A research project has started (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) aiming at: a) establishing a procedure for rearing of *S. pavo* from egg to adult in the laboratory; b) identifying ecological factors (environmental and social) that influence reproductive strategies of *S. pavo.*

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. 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.

The sea bream (*S. aurata*) is a pelagic marine fish of high economic value for which we have collected strong evidence that pheromones play a role in reproduction. Males have an extremely high olfactory sensitivity to water and body-fluids from females, especially intestinal fluid. However, the chemical identity of the active compounds, as well as their functional significance, is unknown.

The next step is the chemical identification of the respective putative pheromones. Once this has been achieved, a number of other questions can be addressed: How is the production/release of the pheromonal components regulated? Which behavioural/physiological responses do they trigger? What are the neuroendocrine pathways involved in the recognition of the chemical signal, as well as in the response(s) that it triggers?

The freshwater cyprinid, *Tinca tinca* (the tench), is a close relative of the goldfish, and often inhabits the same habitats. Given that both species spawn at the same time of year, and under similar conditions, a key question is how do related species maintain species-specificity in their pheromonal systems (if, indeed, they do)? To address this question, we recorded the olfactory responses of male tench to goldfish pheromones. Broadly speaking, tench have similar high sensitivity to these pheromones as goldfish themselves. However, the sensitivity to various metabolites was starkly different, suggesting that it is these, or their ratio, that convey the species-specificity of the message. If true, this would show parallels to pheromonal systems in insects, and would provide an excellent example of convergent evolution.

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 α- and β-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*. We plan to investigate the physiological and/or behavioural consequences of exposure to these compounds.

The influence of humic acids in disrupting pheromonal systems in freshwater fish was also investigated using the goldfish, since in this species both the pheromonal components as well their behavioural and physiological effects are well known. Humic acids are a group of large, complex, organic molecules, which are ubiquitous components of aquatic environments as products of degradation of plant material. In aqueous solution they form microvesicles. As many teleost pheromones are steroidal in nature, and consequently barely soluble in water, we hypothesised that they would preferentially dissolve in the organic, hydrophobic core of these vesicles and therefore be unavailable for detection. Using an electrophysiological approach we showed that humic acids significantly reduce the concentration of steroidal pheromone components (17,20β-P and 17,20β-P-SO_4) available for detection by *C. auratus*, but not the more water soluble pheromone components, *i.e*. prostaglandins ($PGF_{2\alpha}$). In natural water environments, high levels of humic acids may have deleterious effects on the reproductive success of this species. Furthermore, as many teleost pheromones are steroid derivatives, this phenomenon is unlikely to be confined to this pheromone in this species, but may be applicable to chemical communication systems in teleosts in general.

Another 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.

Plan for 2002

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.

Steroid receptor expression and function

A main target tissue for analysis of ER function is the testes, where there are high levels of expression of ERβ 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 approach. Estrogen responsive genes will be isolated and sequenced, and their expression pattern analysed.
Isolation and functional analysis of the ER promoter region will be inititiated.
Isolation and analysis of androgen receptor function will be initiated.

Control of sexual determination and differentiation

1) Isolate by PCR the other target genes so as to have enough sequence to produce specific primers to be used in semi and quantitative PCR;
2) To extract larval and gonad RNA quantify gene expression in the samples from the experiment in which fish were graded according to size and age;
3) To isolate from cDNA libraries and characterize the full clones of target genes already obtained by PCR;
4) To finalize a subtractive library and isolate differentially expressed genes.

Development of biomarkers of endocrine disruption

1) To finalize the construction of the yeast assay. This involves the insertion of estrogen response elements into the yeast chromosome, work which partly completed. This will be followed by specificity and sensitivity tests using steroids and known estrogenic disrupters.
2) Initialize preparation of constructs for development of yeast assays based on the sea bream androgen and thyroid receptors.
3) Finalize setting up the ELISAs for sea bream and mullet vitellogenin. This will entail raising antiserum for mullet vitellogenin and do sensitivity and specificity tests for the assay.

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

1) Chemical identification of the putative pheromones of S. pavo, S. aurata and O. mossambicus and study the mode(s) of action of identified compounds at behavioural and physiological levels.
2) Investigate how olfactory detection of calcium and sodium in the environment influences the neural and neuroendocrine pathways regulating ion homeostasis.
3) Initiate investigations aiming at the identification of attractants associated with natural sources of food in new species for aquaculture, i.e. the Senegal sole.
4) 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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Technicians/ research assistants: Sandra Soares

Summary of activities and progress during 2001

1- Comparative cardiovascular physiology of lower vertebrates

Since 1992, we have been studying several aspects of cardiovascular physiology on a fish experimental model - the Lusitanian toadfish (*Halobatrachus didactylus)*, applying the ultrasonographic technique to the identification and characterisation of cardiac structures and functional indices (Coucelo *et al*., 1996). We adhere to our main objective to have a simple experimental cardiac model to study the different morphofunctional, environmental, pharmacological, experimental and pathological changes.

During 2001 we developed mainly the echocardiographic identification of heart structures and flows of the marine turtle *Caretta caretta* (collaboration with the zoological park Zoomarine). 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. The objective of this work was to apply ultrasound technology to identify and characterize heart chambers and intracardiac flows of the marine turtle loggerhead sea turtle (*Carreta carreta*). Ultrasound images obtained allowed identification and characterization of heart ventricle, distinguishing the *cavuns*, both right and left atrium, valvular structures and outflow vessels – aortic and pulmonary arteries. Doppler flow 2D images and spectrum allowed to define flow direction and its relation to systole and diastole intervals. This new approach is applicable and sensitive to study morphology and cardiovascular physiology of the turtle heart. Studies in development deal with non invasive hemodynamic quantification of filling and ejection flow velocity and determination of functional indices such as cardiac output. The clarification of blood flow dynamics during cardiac cycle in relation with the respiratory frequency in turtles, and particularly the role of anatomical feature such as the partial septation in minimizing and controlling the intraventricular shunt, will help to improve the intervention on some of the human congenital anomalies called "univentricular heart" (two atria, but only 1 ventricle, with, often, the outlet to both systemic and pulmonary circuits via an accessory chamber).

2- Physiopathological responses to toxic metals intoxication

We have been studying the effects of toxic metals, namely cadmium and vanadium, in several tissues of our experimental model – toadfish, with special attention to cardiac tissue. 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 cardiac muscle contraction.
During the past year, several important progress was obtained, in order to determine *in vivo* effects of these metals:

Histological analysis of tissue injury induced by vanadate oligomers on several tissues of *Halobatrachus didactylus*

The effects of an acute exposure (1 and 7 days) to a sub-lethal concentration (5 mM administered trough intraperitonial injection) of a “metavanadate” solution, containing mainly metameric species, and a “decavanadate” solution, containing mainly decameric species, were studied in cardiac, renal and hepatic tissue of *Halobatrachus didactylus* (Schneider, 1801). Samples of ventricular, renal and hepatic tissue were taken, subjected to histological procedures and stained with Haematoxylin-eosin (H&E) staining. Ventricular samples were also subjected to PicroSirius staining. H&E stained sections were examined trough light microscopy and PicroSirius stained sections trough bipolarising microscopy. The relative cardiac and ventricular masses and the area fraction occupied by the ventricular wall structural elements (collagen type I, collagen III and muscular tissue) were calculated. The results evidenced that vanadate affects the cardiac, renal and hepatic morphology of the experimental model *H. didactylus*. The studied oligomeric species of vanadate promoted evident tecidular lesions in the kidney and liver but not in cardiac tissue. However, “decavanadate” induced a dilatation of the ventricle due to a decrease in the myocardial collagen fibers percentage area. In general, “decavanadate” showed to be more toxic than “metavanadate”.

Cadmium and vanadium effects on metahaemoglobin redutate activity

The goal of this preliminary study was to analyse oxidative stress induced *in vivo* by cadmium and vanadium in *Halobatrachus didactylus* erythrocytes. A cadmium chloride solution and two vanadate solutions, “metavanadate” and “decavanadate” (5 mM), were administered intraperitoneoulsy in separated groups. After an acute exposure (1 and 7 days), several haematological parameters were determined. Cadmium and vanadium exposure promoted the reduction of haemoglobin concentration, as well as the increase of erythrocytes cellular volume. However, only decameric species promoted the reduction of erythrocytes count and hematocrit. Cadmium induced the

decrease of methemoglobin reductase activity, while decameric species exhibited an opposite effect. These vanadate species presented an IC_{50} value of 0.5 mM for the activity of this enzyme. This work demonstrated that oligovanadates have a more powerful effect causing haematological changes than cadmium and that different oligomeric species of vanadate act at distinct ways; decameric species presented a stronger effect than meta.

Angiotensin Converting Enzyme activity in *Halobatrachus didactylus*

This work was performed in order to evaluate intraspecific variation of ACE (between sexes, along the cardiac cycle) in several tissues of our experimental model. The ACE is responsible for arterial pressure regulation and will be used as a marker for endothelium dysfunction induced in physiopathological situation as metals intoxication.

Plan for 2002

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

Echocardiographic characterization of heart morphology and function of aquatic turtles (collaboration with Zoomarine and UIC)

Marine turtle have an reptilian heart with a partial divided ventricle in three cavuns and previous studies point out extraordinary functional adaptations to environmental changes such temperature and oxygen. However, the blood flow dynamics during cardiac cycle in relation with the respiratory frequency and particularly the role of anatomical feature such as the partial septation in minimizing and controlling the intraventricular shunt, is far from a clear identification. This project aims to apply echocardiography to characterise several aspects of morphology-physiology relation of the reptilian heart, having as experimental model the *Caretta caretta* heart. The population included in this project is at the zoological park Zoomarine with a temporary statute. A non invasive technique, such as echocardiography, is particularly useful to study these aspects in these endangered species protected by international legislation (Sites). This study will contribute to clarify the cardiorespiratory physiology of marine turtles and may contribute to improve the intervention on some of the human congenital anomalies called "univentricular heart".

Cardiovascular function in flatfishes: environmental influences and control mechanisms (collaboration with the Oceans Science Centre – Memorial University de Newfoundland)

Marine flatfish are a diverse and abundant group, whose biology/physiology has not been extensively studied. However, existing data suggests that there are many unique aspects of their cardiorespiratory physiology and anatomy. Flatfishes are reported to have extremely low rates of active and standard metabolism, low critical swimming speeds, one of the smallest relative ventricular masses among fishes and relatively low arterial blood pressures. However, there are several aspects of their cardiovascular physiology that do not fit this general pattern, and suggest that the hearts of these fish have an elevated pumping capacity. The significance of these physiological traits to cardiac performance in these fish, and the mechanisms that control these parameters under varied physiological or environmental conditions are unknown. The aims of this study are to develop an integrative research approach to examine complimentary aspects of cardiac physiology in flatfish:

1) Define the limits of cardiac function in flatfish;
2) Determine which nervous and endocrine mechanisms are primarily responsible for mediating changes in cardiac performance and vascular resistance;
3) Examine the effects of environmental parameters (hypoxia, temperature, exercise, domestication) on cardiac function and blood flow distribution in this group.

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, UAlg)

This project objective is to analyse oxidative stress induced by exposure to sub-lethal cadmium and vanadium concentrations, in heart ventricle of a teleost fish, Halobatrachus didactylus (toadfish) upon different concentrations and exposition periods. It will be also analysed the role of the oxidation state on toxicological vanadium effects. Toadfish will be used as an experimental model to explore oxidative stress mechanisms induced by these metals in the heart. In vitro studies will be performed to clarify the contribution of metal-protein interactions to their toxic effects. Oxidative stress is one of the main causes of tissue injury and functional failure of the heart and metal contamination may contribute significantly to physiopathological processes in this organ. This study aims to clarify mechanisms underlying the oxidative stress response induced by cadmium and vanadium on cardiac tissue; lipid peroxidation and the protective role of antioxidant enzymes on this tissue; metals targets in subcellular compartments.

Cadmium and vanadium compounds interactions with sarcoplasmic reticulum calcium pump (collaboration with FCT, UAlg)

Cadmium and vanadium are widely know for its toxic effects. However, vanadium is vestigial in muscles and other tissues and is considered an essential oligoelement. It is proposed in the present project to study the interaction of cadmium and different vanadium compounds with a membrane protein responsible for the active transport of ions across biomembranes, calcium pump from the sarcoplasmic reticulum of mammals (rabbit) and fish (toadfish). First, the proteins will be purified and characterized from their natural sources. The effects of cadmium and vanadium on the calcium pump activity, ATP hydrolysis and calcium translocation, will be analysed at different experimental conditions (pH, incubation and pseudosubstrats). The interaction of cadmium and vanadium with the calcium pump will be analysed mainly by differential scanning calorimetry and atomic absorption spectroscopy and also by UV/vis, NMR and EPR spectroscopy. The present project combines spectroscopic and biochemical kinetic studies to clarify the interaction mechanism of two atmospheric toxic pollutants on calcium pump from two different organisms. The results will allow to conclude about cadmium and vanadium interaction with the calcium pump in order to understand the role of these metals on skeletal muscle cells.

Toxic metals effects on cardiac muscle: morphological and functional analysis

The objective of this work is to analyse histopathological and functional effects induced by exposure to sub-lethal cadmium and vanadium concentrations, in heart ventricle of the toadfish, Halobatrachus didactylus upon different concentrations and exposure periods. It is also analysed the role of the oxidation state on toxicological vanadium effects. Toadfish will be used as an experimental model to explore the relation between tissue and functional alterations induced by these metals in the heart. Following the toxic metals exposition, the effects of cadmium and vanadium at tissue and functional levels, will be analysed. Several parameters will be observed: tissue characterisation of myocardium (light, polarized light and electron microscopy) as well as functional indices of the ventricle - heart rate, stroke volume, cardiac output, shortening fraction, ejection fraction, ventricular mass, systolic and diastolic flow velocities - determined by a non invasive technique, echocardiography. This study will clarify cellular injury mechanisms in a vital organ for the animal life by exploring the relation between toxicological effects of metals on cardiac tissue and cardiovascular dysfunction.

3 - Endothelial dysfunction and cardiovascular diseases

Strategically located between blood circulation and vascular smooth muscle, endothelial vascular cells have a major role on cardiovascular function, releasing several vasoactive products. Endothelium dysfunction is involved in the physiopathology of several cardiovascular diseases namely, hypertension and diabetes, where atherosclerosis occurs. On other hand, clinical and

experimental evidence have suggested that free-radical-mediated oxidative processes are involved in the pathogenesis of hypertension and diabetes. However, the mechanisms leading to endothelium dysfunction has not been examined thus far. In this context, it is our objective to study oxidative stress and endothelium dysfunction mechanisms in hypertensive and diabetic patients, and in experimental models (mouse and fish).

Biophysics Group

Leader - Leonor Cruzeiro-Hansson
Paulo Silva, PhD Student

Summary of activities and progress during 2001

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 terciary 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 termodynamic step (energy minimization) wjich 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 pairsand their dynamic and termodynamic stability.

Plan for 2002

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

Group Aquaculture

Research team

leader(s): Maria Teresa Dinis
Post-docs: Luis Conceição, Florbela Soares, Pavlos Makridis
PhD students: Laura Ribeiro, Cláudia Aragão
Technicians/ research assistants: Sofia Engrola, Pedro Cação, Ana Clara Marques

Summary of activities and progress during 2001

The research focused on sole (*Solea senegalensis*), but also in 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.

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 growth of early stages of marine fish. The effect of a low-methionine diet in growth and metabolism of *Solea senegalensis* post-larvae was evaluated. Sole post-larvae (15.1±6.4mg 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, between treatments or between fast and fed larvae in the same treatment. The average value found was 74.7 µmol O2.g-1DW.h-1, being the 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±6.99 µmol NH4+.g-1DW.h-1). In the control treatment fasted larvae had a significant higher ammonia excretion than fed larvae (15.0±3.4 µmol NH4+.g-1DW.h-1 and 9.8±1.1 µmol NH4+.g-1 DW.h-1, respectively). This suggests that sole eating the control diet catabolie more protein for energy proposes 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 sensible 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) used with good results previously was used. Sole were weaned at 20, 30, and 40 days after hatching (DAH) using a “sudden weaning” strategy. Sudden weaning before 40 DAH lead 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±8.75g) 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±1.0°C and 32.0±1.0‰. Dissolved oxygen in water was around 85±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±9.87g) and Day (31.31±12.65g) 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 ± 1.2 g) were grown in recirculated seawater (temperature: 20ºC; 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 abnormalities of sole (Solea senegalensis) larvae and post-larvae

Effect of live food enrichment with two microalgae *Chlorella* sp. and *Dunaliella* in the development of pigmentation in *Solea senegalensis* larvae. Histological and histochemical techniques were used to characterize the pigmentation development.
Influence of two immunostimulants (β-glucan and FMI) in the development of pigmentation in Sole larvae (*Solea senegalensis*). Histological and histochemical techniques were used to characterize the pigmentation development.

Histophatological studies

Two experiments were performed:
Utilization of dietary non-protein energy in Senegal sole (Solea senegalensis) juveniles: histological effect on the liver
Effects of chronic exposure to ammonia on the gill and liver of juvenile Senegal sole, *Solea senegalensis*.

Ongrowing of sole (Solea senegalensis) juveniles

With the cooperation of 3 fish farms, three groups of weaned sole were followed. Growth, as well as shape, and pigmentation abnormalities, were identified.

Microbiology of fish larvae

A first 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. A phenotypic characterisation of the collected bacteria was attempted using a large number of tests. The in vitro inhibition of two pathogenic strains (Vibrio anguillarum and Photobacterium damselae) by the candidate probiotic bacteria strains was examined using the double layer technique.
The ontogeny of the immune system in sole during the first 120 days was examined using histological techniques. The effect of two immunostimulants (β-glucan and mannuronic acid) was examined in the same rearing experiment.
Bioencapsulation of specific probiotic bacteria in live food (B. plicatilis and Artemia franciscana) was examined, as well as the stability of bioencapsulation in seawater added microalgae, in clear seawater and during cold storage. These experiments were run in collaboration with the SINTEF group of Fisheries and Aquaculture, Trondheim, Norway.

Biochemical composition of Phytobloom®, a microalgae live paste for Rotifer enrichment

The research was entirely focused on the characterization Phytobloom® a *Nannochloropsis oculata* based paste produzida pela empresa Necton. This characterization was specifically to the components, which are important for larval rearing: proteins, carbohydrates, total lipids and fatty acids (EPA, DHA and AA).
The biochemical composition of the microalgae biomass was also assessed taking into account type of preservatives and time of storage.
The use of such paste as a culture medium for Rotifers was also tested.
The microalgae biomass tested seems to be suitable to culture rotifers but their biochemical composition doesn't seem to satisfy nutritional requirements of fish larvae due to low levels of DHA/EPA.
The use of preservatives doesn't affect significantly the level of total lipids and DHA/EPA or EPA/AA ratios. Therefore the presence of preservatives will enhance the time storage of the microalgae biomass and will not affect the results in terms of enrichment.
Different time storages tested doesn't affect significantly the level of lipids, DHA, EPA or AA, so it can be assured a constant quality of the microalgae biomass.
The use of this microalgae biomass to enrich rotifers will not provide DHA to rotifers, so it can be assumed that any expected DHA present in their biochemical composition will be provided by addition of other sources of DHA.

Plan for 2002

The research during 2002 will be focus on rearing of two species, sole and grouper, as part of the research projects where the group is involved, as well the development of methodologies for enrichment rotifers and Artemia with algae paste

Sole

1 -Broodstock management: environmental reproduction parameteres, nutrition and quality of eggs and larvae:
Expected results: Identify the principal environmental and nutritional needs, which allow the manipulation of reproduction
2- Optimization of on growing
Expected results:
Development and testing of methods for ongrowing of Senegal sole in earthen ponds.
Adapt techniques developed for ongrowing seabass and seabream in earthern ponds to Senegal sole, and verify its feasability both in monoculture and in polyculture with these species
Determine the effect of grading on social behaviour, feeding behaviour and growth of Senegal sole

Grouper

1 - Catch of brood fish in the wild and production of juveniles in captivity.
Expected results: To develop broodstock management techniques by the identification of the principal needs and conditioning parameters. To describe the sexual cycle of the species and obtain artificial spawning. To characterise the embryonic period of each species.

Division of Living Resources

Group of evolutionary and molecular ecology of malcroalgae and marine plants

Research team

Leader: Ester Serrao
Post-docs: Gareth Pearson, Claire Daguin, Carolyn Engel, Lydia Ladah, Naomi Phillips
PhD students: Filipe Alberto

Technicians/ research assistants: Sara Teixeira, Carla Viegas, Vera Fonseca, Luis Correia, João Ferreira, Marta Valente, Asuncion Lago, Rafael Bermudez, Carla Monteiro

Summary of activities and progress during 2001

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 2001:
1. Development of microsatellite markers for several species of marine plants and algae, as well as endangered animal species.
2. Sampling of the relevant populations and genotyping of individuals and statistical analyses to infer genetic structure and variability and other questions that are specific for each of the populations under study.

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 2001:
1. Confirmation of upregulation of stress-responsive clones isolated in Fucus.
2. Construction of a full-length cDNA library of Fucus exposed to desiccation.
3. 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 2001:
1. Sequencing of the chloroplast genome in target species (ca. 50% complete).
2. Gene expression analysis of various photosynthesis-related genes in response to simulated low and high tide conditions.
3. Development 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, including algae, insects and fish, and to relate this with their biogeographic distribution and the evolution of other characters (e.g., physiological, reproductive, etc) in the group.
Achieved in 2001:
1. Use of rbcL spacer sequences to infer the evolution and phylogeography of fucoid algae.
2. Use of 12S rDNA sequences to infer the evolutionary history of a group of fish.
3. Study of the evolution of bone calcification proteins (BGP and MGP) in vertebrates.

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 2001:

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

Ecophysiology and local adaptation of marine algal populations

Main purpose:
To assess local adaptation of disjunct populations of brown seaweeds along a north-south geographic gradient.
Achieved in 2001:

3. Comparison of photosynthetic responses of different populations of Fucus vesiculosus, cultured under common conditions (in the absence of differential acclimation), using chlorophyll fluorescence analysis.

Plan for 2002

Group Fisheries Biology and Hydrobiology

Research team

Leader: José Pedro Andrade
Principal researchers and post docs: Pedro Domingues, Isabel A. Dias
PhD students: Eduardo Esteves, Jorge Palma
Technicians/ research assistants: Teresa Pina, Catarina Reis, António Sykes

Summary of activities and progress during 2001

Ongoing projects:
Impact of bivalve dredge fishery on juvenile flatfish in the Algarve" (refª DG XIV 99/0057).
Trophic interactions of the cuttlefish, *Sepia offcicinalis* (Cephalopoda, Sepiidae) in the Ria Formosa, Sado Estuary and Ria the Aveiro: a toll for cephalopod resource management (refª PDCTM/C/MAR/15259/1999).

Plan for 2002

Activities will be focussed on the feeding ecology and culture of cuttlefish, *Sepia officinalis*. Efforts will be made to establish cooperative research with private enterprises, which can be interested in the culture of cuttlefish in order to extend pilot-scale experiments that have been the basis of our research to semi-intensive culture.
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.

Externally funded Projects

Division of Aquaculture and Biotechnology

New and ongoing beyond 2001

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 do 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 (Oreochromic 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 avaliable 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 putatative 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 existance. 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: “Desenvolvimento de um modelo de infestação in vitro adaptado ao estudo das interacções 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.
Funding: 98,000 Euro

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: 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 eicosopentaenoic 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 ^{13}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 the 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: 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 substract) 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, Emílio Pascual, Manolo Yúfera.
Total budget: 3.500 Euro; **Funding for CCMAR**: Euro
Web site:

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.
Reference and funding entity: PDCTM/P/mar/15308/1999
Duration: October 2000-2003
Research team: CCMAR: Leonor Cancela, M. Teresa Dinis, ; Ricardo Leite -, - Instituto de Ciències Biomédicas de Abel salazar, Porto: Carlos Azevedo.
Total budget: 125.000 Euro; **Funding for CCMAR**: Euro
Web site:

Title: Development of an in vitro/in vivo infestation model suitable to study the interactions host/parasite between the clam Ruditapes decussatus and Perkinsus atlanticus
Summary and Objectives:
To develop an in vitro/in vivo infestation model suitable to study the interactions host/parasite between the clam Ruditapes decussatus and Perkinsus atlanticus
Reference and funding entity: Praxis/P/BIO/12143/1998
Duration: 2 years, starting date second semester 2001
Research team: CCMAR: Leonor Cancela, M. Teresa Dinis, ; Post doc to be hired. - Instituto de Ciências Biomédicas de Abel Salazar, Porto: Carlos Azevedo.
Total budget: 98.582 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 disrupters 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

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 Cientificas – 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: 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 startegies, 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: 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 comission
Duration: 11/2001-6/2004
Research team:

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

Completed 2001

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
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 trough 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 (policlonal antobody anti-IgM) and the immune system organogenesis (histology) will be characterised. Candidate Probionts 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 probionts 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}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:

Title: New technologies for the controlled administration of hormones for spawning induction in breams: period of administration and quality of eggs and larvae (Praxis 3/3.2/AQ/2020/95).
Summary and Objectives: The main objective is to develop a technology for coontrolled release of ovulation-inducing hormones to be used to induce spawning in sharp snout bream, Diplodus puntazzo, a autochtonous species of high commercial value. Another objective is to obtain the bioeconomic data for the cultivation of sharp snout bream based on an annual study of growth of larvae and juveniles obtained by induced spawning. It is expected to obtain data on growth, food consumption, conversion efficiency and survival which will enable the evaluation of the potential for industrial cultivation of this species. The slow delivery devices to test include polimers, resin implants, and osmotic pumps some of which not yet tested in fishes. The optimum dosages and timing of admistration in relation to gonadal maturity will be studied. The criteria for efficacy will be: i) elevation in levels of sex hormones, ii) volume of spawn(eggs and spermatozoa), iii) percentage of fertilization and biochemical composition of eggs and larvae, iv) survival, fitness and absence of developmental abnormalities and v) larval growth rate.
Duration: 07/1998 to 06/2001
Reference and funding entity: PT National Science Foundation / PRAXIS
Research team: Adelino V. M. Canário (CCMAR), Pedro Pousão (coordinator - IPIMAR), Pedro Ré, Orlando Luís (Guia Marine Laboratory, Lisboa).
Total budget: 45.000 euro **Funding for CCMAR:**
Web site:

Title: Sex pheromone in *Salaria pavo* (Pisces: Blenniidae): Behavioural assays, chemical identification and source of production/release.
Summary and Objectives: 1. The development of a behavioural assay to test the existence of a pheromone released by parental males that attracts ovulated females at a distance, and that the putative pheromone is produced by the anal gland.

2. The development of a behavioural assay to determine whether at close distance visual and chemical signals from parental males combine to elicit female sexual behaviour or only one of these characteristics is sufficient.

3. To test the behavioural effect of 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) and 17,20β-P conjugates on ovulated females.

4. Determine whether 17,20β-P and 17,20β-P conjugates found in the water holding parental males are produced/released by the anal gland or by another organ (e.g. testis).
Duration: 03/1999 to 02/2001
Reference and funding entity: Fundação de Ciência e Tecnologia - PRAXIS XXI
Research team: Eduardo Barata (coordinator) and Adelino V. M. Canário.
Total budget: 29.000 euro **Funding for CCMAR:**
Web site:

Title: Optimisation of the olfactory stimulus of teleosts by sexual pheromones according to chemical characteristics of the environment (Praxis/3/3.2/AQ/2014/95).
Summary and Objectives: Pheromones are substances that are excreted to the outside by an individual and are received by a second individual of the same species in which they receive a

specific reaction, for example a definitive behaviour or developmental process. Teleost fish have a highly developed and sensitive chemosensory and developmental system, but the inherent chemical complexity of natural waters can interfere with this signalling system decreasing its efficiency. The purpose of the project is to study the influence of the chemical characteristics of water on the olfactory stimulus in teleosts caused by pheromones. Goldfish is used as a model for three reasons: i) the biology is well known; ii) it is the fish species where there is more detail on the role and mechanism of action of hormonal pheromones; iii) it is readily available from fish suppliers.

Duration: 05/1998 to 04/2001

Reference and funding entity: PT National Science Foundation / PRAXIS.

Research team: Adelino V. M. Canário, Eduardo Barata and Peter Hubbard (CCMAR), Eurico Melo (coordinator - Instituto de Tecnologia Química e Biológica).

Total budget: 30.000 euro **Funding for CCMAR:**

Web site:

Division of Living Resources

New and Ongoing beyond 2001

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
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.ualg.pt/~rcastil/Flat.htm

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: "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-UAlg: 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-UAlg: 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-UAlg: 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: " 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: Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (southern Portugal)
Summary and Objectives: This two-year project will focus 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 are being sampled on a monthly basis using a 25 m beach seine. In addition, beam trawl, push nets and lift nets are being used to sample specific habitats and a 50 m beach seine is being used to provide data for a comparative study. This project will also permit 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 are also being carried out in order to study spatial and temporal dynamics. This project is currently 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: 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: 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: "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:
To implement a database system with all information from previous projects;
To continue to identify and quantify the by-catch and discards from the main fishing métiers off the Algarve coast;
To continue to study biology aspects of species with low or no commercial value;
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;
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 Tromsoe, 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:

Completed in 2001

Title: ECODREDGE Evaluation and improvement of shellfish dredge design and fishing effort to technical conservation measures and environmental impact
Summary and Objectives: Dredge fishing involves potential disturbance and damage on target bivalve species, non-target species, on the sediment structure and water column characteristics. The amount of the impacts depends on the dredge design. How will the ecosystem be affected by dredging, how disturbance factors will interact and how long thus it take until ecosystem recovers are the aims of this project. Biological, chemical and physical characteristics of the sediment will be analysed prior and after dredge activity. The effect of dredging will be assessed on captured and non-captured organisms, both of target and non-target species. Direct and indirect mortality will be assessed. Effect on bivalve condition and survival rates will be determined. Short, medium and long term changes in ecosystem will be analysed based on benthic, sediment and water analysis.
Duration: 01-12-1998 to 01-12-2001
Funded by: EC- DGXII

Research team: Bill Lart (PI - Seafish UK), Luís Chícharo (PI-U.Algarve, Portugal), Alexandra Chícharo (U.Algarve), Filipe Alves (U.Algarve), João Táta (U.Algarve), Patrick Berthou (IFREMER, France), Gavin Burnell (U. College, Cork), Miguel Gaspar (IPIMAR, Portugal), Hugh Allen (Mallaig & North West Fishermen Association), Ross Campbell (Mallaig Marine World), Peter Allen (Setech), Loriano Ballarin (U. Padova, Italy), Cristina Nasci (CNR, Italy), Andy Brand (U. Liverpool, UK)
Total budget: 1.373.000 euro **Funding for CCMAR:**
Web site:

Title: VALPEG - Guadiana estuary resources valorisation.
Summary and Objectives: Analysis of the abundance and distribution of bivalve species at the estuary and identification of their potential to fisheries. Potential growth of cultivated oysters for commercial use will be analysed. Analysis of the importance of the estuary as a nursery place for fish larvae using RNA/DNA condition index. Bivalve species composition and abundance are analysed in several locations of the estuary. Structures for oyster cultivation are placed in different places of the estuary. Growth of oysters at different densities is determined. Planktonic fish larvae are collected with a plankton 500 µm mesh net. Fish larvae condition is assessed using RNA/DNA ratios determined by spectofluorimetric technique.
Duration: 01-1-1999 to 01-7-2001
Funded by: ODIANA Program
Research team: Luís Chícharo(PI), Alexandra Chícharo (PI), Ana Pereira, Ana Amaral, Carla Graça, Luís Alves (U.Algarve, Portugal)
Total budget: 132.000 euro **Funding for CCMAR:**
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-phisiology, 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 31.01.2001
Funded by: FCT, PRAXIS XXI
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: FANTARED1 A study to identify, quantify and ameliorate the impacts of gear lost at sea.Project ID: FAIR-PL98-4338
Summary and Objectives: This project is a continuation of a preliminary project on ghost fishing financed by the European Union. The main objectives are to quantify gear loss, the reasons for gear loss, the impact of lost gear on living resources, and to investigate ways of mitigating these negative effects. Underwater surveys; Questionnaires; Experiments with lost gear in the field : evolution, catches, colonisation; Ageing studies based on colonisation of lost gear; Studies of mitigating measures (gear construction, materials, escape hatches)
Funded by: European Union, DG XIV, FAIR Programme

Duration: 3 years
Research team: Karim Erzini (coordinator) , J.M.S. Gonçalves, J. Ribeiro; Institution coordinating the project: The Sea Fish Industry Authority, UK; Other participating institutions: Instituto Tecnológico Pesquero y Alimentario (AZTI), Spain, Institute of Marine Research (IMR), Sweden, Institute of Marine Research (IMR), Norway, Instituto Português de Investigação Maritima (IPIMAR), Portugal, IFREMER, France
Total budget: 900.000 Keuro **Funding for CCMAR:** 114.000 euro
Web site:

Title: TRAMMEL Trammel net selectivity studies in the Algarve (southern Portugal), Gulf of Cadiz (Spain), Basque Country (Spain) and Cyclades Islands (Greece). Project ID: DG XIV/C1/ Nº 98/014
Objectives: To evaluate catch composition, catch rates, and species and size selectivity of trammel nets in Portugal, Spain, and Greece.
Summary and Objectives: Characterisation of trammel net métiers in each country; Selectivity trials over a one year period with 6 mesh sizes; Fitting of selectivity curves and data analysis; Evaluation of implications for management of artisanal fisheries
Funded by: European Union, DG XIV, Biological Studies Programme
Duration: 2 years, April 1, 1999 to March 31, 2001
Research Team: Karim Erzini, L. Bentes, M. Oliveira; Institution coordinating the project: CCMAR; Other participating institutions: Aristotle University, Greece, University of Cadiz, Spain, Instituto Tecnológico Pesquero y Alimentario (AZTI), Spain, ANAS, Spain
Total budget: 539.230 euro **Funding for CCMAR:** 147.570
Web site:

Title: SPARIDAE Fisheries biology and assessment of demersal species (Sparidae) from the south of Portugal DG XIV/C/1 Nº 98/082
Summary and Objectives: To obtain the parameters necessary for stock assessment. This will involve age and growth studies, studies of reproduction and maturity, and estimation of parameters (growth, mortality, size at maturity). Yield per recruit models will be used for assessment of 8 to 10 sea bream species. Sampling of catches from artisanal fleet; Sampling of juveniles in the lagoon; Laboratory studies: age and growth, maturity; Estimation of population dynamics parameters; Application of yield per recruit models
Funded by: European Union, DG XIV
Duration: 2 years, April 1, 1999 to March 31, 2001
Research team: Karim Erzini (ccordinator), P.G. Lino, C. Correia
Total budget: 89.740 euro **Funding for CCMAR:** 89.740 euro
Web site:

Title: SURVIVAL - The efficacy of releasing caught Nephrops as a management measure
Summary and Objectives: To evaluate survival rates of discarded Norway lobster off the Portuguese coast and to evaluate the usefulness of introducing measures requiring release of this species for the recovery of this resource. Norway lobster specimens will be collect on board of crustacean trawlers, during regular fishing activity, and placed in cages in the area where in principle they should have fallen is released back to the sea. Survival is determined several days later by retrieving the cages. This experiment will be repeated to understand variability of survival rates with depth, season, water temperatures and handling conditions on board. The estimated survival rates (and its variability) will be used in simulated populations to understand the effect a release measure would have on the structure of the population and on its reproductive success.
Duration: 24 months (1 Mar 1999 to 28 Feb 2001)
Funded by: European Community, DG XIV (DG-XIV/C2/98/081).
Research team: Margarida Castro (coordinator), Artur Araújo (research assistant).
Total budget: 126.790 euro **Funding for CCMAR:**
Web site:

Title: Offshore Oceanography of Cape Saint Vincent: Upwelling and Productivity
Summary and Objectives: Characterisation of upwelling conditions close to the Cape Saint Vincent and their importance for primary and secondary producers (including filter feeding bivalves) in this region. Repeated registration of temperature profiles during an annual cycle at 2 stations east and west of Cape Saint Vincent; sampling of phyto- and zooplankton in weekly intervals close to the Cape; registration of shell and tissue growth of bivalves (mussels and oysters) on long lines under different conditions of upwelling
Duration: 03/1998 to 02/2001
Funded by: Fundação para as Ciencias e a Tecnologia; PRAXIS XXI
Research team: Martin Sprung (CCMAR) and other members of the University of Algarve (general coordination: Maria João Bebianno), Royal Holloway, Queen's University, School of Ocean Sciences, Sagrimarisco (Aquaculture enterprise)
Total budget: 75.000 Euro **Funding for CCMAR:**
Web site:

Title: F-ECTS: feed-backs of estuarine circulation and transport of sediments on phytobenthos
Summary and Objectives: F-ECTS main focus is the interdisciplinary investigation of the ecosystem loops in estuarine environments involving phytobentos communities, hydrodynamics, nutrient cycling and sediment transport. The Lagoon of Venice (Italy) will be considered as a pilot case study. Two major seasonal field campaigns will be carried out and will allow the parameterisation of the main physical and biological processes of the ecosystem, providing a specific background for the assessment of the exportability of the obtained results in other two different European estuarine ecosystems: Laguna della Ria Formosa (Portugal) and Roskilde Fjord (Denmark). Based on the parameterised biophysical interactions, the modelling activities within F-ECTS will enable the set up of linked modules for the simulation of the feed-back loop between the physical processes and the phytobenthic habitat. This loop controlling the survival and evolution of an estuarine ecosystem will be considered from the biological perspective. To accomplish this, biological, hydrodynamic and sediment transport processes will be modelled together in F-ECTS. In particular a new SPM-phytobenthos-reaction model for cohesive sediment and estuarine ecosystems will be developed and used as a common module to which different hydrodynamic models tailored for each specific case study site can be coupled. To demonstrate how the joint exploitation of the field measurements and the model outputs provide support to the production of new "environmental information" on the estuarine territories, GIS-based tools will be developed and implemented in the context of the pilot case study in a way that such new tools can be easily exported to other sites.
Duration: 04/1998 to 03/2001
Funded by: MAS3-CT97-0145 (DG12 – VOMA)
Research team (Portugal): Rui Santos, João Silva e Susana Cabaço (CCMAR), João Alveirinho Dias e Óscar Ferreira.
Funding: 95.000 Euro **Funding for CCMAR:**
Web site:

Title: Reintroduction of ospreys (Pandion haliaetus) to the Portuguese coast.
Summary and Objectives: The project aims at restoring a breeding osprey population on the southwest coast of Portugal, within the boundaries of the Parque Natural do Sudoeste Alentejano e Costa Vicentina. This is a wildlife conservation project, but it also includes a strong research component. The project is still pending, but there was a a preparatory component in 1998. The project includes the annual translocation of about 12 young ospreys, from suitable donor populations in Finland, Corsica and Baleares to the Portuguese coast, where they will be raised and released following well-established hacking techniques. The released birds will be fitted with VHF and satellite telemetry transmitters, giving an unique opportunity to analyse several aspects of osprey biology such as migration patterns, range development, ontogeny of hunting behaviour, etc.
Duration: 5 years

Funded by: Instituto da Conservação da Natureza, ENVIREG, and others
Research team: Luís Palma and Pedro Beja; Other participating institutions: Zoological Museum of the University of Helsinki (Finland), Highland Foundation for Wildlife (Scotland), University of Greifswalsd (Germany), Estacion Biologica de Doñana (Spain), Parc Naturel Regional de Corse (France), Dirección General de Conservación de la Naturaleza de Baleares (Spain).
Total budget: In discussion **Funding for CCMAR:**
Web site:

Cooperações interinstitucionais e internacionais

Division of Aquaculture and Biotechnology

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: Amino acids requirements in diets for larvae and post larvae of *Solea senegalensis*"
Summary and Objectives: Cultivation of fish relies largely on nutrition and feeding, and concerning the larval development these strategies are based on live food, as quality of live food is generaly considered to influence the later acceptance of artificial diets. Protein is the most expensive component in fish diets and then fish culturists try to reduce protein conten in diets in order to reduce costs, but maintaining growth rates.
This project aims to identify the dietary needs in terms of essential amino acids in larvae and post-larvae in *Solea senegalensis* a very interesting new species for aquaculture for the Mediterranean area.
The effect of different amino acid profiles will be analysed on the intermediary metabolic enzymes involved in amino acid metabolism. Besides the determination of indispensable amino acid requirements profile, attempts will be made to determine the quantitative requirement of at least one indicator amino acid, such as lysine, using semi-purified diets.
The activities of enzymes involved in amino acid catabolism (Glutamate dehydrogenase (GDH) and Glutamate oxaloacetate transaminase (GOT)), Glycolysis (Phosphofructokinase (PFK) and/or Hexokinase (HK)), Gluconeogenesis (Fructose biphosphatase (FBPase)), Oxidative catabolism: Citrate synthase (CS), Lipogenesis: Acetyl CoA-carboxylase (ACC) and fatty acid synthetase (glucose and phosphatase dehydrogenase), will be measured
Reference and funding entity: Convénio ICCTI/ IFREMER
Duration: Jan 2000 – Dez 2001
Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Claudia Aragão; IFREMER/ INRA :Sadasivam Kaushik, Jeanine Bregne
Total budget: 1000 Euro; **Funding for CCMAR**: Euro

Title: "Monitoring and Management of European Seagrass Beds (M&MS)".
Funding institution: EU- EnvSD (EVK3-CT-2000-00044).
Duration: Feb 2001-Aug 2005.
Objectes/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-UAlg (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

Division of Living Resources

Acção integrada financed by the CRUP with the Waddensea Station of the Alfred-Wegner-Institut List/Sylt, Germany on the epifauna of seagrass beds (for 2001 and 2002)

MacKenzie, Ken — University of Aberdeen. Zoology Department. 2 weeks for teaching proceedures on the use of gastrointestinal parasites as tags. Financial support given by ICCTI/British Council.

Cooper, Christopher – Ocean Technology Foundation, USA. 2001. Objective: Planning and discussions on the campaigns of Programme SEMMAP. Campaign 2000 and 2001 off Portimão on board the R/V "Donax" from IPIMAR and "Andrómeda" from Instituto Hidrográfico, respectively. (contact person in CCMAR: T.C.Borges)

Babb, Ivar – National Underwater Research Centre, University of Connecticut, USA. 2001. Campaign 2000 and 2001 off Portimão on board the R/V "Donax" from IPIMAR and "Andrómeda" from Instituto Hidrográfico, respectively. (contact person in CCMAR: T.C.Borges)

Frew, Charles – Asiatic Marine, Limited. 2001. Seminar "Shark: Evil fish or king of the sea". 1 October, University of Algarve. Also, meetings aiming future research collaboration. (contact person in CCMAR: T.C.Borges)

CCMAR Seminar programme

Borges, Teresa. (2001). Center of Marine Sciences. Science, Education and Marine Archeology in Portugal Programme (SEMAPP). March, 19th.

Coesel, Sacha and João Varela. (2001). Center of Marine Sciences. Carotenoid Biosynthesis in Microalgae: Molecular Biology and Biotechnological Applications. February, 21st,

Cunha, Alexandra. (2001). Center of marine Sciences. Influence of landscape patterns on spatial dynamics of larval fish in two coastal plain rivers in the southern United States. February, 19th.

Daguin, Claire (2001). Center of Marine Sciences. Phylogeography of the smooth-shelled mussels from the Mytilus edulis species complex. November, 20th.

Deloffre, Laurence. (2001). Center of Marine Sciences. Some factors implicated in the osmoregulation of the leech Theromyzon tessulatum. October, 15th.

Ducheyne, Els. (2001). Laboratory of Forest Management and Spatial Information Technique, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Belgium. Integration of Genetic Algorithms and Geographic Information Systems for forest management. October, 11th.

Ellandoussi, Melle L. (2001). Characterisation of the ATPase activity and study of the chloroquine accumulation properties of purified Plasmodium falciparum plasma membranes. February, 23th.

Fuentes, Xoan. (2001). Center of Marine Sciences. Drinking like a fish. April, 23rd.

Goodfellow, Brian (2001). Structural studies of proteins with Nuclear Magnetic Ressonance. Department of Chemistry, University of Aveiro. November, 5th.Makridis, Pavlos. (2001). Center of Marine Sciences. A method for microbial control in cultures of marine fish larvae. May, 21st.

Marie, Pierre J. (2001). CNRS Research Director, Paris, France. Human osteogenesis: role of FGF / FGFR2 and Twist. July, 26th.

Pinto, Jorge. (2001). Bone Gla Protein in Fish: Molecular Cloning, Expression and Evolution. June, 18th.

Seminars given by CCMAR members in other institutions

Cruzeiro-Hansson, L. How do proteins work?, at the department of Physics of Instituto Superior Técnico, Technical University of Lisbon, Lisbon, Portugal, 25 May.

Cruzeiro-Hansson, L. Protein folding: funnels or multifunnels?, at the department of mathematics, Heriot-Watt University, Edinburgh, Scotland, UK, 31 de Janeiro.

Cancela, Leonor, "Alteraciones del Esqueleto durante el desarrollo larvario en peces: técnicas moleculares e histológicas. " 3 de Abril de 2001. VI Curso de Patologia e Ecotoxicologia Marinas. Cadiz, Espanha. Realizado de 2 a 6 de Abril de 2001

Cancela, Leonor, "Matrix Gla protein and inhibition of calcification: a new function for an old protein. " 20 de Outubro de 2001. Centro de Neurociências de Coimbra, IBILI

Castilho, R. "Biodiversity and Conservation group: ongoing research projects". Museo Nacional de Ciencias Naturales, Madrid, Spain, Outubro 2001.

Cruzeiro-Hansson, L. 2001. Como funcionam as proteínas? no Departamento de Física da Universidade do Porto, 9 de Fevereiro.

Dissemination of scientific culture

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.

Participação no programa da Ciência Viva, tendo recebido três jovens durante o verão de 2000

Projecto de marcação de peixes na Ria Formosa. Folheto de Divulgação nº 7, Centro de Ciências do Mar

Visiting scientists

Geenen, Sophie – University of Antwerp, Belgium. 1 month for running protein electrophoresis of gastropods in the Biodiversity and Conservation Laboratory under supervision of Rita Castilho.

M.Carmen Sarasquete. CSIC.cadiz/ICMAN, Spain. Research collaboration within a bilateral cooperation project CSIC/ICCTI. Abril e Setembro de 2001.

Matzen da Silva, J. (graduate student) – Universidade dos Açores. 1 week for phylogenetic data treatment under supervision of Rita Castilho.Carlos Duarte and Susana Augusti, CSIC, Spain. Research collaboration within a bilateral cooperation project CSIC/ICCTI. July 2001

Myriam Valero and Christophe Destombes, Universite de Lille, France. Research collaboration within a bilateral cooperation project CNRS/ICCTI. April 2001

Organization of Conferences, workshops, courses

4th Southern European School on Physics in Medicine, que teve lugar na Universidade do Algarve de 1-12 Setembro, 2001 (L. Cruzeiro-Hansson, Membro da Comissão Científica e da Organização local)

ICES Working Group on Cephalopod Fisheries and Life History. 28-31 March 2001, F.C.M.A., University of Algarve. 28 participants from 9 countries (organizer: T.C.Borges).

"Modelling: Approaches and Attitudes" by Silvert, William, IPIMAR. 5-9 November 2001, F.C.M.A., University of Algarve. Intensive course to discuss modelling, describing different approaches and methodologies, and focussing on how models are to be used and implemented. The target audience was for advance science students and researchers in the fields of fisheries and marine ecology. 20 participants (organizer: T.C.Borges).

II Forum Internacional de Jovens Investigadores, realizado na Universidade do Algarve de 7 a 11 de Abril de 2001. (L. Cancela Membro da comissão Científica).

27th Meeting of the Federation of European Biochemical Societies (FEBS). Realizado em Lisboa de 30 de Junho a 5 de Julho de 2001. (L. Cancela Membro da Mesa no Simpósio de Biologia do Desenvolvimento (Sessão S5.5).

Financial Report