Centro de Ciências do Mar do Algarve
Algarve Centre of Marine Sciences
Annual Report
2003

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Publications List

Division Aquaculture and Biotecnology

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Caetano, S., Pearson, G.A., Serrão, E.A. 2003. Evolutionary history and phylogeography of the Fucaceae (Phaeophyceae). *38th European Marine Biology Symposium*, Sept 8-12 2003, Aveiro, Portugal.

Carmona, R, Dias, L and Santos R. 2003. Why are tetrasporophytes more abundant than gametophytes in *Gelidium sesquipedale*? European Phycological Congress, Belfast, UK.

Chícharo, L, Chícharo, M. and Morais, P., (2003). Effects of Guadiana river inflow on coastal fisheries. 4th Atlantic Iberian Continental Margin, Vigo, Spain.

Engelen, A, Alves, C, Pearson, G, Serrão and Santos, R. 2003. Demography of the alien species *Sargassum muticum* at its southern European distribution limit. *European Phycological Congress*, Belfast, UK.

Engelen, A., Monteiro, C., Faustino, C., Pearson, G., Santos, R., Serrão, E. 2003. The Portuguese coast as a biogeographic boundary: demography of fucoid seaweeds at their southern European distribution limit. *38th European Marine Biology Symposium*, Sept 8-12 2003, Aveiro, Portugal.

Diekmann, O.E., J.A. Coyer, J. Ferreira, J.L. Olsen, W.T. Stam, E. Serrão. 2003. Population structure of the dwarf eelgrass *Zostera noltii* along the Iberian Peninsula: data from microsatellite analyses. *European Conference on Coastal Marine Ecosystems: A functional approach to coastal marine biodiversity.* May 11-15, 2003, Renessee, The Netherlands.

Diekmann, O.E., J.A. Coyer, J. Ferreira, J.L. Olsen, W.T. Stam, E. Serrão. 2003. Phylogeographic patterns in the dwarf eelgrass *Zostera noltii* along the Iberian Peninsula coast inferred from microsatellites. *38th European Marine Biology Symposium*, Sept 8-12 2003, Aveiro, Portugal.

Daguin, C., Caetano, S., Engel, C., Valente, M., Pearson, G.A., Serrão, E.A. 2003. Ecological and geographic differentiation of *Fucus vesiculosus* populations at the southern limit of distribution. *The third European Phycological Congress*, 21-26 July, Belfast, UK.

Daguin, C., Caetano, S., Engel, C., Valente, M., Pearson, G.A., Serrão, E.A. 2003. Ecological and geographic differentiation of *Fucus vesiculosus* populations at the southern limit of distribution. *38th European Marine Biology Symposium*, Sept 8-12 2003, Aveiro, Portugal.

Engel, C., Daguin, C., Pascal, M., Serrão, E. A. 2003. Hybridization and variation in mating system in two fucoid seaweeds. *The third European Phycological Congress*, 21-26 July, Belfast, UK.

Engel, C., Daguin, C., Pascal, M., Serrão, E. A. 2003. Hybridization and variation in mating system in two fucoid seaweeds. *38th European Marine Biology Symposium*, Sept 8-12 2003, Aveiro, Portugal.

Engelen, A, Espírito-Santo, C ,Simões, T, Santos, R, Pearson, G and Serrão, E. 2003. Timing of gamete expulsion and release in the native brown seaweed *Cystoseira humilis* and the invader *Sargassum muticum*, in southern Portugal. *Encontro Nacional da Sociedade Portuguesa de Ecologia*, Évora.

Faustino, C., Serrão, E.A., Pearson, G.A., Ladah, L. Bermudez, R. 2003. Reproductive ecology in intertidal habitats: comparing different mating strategies in fucoid algae. *38th European Marine Biology Symposium*, Sept 8-12 2003, Aveiro, Portugal.

Lago-Leston, M. A., Serrão, E. A., Pearson, G. A. 2003. Suppression-subtractive hybridization and two-step differential screening to study population differentiation in the desiccation response of intertidal brown seaweeds. *7th International Congress of Plant Molecular Biology*, June 23-28, 2003, Barcelona, Spain.

Lago-Leston, M. A., Serrão, E. A., Pearson, G. A. 2003. Suppression-subtractive hybridization and two-step differential screening to study population differentiation in the desiccation response of intertidal brown seaweeds. *The third European Phycological Congress*, 21-26 July, Belfast, UK.

Machás, R, Cabaço, S, Santos, R, Peterson, B and Kennedy, H. 2003. Effect of urban effluents on the stable isotope signals of organic matter and *Zostera noltii*. *Estuarine Research Federation Conference*, Seattle, USA.

Monteiro, C., Serrão, E.A., Pearson, G. 2003. Reproduction, recruitment and survival of hermaphroditic versus dioecious fucoid algae: *Fucus vesiculosus* L. and *Fucus spiralis* L. *38th European Marine Biology Symposium*, Sept 8-12 2003, Aveiro, Portugal.

Monteiro, P.; D. Ribeiro, J.A. Silva, J. Bispo and J.M.S. Gonçalves. 2003. Underwater surveys of ichthyofauna of the Northwest Bank of Santo Antão (Cape Verde archipelago). "*II Symposium Island Ecosystems*" 5 - 9 October 2003, Funchal, Madeira Island, Portugal. Poster presentation.

Morais, P., Chícharo, M, Chícharo L. 2003. The use of light traps to infer fish larvae retention strategies within an estuary: summer situation in the Guadiana Estuary II Plankton Symposium Vigo October 2003.

Morais,P. Chícharo, M, Faria, A., Chícharo L. 2003. The Role of the Guadiana Estuary in the Early Development of *Sardina pilchardus* (Walbam, 1792): An Hypothesis. II Plankton Symposium Vigo October 2003, Spain.

Pearson, G.A., Viegas, C.S.B., Fonseca, V., Lago-Leston, A., Serrão, E.A. 2003. Intra- and interspecific patterns of plastid gene expression and physiological responses to simulated low-tide exposure in *Fucus vesiculosus* L. and *F. serratus. The third European Phycological Congress*, 21-26 July, Belfast, UK.

Perrin, C., Caetano, S., Pearson G. A., Serrão, E. A. 2003. Genetic structure of *Fucus spiralis* populations at their southern distributional limit in relation to environment changes. *38th European Marine Biology Symposium*, Sept 8-12 2003, Aveiro, Portugal.

Ribeiro, J., L. Bentes, J. Gonçalves, P. Lino, P. Monteiro, R. Coelho, K. Erzini. 2003. Long-term changes in the fish community of the Ria Formosa lagoon (Algarve, S Portugal): a comparison based on two studies made 20 years apart. *38th EMBS European Marine Biology: Marine Biodiversity*, 8-12 September, Universidade de Aveiro, Portugal. Poster presentation.

Schuenhoff, A., Shpigel, M., Lupatsch, I., Ashkenazi, A., Msuya, F.E., Neori, A. 2003. A semi-commercial, recirculating integrated system for the culture of fish and seaweed. Aquaculture Europe, Trondheim, Norway 2003, Book of abstracts pp. 308-309

Schuenhoff, A., Mata, L., Santos, R. 2003. Integrating a new seaweed biofilter with aquaculture effluent: the tetrasporophyte of *Asparagopsis armata* (Harvey) Aquaculture Europe, Trondheim, Norway, Book of abstracts pp. 306-307.

Serrão E. 2003. Assessing genetic diversity of European Seagrass Species. *European Conference on Coastal Zone Research*, March 24 – 27, Gdansk, Poland.

Serrão, E.A., Faustino, C., Bermudez, R., Ladah, L., Pearson, G.A. 2003. Reproductive success of fucoid algae with contrasting reproductive modes: the influence of habitat. *The third European Phycological Congress*, 21-26 July, Belfast, UK.

Serrão, E.A., Pearson G.A., Kautsky L., Lifvergren T., Faustino, C. 2003. Gamete dispersal and recruitment in *Fucus vesiculosus* L. *38th European Marine Biology Symposium*, Sept 8-12, Aveiro, Portugal.

Silva, J, Delgado, R and Santos, R. 2003. Seasonal patterns of productivity in the seagrass *Zostera noltii. Encontro Nacional da Sociedade Portuguesa de Ecologia*, Évora.

Valente, M., Viegas, C. A., Serrão, E.A., Pearson, G.A. 2003. Genomic approaches to studying plastid gene expression in intertidal fucoid algae. *The third European Phycological Congress*, 21-26 July, Belfast, UK.

Veiga, P., Vieira, L., Bexiga, C., Sá, R., Erzini, K. 2003. Fish assemblages of Castro Marim salt marsh (poster). 38º *Simpósio Europeu de Biologia Marinha* (EMBS)- Aveiro, 8-12 de Setembro (poster presentation).

Wise, L, A. Malaquias, P.G. Lino, J.M.S. Gonçalves, K. Erzini. 2003 Colonisation and biofouling of lost octopus traps in the Algarve (southern Portugal). *38º Simpósio Europeu de Biologia Marinha* (EMBS)- Aveiro, 8-12 de Setembro (poster presentation).

Wirtz, Peter "Crustaceans symbiotic with Antipatharia, Gorgonaria, Actiniaria, and Bivalvia at the Cape Verde Islands". Simpósio sobre a biologia de ilhas, Funchal 5 – 10 Outubro

Wirtz, Peter "Mother species – father species: unidirectional hybridization in animals with female choice" Encontro CIIMAR Santarém, 7-8 Novembro.

Project and consultancy final reports

Cristo, M., M. Machado & J. Sala (2003). Identificação dos elementos de conservação (Fauna de Crustáceos Filópodes e Anfíbios) nos charcos temporários do Parque Natural do Vale do Guadiana e áreas limítrofes). Relatório Final do Projecto. Faro. 111 p + 6 anexos.

Prizes and Honours

List of thesis supervised by members of the research unit

Division of Aquaculture and Biotechnology

Theses PhD

Completed

Modesto, Teresa. Hormonal control of the reproductive cycle of the Lusitanian toadfish, *Halobatrachus didactylus* Universidade do Algarve. (supervisor Adelino V. M. Canário).

Ribeiro, Maria Laura. Ontogenic development of *Solea senegalensis*: digestive system and nutritional aspects. Universidade do Algarve, 23 de Junho de 2003 (supervisor Maria Teresa Dinis).

Rueda-Jasso, Rebeca. Interaction between nutrition, oxidative status and condition of *Solea* spp. post-larvae and juveniles. University of Ghent (Belgium). 12 de Novembro 2003 (Supervisors: Patrick Sorgeloos and Luis Conceição).

Simes, Dina Cristina Costa. Purification, biochemical characterization and localization at single cell resolution of Matrix Gla protein (MGP) and Bone Gla protein (BGP) in the teleost fish *Argyrosomus regius.* Universidade do Algarve. 15 de Janeiro de 2003 (supervisors Leonor Cancela).

Ongoing

Aragão, Cláudia. Determinação dos requisitos em aminoácidos na dieta das larvas e pós-larvas do linguado (*Solea senegalensis*) e da dourada (*Sparus aurata*). Universidade do Algarve (supervisors Maria Teresa Dinis and Luis E.C. Conceição). Completion expected in 2004.

Ascenso, Rita Margarida Teixeira: "Identification of *P. Atlanticus* genes differentially expressed in response to parasite-host interaction and development of an in vivo infestation system". (Supervisor: Leonor Cancela). Completion expected in 2007.

Borges, Gisela. Endothelium dysfunction in microvascular diseases. (Supervisor: Josefina Coucelo). Completation expected in 2006.

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 genética dos receptores acoplados a proteínas-G no peixe balão. Universidade do Cambridge, UK (Supervisors: Deborah Power and Melody Clark). Completion expected in 2004.

Carvalho, Suzana. Papel das Comunidades de Macrofauna Bentónica na Gestão de Tanques para Piscicultura (supervisors Maria Teresa Dinis and Luis Fonseca). Completion expected in 2005.

Coesel, Sacha. Isolation and characterization of regulatory and biosynthetic genes involved in carotenogenesis in the microalga *Dunaliella salina.* (supervisors João Varela and Chris Bowler). Completion expected in 2006.

Fagundes, Teresa Ecologia comportamental do blenídeo *Salaria pavo* na Ria Formosa: tácticas alternativas de reprodução e inversão de papéis sexuais (Supervisors Rui Oliveira and Adelino Canário). Completion expected in 2005

Frade, Pedro Alexandre. Chemical identification and function of pheromones in the reproduction of tilapia, *Oreochromis mossambicus* (Pisces: Cichlidae). Universidade do Algarve (supervisors Eduardo N. Barata, Adelino V.M. Canário and Peter C. Hubbard). Completion expected in 2005.

Gavaia, Paulo J. "Functional analysis of osteocalcin (Bone Gla protein, BGP) from bony fish during skeletal development. Universidade do Algarve (Supervisors: Leonor Cancela and Carmen Sarasquete, CSIC Cadiz, Espanha). Completion expected in 2004.

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 2004.

Mira, Sara Maria. Population genetics of an endangered species, the Bonelli's eagle (*Hieraaetus fasciatus*). (Supervisors: Leonor Cancela and Pedro Beja in Portugal; Paula Dias/CNRS – Montpellier, França). To be completed in 2005.

Morais, Sofia. The role of nutrition in the larval quality of flatfishes. (Supervisors Maria Teresa Dinis and Ivar Ronestad). Completion expected in 2005.

Pinto, Patricia Isabel Silvestre - Diversidade, expressão e mecanismo de acção do receptor de estrogénio na dourada, Sparus aurata. Universidade do Algarve (supervisor Adelino V.M. Canário). Completion expected in 2005.

Ramos, Alexandra. Isolation and characterization of the lycopene cyclase gene involved in the carotenoid biosynthetic pathway in the microalga *Dunaliella salina.* (supervisors João Varela and Bertram Brenig). Completion expected in 2007.

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

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

Soares, Sandra Sofia Ganchas. Stresse oxidativo no músculo cardíaco induzido por iões metálicos. (supervisors: Josefina Coucelo, Manuel Aureliano Alves e Carlos Gutierrez-Merino). Completation expected in 2006.

Tiago, Daniel António Martins: Role of IGF1 and IGF2 in the mineralization mechanisms using fish bone derived cell lines. (Supervisors M.L. Cancela and V. Laizé from CCMAR, MA Alves from FCT-UALG) To be completed in 2007.

Vasconcelos, Paulo. Universidade do Algarve (supervisor Miguel Gaspar, IPIMAR and Margarida Castro. Completion expected in 2005.

Theses Master of Science

Completed

Diogo, Maria de Lurdes. Qual o papel da interregnal na diferenciação sexual induzida pela temperatura na tilapia *Oreochromis mossambicus*. MSc Aquaculture, Universidade do Algarve (supervisor Adelino V M Canário).

Martins, Rute. Does the interrenal influence sex differentiation in sea bass, *Dicentrarchus labrax*? MSc Aquaculture, Universidade do Algarve (supervisor Adelino V M Canário).

Vieira, Nuno. Contribuição para o conhecimento da estrutura do gene da POMC na dourada (*Sparus aurata*). MSc Aquaculture, Universidade do Algarve (Supervisor Deborah M Power)..

Pais, Susana Isabel da Silva (2003). A regulamentação portuguesa de segurança biológica como um instrumento de gestão ambiental Mestrado em Gestão e Conservação da Natureza, Univerisdade do Algarve. (supervisor from CCMAR- Leonor Cancela)

Pita, Cristina. O perfil socio-económico de uma comunidade costeira portuguesa: a Fuseta. Mestrado em Estudos Marinhos e Costeiros, Universidade do Algarve (supervisors: Maria Teresa Dinis and Karim Erzini).

Violante, Ana. Estudo da doença de inverno em dourada (*Sparus aurata* L.) de cultivo, a sul de Portugal. Universidade do Algarve (supervisor Maria Teresa Dinis).

Ongoing

Anastassiades, George. Early weaning of sole, *Solea senegalensis*, onto microencapsulated diets, with different levels of protein hydrolysates. MSc student at the University of Ghent (Belgium). (Supervisors: Maria Teresa Dinis, Luís Conceição and Laura Ribeiro). Completion expected in 2004.

Pombinho, António Ribeiro. Effect of extracellular calcium on MGP gene expression. Mestrado em Biotecnologia, Universidade do Algarve. (Supervisors: Leonor Cancela and Vincent Laize). Completion expected in 2004/2005.

Silva, Conceição. Desenvolvimento de ferramentas interactivas para divulgação das aplicações da biotecnologia ambiental em biorremediação. MSc Biotechnology, Universidade do Algarve (co-orientação. (Supervisors: C. Rocha and M. L. Cancela CCMAR/UALG). Completion expected in 2004/2005.

Fonseca Vera. Identification of genes differentially expressed during the mineralization of fish bone-derived cell lines. Mestrado em Biotecnologia, Universidade do Algarve (Supervisors: M.L.eonor Cancela and Vincent Laizé). Completion expected in 2004/2005

Graduation Honours thesis (Estágio de licenciatura)

Completed

Mendonça, Sofia (2003) O efeito das microalgas no desenvolvimento de larvas de peixes marinhos. (Supervisors: Maria Teresa Dinis and Laura Ribeiro).

Ongoing

Alberto, João Carlos Rodrigues. (2003). Efeito do teor em proteína da dieta no crescimento de juvenis de linguado (Solea senegalensis). (Supervisors: Paulo Rema and Luís Conceição). Completion expected in 2004.

Correia, Sónia (2003) Estudo da ontogénese do esqueleto e do sistema digestivo em larvas de pargo (*Pagrus pagrus*) em condições de cultivo. (Supervisors: Maria Teresa Dinis, Luís Conceição and Florbela Soares). Completion in 2004.

Costa, Isabel. Metabolic Capacity and Stress Response of five North Atlantic Teleost species. (Supervisores: Natércia Joaquim, Josefina Coucelo (UAlg) and Kurt Gamperl (Ocean Sciences Centre, MUN, Canada)

Couto, Ana (2003). O efeito das microalgas na actividade dos enzimas digestivos de larvas de peixes marinhos (Supervisors: Maria Teresa Dinis and Laura Ribeiro). Completion expected in 2004.

Dias, Maria de Lurdes Duarte. (2003). Influência da estratégia alimentar na adaptação a alimento inerte e crescimento do linguado (*Solea senegalensis*). (Supervisors: Maria Teresa Dinis and Luís Conceição). Completion in 2004.

Ramos, Rui José Gaspar. (2003). Influência de factores zootécnicos na adaptação a alimento inerte e crescimento do linguado (*Solea senegalensis*). (Supervisors: Maria Teresa Dinis and Luís Conceição). Completion expected in 2004.

Division of Living Resources

Theses PhD

Completed

Costa, M.E. By-catch e rejeições da pesca comercial de arrasto na costa Sul de Portugal. Universidade do Algarve (supervisor Teresa Cerveira Borges)

Ongoing

Alberto, Filipe. Population genetics, clonal structure and phylogeography of the seagrass *Cymodocea nodosa* using microsatellite markers. Univ. Algarve (supervisor E. Serrão, Univ. Algarve, and C. Duarte, CSIC, Spain). Completion expected in 2004.

Amaral, A. "Ecofisiologia de *Ruditapes decussatus* na Ria Formosa" (Supervisor Luís Chícharo Co-orientação com o Doutor Uxio Labarta (CSIC – Vigo) (Espanha).

Aníbal, Jaime. Effects of the seasonal dynamics of green algae of the nutrient flux in the sediments of the Ria Formosa lagoon. Universidade do Algarve (supervisor Martin Sprung). Completion expected in 2004

Beldade, M.R.do Ó. De O. Padrões de recrutamento e estabilidade em comunidades de peixes crípticos das costas rochasas. (supervisors: Emanuel Gonçalves (ISPA) and Karim Erzini). Completion expected in 2006.

Berecibar, Estibaliz. "Global related changes in the Portuguese marine flora". Universidade do Algarve (Supervisors: Rui Santos, CCMar and Christine Maggs, Universidade de Belfast).

Billard, Emmanuelle. Evolution of reproductive strategies in four closely related brown seaweeds, *Fucus spiralis, F. vesiculosus, F. ceranoides* and *F. serratus*. Univ. Algarve and Univ. Paris VI, France. (Supervisors: Ester Serrão and Myriam Valero, CNRS, France). Completion expected in 2007.

Borges, R. “Processo de retenção do ictioplancton na costa da Arrábida (supervisors: Emanuel Gonçalves-ISPA, Alexandra Chícharo Universidade do Algarve) - Bolseiro da FCT (started in 2003).

Cabaço, S. “Population dynamics of *Zostera noltii* along a nutrient gradient”. Universidade do Algarve (supervisors: Rui Santos, CCMar and Carlos Duarte, Universidade das Ilhas Baleares).

Campos, Aida. (2003). The estimation and improvement of the selectivity in crustacean and fish trawls. (Supervisor: Karim Erzini). Completed in January 2004.

Candeias, A. “The Processes Of Feeding In The Physiological Energetics Of Coastal Meroplankton” (Supervisor Alexandra Chícharo em co-orientação com Doutor Andrew Bruce Yule - School Of Ocean Sciences, University of Wales).

Coelho, R. Biologia, dinâmica espacio-temporal, gestão e conservação de tubarões de profundidade. (Supervisor: Karim Erzini). Completion expected in 2005/2006.

Dias, Ana Natália. Ecology of the saltmarsh fauna in the Ria Formosa lagoon. Universidade do Algarve (supervisor Martin Sprung). Completion expected in 2004

Díaz-Almela, Elena. “Population dynamics and reproductive ecology of *Posidonia oceanica* (Delile)”. Univ. Illas Ballears, Spain (Supervisors: Ester Serrão and Carlos Duarte, CSIC, Espanha). Completion expected in 2005.

Esteves, Eduardo Bruno Oliveira. Recrutamento e condição larvar de savelha, *Alosa fallax fallax*, nos rios Mira e Guadiana. (Supervisor: J. Pedro Andrade).

Fonseca, P. J. M. R. da. Selectividade de redes de arrasto e emalhar na costa continental Portuguesa. (Supervisor: Karim Erzini). Completion expected in 2006.

Godinho, C. “The impact of anti-fouling paints on seagrass populations of *Zostera noltii* (Hornem., 1832) in the Ria Formosa Lagoon”. Universidade do Algarve (supervisors: Rui Santos, CCMar and Murray Brown).

Hazimn, H. Influência das variáveis oceanográficas na dinâmica populacional do espadarte, *Xiphias gladius*, no Oceano Atlânico. (Supervisor: Karim Erzini). Completion expected in 2005.

Lago-Leston, Asuncion. The Molecular Basis for Differential Stress-Tolerance in Co-Existing, Ecologically Similar Algal Species, Univ. Algarve (supervisor G. Pearson, and E. Serrão). Completion expected in 2006.

Leitão, F.M. de S.- “Contribuição dos recifes artificiais da costa Algarvia na ecologia trófica de sparídeos.” (Supervisors: Miguel Neves dos Santos (IPIMAR) and Karim Erzini). Completion expected in 2004.

Mata, Leonardo - “Estudo da fisiologia de *Falkenbergia rufolanosa* para a optimização da produção e valorização da biomassa cultivada com os efluentes de uma piscicultura.” Universidade do Algarve (Supervisor: Rui Santos).

Machás, Raquel. “The role of *Zostera noltii* on the food web of Ria Formosa”, Universidade do Algarve (supervisor: Rui Santos).

Marques, Alexandra. Population dynamics of dominant copepod species in the Ria Formosa lagoon. University of Bangor (supervisors Andy Yule and Martin Sprung). Completion expected in 2004

Mendes, J.C “Long-Term Time Series of Continuous Plankton Recorder Survey off Portuguese Coast” (Supervisor Alexandra Chícharo em co-Orientação com o Doutor Miguel Santos do IPIMAR e com o Doutor Chris Reid, do SAPHOS (Plymouth).

Morais, P. - “*Engraulis encrasicolus* (Linnaeus, 1758) population dynamics in the Guadiana estuary and adjacent coastal area” (Supervisores Alexandra Chícharo e Luis Chícharo).

Moschino, V. - “Impact of fishing activity on the morphology, physiology and biochemistry of the bivalves *Chamelea gallina* and *Tapes philippinarum* from coastal and lagoon areas of the Northern Adriatic Sea (Italy)” (Supervisor Luis Chícharo em co-orientação com a Dra. Maria Gabriella Marin da Universidade de Padova (Itália).

Pais, MC. Use and selection of habitats by non-breeding Bonelli’s eagles in southern Portugal. (supervisors Pedro Beja and Leonor Cancela). Completion expected in 2006.

Ribeiro, J. Ecologia e dinâmica da ictiofauna da Ria Formosa. Universidade do Algarve (supervisor: Karim Erzini). Completion expected in 2006.

Santana, J. - “Comparação bioeconómica das pescas no rio Tocains Amazónia-Brasil”, (Supervisor Luis Chícharo em co-orientação com o Doutor Miguel Petrere da Universidade de Pernambuco (Brasil).

Schuenhoff, Andreas - “The application of *Asparagopsis-Falkenbergia* as a commercially viable biofilter for water re-use”. Universidade do Algarve (Supervisors: Rui Santos, CCMar and James Muir, Universidade de Stirling).

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

Silva, J. Carbon acquisition and nitrogen uptake in the seagrasses of Ria Formosa. Universidade do Algarve (supervisor, Rui Santos).

Stobberup, K. Study of community structure, trophic interactions and exploitation pattern in the Cape Verde coastal ecosystem. (Supervisor: Karim Erzini).

Teodósio, J. "Dinâmica populacional e caracterização do estado fisiológico e bioquímico da ameijoa asiática Corbicula fluminea na bacia hidrográfica do rio Guadiana". (Supervisores Alexandra Chícharo e Luis Chícharo).

Valente, Marta. Environmental and functional genomics of chloroplast gene expression in *Fucus*. Univ. Algarve (Supervisors G. Pearson, E Serrão). Completion expected in 2006.

Theses Master of Science

Completed

Carvalho, Mário. Cartografia do ecosistema intermareal no Cabo Delgado, Moçambique. Univ. Algarve (supervisors: E.Serrão and T. Boski, CIMA). March 2003.

Mata, Leonardo. (2003). Cultivo da alga *Ulva rotundata* (Ulvales, Chlorophyta) em efluentes de uma piscicultura semi-intensiva: productividade e biofiltração. Mestrado de Aquacultura, Universidade do Algarve.

Oliveira, M. (2003) Contribuição para o estudo da pesca recreativa de costa em Portugal. MSc Thesis in Marine Science – Marine Resources, Instituto de Ciências Biomédicas de Abel Salazar – IPIMAR, 100p + anexos. (Supervisor: Karim Erzini).

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.

Alexandre, Ana. Impacto do marisqueio na reprodução da fanerogâmica marinha *Zostera noltii*. Univ. Algarve (supervisor: R. Santos). Completion expected in 2004.

Cruz, Joana. Induction of anti-herbivore defenses by direct amphipod consumption and water borne grazer cues in southern Portuguese red and brown marcoalgae. Univ. Algarve (supervisors: E. Serrão, M. Molis and M. Wahl, Inst. Meeresk., Kiel, Germany). Completion expected in 2004.

Barata, F. *A avifauna aquática das zonas húmidas da costa leste da Ilha Terceira – um contributo para a sua conservação*. Mestrado em Gestão e Conservação da Natureza, Universidade dos Açores, Departamento de Ciências Agrárias. Completion expected in 2002.

Bentes, L. Identifying essential fish habitat. (Supervisor: Karim Erzini) Completion expected in 2004.

Ferreira, R. Influência de parâmetros oceanográficos e pesqueiros na captura acidental de tartarugas marinhas pela pesca de espadarte nos Açores. Completion expected in 2004 Supervisor: Karim Erzini.

Gonçalves, I.A. Caracterização morfológica e histoquímica do tracto digestivo de paralarvas de *Octopus vulgaris*. (supervisor Teresa Cerveira Borges) Completion expected in 2003.

Malaquias, A.A.E. Contribuição para o conhecimento da sistemática e da ecologia do género Haminoea Turton and Kingston, 1830 (Mollusca: Gastropoda: Cephalasidea). Mestrado em Ecologia, Faculdade de Ciências e Tecnologia da Universidade de Coimbra. Completion expected in 2003

Olim, S. Biologia e morfometria de várias espécies da família Triglidae na costa Sul de Portugal. (supervisor Teresa Cerveira Borges) Completion expected in 2003.

Saldanha, H. Ghost fishing of hake gill nets in the Algarve. (Supervisors: Karim Erzini and Miguel Neves dos Santos (IPIMAR)). Completion expected in 2004.

Santos, V. A socio-economic study of a fishing community in India. (Supervisors: Karim Erzini and Maarten Bavink). Completion expected in 2004.

Vieira, V. Modelos populacionais da alga *Gelidium sesquipedale*. EMAC, Universidade do Algarve.

Graduation Honours thesis (Estágio de licenciatura)

Completed

Carvalho, G. (2003). Caracterização da ictiofauna dos esteiros e enclaves do Sapal da Ria Formosa. Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve 60p. + anexos. (Supervisors: Karim Erzini + Jorge Gonçalves).

Corado, M. (2003). Dispositivos de selectividade experimentais para a redução das pescas acessórias e das rejeições no cerco demersal. Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve 54p. + anexos. (Supervisors: Karim Erzini + Jorge Gonçalves).

Constatino, R. (2003) Impacte ambiental da ganchorra na macrofauna bentónica. Relatório de estágio da Licenciatura em Biologia Marinha e Pescas (FCMA-UALG) (Supervisor: Luis Chícharo)

Ferreira da Silva, M.A. (2003) Caracterização da macrofauna de micro-habitats intertidais da Ria Formosa. Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve 43p. + anexos. (Supervisors: Karim Erzini + Jorge Gonçalves).

Frutuoso, A. L. (2003). Characterization of wild mushroom exploitation in S. Marcos da Serra and Monchique: a study of the conditions for sustainable management. Univ. Algarve, (Supervisors: E. Serrão and T. Panagopoulos, FCT).

Machado, D. (2003). Contribuição para o estudo da Fauna Ictiológica do Estuário do Rio Arade. Estágio de Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve 62p. + anexos. (Supervisors: Karim Erzini + Jorge Gonçalves).

Morais, S.M. R. da S. (2003) Contribuição para o estudo da influência das "marambaias" numa população de tartarugas verdes, *Chelonia m*ydas L., no litoral Cearense, Brasil. (Supervisors: Luís Fonseca and Karim Erzini).

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Silva, P. (2003). Estudo da predação da âmeijoa-boa na Ria Formosa Relatório de estágio da Licenciatura em Biologia Marinha e Pescas (FCMA-UALG) (supervisor: Luis Chícharo).

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Ongoing

Afonso, Sónia. Education for sustainability. (Supervisors: E. Serrão and H. Barracosa). Univ. Algarve, Completion expected in 2004.

Barradas, Ana. Biofiltração de efluentes de aquários do Zoomarine. Universidade do Algarve. (Surpervisor: Rui Santos).

Barradas, António. Estudos de Reprodução do Carapau do Cunene (*Trachurus trecae*) nas águas de Angola. Universidade Agostinho Neto, Angola (Supervisors: Filomena Vaz-Velho from the Instituto de Investigação Marinha of Angola; Isabel Afonso-Dias, CCMar; Vitório Samuel from the Faculdade de Ciências da Universidade Agostinho Neto). Completion expected in 2004.

Delgado, Rodrigo. Metabolismo dos carbo-hidratos de reserva não estruturais de *Zostera noltii*. Universidade do Algarve. (Supervisor: Rui Santos).

Espírito Santos, Cristina. Datação de folículos pós-ovulatórios de Sardinha (*Sardina pilchardus*). Universidade do Algarve. (Supervisors: Manuel Afonso Dias from Faculdade de Ciências do Mar e do Ambiente; Isabel Afonso-Dias from CCMar; Cristina Nunes from Instituto de Investigação das Pescas e do Mar). Completion expected in 2004.

Gouveia, Licínia. Spatial genetic autocorrelation analyses of the seagrass *Cymodocea nodosa*. Univ. Algarve (supervisors: F. Alberto and E. Serrão). Completion expected in 2004.

Monteiro, Carla. Reproductive ecology of *Fucus vesiculosus* and *Fucus spiralis* in Northern Portugal. Univ. Algarve (supervisors: G. Pearson and E. Serrão). Completion expected in 2004.

Raposo, Vânia. Estudo da contribuição das macrófitas do intertidal do sapal de Castro Marim para a cadeia trófica do estuário do Guadiana. Universidade do Algarve. (Supervisor: Rui Santos)

Description of the research activities

Division of Aquaculture and Biotechnology

Group: Molecular Biology of Marine Organisms

Research team on Evolution, Differentiation and Gene Expression (EDGE)

Leader: Leonor Cancela
Visiting Scientist: Ivar Ronnestad
Researchers and post docs: Vincent Laizé, Laurence M. Elandalloussi, Jorge P. Pinto, Juan B. OrtizDelgado, Pedro M. Rodrigues, Natércia Conceição, Dina Simes, Sandra P. Marques
PhD students: Paulo Gavaia, Nuno Henriques, Sara Mira Silva, Daniel Tiago, Rita Ascenso
MSc students: António Pombinho, Vera Fonseca
Technicians/ research assistants: Carla Viegas, Ricardo Leite, Ricardo Afonso, Carla Cruz, João Fidalgo
Undergraduate students: Brian Schaff, Susana Domingues, Bruno Pardelha, Brigite Simões, Sofia Cavaco, Marta Rafael, Anabela Brito, Lionel Araújo

General description of the major objectives:
The major theme of our group research relates to study of regulatory pathways envolved in bone and cartilage cellular differentiation and molecular adaptations to physiological and environmental stress. Various aquatic organisms are used as models systems with an emphasis on fish and amphibians. Specific genes currently being used as molecular markers for bone and cartilage have been cloned from all model organisms (non-mammalian) presently in study in our laboratory and their sites of gene expression and protein accumulation identified. Functional analysis of promoter regions through construction of specific deletion mutants, site-directed mutagenesis and electrophoretic mobility has led to the identification of previously undetected DNA regulatory regions in selected genes of interest. These results will further permit the identification of the nuclear factors involved in specific gene regulation. Following the recent development of fish bone and cartilage-derived cell lines in our laboratory, major emphasis is presently directed towards understanding mechanisms involved in bone and cartilage cell differentiation, role of Gla protein in extracellular matrix mineralization and response to environmental parameters through the use of integrated genomic/proteomic approaches.

A second project was initiated more recently on host–parasite interaction using as model organism the parasite *Perkinsus atlanticus* and its natural host the clam *Ruditapes decussatus*. A clonal cell culture of *Perkinsus atlanticus* was developed and good progress was made towards initiating studies on the biology of the parasite and host-parasite interactions using an integrated approach envolving histological, molecular and cellular biology techniques. Molecular diagnostic tools were developed to detect parasite infection in host tissues and environmental factors affecting parasite growth and development. An epidemiological study was initiated three years ago to monitor the extent of the infection in the Portuguese coast and is currently focussing in the southern coastal region.

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

Detailed description of the research objectives, major achievements in 2003 and plan for 2004

1. Molecular determinants of extracellular matrix calcification

Main purposes: Research focus is primarily on i) understanding molecular pathways of tissue mineralization and its regulation in adult life and during development, using as model organisms fish (gilthead seabream and zebrafish) and amphibian (xenopus), and ii) the role of vitamin K dependent proteins (matrix Gla and Bone Gla proteins) in this process. Additional goals include iii) studies on the effect of genetic or environmental factors on alterations of the normal pattern of tissue calcification and its relation with altered expression of vitamin K-dependent proteins and iv) molecular adaptations of mechanisms that control extracellular matrix mineralization throughout evolution. In order to perform studies on bone- and cartilage-related protein function, gene expression/regulation and functional promoter analysis, it was imperative to obtain bone and cartilage derived cell lines from fish and amphibian, not available at present. For this purpose fish and amphibian derived cell lines were developed and transfection conditions for these cell lines as well as primary cells cultures were optimized in order to be able to analyse specific gene expression in vitro.

Major results in 2003 included studies on the comparison of sites of BGP/MGP gene expression and protein accumulation in adult teleost fishes and during larval development. 2) Extend studies on the functional analysis of MGP gene promoter in amphibians and fish 3) The elucidation of the 3D structure of BGP from a teleost fish with emphasis on its functional implications. Comparative studies between fish and mammalian BGPs through protein modeling analysis. 4) Identification of signal transduction pathways involved in the mechanisms of regulation of expression of BGP/MGP genes 5). Development and characterization of bone and cartilage derived cell lines from fish and amphibian. 6) Studies on environmental factors affecting bone biology

Plan for 2004:

Major expected goals include integrated multidisciplinary approaches to perform in vivo/ in vitro functional analysis of bone/cartilage specific genes through techniques of overexpression and knock down. Evolutionary studies on specific gene function. Effect of environmental parameters on bone biology.

2. Characterization of the infection of the clam Ruditapes decussatos by the parasite Perkinsus atlanticus. Studies on the biology of the parasite Perkinsus atlanticus and development of new drug therapies.

Main purposes:

In this project, we follow bivalve infection patterns and levels by Perkinsus sp along the Portuguese and Galicia coast as part of an epidemiology study. A cell culture of Perkinsus atlanticus was also developed and characterized. This allow us not only to analyze in vivo and in vitro parasite-host interactions, but also porsue the molecular characterization of specific parasite genes involved in host infection and look further into the metabolic pathways of the parasite in an effort to develop new drug therapies.

Achieved in 2003:

Major results included the validation of a molecular diagnostic assay for *P. atlanticus* and *P. marinus*, an integrated two year survey study along the Portuguese coast and Galicia using both histological and molecular techniques to detect parasite infection. In vitro screening of various drugs for therapy of perkinsiosis and, investigation of their inhibitory effect on a clonal culture of *P. atlanticus*.

Plan for 2004

Identification of specific genes involved in host-parasite interaction and studies on its regulation of expression through a genome/proteome approach. Identification of environmental parameters capable of modulating parasite growth in vivo and in vitro. In vivo studies of the different

environmental pollutants effect on the proliferation of *P. atlanticus*. Genetic expression of the responsible stress genes in *P. atlanticus* according to the presence of different pollutants. Development of a transfection method for *P. atlanticus* cells. In vivo tests in infected *Ruditapes decussates* clams, with drugs capable of acting as new therapy agents.

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

Main purposes:

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

Achieved in 2003:

For the Bonelli's eagle: 13 polymorphic microsatellites were successfully developed and are currently being used to determine degree of polymorphism and initiate genotyping of the population. Method for DNA extraction from feathers is currently being used to increase our population sampling.For otters, DNA from faeces was successfully purified and proven to be from otter using an additional marker from mitochondrial DNA, cytochrom b. DNA was also extracted from tissues collected from dead animals kept frozen or currently in captivity in order to optimize DNA amplification for microsatellite detection and compare it with results obtained from faeces. A monitorization of individuals resulting from faeces collection in the wild was initiated and provided already some information on the genetic variability and effective population size in the coastal area of Southern Portugal.

Plan for 2004:

Obtention and charaterization of additonal microsatellites for the Bonelli's eagle. For this an application to a Marie Curie training center in UK was submitted and awarded and as a result, work is being performed to extend the number of polymorphic microsatellites from this species through analysis of our pre-existing genomic libraries enriched in repeatrd sequences. The genetic variability and paternity studies of the Bonelli's eagle population from Southern Portugal is being comparedwith those from populations found in different sites in Europe and from older populations previously collected and kept in storage in museums. Studies on otters will be porsued to extend the area analysed and compare results obtained with those already aquired.

Group: Biotechnology and Molecular Biology of Microalgae

Research team

Leader - João Varela
Post-Doc Fellow: Alexandra Cordeiro
PhD students: Nuno Henriques, Sacha Coesel, and Alexandra Ramos.
Technicians: Ana Rita Marques and Vanessa Duarte

Summary of activities and progress during 2003

Biotechnology and Molecular Biology of Microalgae (BMBM)

The research carried out in 2003 is the natural continuation of the study of the carotenoid biosynthetic pathway in the halotolerant microalga *Dunaliella salina.* After having cloned the cDNAs encoding the first two enzymes of this pathway, namely phytoene synthase (*Psy*) and phytoene desaturase (*Pds),* the BMBM group performed northern analysis to determine the respective steady-state levels of messenger RNA (mRNA) either in green or carotenizing cells. This analysis showed that there is no absolute correlation between *Psy* and *Pds* transcript levels and carotenoid accumulation, suggesting the existence of additional rate-limiting factors. In order to investigate such a possibility three strategies were used: 1) targeted RT-PCR cloning of genes coding for carotenoid biosynthesis pathway enzymes yet to be isolated; 2) screening and characterization of a subtractive cDNA library constructed from carotenizing *D. salina* cells; and 3) screening for carotenoid-hyperproducing *D. salina* strains by chemical mutagenesis and resistance to carotenoid biosynthesis inhibitors.

Targeted RT-PCR cloning of genes coding for carotenoid biosynthesis pathway

The first strategy yielded the cloning of a cDNA encoding yet another enzyme of the pathway, namely lycopene cyclase (Lcy), the enzyme catalysing the cyclisation of the two ends of lycopene, giving rise to beta-carotene. The cloning of the cDNA encoding this enzyme allowed us to conclude that *D. salina Lcy* gene is in the same evolutionary branch as its homologues from higher plants. However, it showed some similarities with those found in (cyano)bacteria. This result indicates that this gene represents a middle term in the evolutionary scale between higher plants and a common ancestor with strong similarities with prokaryotic carotenoid biosynthetic enzymes (S. Coesel, A. Ramos, A. Cordeiro & J. Varela, to be submitted).

The cloning of the *Lcy* cDNA allowed us to determine that this gene has an expression pattern very similar to that of *Psy* and *Pds* genes. When *D. salina* cells are exposed to a shift to high salinity (9→18% NaCl) in medium with nutrients, carotenoid accumulation seems to be partially inhibited as compared with non-shifted control cells (9% NaCl). Conversely, northern analysis suggest that *Psy, Pds* and *Lcy* transcripts levels are higher in cells submitted to the salinity upshift when compared with cells remaining at the same salinity. Interestingly, we have found out that the main factor for efficient biosynthesis and accumulation of carotenoids is nutrient depletion. In other words, when *D. salina* cells are starved for nitrates, a stronger accumulation of carotenoids is observed than that found in non-starved cells. This higher carotenoid content is accompanied by increased levels of *Psy, Pds* and *Lcy* transcripts, if compared with those found in non-starved cells challenged with a salt upshift (9→18% NaCl). This apparent correlation between carotenoid biosynthetic enzyme-encoding transcripts levels and carotenoid accumulation is not observed in cells simultaneously starved for nitrates and subjected to a similar salinity upshift (9→18% NaCl). In these cells, levels of *Psy, Pds* and *Lcy* mRNAs are the highest when compared with the aforementioned growth conditions; however, *D. salina* usually fails to accumulate high levels of carotenoids in the first 5 days upon the salt upshift. These results strongly indicate that a sudden upshift in salinity prevents *D. salina* cells from accumulating carotenoids even though the molecular machinery for their production is induced. These results are very interesting as they suggest that additional factors are inhibited by the salinity upshift, which prevents the higher levels of enzyme transcripts to be translated into higher levels of carotenoids (S. Coesel, N. Henriques, L. Cancela & J. Varela, to be submitted; S. Coesel, A. Ramos, A. Cordeiro & J. Varela, to be submitted).

Concomitantly, a phototropin-like blue-light receptor has been cloned in an unrelated screen. Recent results suggest that blue light seems to be an important factor for carotenoid biosynthesis induction in algae as well as for the early steps of chlorophyll biosynthesis, a pathway also dependent upon the isoprenoid metabolism. Moreover, recent data from our lab has shown that this *D. salina* homologue contains a serine-threonine kinase domain as well as a LOV2 (Light, Oxygen, or Voltage type 2) domain. This LOV2 domain binds to a FMN molecule, which acts as a

co-factor for this light-activated receptor. The LOV2 motif share homology with the PERT-ARNT-SIM (PAS) domain, indicating that this *D. salina* clone is part of the PAS superfamily of sensor proteins. Northern analysis has shown that the levels of the mRNA coding for *D. salina* phototropin-like protein are largely insensitive to salinity shifts; conversely, nutrient stress seems to reduce this transcript levels. The only condition that appears to induce this mRNA is when non-starved cells are grown under low light conditions (S. Coesel, A. Cordeiro, C. Bowler & J. Varela, to be submitted). These results open a new line research that will attempt to elucidate the molecular mechanism by which light stress (via intense blue light) induces carotenogenesis in *D. salina* and other algae.

Screening and characterization of a subtractive cDNA library

Strategy 2) became an important research line for finding additional factors regulating or participating in carotenoid biosynthesis in this alga. The screening of the cDNA subtractive library isolated from carotenizing *D. salina* cells resulted in the cloning and characterization of several genes apparently involved in carotenoid accumulation (A. Cordeiro, N. Henriques, L. Cancela & J. Varela, to be submitted). For instance, a *D. salina* homologue of the *LytB/IspH* gene found in cyanobacteria was isolated from this subtractive library. This result is very significant as this gene seems to play a pivotal role in the accumulation of carotenoids in cyanobacteria as well as in higher plants. The *IspH* gene codes for (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase, a [4Fe-4S] protein involved in the biosynthesis of the carotenoid precursor isopentenyl diphosphate (IPP). Interestingly enough, homologues of the *IspH* gene were also found in similar screens in carotenoid-accumulating prokaryotes. Northern analysis indicates that a heat-shock (21C→37ºC) stimulates the induction of this gene in nitrate-depleted medium, whereas in nitrate-containing medium the levels decrease steadily with time upon heat shock. This result suggests that the absence of nitrates is able to stimulate *IspH* gene expression in *D. salina* subjected to heat stress. Further analysis is needed to establish whether *IspH* mRNA levels correlate fully with carotenoid accumulation rates in *D. salina* cells challenged with nutrient and / or salt stress (N. Henriques, A. Ramos, A. Marques, L. Cancela & J. Varela, to be submitted).

A second gene that was pulled out of the subtractive cDNA library corresponds to a *D. salina* cDNA coding for a homologue of ClpC, a protein belonging to the ATP-dependent ClpC/HSP100 family. ClpC is thought to be a nuclear-encoded regulatory ATPase involved in protein unfolding and polypeptide trafficking between the chloroplast and the cytoplasm. It may also play a role in the proteolysis of misfolded proteins inside of the chloroplast. Preliminary gene expression analysis indicate that cells exposed to a salinity upshift (9→18% NaCl), either nutrient-starved or not, display increased *ClpC* transcript levels than that of non-shifted control cells. Heat shock experiments suggest that *ClpC* is indeed a *bona fide* heat-induced gene (A. Ramos, N. Henriques, A. Marques, L. Cancela & J. Varela, to be submitted). However, confirmation of these results is still needed.

Usage of macroarray hybridisation technologies have shown that most genes isolated from the subtractive cDNA library are indeed induced in environmental conditions favouring carotenogenesis. For instance, this technology enabled us to isolate a *D. salina* homologue of the protein synthesis e1F-alpha elongation factor, which is induced in conditions stimulating carotenogenesis as, for instance, nutrient depletion. Likewise, salt stress seems to increase e1F-alpha transcript levels (A. Cordeiro & J. Varela, unpublished results). This is in agreement with results obtained by other labs, as cDNAs encoding this elongation factor have been found in similar screenings for salt-induced genes in other eukaryotes. This result suggests that the protein synthesis machinery is altered in other to adapt to the new environmental conditions. How this influences carotenogenesis is still a question that remains unanswered.

A second cDNA, coding for a starch phosphorylase cytoplasmic isoform, has been isolated. Once again northern analysis showed that this *D. salina* homologue is induced by nutrient starvation as well as salt stress. However, nutrient starvation seems to be the main factor for accumulation of high levels of the respective transcript (A. Cordeiro & J. Varela, unpublished results). In other words, this gene is induced in the very same conditions that stimulate carotenogenesis. The significance of this result is still unclear, as the starch metabolism in plants is largely to be elucidated. One can speculate that the isoprenoid metabolism leading to carotenoid biosynthesis needs carbon skeletons from glycolysis. In turn, this metabolic pathway, especially in cells under stress conditions, may induce mobilization of the starch present in the chloroplast. However, this simplistic scenario is unlikely as (1) the *D. salina* clone seems to encode a cytoplasmic isoform of the enzyme, instead of a chloroplast isoform; and (2) the most prominent enzymes involved in starch catabolism are amylases whereas starch phosphorylases seem to play only a minor role. It is therefore clear that further work is needed in order to ascertain the role of this enzyme in the overall metabolism of carotenizing *D. salina* cells and its effect on carotenogenesis (A. Cordeiro & J. Varela, unpublished results).

Other cDNAs induced in carotenizing *D. salina* cells have been isolated and sequenced. Although their induction has been confirmed by northern analysis, BLAST searches have revealed no significant homology with a large proportion of them. Interestingly, some of these clones are conserved from *D. salina* to man, but their function remains unknown (A. Cordeiro, N. Henriques & J. Varela, unpublished results).

Screening for carotenoid-hyperproducing D. salina *strains*

This strategy has two goals: (1) generate carotenoid-overproducing *D. salina* strains more suitable for biotechnological applications such as aquaculture feed; and (2) a third way of elucidating the carotenoid biosynthetic pathway in this alga by studying alterations of the gene expression patterns in the mutants strains as compared with those of wild-type cells. Basically, the first step of this procedure consists of chemical mutagenesis by means of EMS (ethyl methane sulphonate). In a second step, the screening for carotenoid-overproducing mutants is carried out by cultivating *D. salina* mutagenized cells in the presence of diphenylamine (DPA). DPA is known inhibitor of beta-carotene oxygenase, an enzyme that catalyses the conversion of beta-carotene into canthaxanthin.

Although the major carotenoid in *D. salina* is beta-carotene, the original goal of this screening was to find mutants with increased canthaxanthin levels, a precursor of a highly valuable carotenoid, astaxanthin. After screening a population of 5000 putative carotenoid-overproducing strains, so far only one clone (VD3A) has shown to produce higher levels of alpha-carotene than non-mutagenized cells. Although unexpected, in biotechnological terms this result is interesting in itself as it has been shown that alpha-carotene is a more potent anti-oxidative than beta-carotene (V. Duarte, H. Lobo & J. Varela, unpublished results). At this moment, the VD3A strain is under pilot-scale testing at Necton, our industrial partner.

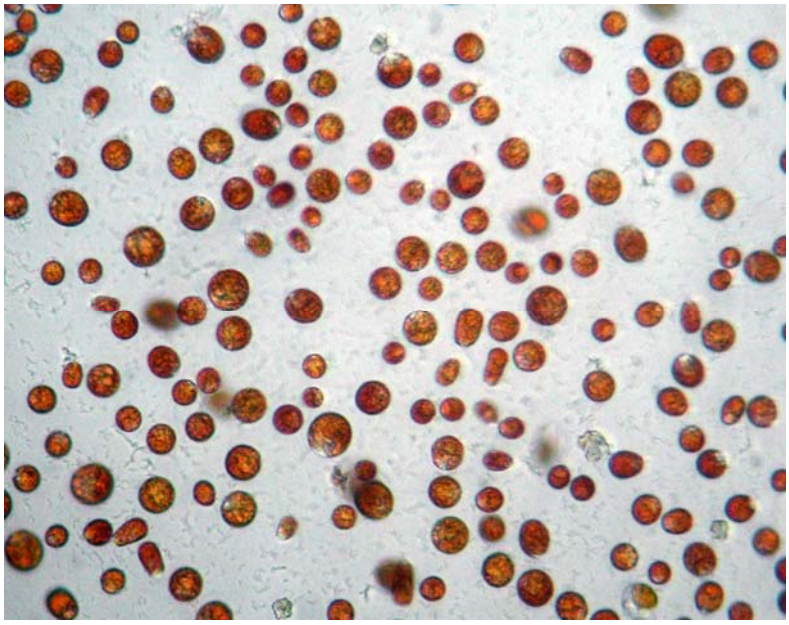

Figure 2. Carotenoid-overproducing *Dunaliella salina.*

Plan for 2004

In 2004 we intend to obtain the full-length cDNA clones encoding Lcy, ClpC, IspH and the phototropin-like gene products. This has been the major obstacle that is preventing BMBM from publishing the work described above. However, papers about the *Pds* and *Psy* gene expression patterns and the subtractive cDNA library construction and screening are being written at the moment and will be submitted soon. Once it is obtained the full-length cDNA clones of the remaining genes, the gathering of functional data and expression levels at the protein level will begin. Functional data will be obtained in three ways: (1) genetic complementation in modified *E. coli* and/or diatom mutant strains lacking a specific *D. salina* homologue; (2) determination of sub-cellular localization; and/or (3) anti-sense gene-specific silencing. The latter research line will only be performed if a DNA transformation method is available for *D. salina*. In order to study the second research line antibodies will be raised against the aforementioned polypeptides. These antibodies will also be used in the study the gene expression patterns at the protein level by means of western analysis.

Another priority for 2004 is the development of a DNA transformation procedure for *D. salina.* With that goal in mind, Sacha Coesel has moved to Chris Bowler's lab at the Stazione Zoologica, Napoli, Italy. *D. salina* is known to be quite refractory for the isolation of stable transformants. The first main obstacle to this end was to find a proper way of selecting transformed cells. During 2003, it was thought that the glufosinate-containing agar plates would be a suitable procedure for selecting transformants. However, it seems that alternative protocols will have to be used, due to the slow growth of the alga on agar plates as well as the appearance of a significant background of untransformed cells in glufosinate-containing medium (S. Coesel, C. Bowler & J. Varela, unpublished results). Apparently, phleomycin seems to yield better results.

For the purpose of obtaining additional carotenoid-overproducing strains, nicotine will be used in the second step of mutant screening instead of DPA. Nicotine is a known lycopene cyclase inhibitor and it seems to be quite toxic to *D. salina* cells even at low concentrations (in the µM range). Other carotenoid biosynthesis inhibitors are being considered, though. Our goal would be to find a *D. salina* mutant able to accumulate high levels of lycopene or a carotenoid other than beta-carotene. Mutants resulting from this screen will be characterized in several ways: (1) growth

rate; (2) nicotine resistance; (3) carotenoid profile (in collaboration with Dr. Rui Mendes at ESB-UC); (4) ease of carotenoid extraction (in collaboration with Dr. Rui Mendes at INETI); (5) gene expression patterns by means of macroarray hybridization technology; (6) levels of protein and lipid oxidation in normal growth conditions, upon salt and / or nutrient stress, and in the presence of sub-lethal concentrations of nicotine. The latter research line will provide important data regarding the *in vivo* anti-oxidative properties of the accumulating carotenoid(s).

Group: Comparative and Molecular Endocrinology

Research team

Leader(s): Adelino V. M. Canário and Deborah Power
Principal investigators and Post-docs: Eduardo N. P. Barata, Juan Fuentes, Ana Lúcia S.de Passos, Josep Rotllant, Peter C. Hubbard, Ana Freitas, Laurence Deloffre, Begoña Redruelo, Pedro Miguel Guerreiro da Costa Guerreiro, Teresa Isabel Mendonça Modesto, João Carlos dos Reis Cardoso.
PhD students: Lília Brinca, Mário Cruz, Dulce Estevão, Pedro Alexandre Carraxís Duarte Frade, Patrícia Pinto, Isabel Morgado, Rui Manuel Lanceiro Serrano,.Natalia Moncaut.
MSc students: Rute Sofia Tavares Martins, Maria de Lurdes Borges Diogo, Nuno Vieira.
Technicians/ research assistants: Liliana Isabel Tomé dos Anjos, Elsa Maria Anjinho do Couto, Natália Maria Campos Teixeira de Moura, Sara Almeida, Bruno Louro, , François Noel Hubert, Zélia Cristina Pereira Velez.
Undergraduate students: Alexandra Maria Patrício Pereira, Marco António Nascimento Sequeira Jesus Campinho, Antonio Luis Miranda, Dulcineia Azinheira, Olinda Gomes de Almeida, Joana Guimarães, Joana Guilhermino, Patrícia Guerreiro..

Summary of activities and progress during 2003

The main topics of the group are the molecular mechanisms underlying hormone action and the physiological response of the whole animal. The processes that are the focus of attention are growth and development (with particular emphasis on cartilage, bone and muscle metabolism), reproduction (with recent emphasis on sex determination), endocrine disruption in the wild and the stress response to normal physiological challenges (with emphasis on ion regulation). An integrated approach is being taken and genomics, biochemistry, cell biology and whole animal physiology are deployed in order to give an overview of hormone function. The approach encompasses studies of gene regulation, gene expression, post-translational and post-secretory processing, receptor binding, signal transduction and finally the response at a cellular level and also the whole animal response.

Parathyroid hormone-related protein and calcium homeostasis

Mechanisms of ionic calcium homeostasis in fish are different from those in terrestrial vertebrates because there is an almost constant supply of calcium ions from the surrounding water, whilst terrestrial vertebrates rely on dietary sources of calcium, which are very variable and intermittent. In higher vertebrates there are three hypercalcaemic hormones involved in calcium homeostasis, vitamin D, calcitonin and parathyroid hormone (PTH). In fish, the hormones regulating calcium homeostasis are poorly studied and the hypocalcaemic hormone stanniocalcin is assumed to be the principal regulatory factor. In fact PTH has not been identified in fish and the parathyroid gland which produces the hormone in terrestrial vertebrates is absent.
The program of work aims to characterise hypercalcaemic hormones in fish and to establish at the whole organism and cellular level the mechanisms that regulate calcium homeostasis. The organisms studied are a marine teleost, the sea bream (*Sparus aurata*), and a euryhaline species tilapia (*Oreochromis mossambicus*). The 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. New PTH-like genes have

been characterized both at the moleciular and physiological level. One of the PTH-like peptides is very active in calcium regulation and acts through a PTH receptor. In vivo and in vitro studies on the interaction between PTHrP cortisol and estradiol in sea bream have been carried out. Unidirectional ^{45}Ca fluxes (mucosal-to serosal / serosal-to-mucosal) were measured *in vitro* in isolated duodenum, hindgut and rectum mounted in Ussing chambers. In the absence of osmotic gradient the addition of (1-34) Piscine PTHrP to the serosal surface resulted in increased calcium movements mucose to serose and/or inhibition of calcium movements from serose to mucose, indicating effects of PTHrP in either calcium uptake and/or efflux. In the presence of osmotic gradient (with saline mimicking the intestinal fluid composition in the mucosal side) the addition of (1-34) Piscine PTHrP to preparations of all intestinal regions resulted in increased net calcium uptake nearly 2 to 4 fold in all regions. Taken together these results indicate that PTHrP acts not only in the transcellular pathway but probably (and more importantly) in the paracellular calcium movements. Bioelectrical characterisation of the different intestinal regions (i.e. voltage, short circuit current and resistance) in response to physiological doses of PTHrP is under way at present. Preliminary results show little deflections in trans- epithelial voltage and short circuit current in response to Piscine PTHrP indicating that the action on calcium movement is probably specific and does not dependent on more abundant ions such as chloride or sodium.
Several genes regulated by PTHrP have been identified and are being subject of detailed characterization.

Hormonal control of development and growth of fish eggs and larvae

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

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

4. Good progress has been made in the development of methodologies for gene expression. Several expression vector constructs have been generated using different vectors and strategies and they are currently being tested in vitro and in vivo. Methodologies are now available in the group permitting the application of this technique to a range of genes currently under investigation.
5. The hardware for most of the physiological studies 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.

Sea bream genome mapping

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

Steroid receptor expression and function

The estrogen receptor (ER) is a transcription factor of the nuclear receptor family with a wide range of functions in vertebrates. The objective of the research is to study the function of estrogen receptor in relation to reproduction in sea bream. Several estrogen responsive genes have been identified in the testis and are being characterized, their expression compared in testis and liver..

Control of sexual determination and differentiation

Environmental conditions in fish farms have a strong influence on the differentiation of sex in seabass. One of the objectives of the work is to identify the sex determining genes and emphais has been put on DAX-1 and DMRT-1, among others. Individual profiles of gene expression has been obtained from embryo and encompassing the period of sex differentiation and under different environmental conditions. Analysis of the effect of rearing rearing density on sex differentiation indicates a possible effect only in the first 60 days of life.

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. A screen for Estrogenic substances has been developed and is operacional. This will have immediate application to the measurement of drinking and waste water, which analysis is compliant following a recent European Directive.

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 Senegal Sole

(*Solea senegalensis*), the goldfish (*Carassius auratus*), the Mozambique tilapia (*Oreochromis mossambicus*) and the tench (*Tinca tinca*).
During the breeding season, *S. pavo* males develop an androgen-dependent anal gland (AG) from the first two rays of the anal fin. Behavioural experiments have shown that a putative pheromone from the AG promotes female attraction to nesting sites and influences female mate choice, thereby affecting male reproductive success. However, this putative pheromone is not involved in sex recognition, since male visual cues are sufficient to trigger female courtship behaviour. This is the first demonstration of an external structure specialised in the production/release of sex pheromones in teleosts. Research work has continued in collaboration with the research team lead by Prof. John Pickett at Rothamsted Research (U.K.) aiming at the identification of the putative sex pheromone. HPLC fractionation of extracts of water conditioned by males with and without anal glands and of anal glands combined with EOG recordings in females have highlighted four HPLC fractions that should contain the active compounds. This research activity has been partially supported by a British Council/CRUP protocol (Treaty of Windsor, ref. B-14/03). Knowledge of the pheromonal system(s) in *S. pavo* is also important for management of its wild populations. The only habitat of this species in Portuguese waters, classified as vulnerable in the Red List of Portuguese vertebrates, is the Natural Park of Ria Formosa (southern Portugal). The research project, Reproductive Biology of the Blenny *Salaria pavo* from Ria Formosa: Knowledge for Population Management and Conservation – (PNAT/1999/BIA/15090; 01/3/2001 – 01/2/2003) was suspended since the supporting institution (FCT – PNAT programme) did not follow the funding agreement . Nevertheless, the results of the experiments conducted in 2002 have been analysed and environmental and social factors that influence reproductive strategies of *S. pavo* were identified. The results have been presented in national and international scientific meetings, have contributed to a Master thesis, and should be submitted for publication in 2004.
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. In addition, the urine of territorial males showed higher olfactory potency than that of non-territorial males. Furthermore, the urination rate of males is highly dependent on social context, being markedly increased in the presence of pre-ovulatory females. In addition, it was shown that males have the ability to descriminate via olfaction between pre- and post-ovulatory females. This suggests that both sexes are transmitting chemical information about their reproductive status. Our aim is to identify these chemicals, and to establish their biological roles. Progress has been made in such direction, as unique HPLC fractions of urine from territorial males were shown to activate olfactory receptor neurones in females. These fractions were isolated in collaboration with Prof. Peter Sorensen (Univ. Minnesota, USA). In addition, HPLC fractionation of extracts of water conditioned by females and screening of their olfactory potency via EOG recordings on males have highlighted three fractions that showed high olfactory potency. These results were obtained in collaboration with Prof. John Pickett *et al*. and are the ground for further chemical identification of the active compounds. This research has been supported by the project, “Chemical Identification and Functional Roles of Reproductive Pheromones in the Tilapia, *Oreochromis mossambicus*” (POCTI/BSE/38815/2001). The results have been presented in national and international scientific meetings and should be submitted for publication in 2004.
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*. The results have been published in Chemical Senses. The physiological and behavioural consequences of exposure to these compounds have been investigated and the experimental data are being analysed.

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

Finally, research has been initiated on the role of olfaction in the feeding behaviour of *Solea senegalensis* (POCTI/CVT/38831/2001) aiming at the identification of attractants in natural preys. A behavioural assay has been developed and the preliminary results showed that food-related chemical stimuli affect the locomotion pattern of sole specimens. In addition, electrophysiological methods (EOG and EEG) have been implemented to study the olfactory detection cabability of sole. In contrast to most fish species, the two olfactory epithelia of the family Soleidae are essentially in contact with two different environments; the upper (right) side samples open water whilst the lower (left) side samples interstitial water. The upper epithelium was significantly more sensitive to the aliphatic amino acids L-alanine, L-glycine, L-threonine and L-serine than the lower epithelium. The lower epithelium was significantly more sensitive to aromatic amino acids such as L-tryptophan, L-tyrosine and L-phenylalanine. Both epithelia had similar sensitivity to basic amino acids (L-arginine and L-lysine) and sulphur-containing amino acids L-cysteine and L-methionine. Neither side was sensitive to acidic amino acids (L-aspartate and L-glutamate) nor the D-isomers of any amino acid tested. The upper side was much more sensitive to conspecific-derived stimuli (bile and intestinal fluid) than the lower side. We suggest that these differences in sensitivity are related to different functional roles in the location (upper) and identification (lower) of potential food items; the upper olfactory epithelium is likely to be more involved with chemical communication than the lower.

Plan for 2004

Molecular evolution of hormones and receptors

The genome of the model species *Fugu rubripes*, a teleost with a remarkably small genome (400Mb), was fully sequenced in 2001. This resource has opened the door for comparative studies

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

Parathyroid hormone-related protein and calcium homeostasis

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

Steroid receptors and development of biomarkers of endocrine disruption

A main target tissue for analysis of ER function is the testes, where there are high levels of expression of 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, macroarray and quantitative PCR approach. The estrogen-responsive genes which have been isolated are now being characterized and some will be chosen for detailed anaylsis. During 2002 the analysis of samples to screen for signs of endocrine disruption in Portuguese estuaries should be finalized.

Control of sexual determination and differentiation

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

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

1) Determination of how many odorants (putative pheromones) are present in the urine of territorial and non-territorial male tilapia (*O. mossambicus*) and comparison of their concentrations. This will be achieved by combining HPLC fractionation and EOG recordings for screening the olfactory potency of HPLC fractions. The results of this work in collaboration with the team lead by Prof. Peter Sorensen (Univ. Minnesota) will be the ground for tentative chemical identification of the putative male pheromones. In addition, a behavioural and/or physiological assay should be developed to test the effect of the putative pheromone on females.
2) Investigate the role of chemical communication in aggressive interactions between males of *O. mossambicus*.
3) Continue the collaboration (Treaty of Windsor, ref. B-14/03) with the research team lead by Prof. John Pickett at Rothamsted Research aiming at the chemical identification of the putative pheromone from the anal gland of male *S. pavo*. The behavioural effect of extracts of water conditioned by males, of anal glands and of identified compounds (if made available) will be tested.

4) Continue the collaboration with Prof. John Pickett *et al*. aiming the tentative identification of putative pheromones released by female tilapia.
5) Initiate a comparative study of the pheromonal system in two related species of blennies: "Identification of Sex Pheromones from the Anal Gland of Male Blennies, *Salaria pavo* and *S. fluviatilis* (Pisces: Blenniidae)"; ref. POCTI/BSE/45843/2002.
6) Investigate if and how olfactory detection of calcium and sodium in the environment influences the neural and neuroendocrine pathways regulating ion homeostasis.
7) Continue investigations aiming at the identification of attractants associated with natural sources of food of the Senegal sole (*Solea senegalensis*). This will be carried out in collaboration with Prof. Joerg Hardge (Univ. Hull, U.K.) under a Treaty of Windsor programme (ref. B-71/04).
8) 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: Physiopathology

Research team

Leader: Josefina Coucelo
Post-doc: Natércia Joaquim
PhD students: Gisela Borges; Sandra Soares
Undergraduate students: Mariana Palma, Sara Barrento, Rita Bicho

Summary of activities and progress during 2003

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

Our main research goal is to contribute to clarify mechanisms of vertebrate cardiac function regulation, with special attention to strategies of species adaptation to specific habitat conditions and way of life. During 2003, our progresses regarding this objective were:

Non-invasive study of heart morphology and function of aquatic turtles (collaboration with Zoomarine and UIC)
This work aims to contribute to clarify the mechanisms that regulate cardiac function in marine turtles, in particular the dynamics of blood flows during cardiac cycle in relation with the respiratory frequency. During 2003 we did several echocardiographic exams along the year in order to evaluate seasonal variation of cardiovascular function in *Caretta carreta*. Haematologic parameters were also determined in order to evaluate the general metabolic/physiologic conditions of the animals. This work will proceed in the following years.

Cardiac Performance in the Atlantic Wolffish (*Anarhichas lupus*) Following Acute Changes in Temperature and Oxygen (collaboration with the Oceans Science Centre – Memorial University de Newfoundland)
An *in vivo* study was performed to examine the effect of increased environmental temperature and hypoxia (reduced water oxygen levels) on the cardiac performance of the Atlantic wolffish. These environmental factors are known to strongly affect the cardiac physiology of pelagic (active) fish species, but few studies have been conducted on benthic (sedentary) marine fishes. Cardiac function was measured by implanting an electromagnetic flow probe (2 or 2.5SB, Transonic®) around the ventral aorta, which allows for direct measurements of cardiac output (Q), heart rate (f_H) and stroke volume (SV) in conscious unrestrained individuals. These parameters were measured while at rest at 6ºC, during temperature challenge - water temperature was increase from 6 to 16 ºC, at the rate of 2 ºC/hour, and then rapidly decreased (in 1 hour) to 8 ºC; and during an hypoxia

challenge - initiated by lowering oxygen saturation from 100% to 80% (maintained at this value for 20 min), and then the water oxygen content was lowered by 10%, every 20 min, until 20% saturation was achieved; this level of hypoxia was maintained for 30 min., and the the hypoxia challenge was finished by gradually increasing the water oxygen content back to 100% saturation over a 30 min period. The results obtained clearly show that acute changes in water temperature (10 ºC increase) and severe hypoxia (O_2 content = 20%) significantly alter cardiovascular performance in the wolffish. An acute increase in temperature from 6 to 16ºC resulted in an increase of cardiac output, with a Q_{10} of 1.59. This increase was mediated through heart rate, which increased about 2-fold (Q_{10} of 1.83), while stroke volume showed a slight, but non-significant,decrease. The results from the present study indicate that for the wolffish Q increases are mediated through f_H and not SV, in the temperature range from 6 to 16ºC. This study also demonstrates that exposure to severe hypoxia induces a marked bradycardia in the wolf-fish, with a f_H decrease from 25.8±0.9 beats min^{-1} at rest, to 12.0±1.2 beats min^{-1}, at 20% saturation. This drop in f_H also resulted in a strong decrease of cardiac output, from 18.1±2.1 at 100% saturation to 9.0±1.2 ml min^{-1} kg^{-1} at 20% saturation, as stroke volume remained constant. No changes in cardiac variables were observed when water oxygen content was decreased from 100% to 70% saturation, however, at 60% saturation both Q and f_H had fallen significantly. These data suggest that that the critical oxygen level for is 60-70% saturation, and that after this level physiological adjustments are required so that this species can maintain homeostasis.

2- Physiopathological responses to toxic metals intoxication (collaboration with FCT – UAlg and Universidad de Extremadura, Badajoz)

We have been studying the effects of toxic metals, in several tissues of our experimental model, the toadfish. During 2003 our afford was applied to study oxidative stress induced by metal ions in cardiac muscle. Cardiovascular diseases affect a great part of the population in developed countries. The increasing release of toxic pollutants into the environment has been indicated as one of the possible causes of the increase of cardiovascular pathologies. However, its biochemical mechanisms remain unknown, making necessary to define the tolerance limits, as well as the preferential biological targets. The aim of this study is to evaluate the oxidative stress responses induced by an acute exposure to a sub-lethal concentration of a cadmium or two vanadium solutions (metavanadate and decavanadate) administrated intravenously, on the cardiac muscle of a marine teleost, *Halobatrachus didactylus* (toadfish). Antioxidant enzymes activities, lipid peroxidation, metals subcellular distribution and the overall prooxidant and antioxidant activities, in heart tissue and plasma, were measured in control and in intoxicated fishes. Our studies showed that both metals accumulate primarily in the blood (preferentially in plasma), with higher accumulation for vanadium. The obtained results also point out for an increase in glutathione peroxidases activity as a response to cadmium intoxication, while vanadate oligomers seem to have an *in vivo* antioxidant effect and an *in vitro* prooxidant ability to induce oxidative stress in the toadfish heart, although no stress responses were detected in plasma.

3 - Endothelial dysfunction and cardiovascular diseases (collaboration with UIC)

Type 2 diabetes mellitus (non insulin-dependent diabetes) and atherosclerosis are complex and progressive conditions that share several common antecedents. Recent data suggest that inflammation may play a central role in the origins and complications of cardiovascular diseases and, possibly, type 2 diabetes mellitus. The toxicity of oxygen free radicals is implicated in the etiology of a number of disorders, including diabetes mellitus. Clinical studies and experimental evidences suggest a causal pathophysiological role of increased oxidative stress in the progression of atherosclerosis. The objective of this work is to study oxidative stress and endothelium dysfunction mechanisms in hypertensive and diabetic patients, and in experimental models (rabbit and fish). During 2003, diabetic (type II) patients and controls subjects were referred and blood samples were collected in order to determine: metabolic status (glucose and cholesterol content in serum), cardiovascular risk indicators (C-reactive protein, homocysteine, creatinine), antioxidant enzymes activities, lipid peroxidation products, angiotensin-converting enzyme and vanadium concentration. Although previous studies describe increased oxidative

damage in NIDDM related to deficits in antioxidant defence, in this study, we found increased lipid peroxidation as well as increased SOD activity in NIDDM patients. Our results also confirm that NIDDM patients are exposed to both oxidative stress and cardiovascular risk, however, these features seem not to be quantatively correlated. Moreover, glucose levels in NIDDM patients were not correlated with the presence of oxidative stress or cardiovascular risk.

Plan for 2004

It is our purpose to continue the work developed during the past years, specifically:

Cardiovascular function of aquatic turtles

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

Oxidative stress induced by metal ions in cardiac muscle (collaboration with FCT – UAlg and Universidad de Extremadura, Badajoz)

1) Antioxidant enzymes activities and expression, lipid peroxidation products, glutathione content, NADH and NADPH, overall antioxidant and prooxidant activity (reactive oxygen species – ROS) measurements in *Sparus aurata* (gilthead seabream) cardiac muscle from control and vanadate intoxicated individuals.
2) Metal subcellular distribution studies in fish blood and cardiac tissue from control and vanadate intoxicated individuals.
3) Total plasma antioxidant capability and lipid peroxidation products assessment in fish plasma from control and vanadate intoxicated animals.
4) Analysis of an environmental pollution marker and a cardiovascular risk indicator (C-reactive protein) and immunodetection of protein nitrosylation level in fish plasma from control and vanadate intoxicated individuals.
5) Ca^{2+}-ATPase activity and expression in cardiac homogenates from control and vanadate intoxicated fishes.
6) Cardiac miocites culture setup from fish and mouse to calcium homeostasis studies.

Endothelial dysfunction and cardiovascular diseases

1) Antioxidant enzymes activities, lipid peroxidation products, endothelial dysfunction markers, metabolic status (glucose and cholesterol content in serum) and cardiovascular risk indicators (C-reactive protein, homocysteine, creatinine) analysis in blood samples from hypertensive patients and controls.
2) Endothelial dysunction markers analysis in blood samples from hypertensive patients and controls.
3) Antioxidant enzymes activities, lipid peroxidation products and endothelial dysfunction markers analysis in isolated microvascular endothelial cells from the heart of diabetic and hypertensive rabbits.
4) Antioxidant enzymes activities, lipid peroxidation products, endothelial dysfunction markers, metabolic status (glucose and cholesterol content in serum) and cardiovascular risk indicators (C-reactive protein, homocysteine, creatinine) analysis in blood samples from diabetic and hypertensive rabbits.

Group: Biophysics

Leader - Leonor Cruzeiro-Hansson
Paulo Silva, PhD Student

Summary of activities and progress during 2003

The work on protein folding and function is being pursued in two main directions: 1) is the simulation of vibrational energy transfer in specific proteins, such as the F1 F0 ATP synthase, myoglobin and prions. The aim is to establish, both theoretically and experimentally, that vibrational energy transfer is an important step in the way proteins works. 2) A second direction is the derivation of a model that can describe the transfer of vibrational energy to the conformational degrees of freedom. Paulo Silva is working along this line and has derived a set of equations that can be applied to the dynamics of a large variety of models. Its application to a specific case has already led to one publication and four communications in international meetings.

Plan for 2004

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: Maria Teresa Dinis
Researchers: Luis Conceição
Post-docs: Florbela Soares, Pavlos Makridis, Laura Ribeiro, Neil Ruane
PhD students: Cláudia Aragão, Sofia Morais
Research assistants: Sofia Engrola, Pedro Cação, Marc Lacuisse, Silvia Martins, Cristina Pita, Rui Rocha, Céline Paul
Undergraduate students: Rita Colen, Sofia Mendonça, Ana Couto, Sónia Correia
Aquaculture technicians: Helena Damásio, Helena Teixeira

Summary of activities and progress during 2003

The central aim of the group is to contribute to the sustainable development of the aquaculture industry, through basic and applied research directed to the optimisation of cultivation techniques and to the bottlenecks identified in the cultivation of new species for aquaculture. Ongoing projects involve nutrition physiology, morphology, stress physiology and microbiology studies, in particular during the early stages of marine fish, but also the characterization of the aquaculture industry in the Algarve region, both in technical and socio-economic terms. In 2003 research was mainly focused on broodstock management, aspects of larval and juvenile rearing of sole (*Solea senegalensis*), seabream (*Sparus aurata*), red porgy (*Pagrus pagrus)* and grouper (*Epinephelus marginatus*), as well on methodologies for enrichment of rotifers and Artemia, as part of the research projects where the group is involved. Effort was also directed towards some experiments concerning digestive capacity of sole juveniles fed microdiets and lipid metabolism.

The effect of dietary amino acid profiles on larval metabolism
The effect of a balanced and of an imbalanced dietary AA profile on growth, survival, and AA metabolism of seabream (*Sparus aurata*) larvae was studied. Two experimental microencapsulated diets were prepared, with the same basal formulation, but while one was

supplemented with indispensable amino acids in order to obtain dietary A/E ratios balanced to the larval seabream (balanced diet), the other was supplemented with dispensable amino acids in order to obtain imbalanced dietary A/E ratios (imbalanced diet). Larval seabream were reared according to standard procedures until 32 days after hatching (DAH). At 33 DAH, each microencapsulated diet was randomly assigned to triplicate tanks. In other 3 tanks (used as a control), larvae were fed *Artemia* metanauplii. Survival, growth, oxygen consumption, and ammonia excretion were determined after 10 days of experiment. Survival was similar in larvae fed the balanced diet (75±6%) or *Artemia* (87±5%), but was significantly lower in larvae fed the imbalanced diet (52±9%). Larvae fed both microcapsules finished the experiment with similar weight (2.3±0.7 and 2.0±0.7 mg, for the balanced and the imbalanced diet, respectively), but this was significantly lower than that attained by larvae fed with *Artemia* (3.2±1.3mg). The oxygen consumption measurements suggest that larvae fed the imbalance diet had higher metabolic maintenance requirements than the larvae fed the balanced diet. The ammonia excretion results suggest a higher utilisation of protein as an energy source in fish fed the imbalanced than the balanced diet. Although *Artemia* was imbalanced in terms of amino acids for the seabream larvae, it is suggested that the higher ingestion of *Artemia* than of microcapsules might be sufficient to compensate those imbalances, resulting in higher growth and lower metabolic costs. A better nitrogen utilisation in larvae fed the balanced diet is apparent, although it did not improve growth.

Absorption and metabolism of fatty acids amino acids in response to dietary protein/lipid ratios in Senegalese sole larvae

Thia study examined the effect of dietary protein/lipid ratio on fatty acid (FA) and amino acid (AA) absorption and metabolism on larval Senegalese sole. Additionally, different lipid-bound (triolein and phosphatidylcholine 1,2, di-oleoyl) and free 14C-FA's (stearic acid, oleic acid and docosahexaenoic acid) were tested. Two experiments were conducted in which larvae were fed non-enriched Artemia (NEA) or Artemia enriched on soybean oil emulsion (EA) since settlement. These larvae were tube fed 14C-FA's in the first experiment and fed AA-labeled Artemia in the second. In general, a higher growth was obtained with the NEA diet. Larvae fed EA exhibited neutral lipid accumulations within the gut epithelium and lower absorption of the FA label due to loss through evacuation. On the other hand, total AA absorption was similar, although larvae fed NEA appeared to have a faster absorption and showed a higher AA catabolism and therefore lower retention. The higher growth achieved with NEA in this species may be due to a low digestibility and absorption of high neutral lipid diets but an increased ingestion of a diet containing a lower lipid level may also explain the results. Finally, the tested FA's showed intrinsic digestive and metabolic properties, independently of dietary protein/lipid ratio.

The effect of green water on larval development

The effect of microalage on the development of seabream and Senegalese sole larvae was analysed by the determination of dry weight, protein and lipid content as also histological techniques.

Three treatments were used: 1) Microalgae, addition of *Tetraselmis suecica* to the rearing tanks at a density of 40000 cells /ml; 2) Fitobloom, addition of microalgae concentrate to the rearing tanks at a density of 65000 cells /ml; 3) Control, clear water. The experiment lasted until 15 days after hatching. as Rearing was run in triplicate in individual tanks, which involved an adaptation of the exixting rearing systems. Treatments were added after fish larvae opened their mouth. Sea bream larvae were fed with enriched rotifers (*Brachionus rotundiformis*) and Senegalese sole was fed initially with enriched rotifers but at 4 days after hatching Artemia sp. was also introduced in the diet of this fish species. Live food enrichement was done with DHA Protein Selco (Inve). Pooled samples of fish larvae (varying number according to age and parameter being analysed) were colleted at mouth opening (2 to 3 days post hatching – dph), 10 and 15 dph. Ten larvae were also sampled for histological analysis. Five larvae were fixed in Bouin and five were fixed in buffered formaldeide, posteriorly these larvae were embbed in paraffin and metachrylate, respectively. Amonia and nitrates analyses were also carried out to assess the quality of rearing water for the different treatments.

The growth of larvae was not significantly affected by the presence of microalgae in the rearing water, although seabream larvae from Fitobloom treatment exhibited a smaller length at 15 dph when compared with the other treatments. Concerning the biochemical and histological analysis no significant differences were observed among treatments.

Health management during early developmental stages of marine fish

The use of probiotic bacteria and immunostimulants is early stages during the rearing of Senegalese sole and seabream has been studied. The survival rates of sole during the larval stages are exceptionally high, compared with other cultured species. The research will therefore further focus in the weaning of sole to an artificial diet which often represents a phase with high mortalities during the rearing of this species.

Candidate bacterial strains for use as probiotics have been screened by use of both in vitro and in vivo tests. In the case of sole, the gut microflora of juvenile and pre-adult fish fed on artificial diet has been compared to the microflora of fish fed a natural diet. In several experiments, it has been found an increased number of strains showing inhibition against common pathogens. In the case of seabream, a group of bacteria have been isolated from successfully weaned seabream juveniles. Another area where we search for beneficial bacteria is the microalgae.

DNA analysis by use of 16S rRNA PCR has been applied for the identification of the different bacterial strains. The use of both classical approach microbiology and molecular techniques are used for the description and comparison of the microflora of fish fed artificial diet and natural diet.

The ontogenetic development of the immune system in sole and the influence of immunostimulants on the non-specific and specific immune defenses is also been investigated by use of histology and other approaches. Several genes related to the immune system (Nramp, C3, and TGF b) were isolated from sole postlarvae after collaboration with IBMC, Porto.

Pigmentation abnormalities in sole

α-Melanophore-stimulating hormone (MSH) is a major melanotropic pituitary hormone involved in colour change and background adaptation in fish. Using an antibody specific for three isoforms of α-MSH (mono-, di- and des-acetyl-α-MSH) a radioimmunoassay was set up to measure MSH levels in plasma, larval homogenates and in *in vitro* superfusion experiments. This assay was then used in experiments investigating background adaptation in sole involving the rearing of fish in white, green and black tanks. Adaptation in normally pigmented juvenile sole (ca. 20 g) was compared by measuring plasma α-MSH levels, plasma cortisol, tyrosine kinase activity and histological examination of the skin melanophores. Fish adapted to black tanks showed a higher basal cortisol level and reduced α-MSH levels (despite changing colour). Histological examination of the skin showed increased amounts of melanin and a greater number of mucous cells in the skin of black adapted sole. These results were further supported by data on black-adapted juveniles (59 days-after-hatch) showing higher cortisol and lower α-MSH levels.

In vitro tests are being developed, using larvae and scale slips, to determine the chemical signals involved in the aggregation/dispersion of melanin within the melanophores. Preliminary results show that melanin-concentrating hormone and melatonin both have aggregating effects while α-MSH has a dispersing effect. Further work has shown that α-MSH works through G-protein coupled receptors increasing intracellular cAMP and IP3 levels.

Another pigmentation-related phenomenom is the occurrence of dark larvae within groups of larvae reared in bright tanks. This has often been regarded as a sign of poor quality. Results with sole show that cortisol levels in these dark larvae are 5 – 10 fold higher than in the normal-pigmented larvae. However, in agreement with data from black adapted fish, α-MSH levels were either not different or lower in these fish. Such findings raise the question as to the role of α-MSH in pigmentation in these fish.

Feeding trials for enrichment feed with microalgae paste and SCO

Trials with the enrichment protocols development in previous years with Single Cell Oil (produced by heterotrophic growth of microalgae *(Crypthecodinium cohnii)*, a DHA rich biomass, incorporated

in a microalgae biomass of *Nannochropsis occulata*, were performed with seabream (*Sparus aurata*) and sole larvae (*Solea senegalensis*).

The results are summarized below:

1) Rearing of *Sparus aurata* larvae fed with rotifers enriched in SCO rich biomass
Main conclusions: seabream larvae can assimilate the DHA available in the biomass used for rotifer enrichment without significant differences, when compared to a commercial emulsion

2) Rearing of *Solea senegalensis* larvae fed with zooplankton enriched in SCO rich biomass (lacking some data)
Main conclusions: First results showed that sole larvae growth is negatively affected by the use of DHA rich biomass as enrichment of live feeds, when compared to a commercial emulsion. Biochemical analysis (still running) will provide information regarding fatty acids assimilation.

3) Zooplankton enrichment in EPA rich biomass
Main conclusions: it might be assumed that this EPA rich biomass can be used for rotifers enrichment and the levels of EPA and DHA present can be manipulated according to the enrichment time. Biochemical analysis (still running) will provide information regarding fatty acids assimilation on Artemia.

4) Evaluation of Rotifers long term enrichment in EPA and SCO rich biomass
Main conclusions: EPA rich biomass does not seem suitable for rotifers growth. DHA rich biomass also does not seem suitable for rotifers growth because it is not possible to achieve high densities, probably due to the pollution in culture medium. Biochemical analysis will provide information on assimilation of fatty acids levels checking then the performance on different biomass.

5) Rotifers short term enrichment in two different types of SCO rich biomass (SCO in powder or in flakes)
Main conclusions: Both types of pastes do not seem adequate for maintaining cultures of rotifers due low growth rates observed.

Broodstock management

The objectives for 2003 were the were the identification of the parameters responsibles for sole maturation and reproduction, one important bottleneck on sole cultivation. Spawnings with good quality eggs were obtained from two groups, kept under natural photoperiod and temperature. A closed analysis of data of the environmental parameters during previous spawning periods (1996-2000), were compared with 2003 data, specially on what concerned temperature, photoperiod and salinity. The temperature was identified as one of the triggering environmental parameters for the maturation and spawning of sole broodstock. The spawning performance of the 4 groups in captivity was evaluated based on the egg quality and egg hatchability.

A new group of sole broodstock were established. All fish were tagged and sexes were determined. The existing four groups of fish were maintained in a open system at the Experimental Station of Ramalhete. One group was feeding with dry feed and the three remain groups was feeding with squid, worms and mussel.

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 at CCMAR, additional experimental diets and weaning strategies for sole were tested together with the study of sole digestive capacity. The goal was to test two different kinds of microdiets (containing different levels of fish protein hydrolysates or di-peptides) both in larval and post-larval sole. Length, dry and wet weight, enzymes activities (amylase, alkaline phosphatase and trypsine) were monitored at the end of each experiment. Relative growth rate of sole fed microdiets from both kinds was lower (ranging from -1.5 to 7.4% DW/day) than the control fed frozen metanauplii (11%DW/day average). Trypsine specific activity was significantly reduced by both microdiet treatments, lowering the ability of sole to use efficiently the feed. Abrupt weaning

affected significantly the activity of alkaline phosphatase. Results from this study suggest that digestive capacity of S. senegalensis is affected by weaning into inert diets. In addition, size at weaning and duration of the co-feeding period with Artemia also seem to influence digestive capacity. The different dietary levels of protein hydrolysates and di-peptides used in these experiments seem to have little effect on digestive capacity or growth.

Effect of dietary carbohydrate and lipids ratios on growth and oxidative status of Senegalese sole post-larvae

[Study in cooperation with researchers from the Laboratory of Aquaculture & Artemia Reference Center (Ghent University, Belgium)] The effect of dietary lipid levels and carbohydrate nature on growth, condition and oxidative status of Senegalese sole post-larvae was studied. Three iso-nitrogenous diets and with similar digestible energy contents (13% digestible starch and 15% lipid (D13/15), 13% raw starch and 21% lipid (R13/21) and 7% digestible starch and 21% of lipids (D7/21)) were tested in post-larvae of 78 days after hatching for a period of 76 days. Fish fed R13/21 recorded significantly higher final body weight and relative growth rate. A more efficient feed conversion ratio and lower hepatosomatic index were also observed on fish fed on R13/21. Higher lipid contents were observed in muscle samples of fish fed diets with high lipid levels. Energetics, oxidative test and antioxidative enzymes responses were higher in liver than in muscle samples. The significant differences noted in energy available values (liver and muscle samples) were mainly related with differences of dietary lipid levels. As a consequence, more energy available for growth was observed in fish fed high dietary lipid levels. However, these diets also evidenced a significantly higher energy consumed. The antioxidative enzymes catalase, superoxide dismutase and gluthatione peroxidase evidenced higher activities in samples of fish fed with high lipid contents and raw starch. Diet R13/21 resulted in higher oxidative stress possible related to the high mortality recorded in fish fed this diet. The inclusion of high level of lipid and raw starch affected positively the growth of Senegalese sole post-larvae. However, the addition of raw starch also increased the oxidative level in muscle and liver tissue, which can produce deterioration of the flesh quality.

Plan for 2004

The research during 2004 will continue to focus on broodstock management and aspects of larval and juvenile rearing of sole (*Solea senegalensis*), seabream (*Sparus aurata*), red porgy (*Pagrus pagrus)* and grouper (*Epinephelus marginatus*), digestive physiology of marine fish larvae, as part of the research projects where the group is involved.

The effect of green water on larval development

Within the aim of this project we will start an experiment to observe the protein and lipid content of fish larvae when Isochrysis galbana is added to the rearing water. Also within this project we will determine the effect of microalgae addition to the rearing water on the activity of digestive and metabolic enzymes of marine fiah larvae.

Neuroendocrine system of marine fish larvae

a) Study the development of the nervous system associated with digestive system of marine fish larvae through histological and histochemical analysis.
b) Study the development of neuropeptides associated with the gastro-entero-pancreatic tract through imunohistochemical techniques.

Broodstock management

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

Nutritional and zootechnical aspects of larval and juvenile rearing

a) The effect of dietary composition and supplementation with key nutrients (e.g., essential faty acids and amino acids) will be studied both in larval and juvenile fish. Growth trials and tube-feeding assays will be used, together with measurements of respirometry, biochemical contents, activities of enzymes of intermediary metabolism and tracer studies.
b) Determine the influence of dietary natural zooplankton, larval density and rearing volume in the growth performance, survival rates and fish quality for grouper and red porgy. Fish quality will be assessed based on morphological (e.g., skeleton malformations, pigmentation, histology) and biochemical (e.g., RNA/DNA ratios, free amino acid contents, enzyme activities) criteria.
c) Determine whether a feeding hierarchy exist during weaning of Senegalese sole, how this eventual hierarchy is affected by diffeent zootechnical conditions, and how it relates with larval metabolism. This will be done using tracers in the food.
d) Preliminary work on the relation between stressfull conditions and amino acid requirements will be done. Stress levels will be assessed through plasma/whole body cortisol levels. Amino acid requirements will be studied studying plasma free amino acid levels and also tracer studies.
e) Preliminary work will be conducted on the effect of nutrition and feeding on fish proteome expression. Two-dimension gel electrophoresis will be followed by proteome comparision and identification of differentially expressed proteins.

Pigmentation abnormalities in sole

a) Development of *in vitro* tests to determine the chemical signals involved in the aggregation/dispersion of melanin within the melanophores. Characterization of the intracellular signalling pathways involved and binding studies of α-MSH to skin melanophores will be further elucidated.
b) *In vitro* superfusion studies to compare the release of α-MSH from the pituitaries of normal and malpigmented sole adapted to different backgrounds.
c) Morphological and histological characterisation of the endocrine system (pituitary, thyroid, head-kidney and pineal) during ontogeny in sole larvae. This study will be supported by hormonal measurements in the developing larvae and immunohistochemistry of the developing organs with specific antibodies.

Division of Living Resources

Group: Ecology and Evolution of Marine Organisms

Research team

Leaders: Ester Serrão, Gareth Pearson

Post-docs: Sophie Arnaud, Martin Billingham, Cecile Perrin, Onno Diekmann, Elena Varela

PhD students: Filipe Alberto, Asuncion Lago-Leston, Emmanuelle Billard, Marta Valente

Technicians/research assistants: Sara Teixeira, Carla Viegas, Vera Fonseca, Luis Correia, Tiago Simões, Carla Monteiro, Cláudia Faustino, Sofia Caetano, Mirjam van de Vliet, Dulcineia Azinheira.

Summary of activities and progress during 2003 and plan for 2004

Genetic Structure of Seagrass Populations

Main purpose:

Large–scale panmixis has long been the leading hypothesis for marine species, as marine habitats are often viewed as having few strong barriers to dispersal, possibly due to poor understanding of the nature of limitations to gene flow in marine environment. However, an increasing number of studies report high differentiation and restricted gene flow between marine populations, despite high dispersal potential. The elucidation of the factors responsible for such divergence is difficult but essential to understand evolutionary and speciation processes. In this topic we aim at inferring population genetic variability and differentiation at different spatial scales in order to provide insight in the predominant causes of genetic structure, allowing the identification of physical and biotic factors limiting gene flow. We use all four European seagrass species as model species for these questions because i) these are keystone ecosystem structuring species, ii) can have contrasting clonal and sexual colonization strategies and iii) there is widespread regression and loss of seagrass meadows and management predictions as well as restoration efforts should take into account genetic differentiation within and between populations and the factors inferred to influence these.

Achieved in 2003:

- Development of new microsatellite markers for the seagrasses *Posidonia oceanica* (ms published in 2003), *Cymodocea nodosa* (ms published in 2003) and *Zostera noltii* (ms in prep.).

- Analysis of the evolution of genetic diversity during recolonization of disturbed meadows of *Cymodocea nodosa*. Seagrass colonization proceeds from the initiation of patches, from seed, followed by clonal growth. It is as yet unclear how genetic diversity evolves during the colonization process. We examined the development of genetic diversity during seagrass colonization by examining how genetic diversity varies across a gradient of patch age and size of *Cymodocea nodosa* meadows arrested in a permanent recolonization state due to patch extinction-recolonization derived from disturbance by sand waves. We have described the relationship between genet richness (number of genotypes) and clonal demography, patch age and size and compared these with model expectations. Assignment tests and patternity exclusion tests for the seedlings surrounding the patches revealed higher than expected dispersal capacity for this species (ms in prep.).

- Phylogeography of *Posidonia oceanica*: Vicariance patterns in the Mediterranean Sea (Figure 7).

Our results showed a sharp East-West cleavage in the Mediterranean, likely to represent the footprint of vicariance due to the last Pleistocene ice age, maintained by a present day low gene flow. Although apparently no allopatric speciation was initiated, the mixing of divergent gene pools

seems to be still very limited by some physical or biological factors. Different factors were revealed by changes in population differentiation observed at different spatial scales. At the intra-meadow scale, the trade-off between survival and migration may explain the advantage of low dispersal and small genetic neighborhood and at the intra-basin scale; the vagaries of currents and the low turnover can be responsible for the low effective migration rate (ms submitted).

Figure 7- Sampling the seagrass *Posidonia oceanica* for genotyping with microsatellite markers for a phylogeographic study of the Mediterranean Sea.

- Increasing asexual reproduction at species' limits inferred from population genetic structure: the case of *Zostera marina* in the Ria Formosa. In populations of species that are able to reproduce both sexually and asexually, there may be local differences with regard to the relative importance of the two modes of reproduction for population maintenance, and such differences can be accentuated at geographical margins, where higher selective pressures can lead to faster evolutionary rates. This hypothesis was examined for the seagrass *Zostera marina* at its eastern Atlantic southern limit in the Ria Formosa. Genotypic diversity was used as an indicator of the relative importance of vegetative and sexual reproduction. Results from this study showed reduced sexual reproduction, genotypic diversity and among-population gene flow at this species' limits. (ms published in 2003)

– Genetic substructure and optimal outcrossing distance in *Zostera marina*.

Genetic structuring of a species may have important genetic, evolutionary and ecological consequences including distance dependent mating success. In this study clones of *Zostera marina* within four metres of each other exhibited significant and positive kinship values (inferred from microsatellite data), reflecting limited seed dispersal. Hand-pollinations between near, intermediate, and far individuals resulted in seeds from intermediate distance pollinations having significantly greater mass, higher proportion of germination and lower time required to germinate

than both the near and far seeds. Similarly, the average number of seedlings produced per pollination, used as an overall measure of fitness, was significantly greater for the intermediate distance when compared to both near and far pollinations. These results suggest that the genetic structuring observed may result in both inbreeding and outbreeding depression, which gives rise to an optimal outcrossing distance. (ms submitted).

- Participation in a global phylogeographic analysis of *Zostera marina*, coordinated by the Univ. of Groningen. As the most widespread seagrass in temperate waters of the northern hemisphere, *Zostera marina* provides a unique opportunity to investigate the extent to which the historical legacy of the last glacial maximum is detectable in modern population genetic structure. A near absence of DNA sequence variation between Pacific and Atlantic populations (including the Mediterranean and Black Sea), combined with tightly associated biogeographic groupings based on microsatellite data, suggests that the trans-Arctic connection is still open. The E Pacific and W Atlantic are more connected than either is to the E Atlantic. Allelic richness was nearly two-fold higher in the Pacific. Populations from putative Atlantic refugia, like the Iberian Peninsula, now represent the southern edges of the distribution and are not genetically diverse. Links between historical and contemporary processes offer insight into the projected effects of climate change on coastal marine plants (ms submitted).

- Population genetic structure of *Zostera noltii* along W Iberia: consequences of small effective population size, habitat discontinuities, and current patterns. The effects of oceanographic patterns on marine genetic biodiversity along the SW Iberian Peninsula are poorly known. We addressed the question of whether gene flow in this region depends solely on geographic distance between isolated patches of suitable habitat or are there superimposed effects correlated with other factors such as current patterns. *Zostera noltii*, is the keystone habitat structuring seagrass species on intertidal mudflats along the Iberian west coast. We used nine microsatellite loci to analyze all existing 8 populations from Ria de Vigo to Cadiz, Spain. Populations are highly genetically differentiated, with a sharp split between those located North versus South of the Tagus river. This pattern of differentiation can be explained by 1) ocean surface current patterns (the Cadiz poleward current) present during *Z. noltii* reproductive season causing a barrier to gene flow and dispersal between the northern and southern populations of this region, 2) habitat isolation preventing frequent gene flow, 3) small effective population sizes, causing high drift and thus faster differentiation rates (ms in prep).

Plan for 2004: (List of manuscripts that will either be completed or in progress during 2004):

- Review paper on methodological problems in the analyses of population genetic structure in clonal plants, and on new methods for describing genetic structure in clonal plants.
- Methodological paper on combining power and efficiency in selecting the genetic markers.
- Phylogeographic analysis of *Cymodocea nodosa* at the Atlantic-Mediterranean transition zone: sources and sinks, geographic isolation and current patterns.
- Inferring genetic neighborhood size and dispersal distances in *Cymodocea nodosa*.
- Spatial variation in patterns of clonal growth across meadows of *Posidonia oceanica* and its relationship with demographic status of the meadow.
- Mating system in *Posidonia oceanica*: Description of sexual reproduction: allo vs auto fertilization and respective survival of progeny issued from both.
- Phylogeny of the genus *Posidonia*: How did *Posidonia oceanica* happen to be endemic to the Mediterranean Sea? Retracing the history of the *Posidonia* genus, and confirming morphological taxonomy of Australian species.
- Do aquaculture cages have an effect on population genetic composition of adjacent seagrass meadows versus non-adjacent ones?
- The impact of fish herbivory on genetic diversity of *Zostera marina*.
- Participation on a global phylogeographic analysis of *Zostera noltii*
- How does population genetic structure of *Zostera noltii* differ from stable continuous meadows versus patchy meadows under frequent disturbance, in a permanent state of recolonization?

Population genetic structure of natural mangrove forests of Avicennia sp. in Vietnam: the recovery of genetic diversity 3 decades after Agent Orange

Main purpose:

Mangrove forests, together with seagrasses, are major keystone ecosystem structuring species along the coastlines of the world's tropical regions, and in addition to their ecological role their ecosystem services have high economic value, particularly as nurseries for commercailly important species (around 10,000 US$ per ha, Costanza *et al*. 1997). However, there has been widespread mangrove reduction throughout SE Asian coastlines, associated with human activities. The aim of this research theme is the assessment of population genetic structure in disturbed mangrove forests in SE Asia. *Avicennia* spp were chosen as model species because these are not artificially planted and thus reflect ecosystem history.

The destruction of the Mekong Delta mangrove forests by Agent Orange is possibly the largest human disturbance event experienced by any ecosystem. The first objective of this research theme is the evaluation of the genetic composition of the mangrove forest recruited following the Vietnam war.

Achieved in 2003:

- Development of microsatellite markers for *Avicennia alba* (ms published).
- Mangrove Genetic Diversity Still Recovering 30 Years After Agent Orange: Widespread spray of Agent Orange over Southern Vietnam by United States Forces led to the decimation of mangrove forests over the Mekong Delta. Whereas Rhizophora stands have been partially recovered through large scale reforestation programs, recovery of other mangrove species is dependent on propagule dispersal from external sources. We described the genetic recovery of the mangrove *Avicennia alba* population in the Mekong Delta during three decades following the end of the war (ms in prep).

Plan for 2004:

- Analysis of population genetic structure in *Avicennia marina* mangroves in Northern Vietnam and the Phillippines. Consequences of selfing and population size.

Evolution of reproductive strategies in fucoid algae: Population genetic structure of dioecious versus self-compatible hermaphrodite species and their hybrids.

Main purpose:

The reproductive system shapes mating patterns thereby determining the organisation of genes in populations. Changes in the reproductive system can thus function as the drive for reproductive isolation and eventually for speciation. Population genetic studies can reveal the consequences of a particular reproductive strategy. In this theme we explore the relationship between reproductive strategies and population genetic structure in two closely related seaweed species, *Fucus spiralis* and *F. vesiculosus* that have broad areas of sympatry along their distribution. The opportunity to study alternative reproductive strategies in closely-related taxa is rare. The comparison of population genetic structure in *F. spiralis* and *F. vesiculosus* provides an excellent opportunity for the study of the evolution of reproductive strategies.

Hybridization between these two species has been suggested by reports of individuals with intermediate morphologies. Another goal was thus to use microsatellite data to gain accurate estimates of hybridisation frequency. Hybrid zones are excellent models for the study of evolution and maintenance of contrasting reproductive systems, as they are stabilized by the balance between introgressive gene flow and selection against hybrids contributing to maintenance of parental species integrity.

Achieved in 2003:

- Mating system. To determine if the different reproductive strategies adopted by each species correspond to mechanisms promoting selfing or outcrossing, we have studied the mating system of each species. Results revealed a high degree of self-fertilisation in *F. spiralis*,

suggestive of the selective advantage of autogamy for increasing mating probability in this externally fertilizing species, and also revealed a significant degree of biparental inbreeding in *F. vesiculosus*, suggestive of short gamete dispersal (ms in prep).

- Study of the sexual phenotypes: Since the cosexual/unisexual reproductive character has changed several times during the evolutionary history of the Fucaceae, the change from one reproductive system to the other may be relatively simple. In the evolution from one reproductive strategy to another, while sexual differentiation is still incomplete one may expect to find populations of mixed sexual phenotypes, e.g. female or male and hermaphroditic morphs are observed. To understand the evolutionary mechanisms underlying the transition from one reproductive strategy to another, we have identified and documented the sexual phenotypes of individuals in populations, and assessed the investment into male and female function of each sexual phenotype. Polymorphism was observed in the sexual phenotypes of both parental species, and the hybrids presented all possible sexual phenotypes. The maintenance of great differences in the investment in male and female functions between the dioecious and the hermaphroditic species despite the possibility of gene flow between *F. vesiculosus* and *F. spiralis* suggests a strong counter selection of hybrids, even though they look fertile (ms in prep).

- Population genetic structure in *Fucus spiralis*. By assuring its reproduction through self-fertilization, *F. spiralis* shows a higher capacity as founder of new sites. In this case, loss of genetic variability through drift in these founder-type populations will be stronger than the genetic drift that occurs as a result of selfing in otherwise demographically stable populations. However, our analyses using five microsatellite markers revealed that contrary to expectations, *F. spiralis* from South Portugal do not show significantly less genetic diversity within populations or more genetic divergence among populations than *F. spiralis* from more central distribution areas. However, in locations where *F. spiralis* co-occurs with the dioecious species *F. vesiculosus*, *F. spiralis* populations tend to display higher genetic variability maybe due to events of hybridisation with F. vesiculosus. In conclusion, for *F. spiralis*, a predominantly autogamous mating system, potential hybridization with its sister species and/or other processes might be more important than the effects of the biogeographic boundary for genetic partitioning (ms in prep).

Plan for 2004:

- Development of new microsatellite markers specific for *Fucus spiralis*.

- Comparisons between *F. spiralis* and *F. vesiculosus* will be extended to the worldwide distribution of these species. The rate of self-fertilization in F. spiralis populations will be estimated by adding five new microsatellite markers. The comparison of population genetic structure of *F. spiralis* and *F. vesiculosus* will allow us to assess how mating strategies might result in different patterns of genetic structure. This will be analysed in locations where species occur together in sympatry and where species occur separately in allopatry in order to assess the effect of spatial isolation versus sympatry.

- Genetic diversity and the amount of gene flow at various spatial scales within and between populations will be compared between the hermaphroditic and dioecious species. Spatial autocorrelation analysis will be used to compare the dispersal of gametes and zygotes within populations. Second, gene flow between populations within and among regions will be quantified for each species and compared. Third, the amount of gene flow between populations of different species will be assessed. Fourth, the pattern of genetic differentiation between species, among regions will provide clues as to the maintenance and evolution of the different reproductive strategies of the two species.

- Laboratory experiments and transplants prepared in parallel with field experiments will be designed in order to study the rate and potential mechanisms of self-fertilization in *F. spiralis* and the mechanisms of reproductive isolation between *F. spiralis* and *F. vesiculosus*.

- Search for cytoplasmic discriminant markers between *F. vesiculosus* and *F. spiralis* in order to resolve the direction of hybridization. Sexual competition experiments between these species and their hybrids.

Local adaptation and population genetic structure in intertidal algae

Main purpose:
Populations isolated near their limits of distribution experience strong selection pressure from abiotic stress and undergo accelerated, environmental stress-driven evolution. Such populations may thus become genetically differentiated, as a consequence of both limited interpopulational gene flow due to geography, and local adaptation to their environment. In order to test these predictions, the project addresses two questions 1) has stress-driven evolution occurred in populations along an environmental stress gradient (local adaptation)? 2) What are the consequences of local adaptation to stressful environments on population structure? Local adaptation may indeed change genetic characteristics of a population, such as mating system, or dispersal, which are reflected in population structure. We aim at combining information from selective traits and neutral genetic markers to study the evolution of local adaptation of edge populations of the intertidal alga *Fucus vesiculosus*.

Achieved in 2003:

- In order to identify partial genes involved in desiccation tolerance four subtracted cDNA libraries were constructed following desiccation and recovery experiments for *F. vesiculosus* by suppression subtractive hybridization (SSH) (2 libraries for up-regulated genes and 2 for down-regulated genes). A full-length cDNA library was constructed for *F vesiculosus* that contains up-regulated genes for desiccation and recovery processes. Subtracted library was screened to identify differentially expressed genes. Approximately 2000 clones were screened from both desiccation and recovery subtracted libraries and we found close to 700 positive clones after "primary" screening.
- Using microsatellite markers, we have identified strong differentiation within the species *Fucus vesiculosus*, where populations were shown to cluster not only according to geographic separation of central versus southern limit locations, but also according to habitat characteristics. There is evidence for either sympatric ecotypic differentiation or multiple colonizations by differentiated sources, and these are still maintained as distinct despite being sympatric.

Microsatellite data have also shown that genetic diversity decreases from the center towards the species margin, whereas the genetic variation between populations increases in populations at the limit, as would be expected from the balance between selection, migration and drift within marginal populations as compared to central populations (ms in prep).

Plan for 2004:

- Positive clones obtained from screening cDNA libraries will be sequenced, and if possible identified, based on homology with known sequences in the databases (blastn and blastx, NCBI).
- Crosses between populations that are highly differentiated in order to evaluate their cross-fertility and their fitness relative to within-population crosses (testing for inbreeding/outbreeding depression).
- Microsatellite analysis of *Fucus ceranoides*, a sister species to *F. vesiculosus* but that is restricted to estuarine habitats, in order to evaluate the taxonomic position of the estuarine types relative to this species.

Circadian and circatidal cycles and signals for the chloroplast: photosynthetic physiology and gene expression responses in *Fucus*

Main Purpose:
Recent investigations of gene expression by our team, have shown that chloroplast-encoded ribulose-1,5-bisphosphate carboxylase (rbcL, rbcS), ATP synthase (atpH, atpI), and photosystem I chl a binding protein (psaA) mRNA abundances vary in response to irradiance and immersion/emersion in *Fucus vesiculosus*. We now aim to evaluate whether the variability in photosynthetic parameters and gene expression in *Fucus vesiculosus*, detected in our previous studies, is related to circadian and/or circatidal signals and/or endogenous rhythms. The work involves determining gene sequences from macroalgal chloroplasts (plastids) to study their regulation and control of expression in photosynthetic organisms living in intertidal environments.

Patterns of chloroplast-encoded gene transcription and adundance in intertidal fucoid algae in relation to circadian and tidal rhythms will be revealed for the first time. Environmental and/or endogenous cues with a role in regulating chloroplast mRNA abundance will be identified

Achieved in 2003:

1. Sequencing of 90% of the chloroplast genome in *Fucus vesiculosus*

2. Gene arrays for large scale plastome gene expression analysis Specific primers were designed for 80 protein-coding genes in *Fucus vesiculosus* that had been sequenced in the lab. From those, 60 were amplified for *Fucus vesiculosus* and *F. serratus* and cloned to produce a nylon-based probe macroarray by spotting all the cDNAs. Technical optimizations were done in the cDNA/RNA labelling methodology. Radiolabelled cDNA/RNAs from different experimental conditions were hibridized to the probe nylon membranes to study the differences in gene expression.

Plan for 2004:

- Ecophysiological experiments to determine whether the chloroplasts have circadian and/or circatidal rhythms. To which environmental cues do abundance/transcription of chloroplast genes respond during immersion/emersion cycles? Irradiance level, water stress (desiccation), and inorganic carbon supply will be experimentally manipulated in culture.
- Gene expression analysis of new photosynthesis-related genes in response to simulated low and high tide conditions, manipulating light and desication levels.
- Finish the amplification and the cloning of the probes that were not amplified and cloned already. Achieve the reproducibility of the labelling methodology for cDNA/RNA. Sample collection, acclimation, and experiments manipulating light and tidal cycle conditions to analyze the different patterns of gene expression for both *Fucus vesiculosus* and *F serratus*. Determination of the transcription start sites using primer extension to map the 5' termini of mRNAs for the genes rbcL and psaA.

Signals for gamete release by intertidal species with external fertilization

Main purpose:

Fucoid algae, like many marine organisms, rely exclusively on external fertilization in the water column for successful reproduction. We use these as model species to investigate how intertidal species with external fertilization can achieve high reproductive success despite the gamete diluting effects of high water motion and whether external fertilization takes place at either slack high tide or as soon as organisms are reached by the incoming tide, as these are the phases that minimize gamete dilution. It is also asked whether for these intertidal populations gamete dispersal is restricted and whether the bottleneck between the large amounts of gametes that are released and the few juveniles that are found is not fertilization success but is instead early post- settlement survival to recruitment.

Achieved in 2003:

-The monthly periodicity of gamete release has been estimated in *Fucus vesiculosus* and *F. spiralis*, by sampling egg settlement daily with artificial substrates shown to trap the eggs (the eggs settle as soon as they are released). The success of fertilization was estimated to be high for both species. Recruitment was estimated as the density of embryos found on artificial substrates at the end of each reproductive season, which was low on exposed coast habitats and high in sheltered areas (ms published).

- We determined the spectral light signals for synchronous gamete release in *Silvetia compressa*. The spectral characteristics of light-induced gamete release suggest that the blue-green light environment in many coastal waters may induce gamete release from receptacles at high tide. The proximity of the effective wavelengths for the opposing processes of light-induced gamete release (effective in blue and blue-green to at least 515 nm) and de-potentiation (maximum measured effectiveness at 530 nm) suggest that the ability of receptacles to de-potentiate in green light reflects the relationship between turbidity (and therefore often in turbulence) and a shift in light

transmission in the water column towards longer wavelengths, functioning as a second sensing mechanism for water motion, in addition to the sensitivity to inorganic carbon supply detected in our previous studies (ms published).

Plan for 2004:

- *Fucus* egg and sperm dispersal shadows will be estimated and compared. The dispersal role of unattached algae carrying reproductive structures will be evaluated.
- The tidal timing of gamete release will be estimated in order to test the prediction that it coincides mostly with slack high tide or low tide.

STRESSREG The molecular basis for differential stress-tolerance in coexisting, ecologically similar algal species.

Main purpose:
The intertidal zone of rocky shores is a severe habitat in which, over a small spatial scale, an extremely strong gradient of changing environmental conditions for marine organisms occurs. Intertidal seaweeds of the genus *Fucus* contain several similar and recently diverged species, with vertical distributions correlated with their desiccation-stress tolerances. However, despite a large amount of ecological literature on this topic, there is yet no mechanistic understanding of the biochemical and molecular basis of desiccation tolerance in fucoid algae. In this research theme we intend to follow a gene isolation strategy, by using PCR-based subtractive hybridization techniques established in our lab to isolate and identify desiccation- and rehydration-responsive genes in *F. vesiculosus*. The high degree of relatedness between *Fucus species* (*F. vesiculosus*, *F. spiralis*, *F. serratus*) will be exploited to isolate full-length cDNA for desiccation/rehydration-responsive transcripts from cDNA libraries of each species. The intention is then to focus more narrowly on an individual gene(s) to identify cis-acting factors involved in transcriptional control. This will involve cloning gene promoters from different species of *Fucus*, and screening using a one-hybrid system to identify proteins that interact with (activate) the promoter.

Achieved in 2003: planning only - project starts in 2004.

Plan for 2004:
We will compare homologous desiccation-responsive genes from the 3 species, their cis-regulatory regions, and transcriptional activation pathways. Results from current work in isolating desiccation- and rehydration-specific transcripts using suppression subtractive hybridization will be used to screen cDNA libraries for 3 species to obtain full sequences of homologous genes, and screen for species-specific variations in gene expression in response to desiccation using Northern analysis. Candidate genes will be selected for further analyses.

Colonization strategies and population genetic structure of invasive algae: the genus Caulerpa in the Mediterranean Sea

Main purpose:
Caulerpa spp are clonal marine algae, which have been shown to act as invasive species, and reported to outcompete seagrasses. In the Mediterranean, two exotic *Caulerpa* species, *Caulerpa taxifolia* (probable introduction via aquaria from Australian sources) and *Caulerpa racemosa* (migrant from the Red Sea) have spread into areas formally occupied by seagrasses, and there is concern about the potential environmental and economic negative effects on the ecosystem. There is also a native species, *Caulerpa prolifera*, which opportunistically occupies disturbed *Posidonia* meadows. Very little is known about the reproductive strategies, clonal diversity and population structure of these species, despite growing concern about their spread as invasive species. The purpose of this project is to examine patterns of clonal growth, clonal structure and genetic composition of recently established populations of *Caulerpa taxifolia* and *Caulerpa racemosa* with populations of the native *Caulerpa prolifera* in the Mediterranean.

Achieved in 2003: planning only - project starts in 2004.

Plan for 2004:
Development of microsatellite markers for *Caulerpa prolifera*. Field studies of population demography to estimate the spread of this species by asexual or clonal reproduction. Sampling of populations for genotyping in 2005.

Group: ALGAE - Marine Plant Ecology

Research team

Leader: Rui O. P. Santos
Post-docs: Raquel Carmona, Alexandra Cunha
PhD students: João Silva, Raquel Machás, Susana Cabaço, Cecile Godinho, Leonardo Mata, Andreas Schuenhoff, Estibaliz Berecibar
Master's students: Ana Alexandre, Vasco Vieira
Research Assistants: Aschwin Engelen, Helena Barracosa
Technicians: Catarina Alves, Luís Dias, Carla Domingos, Rui Candeias
Honours Students: Ana Barradas, Rodrigo Delgado, Vânia Raposo
Diving officer: Pedro Neves

Summary of activities and progress during 2003

THE METABOLISM OF COASTAL LAGOONS

Our research on the metabolism of Ria Formosa aims the role of biota, particularly of plants, in the organic matter, nutrients and gas (e.g. O_2 and CO_2) fluxes in the ecosystem. The understanding of ecosystem function will allow addressing important processes, such as the human utilization of the ecosystem, the regulation of atmospheric CO_2 concentrations, or global change scenarios.

The specific lines under research and a summary of achievements of 2003 were:

Seagrass carbon and nitrogen uptake
Zostera noltii plants from the upper intertidal have a higher capacity to use HCO_3 as inorganic carbon source for photosynthesis than lower intertidal plants (Mercado *et al*., 2003).
Nitrate reductase activity of *Zostera noltii* is always higher in the upper intertidal at all temperatures tested. NR activity is higher in the leaves than in the roots (Alexandre *et al*., submitted).
Photosynthetic ecophysiology of seagrasses
Zostera noltii presents shade- and sun-type plant behaviour, at its lower and upper vertical distribution limits, respectively (Silva and Santos, 2003).
As opposed to what is described for other locations, the net photosynthetic capacity of *Zostera noltii* in Ria Formosa is lowest in the summer and highest in the winter months, both in the upper and lower intertidal. During the summer, the plants dissipate excessive inhibitory energy and reduce the productivity. In winter, plants are exposed to ideal conditions for photosynthesis and growth (Silva and Santos, in prep.)
The use of chlorophyll *a* fluorescence as a valid proxy for photosynthetic production was validated for *Zostera noltii* (Silva and Santos, submitted)
Organic matter fluxes
Primary producers with a minor contribution of microalgae (5%). Digestion of lagoon seston is selective; the filter feeders are assimilating a mixture of phytoplankton and microphytobenthos.

Stable isotopes values did not differ between mussels collected on buoys and clams collected in the sediment, suggesting a considerable mixture of benthic-pelagic organic matter throughout the water column (Machás *et al*., 2003)

Algae-sediment relationships

The best predictors for surface sediment stability are chlorophyll a, colloidal carbohydrate, water and organic content. Typically, critical erosion thresholds decreased seawards, reflecting a change from biostabilisation by cyanobacteria (summer) or *Enteromorpha clathrata* (winter) in the upper intertidal areas, to biostabilisation by diatoms on the bare substrata of the channel edges (Friend *et al*., 2003).

Metabolism and carbon fluxes of Ria Formosa lagoon

The most productive community of Ria Formosa is the seagrass *Zostera noltii*, but benthic microalgae are responsible for most of the net production of the ecosystem. The western sector of Ria Formosa is autotrophic, with a P/R ratio of 1.39, the highest yet reported for estuarine ecosystems. The daily net export rate to the adjacent coastal waters represents 14 % of the net ecosystem production, suggesting that the bulk of the ecosystem net production accumulates within the ecosystem. The organic carbon retention is higher than net production, as the allochthonous carbon inputs from urban sewage enter the carbon mass balance with about 40% of the autochthonous processes. The western sector of Ria Formosa sinks every year about 46.4 tons of organic carbon. Most of this is removed in the form of clams (Santos, R., *et al*., submitted).

THE HUMAN IMPACTS ON COASTAL LAGOONS

This research line focuses on the effects of the human-related disturbances on the seagrasses and salt marshes of the most important costal systems of southeastern Portugal, the Ria Formosa lagoon and the Guadiana estuary. Our aim is to contribute with sound scientific inputs to the conservation and management of these systems.

Impacts of urban effluents on Ria Formosa lagoon

Manuscripts in preparation:

Cabaço, et al. Porosity of belowground tissues of the seagrass *Zostera noltii* under sediment anoxia conditions

Machás, R, et al. Urban effluent distribution and biological uptake in Ria Formosa

Impact of inlet relocation and inlet migration on the landscape-scale dynamics of seagrasses in Ria Formosa lagoon

Manuscripts in preparation:

Cunha, A., et al., M. Landscape-scale changes in response to disturbance by barrier-islands dynamics.

Cunha, A.and Santos, R The use of fractals to assess seagrass landscape stability.

ECONOMIC VALORIZATION OF SEAWEEDS

The research on the economic valorization of seaweeds focus on the red alga *Gelidium sesquipedale*, which is harvested in Portugal for the extraction of agar and on the integrated aquaculture of seaweeds in fish farms, in order to biofiltrate their effluents and to produce economically valuable biomass.

Ecophysiology and resource management of *Gelidium sesquipedale*

Vegetative growth of erect fronds from the prostrate system is the main process of *Gelidium sesquipedale* recovery from disturbances, particularly commercial harvesting. Prostrate axes store considerable amounts of carbon and mainly nitrogen, besides being highly independent on environmental changes, which makes them crucial for perennation (Silva and Santos, 2003).

Divers operating from small boats hand-pluck *Gelidium sesquipedale* along the Portuguese coast for the extraction of agar. The relationship of the catch per unit of effort with the cumulative harvest

throughout the harvest season was used to estimate the species catchability, the pre-harvest standing stock and the annual exploitation rates. We showed that the combined use of these indexes is a powerful tool to manage this resource (Santos *et al*., 2003).

Seaweed-based integrated mariculture

The optimum cultivation density of Ulva rotundata ranged between 0.8 kg m-2 and 1.3 kg m-2. Ammonium-N uptake, tissue N content (% DW) and yield as a function of ammonium-N flux indicated that below fluxes of 9 µmol NH4+-N L-1 h-1, *U. rotundata* was N-limited. At this ammonium-N flux, the NH4+-N removal efficiency was 75% during daytime, but decreased to 52% during the night (Mata and Santos, 2003)

No photoinhibition was observed in *Falkenbergia rufolanosa* at biomass densities higher that 4 g FW l-1, except when cultivated at a very low ammonium flux (5.8 µM h-1). Even when exposed to full solar light for one hour, *F. rufolanosa* showed a fast recovery of photoinhibition after two hours in the shade, suggesting a high photosynthetic capacity under a wide range of irradiances. Maximum net photosynthesis peaked at 15 ºC, but was still high until 24 ºC and only at 29 ºC oxygen exchange was significant reduced. This suggests that the mass cultivation of this species in southern Portugal will be high over most of the year, particularly at lower temperatures, but it will be difficult to maintain during hot summer periods (Mata et al., submitted)

Manuscripts in preparation:

Schuenhoff, A *et al*. R. *Falkenbergia rufolanosa* - a novel seaweed biofilter for mariculture effluent.

Plan for 2004

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

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

Financial support was obtained from FCT to develop this project. The project started in December 2003.

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

biodiversity for conservation purposes, but the available data on the Portuguese algal communities are very scarce.

Environmental Education
Financial support was obtained from the Environmental Institute (Instituto do Ambiente) to develop a project to evaluate the environmental education equipments in Portugal. We aim to assess the environmental equipments in Portugal, their implementation and development. Criteria will be established to evaluate them. This project started in December 2003.

Group: Ecologia dos Recursos (EcoRecursos)

Research team

Leader: Luis Chícharo, Alexandra Chícharo
Visiting scientist: Suwanna Panatrakul
Post-docs: Marina Delgado
PhD students: Pedro Morais, Joaquim Teodósio, Ana Amaral, Rita Borges, Vanessa Moschino, Adriana Candeias
Master's students: Isabel Gouveia
Honorous thesis students: Ana Faria
Research Assistant: Isabel Marques

Summary of activities and progress during 2003

Main purpose:
1 – To study how environmental impacts caused by fishing gears affect organisms' condition
2- To evaluate how the environmental factors affect the biochemical condition and recruitment success of several meroplanktonic larvae
3- To analyse changes in benthic communities due to antropogenic disturbances
4- To study the impact in river inflow in estuarine and coastal biodiversity. Within this aim, the group collaborated with UNESCO IHP (International Hydrologic Programme) under the Ecohydroloy programmne

Achieved in 2003:

1 – Publication of several papers related to this subject: Chicharo, M. A., Amaral, A., Condinho, S., Alves, F., Regala, J., Gaspar, M., and Chicharo, L. (2003). Adenylic-derived indices and reburying time as indicators of the effects of dredging-induced stress on the clam Spisula solida. Marine Biology 142, 1113-1117.
Chícharo, M. A., Chícharo, L., Amaral, A., Condinho, S., and Gaspar, M. (2003). Chronic effects of dredging-induced stress on the clam (Spisula solida): nucleic acid and lipid composition. *Fisheries Research* 63, 447-452. Chícharo *et al* 2003a, b.

2- Publication of the paper related with this subject: E., Santos, A. M. P., dos Santos, A., Peliz, A., and Ré, P. (2003). Are sardine larvae caught off northern Portugal in winter starving? An approach examining nutritional conditions. *Marine Ecology-Progress Series* 257: 303-309.
3- Publication of several papers related to this subject: Alves, F., Chícharo, L., Nogueira, A., and Regala, J. (2003). Changes in benthic community structure due to clam dredging on the Algarve coast and the importance of seasonal analysis. Journal of the Marine Biological Association of the United Kingdom 83, 719-729. Gaspar, M. B., Leitão, F., Santos, M. N., Chícharo, L., Dias, M. D., Chícharo, A. and Monteiro, C. C. (2003). A comparison of direct

macrofaunal mortality using three types of clam dredges. Ices Journal of Marine Science 60: 733-742.
Gaspar, M. B., Leitão, F., Santos, M. N., Sobral, M., Chícharo, L., Chícharo, A. and Monteiro, C. C. (2003). Size selectivity of the Spisula solida dredge in relation to tooth spacing and mesh size. Fisheries Research 60: 561-568.
Gaspar, M. B., Santos, M. N., Leitão, F., Chícharo, L., Chícharo, A. and Monteiro, C. C. (2003). Recovery of substrates and macro-benthos after fishing trials with a new Portuguese clam dredge. Journal of the Marine Biological Association of the United Kingdom 83: 713-717.

4- Participation in UNESCO Working Group on Ecohydrology as a tool for restoration and management of the coastal zone, meeting in Hel Poland Abril 2003. Participation in "Integrated watershed management" (sessão 1-4.16) no âmbito do World Water Forum-3, entre 16 e 22 de Março de 2003, em Kyoto (Japão). Programa Ambiental das Nações Unidas UNEP-IETC.
Submitted paper on Estuarine, Coastal and shelf Science: Are there evidences of changes in the estuarine fish communities due to changes in fluvial outflow in Guadiana river (South Portugal)?

Plan for 2004

To continue research lines started in 2003, with particulary emphasis in:

1- Remediation and mitigation studies of the impacts of anthropogenic activities on the ecosystems
2- Ecophysiological and biochemical studies of exotic versus indigenous species
3- Condition and retention mechanisms of estuarine and coastal fish larval
4- Organising an international whorkshop on Ecohydrology, under the support of UNESCO.

Group: Crustacean Biology and Fisheries

Research team

Researchers: Margarida Castro, Margarida Cristo and Margarida Machado.
PhD students: Paula Serafim.
Technician: Artur Araújo.

Non-cladoceran branchipods in temporary ponds
Main Purpose:
Obtain an inventory of species present in ponds form the South of Portugal. To study of the biology ans population dynamics of the most important species:
Achieved in 2003:
1. Identification of species present in 19 ponds.
2. Registration of 1 new species for Portugal.
3. Registration of one new species worldwide.
4. Following of the biological cycles of 4 species in 7 ponds.
5. Study of the accompanying fauna, mainly amphibians.

Survival of invertebrate non-target species of crustacean trawling, discarded on board
Main Purpose:
To obtain estimates of survival rates of discarded invertebrates. To assess stress and damage cause by capture and release.
Achieved in 2003:
1. Field experiments in the laboratory and on boards crustacean trawlers.

Spiny lobster biology and management

Main purpose:

To update the information of the biological cycle of *Panulirus elephas*.
Characterization of the fishing activity of the artisanal fleet in the port of Sagres.
Set up of lobster larvae collectors for estimation of recruitment.

Achieved in 2003:

1. Testing of larval collectors
2. Definition of trap models

Plan for 2004

- Continuation of the ecological studies in temporary ponds. A sample of the previously studied ponds will be observed for understanding year-to-year variation. Submission of the newly recorded species to international zoological boards.
- Continuation of the feeding ecology of deep water decapods
- Seting of *puerulus* collectors for estimation of temporal and spacial paterns of settlement for the rock lobster *(Panulirus elephas)*
- Construction of traps for testing selectivity for the rock lobster *(Panulirus elephas)*
- Microscopic evaluation of intermolt stages in Norway lobster (*Nephrops norvegicus*) evaluated in resin preparations of abdomen segments.
- Data analysis of field and laboratory studies to quantify survival of discarded invertebrate species.

Group: Fisheries Biology and Hydrobiology

Research team

Leader: José Pedro Andrade
Principal researchers and post docs: Pedro Domingues, Jorge Palma
PhD students: Eduardo Esteves,
Technicians/ research assistants: Rita Sá, António Sykes

Summary of activities and progress during 2003

Cuttlefish culture:

The European cuttlefish (*S. officinalis*) has been cultured throughout the life cycle in the University of the Algarve for the past 4 years. In 2003, a 6th consecutive generation has been cultured in the Ramalhete field station, all from a batch of 50 reproducers from the first generation (F1). Cooperation with a private company has been initiated this summer to produce cuttlefish in semi-intensive aquaculture in earth ponds. Cuttlefish has been cultured at a rate of two generation per year.

Effects of inbreeding with consecutive generations have also been addressed and showed that size, duration of life cycles, mortality, fecundity and other aspects of the life cycle have been mainly affected by temperature.

Cuttlefish feeding ecology:

Research during 2003 was directed to daily ration estimates. These estimates were based on gastric evacuation rates combined with data on weight of stomach contents at intervals, obtained during 24 hour sampling of wild animals. This information was collected at two-hour intervals

during 4 (one in each season) 24 hour cycles. Information on gastric evacuation rates was combined with field data on stomach fullness to estimate daily ration.

Plan for 2004

The experimental culture of cuttlefish, Sepia officinalis, will be, during 2004, the main research area of this group. During 2004, research will be focused on the use of nucleic acid derived indices and instantaneous growth rate as tools to determine different nutritional condition in cuttlefish hatchlings
Groups of cuttlefish hatchlings will be used to determine the duration of the yolk reserves, during which growth can be obtained with no food supply. These will be fed live grass shrimp ad libitum from the third day of life onwards Nucleic acid derived indices and instantaneous growth rates (IGR) will be used as a way to describe their condition, when all the yolk reserves would be exhausted and to determine to most accurate tool to express growth and condition.

Group: Coastal Fisheries Research

Research Team

Leader: Karim Erzini
Post Doc: Jorge M.S. Gonçalves
PhD students: Joaquim Ribeiro, Rui Coelho and Humberto Hazin.
MSc: Pedro Monteiro
MSc Students: Luís Bentes, Hugo Saldanha, Sara Vanessa Santos, Rogério Ferreira
Technicians/ research assistants: Pedro Veiga, Margarida Corado and Daniel Machado

Summary of activities and progress during 2003

Ongoing Projects:

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

New Projects:

1. Recruitment of Fish Species of Commercial Interest in the Arade River Estuary. MARE Programme - P.O. Pescas (Cód. 22-05-01-FDR-00017)
2. The experimental evaluation of marine biocenoses associated with the extraction of sand and gravel off the central Algarve (CCMAR-DRAOTA)
3. Scientific bases for the management of fisheries resources of common interest (Project GESTPESCA – INTERREG III)

Plan for 2004

Project BRD: The research group will focus on the analysis of data and the preparation of reports and papers based on the experimental fishing trials carried out with BRDs for the coastal seine fishery. The implications for discarding will be evaluated, especially in terms of survivorship of fish that escape through these devices.

Project RENSUB: In this line of research, the first phase of sampling by divers, dredging and trawling will be finished and attention will be focused on the data analysis for this phase of the project concerning the evaluation of the impacts of extraction of sand and gravel on the subtidal biological communities. A GIS will be implemented, integrating geological, biological and statistical

information in a geo-referenced framework. This will constitute the first mapping of a biological nature of the marine bottom of the Central Algarve.

Project ARADE: In this project monthly sampling will take place in the estuary of the Arade River over a one year period with five different fishing gears with a low selectivity. The sampling design will take into consideration factors such as the tide, salinity, depth, type of bottom and proximity to man-made structures.

Project GESTPESCA: The spatio-temporal distribution and habitat use of several species of sea bream (family Sparidae) will be studied by telemetry. VEMCO receivers, hydrophones and pingers will be used to track tagged fish over short and long periods in the vicinity of artificial reefs located off the south coast of the Algarve.

Underwater sea mounts: The research group will continue to participate and provide logistical support to the expeditions exploring the underwater sea mounts of the Atlantic Ocean. In addition, data from the last expedition (Gorringe 2003) will be analysed.

RIA FORMOSA: The long term monitoring of the fish fauna of the Ria Formosa will continue with sampling with beach seine scheduled for the spring / summer of 2004.

Within the framework of PhD programmes, work will continue on the study of the relationships between swordfish fisheries and oceanography in the Atlantic as well as the fisheries biology, ecology and population dynamics of deep water sharks.

In addition to the above mentioned research, the research group will continue to be available to provide information and support to requests for scientific dissemination from parks and nature reserves, media, museums, municipalities, federations and sport clubs and associations of fishermen and vessel owners.

Group: Biodiversity and Biology in Fisheries (BIOPESCAS)

Research team

Leader: Teresa Cerveira Borges
PhD students: Maria Esmeralda Costa
MSc students:.Inês Gonçalves, Sónia Olim
Technicians/ research assistants: João Sendão

Group: Fish Parasitology and Reproduction

Research team

Post-doc: Isabel Afonso-Dias (researching in the Faculdade de Ciências do Mar e do Ambiente under the responsibility of Manuel Afonso-Dias) with Ken Mackenzie acting as a consultant regarding Ichthyoparasitology.

Summary of activities and progress during 2003

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

The work under this Post-doctoral grant was carried out as previously stated in the last report. A summary of the current status of this work was provided by Dr Ken Mackenzie to be published (http://www.diplectanum.dsl.pipex.com/newsletter/2004/newsl11.htm#upd) in the International Ichthyoparasitology Newsletter, No. 11, January 2004, under the Current Research Activities in the United Kingdom.

As previously stated in last year's report, the identification at species level within some groups of parasites required the collaboration of specialists. The time required to process and analyse this set of date, is beyond our control, therefore the specimens of *Anisakis* spp. retrieved from both host species were sent to Prof. Simonetta Mattiuci (Dipartimento di Scienze di Sanità Pubblica Sezione di Parassitologia Università di Roma "La Sapienza") for identification to species level using genetic analysis. In the case of the three new species of Myxosporea found in collaboration with Prof. Kalavati and Dr. Mackenzie, this work should have been published by now, if it did not suffer two setbacks. 1- The gallbladders were examined and infections found classified in a binary way, presence or absence of parasites. Since two species of Myxosporea were found in *L. piscatorius,* the samples have to be all re-examined to estimate the prevalence of each parasite. The second setback was the fact that to publish a new species record, it is not 'convenient' to work with frozen material. In order to obtain good results new contacts with fishermen to get fresh samples had to be made to get fresh samples. In the case of *L. budegassa* that was already done, the problem now is getting the samples from the west coast of Scotland and preserve them in gluteraldehyde so that the description of the new myxosporeans can be made according to the guidelines and recommendations given by Lom and Arthur (1989).

The difficulties found in previous years tracing down dispersed papers and host-parasite records were felt again during research activities in 2003.

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

This research activity consists of occasional consultancy and data analysis regarding routine histological examinations. Some of the activities performed included preparation of resin-embedded tissue (sardine's gonads), cutting of sections, staining and interpretation of germinal cells development. These activities are integrated in different projects carried out at the Instituto de Investigação das Pescas e do Mar (IPIMAR), in Lisbon, to describe temporal and regional variation in sardine maturation patterns. This collaboration produced a working document with an oral presentation presented to the ICES (International Council for the Exploration of the Sea) working group meeting in Malaga (Study Group on the estimation of Spawning Stock Biomass of Sardine and Anchovy, Malaga, Spain, 23–27 June 2003).

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

During 2003 the work under the above-mentioned title was submitted for publication in a shape of a scientific paper to 'Fisheries Research'. Although both referees accepted it with changes, the editor found it 'beyond the scope of the journal'. This work was revised, changed and re-submitted to 'Scientia Marina'.

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

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

caused by *Eimeria sardinae*. How serious in terms of male reproductive success? During the 2003, smears from fresh material were made to try to assess the extent of this infection in sardine, a very important Portuguese fish resource.

"Reproductive aspects of the Cunene horse mackerel *Trachurus trecae*, Cadenat, 1950 from the Coast of Angola"
This study is inserted in the existing cooperation between the University of Algarve and the Marine Institute of Research (IIM) of Angola. This action in particular, aims to transfer knowledge regarding fish reproductive studies. This species was selected because it is a very important fish resource that seems to be overexploited and of which little is known regarding its reproductive biology. During May-June we received the visit of Mr. António Barradas to do the laboratory procedures necessary to his honours project.

"Comparing the parasite fauna of *Lophius* spp. with that of another top predatory fish, the halibut *Hippoglossus hippoglossus*"
Considering the interesting results obtained regarding the gastrointestinal parasites of the two species of *Lophius*, it seemed appealing to compare the parasite fauna of these species with that of another top predatory fish, such as halibut *Hippoglossus hippoglossus*, and to look for links between the diets and parasite faunas of the two fish. The opportunity arose when Prof. W. Hemmingsen (University of Tromsø) proposed the participation of Isabel Afonso-Dias in a multidisciplinary research vessel survey along part of the coast of North Norway from 17 October to 3 November 2003. During this survey (Figure 14) 22 specimens of halibut and 2 anglerfishes were surveyed for parasites. The halibut harbours different species of parasites, which pass through a series of unrelated hosts in their life cycle. The parasites retrieved from these hosts were identified to genus and species level.

Figure 14- Retrieving parasites from a small halibut on board the RV "Jan Mayen". I. Afonso-Dias (front) and K. Mackenzie (back).

Plan for 2004

***"*Anglerfish (*Lophius piscatorius and L. budegassa*) Parasites: Study to assess the possibility of using anglerfish parasites as biological tags"**
At this stage, it is possible to say that the amount of work involved in the collection of host samples, recovery and identification of parasites was greatly underestimated. The literature collation and research in this field of research is very time-consuming for just one person. However, with all the information collected it is possible to perform follow two lines of research:

1- Two different species of the digenean genus *Stephanostomum* have been found in the Northeast Atlantic species of *Lophius* (monkfish). *Stephanostomum cesticillum* was reported by Bray and Bartoli (2001) from *Lophius piscatorius* caught in the Gulf of Marseilles, France, and was also found by Afonso-Dias and Mackenzie (in preparation) in *Lophius budegassa* caught off the Algarve coast. Another species, *Stephanostomum lophii*, was described from *L. piscatorius* caught off the north coast of Spain by Quintero et al. (1993). One of the main diagnostic features of these digeneans are the oral spines, but these tend to disappear in frozen specimens so, although we found specimens of *Stephanostomum* in deep-frozen samples caught to the west of Scotland, it was not possible to identify them to species. Eydal and Ólafsdóttir (2003) described the same problem in frozen material from Icelandic waters. We suspect that these *Stephanostomum* species may have different geographical distributions, in which case they could make useful biological tags in population studies of *Lophius* spp. To investigate this we plan to examine fresh samples of *Lophius* from areas to the north of Algarve and Norway.

2. Three different species of myxosporeans were found in the gall bladders of *Lophius piscatorius* and *L. budegassa* from west of Scotland and off the Algarve coast. When samples of these were sent for identification to an Indian colleague, Professor C. Kalavati, they all proved to be new species. Detailed descriptions are now being prepared for publication. Myxosporeans have been successfully used as biological tags in several previous studies, so we plan to investigate the geographical distributions of these parasites by examining *Lophius* gall bladders from other areas, particularly in northern waters.

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

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

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

The activities planned in this field of research for the coming year include the preparation of written material for publication, may be in the IX EUROPEAN MULTICOLLOQUIUM OF PARASITOLOGY (EMOP IX), Valencia, Spain.

"Reproductive aspects of the Cunene horse mackerel *Trachurus trecae*, Cadenat, 1950 from the Coast of Angola"

This study is expected to be finished during 2004.

"Comparing the parasite fauna of *Lophius* spp. with that of another top predatory fish, the halibut *Hippoglossus hippoglossus*"

During 2004 we intend to analyse the data collected last year and discuss the viability of further developing this investigation.

***"*Gastrointestinal parasites of Sardine (*Sardina pilchardus, Walbaum 1792*): Study to assess the possibility of using sardines' parasites as biological tags"**

This study will start during February and intends to identify the parasites of the Atlantic Sardine collected by IPIMAR (Instituto de Investigação das Pescas e do Mar) for morphometric studies. The samples are frozen, which can cause some problems in the parasites identification, but since they were available it was a waist not to use them. The parasitic study intends to complement the already in placement study of stock identification of this host species.

Externally funded Projects

Division of Aquaculture and Biotechnology

New and ongoing beyond 2003

Title: "ARRESTED DEVELOPMENT: The molecular and Endocrine Basis of Flatfish"
Reference and funding entity: Q5RS-CT-2002-01192
Summary and Objectives: Flatfish species form a major focus of the diversification of European marine aquaculture industry. However, production has been severely hampered by biological problems in larval rearing. This project focuses on solving the major problem of arrested metamorphosis, in order to reliably control the resulting juvenile quality and production quantity. This will be achieved by addressing key questions regarding the morphological, endocrine and molecular basis of metamorphosis in the Atlantic halibut as a model species. The ultimate goal of the project is therefore to strengthen European aquaculture of marine flatfish species, an important emerging industry in many rural areas, by facilitating the production of fully functional juveniles.

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

Description of the work
1) The events of metamorphosis in halibut will be analysed by monitoring biochemical and morphological markers of metamorphosis. These markers are skeletal development, erythrocyte morphology, expression of troponin-T isoforms, production of gut enzymes, and expression of immunoglobulins.
2) Differential gene expression will be analysed by screening of a gene microarray based on genes induced during metamorphosis, This will allow high throughput analysis of an extensive panel of genes involved in metamorphosis.
3) Endocrine regulation of metamorphosis will be analysed in terms of hormone levels and hormone responsiveness. Tissue and plasma levels of thyroid hormones, cortisol, growth hormone and IGF-1 during metamorphosis will be determined by RIA. Prolactin expression will be monitored by assaying its mRNA. The genes encoding receptors for prolactin, cortisol and growth hormone will be cloned. The temporal and spatial expression of these genes, along with the already-cloned thyroid hormone receptor will be analysed during metamorphosis by competitive RT-PCR and in situ hybridisation.The role of individual hormones during metamorphosis will be further investigated through hormone treatment experiments on pre-metamorphic and metamorphosing larvae.
4) Abnormally metamorphosing larvae will be collected, grouped according to type of abnormality (arrest of metamorphosis, pigmentation defect, inappropriate eye migration etc). These larvae will be compared with normally metamorphosing larvae from the same culture in terms of expression of biochemical and morphological markers of metamorphosis, differential gene expression, expression of hormone receptors and hormone content. This will indicate the underlying molecular and/or endocrine bases of the various abnormalities.
5) In vivo treatments with hormones and nutritional supplements will be carried out to establish functional relationships.
Milestones
A staging scheme for metamorphosis, based on biochemical, morphological endocrine and genetic

markers. A ""metamorphic"" microarray of 700 genes to identify endocrine and molecular basis of metamorphic arrest. Larval treatments with hormones, iodine arid selenium to establish functional relationships between nutritional factors, endocrine axes and metamorphosis, establishing ""functional feeds"" and scientific tools for effective metamorphic larval aquaculture
Duration: 1/10/2002 – 31/09/2005
Research team: D. M. Power
Total budget: Euro; **Funding for CCMAR**: 217 536 Euro

Title: "Bridging genomes: an integrated genomic approach toward genetic improvement of aquacultured fish species"
Reference and funding entity: EC Q5RS-CT-2001-01797
Summary and Objectives: The project will use a far reaching but parsimonious approach to:

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

The project workplan is centered round the application of modern biotechnological methods to aquaculture. It is subdivided into 7 main workpackages, each of which is co-ordinated by the partner with the relevant expertise, and the completion of which will contribute to the attainment of the project objectives.
The workpackages articulate with each other and can be subdivided into 3 main groups according to the nature of the methods they use:

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

Duration: 1/11/2001 – 31/10/2005
Research team: D. M .Power, A. Canário.
Total budget: Euro; **Funding for CCMAR**: 211 906 Euro

Title: "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

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
Duration: 1/09/2002 – 31/08/2004
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

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

Summary and Objectives: *Salaria pavo* is small bottom living fish in the littoral zone of the Mediterranean and adjacent Atlantic coast. The closely related freshwater species, *S. fluviatilis*, inhabits rivers and lakes in the vicinity of the Mediterranean. In both species, the mating system is promiscuous. Males occupy holes or crevices in rocks where females come to spawn and the males subsequently guard the eggs. These are a good model fish species to investigate specialization in sex pheromone production. The males develop anal glands from the epidermis of the first two rays of the anal fin concurrent with development of the gonads. The research team has previously shown that the anal gland of *S. pavo* is a source of substances that attract pre-ovulatory females and promotes male reproductive success. This suggests that male blennies are "active signallers" in contrast with known pheromone systems in teleosts, where receivers are "chemical spies" detecting gonadal steroids or prostaglandins passively excreted by females into the water *via* the urine or gills.
The proposed work aims to identify the chemical structures of sex pheromones in both species. The marine origin of *S. fluviatilis* is well established and, as with *S. pavo*, it is plausible that the anal gland has a pheromonal function in female attraction. If so, one would expect the pheromonal components of the two species to have similar or closely related chemical structures. In addition, the inclusion of *S. fluviatilis* in the project will simplify the chemical identification, since recording of electro-olfactograms (EOG) is technically easier in freshwater, and can be combined with chromatographic separation of anal gland-produced substances.
The research will involve: 1) testing a pheromonal function for the anal gland of males *S. fluviatilis* in female attraction, through behavioural assays in the laboratory; 2) evaluation of the specificity of pheromonal action through test of the behavioural activity of substances from the anal gland of male *S. fluviatilis* and the marine fish, *S. pavo*, on females of the other species; 3) solid phase extraction of substances released by the anal gland of both species, fractionation of extracts by vacuum distillation and chromatographic techniques, and evaluation of biological activity of each fraction through behavioural assays and EOG recordings; 4) chemical identification of active substances, and synthesis of putative pheromones; 5) confirmation of biological activity of synthesised chemicals.
This research project will likely provide empirical evidence to fill a gap in the current leading hypothesis on the evolution of fish sex pheromones. Known pheromonal systems in teleosts indicate that males are "chemical spies" of information excreted by females. Evidence of true chemical communication is lacking, where senders should have evolved a specialization in the way they produce and release a signal. The results will also add to the knowledge on *S. pavo* biology, grounding management strategies of the populations in the nature park of Ria Formosa (Algarve).
Reference and funding entity: POCTI/BSE/45843/2002, FCT
Duration: 1/02/2004 – 31/01/2007
Research team: Eduardo N. Barata (Coordinator), Peter C. Hubbard, Adelino V.M. Canário.
Total budget: 59.706 Euro; **Funding for CCMAR**: 59.706 Euro

Title: "Design and development of commercial scale farming technologies for sole – **SOLEMATES**"
Summary and Objectives: The overall objective of this project is to design and develop technologies for commercial scale farming of the sole species Solea solea and Solea senegalensis. The project will include the development of a commercial scale fingerling production system, namely optimisation of weaning zoo technical conditions and feeding regimes. It will obtain data on feeding and growth of both species of sole, including a comparison of growth potential in different conditions between the two, and development of an optimised feed for sole. This project

will also work in the development of ongrowing and feeding systems suited for different areas within Europe, more specifically: earth ponds, shallow raceways, circular tanks and terraces.
Reference and funding entity: European Community - CRAFT Project Q5CR-2002-71039
Duration: Nov 2002 – Out 2004
Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Florbela Soares, Sofia Engrola; CIIMAR: Emidio Gomes, Paulo Rema, Luisa Valente; RIVO (NL): Andries Kamstra, Edward Schram; Akvapna Niva (NO): Patrick White, Albert Imsland; DVZ-CLO (BE):Daan Delbare.
Total Budget: 1.801.857 Euros **Funding for CCMAR:** 165.480 Euros

Title: "Development of Virtual Learning Environment in Environmental Science, with Online Re-usable Interactive modules for remote users in marine pollution and ecology, with self learning languages packages in English, Greek, Portuguese and Swedish – **ORION**"
Summary and Objectives: ORION will develop a state-of-the-art Virtual Learning Environment, housing a Resource Repository for a 4-language glossary, self-instruction language modules in English, Greek, Portuguese and Swedish, with environmental science course materials (marine pollution studies, ecology, marine biodiversity, etc.) suitable for the vocational and tertiary sectors at various levels, prepared by university departments and research organisations in Greece, Ireland, Portugal and Sweden. The major goal of ORION is the development of a powerful state-of-the-art ICT learning/teaching tool, to be used within a Virtual Learning Environment and delivered by means of a Distributed Network, in the subject area of the marine environment (pollution studies, ecology, biodiversity, aquaculture, etc.) relevant to both the vocational and tertiary sectors at various levels. This is an area of great importance, subject to increasing legislative and regulatory demands affecting a range of coastal zone users and decision-makers. These multi-disciplinary materials, prepared by top-level trainers in native English, Swedish, Portuguese and Greek, will be developed as innovative re-usable learning objects which can be shared and re-used by teachers and target group users situated in remote areas of Europe: Stockholm in the North, Cork in the north-west, Portugal in the south-west and Greece in the south.
To underpin the training in language which will be needed to maximise the benefits from this innovative methodology, an online marine environmental glossary in English, Greek, Portuguese and Swedish will be created, to form a major resource freely available to partners only, with accompanying modules in ESP (environmental science) and basic Greek, Portuguese and Swedish language modules, to reflect the needs of the target users.
Reference and funding entity: European Community Programme LEONARDO DA VINCI, Community Vocational Training Programme Project nº EL 2001 BP LA 114443**,**
Duration: April 2002 – April 2005
Total Funding: 404.672 Euros
Partners: FEAP (Federation of European Aquaculture Producers), IMBC, Crete, Greece, EKTHE (National Centre for Marine Research, Athens), TEREUS, SA, Athens, Department of Zoology, University of Cork, Ireland, AQUALEX Multimedia Consortium Ltd Ireland, Centre for Marine Sciences, University of Algarve, Portugal, Department of Systems Ecology, University of Stockholm, Sweden

Title: "Feed for aquatic animals that contain 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

Title: "Fish restocking associated to the Algarve artificial reefs: environmental mitigation, biodiversity and fisheries management – **RESTOCKING**"
Summary and Objectives: The project objectives are related to: (i) evaluate the efficiency of restocking of finfish species associated with artificial reefs, using native species where the artificial reproduction and juvenile production are standard procedures, and (ii) develop the methodology necessary for the production in captivity of juveniles of other native with interest for restocking of artificial reefs, and whose populations are depleted as a result of a long and intense fishing effort.
Different methodologies will be developed in order to optimise tagging and releasing techniques. Initially, this will be done for available juveniles of *Sparus aurata* (gilt head seabream) and *Diplodus sargus* (white seabream). In a later stage, this will be done for juveniles of species which production methods will be developed during the project, (grouper *Epinephelus* sp. and the red porgy *Pagrus pagrus*). It should be noted that these are species with 1) ecology interest in relation to artificial reefs; (2) economical interest in terms of fisheries; and (3) interest for the revitalisation of over-exploited marine resources.The evaluation of restocking of fish in artificial reefs areas will be done in function of the size of the specimens, the season and the types of reefs. Classic methodologies will be used in this project, based on analysis of catch (recapture) and direct observation (underwater surveys) carried out in a regular basis. This restocking initiative will be advertised to the general public, in particular fishermen and anglers, to maximise the recapture records. Standard procedures for juvenile production in captivity of species that are not produced in Portugal and of interest for restocking (i.e., grouper and red porgy) will be developed. In particular, methodologies for induction of spawning in captivity and adequate feeding regimes and zootechnical conditions will be studied. This will be done using the methodologies available for other species as reference. This project expects to contribute for an integrated coastal management, promoting biodiversity, biological production and environmental mitigation. It will also contribute to the management of the local fisheries in order to promote their sustainability, with the subsequent socio-economic effects.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI 35608/99
Duration: Jul 2001- Jun 2004
Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Marc Lacuisse, Florbela Soares; IPIMAR: Carlos Costa Monteiro, Miguel Neves dos Santos, Miguel Gaspar, Pedro Pousão-Ferreira.
Total budget: 149.639 Euro; **Funding for CCMAR**: 74.221 Euro

Title: "Hormones and life-history trade-offs and plasticity: a study on alternative reproductive tactics in blenniid fish"
Reference and funding entity: Fundação para a Ciência e a Tecnologia POCTI/BSE/38395/2001
Summary and Objectives: Diversas espécies apresentam histórias vitais alternativas. Contudo, os mecanismos causais subjacentes à plasticidade das histórias vitais e aos "trade-offs" (e.g. investimento na reprodução presente vs reprodução futura) só recentemente têm sido tema de investigação.
Numa população de Salaria pavo (Blenniidae) da Ria Formosa (Portugal) ocorrem dois tipos de histórias vitais alternativas. De entre os machos recrutados no próprio ano, os mais pequenos reproduzem-se como "sneakers", enquanto os maiores continuam a crescer, apenas se reproduzindo na segunda época de reprodução, no ano seguinte, como "nest-holders". Assim, nesta população existem dois tipos de machos sexualmente activos: machos mais velhos e maiores que defendem ninhos e cuidam dos ovos (CT »14 cm; idade ³ 2 anos) e machos mais

pequenos e mais jovens (CT » 10 cm; idade < 1 ano) que imitam o comportamento e a coloração nupcial das fêmeas na tentativa de se aproximarem dos machos que defendem ninhos e fertilizarem parte dos ovos (Gonçalves *et al*. 1996). Os machos que se comportaram como "sneakers" durante a sua primeira época de reprodução podem tornar-se "nest-holders" em épocas de reprodução subsequentes. Deste modo, o comportamento de "sneaking" parece ser uma estratégia condicional nesta espécie.
Neste projecto tencionamos investigar os mecanismos causais que permitem a expressão das histórias vitais alternativas acima mencionadas e dos "trade-offs" envolvidos. Os principais objectivos são os seguintes: 1) caracterizar as vias ontogenéticas alternativas e a plasticidade do comportamento de acasalamento dos machos jovens (i.e. classe etária 0+/1), através de um programa de marcação e recaptura intensivo e de longa duração (3 anos), que recorrerá à implantação de marcas magnéticas; 2) caracterizar os perfis hormonais dos diferentes tipos de machos recém recrutados (i.e. "sneakers" vs. machos não reprodutores), assim como dos "nest-holders" que servirão de referência para os parâmetros de reprodução. Proceder-se-á à quantificação das seguintes hormonas: esteróides sexuais em circulação (e.g. testosterona, 11-Cetotestosterona); ii) neuropéptideos da família da prolactina (e.g. hormona do crescimento, somatolactina, prolactina); iii) gonadotrofinas (GtH I e II); e iv) GnRH e AVT na área pré-óptica; 3) testar experimentalmente os efeitos do tamanho relativo, da densidade e do estatuto social nas decisões da história vital dos jovens machos, manipulando estas variáveis em grupos mantidos em cativeiro e avaliando o impacto da manipulação experimental nos parâmetros referidos em 1); 4) testar de que forma a presença de machos grandes, defensores de ninhos afectam a direcção da via ontogenética dos machos jovens, avaliando o efeito da exposição dos machos recém recrutados aos machos defensores de ninhos.
O conhecimento da biologia reprodutora constitui um excelente modelo para o estudo integrado dos mecanismos causais da plasticidade sexual e das histórias vitais dos teleósteos.
Duration: 2002-2004
Research team: Rui Oliveira (Ispa- coordinator), A. Canário, D. Power
Total budget: 103.080 Euro; **Funding for CCMAR**: Euro

Title: "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

Title:" Isolation of carotenoid-overproducing *Dunaliella salina* strains. **OVERCAROTEN**."
Summary and Objectives: To isolate novel strains of microalgae, namely *D. salina* and *H. pluvialis able to accumulate higher levels of carotenoids at early stages of growth.* This will be accomplished by several strategies: 1) generation and screening of mutants by chemical mutagenesis and carotenoid biosynthesis inhibitors; and 2) metabolic engineering. The latter strategy will be implemented by the development of genetic transformation procedures and expression of homologous and heterologous genes associated with the carotenogenesis in these microalgae.
Reference and funding entity: FCT, PDCTM / MAR / 15237 / 99
Duration: February 2002 – February 2005
Research team: CCMAR: João Varela, Vanessa Duarte, Sacha Coesel, Nuno Henriques. **INETI:** Rui Mendes e Luísa Gouveia; **NÉCTON:** João Navalho e Vítor Duarte; **ESB-CU:** Rui Morais.

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

Title: "Microalgae as cell factories for chemical and biochemical products. **ALGINET**."
Summary and Objectives: Microalgae are microscopic photosynthetic organisms that form the base of the food chain. They have long been proposed as possible "cell factories" for obtaining chemical and biochemical substances. There has been considerable interest in the field in recent years and a number of small companies have been founded to market microalgae and microalgal products. The main products are microalgae used as animal feed or as human health supplements, but a number of companies are marketing products directly obtained from microalgae (e.g. 3R,3'R-zeaxanthin, an important carotenoid). This proposed thematic network aims to overcome these problems by improving communication between researchers and manufacturers in the microalgal field, and by encouraging further development in the field. Its main goals are: 1) Improve communication between workers in the field of microalgae; 2) Focus the direction of European research, to open new markets for microalgal products; 3) To provide a standard reference portal for workers in the field; 4) Enable rapid dissemination of research results, to speed the uptake of new technologies; 5) To attract new interest in the field; 6) To develop an ongoing 'virtual institute' model and lay the groundwork for future RTD projects.
Reference and funding entity: European Union, QLK3-CT-2002-02132
Duration: February 2003 – February 2005
Research team: CCMAR: João Varela Ana Ramos e Ana Rita Marques.
Total budget: 1221878 Euros**. Funding for CCMAR:** 28656 Euros.
Site: www.algi-net.org

Title: "Nutritional requirements and feeding of blackspot seabream (*Pagellus bogaraveo*), a new species for aquaculture – **GORAZ**"
Summary and Objectives: In order to ensure a sustainable growth of the Portuguese mariculture, it is necessary to diversify the offer of cultivated species to avoid market saturation, competition among producers and to increase the efficacy of production facilities, namely hatcheries. The marine teleost, blackspot seabream (*Pagellus bogaraveo*), has a high market price and is considered as a strong candidate species for intensive aquaculture in Atlantic coasts. Up to now, studies with blackspot seabream under captivity are extremely scarce and have dealt mainly with the control of reproduction, larvae and juveniles cultivation techniques. To our knowledge, blackspot seabream has been fed with diets developed for other marine fish, namely gilthead seabream, and no available literature data exists on the specific nutritional requirements of this species. Therefore, the overall objective of this project is to contribute towards a better knowledge of the nutritional requirements and feeding strategies of the larvae and juveniles of blackspot seabream. Concerning the larvae, studies will cover: 1) optimisation of feeding strategies with live preys; 2) evaluation of precocious feeding strategies with micro-particulate diets; 3) optimisation of the dietary composition of micro-particulate feeds (i.e. dietary lipid, protein and energy level and source, adequate dietary PUFA level and DHA/EPA ratio). Regarding the juveniles, studies will evaluate: 1) the dietary protein requirements; 2) the relative potential of proteins, fats and carbohydrates as energy donors; 3) the optimal dietary DP/DE ratio. Given the economic importance and ecological implications of man-made feeds and feeding in aquaculture, the development of nutritionally balanced and environmental friendly diets is of utmost importance for a future establishment of blackspot seabream as a consolidated species in intensive aquaculture.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI 39239/2001.
Duration: Out 2002 – Set 2005
Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Florbela Soares, Laura Ribeiro; CIIMAR: Emidio Gomes, Paulo Rema, Luisa Valente; DAM-SRP da Madeira: Carlos Andrade, Nuno Gouveia

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

Title: "Probiotics and immunemodulation in marine fish larvae and juveniles – **PROBIMU**"

Reference and funding entity: FCT Project nº 38781/BSE/2001
Duration: Mar 2002 – Feb 2005
Summary and Objectives: Most bacteria causing disease in marine fish are opportunistic pathogens that are present as part of the normal seawater microflora. Environmental stress may weaken the immune system of the larvae and allow opportunistic pathogenic bacteria to invade the fish tissues, leading to disease.
Fish larvae drink water soon after hatching, and ingest bacteria associated with the water. Members of the "pioneer" microflora established in the larval gut may acquire a competitive advantage and become part of a persistent flora at the juvenile stage. Therefore, the early colonisation with non-pathogenic bacteria seems to be essential and has been shown to reduce mortalities after infection with pathogenic bacteria. Beneficial effects of probiotics in growth promotion and disease prophylaxis have also been reported. During the early life stages, fish have a very limited specific immune system, making difficult the use of vaccines. Successful use of immunostimulants in marine fish larviculture has been reported in recent years. Use of immunostimulants may aid the fish larvae to overcome stressful situations and critical stages during the early phase of rearing. Immunostimulants may act, either by stimulation of the non-specific mechanisms, or by acceleration of the development of the specific immune system.
This project aims at improving growth and survival of larval and juvenile marine fish using both probiotics and immunostimulants, as well as to find possible interactions between the two approaches. The Senegal sole (*Solea senegalensis* Kaup) and gilthead sea bream (*Sparus aurata* L.) will be used as models. In particular, it is intended to verify to what extent the immune system can be influenced by potential immunostimulants, and whether growth and survival can be improved by manipulation of the species composition of the gut microflora. The project also intends to study the effect of exposure of fish larvae and juveniles to virulent pathogens after immune stimulation and/or modulation of the water microflora.
Ontogeny of the immune system organogenesis, non-specific cellular immunity and humoral immunity will be characterised in sole. Candidate probiotic strains will be isolated from the larval gut microflora based mainly on their inhibitory activity on virulent bacteria. The relative virulence of different pathogenic bacterial strains will be determined through challenge tests.
The effects of immunostimulants and selected probiotics on the development of the immune system, on the immune response, and on fish growth and survival will be assessed. This will be carried out by using standard microbiological, immunological and physiological techniques, both in normal conditions and when exposed to virulent pathogens.
Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Pavlos Makridis; IBMC: Pedro Rodrigues.
Total budget: 71.500 Euro; **Funding for CCMAR**: Euro

Title: "The Role of Olfaction in the Feeding Behaviour of *Solea senegalensis* – **SOLFACTO**"
Summary and Objectives: Food consumption is the primary determinant of growth in fish. Many fish species, in particular those with nocturnal activity and/or in habitats with frequent high water turbidity, rely mostly on chemo-sensory mechanisms for food detection and location. In different species, feeding behaviour is triggered by different chemical substances, some of which may act as attractants via olfaction, and others may act as promoters or enhancers of food consumption via both olfaction and gustation. In general, free amino acids, nucleotides, nucleosides and quaternary ammonium bases have been identified as feeding stimulants in some species.
The Senegal sole (*Solea senegalensis*) is a good model species to investigate olfactory mechanisms underlying feeding behaviour, due to its feeding strategy, and a well-developed olfactory system accessible to electrophysiological recordings. The central aim of this project is to identify substances released by natural food sources that act as olfactory cues involved in food-search behaviour, and ingestion of food. This objective will be achieved by an integration of electrophysiological, behavioural and food ingestion measurements in response to candidate olfactory stimuli.
A method for the electrophysiological recording from the olfactory system of juvenile sole will be employed, based on electro-olfactogram and/or multi-unit olfactory nerve recordings. This will give quantitative measurements to define relative olfactory sensitivities to various chemical stimuli.

Stimuli to be tested include water containing live natural food items (e.g. polychaetes), crude filtered macerates of food items, chromatographic fractions of water containing the food items and of its macerate, and a range of amino acids, nucleosides and nucleotides. The most potent olfactory stimuli identified will be used in behavioural assays.
Two types of behavioural assays will quantify how a given chemical stimuli affect the patterns of food-search behaviour in juvenile sole. The first will employ Y-maze aquaria to quantify the end result of food-search behaviour. The second assay will quantify specific behavioural acts during food-search behaviour observed in single fish. In both assays, sole behaviour will be videotaped for a fixed period of time and subsequently analysed for pre-defined behavioural responses. The strength of these responses will be related to stimulus quality and intensity.
Food consumption measurements and growth trials will be conducted to test the effect of olfactory stimuli shown to improve food-search behaviour on the ingestion of inert food pellets by juvenile sole. Measurement of ingestion will be obtained by a method employing X-radiography.
Finally, we will examine whether olfactory stimuli that affect feeding behaviour of juvenile sole also act in stimulating behavioural elements associated with feeding in early juveniles (after metamorphosis) during the weaning period.
Ultimately, the project will identify the chemical cues that are important in the initiation of food-search behaviour, food ingestion, and therefore growth. This may be the ground for future technological development of new inert food diets that enhance the farming of this commercially important species.
Reference and funding entity: POCTI/CVT/38831/2001, FCT
Duration: 1/01/2003 – 31/12/2005
Research team: Eduardo N. Barata (Coordinator), Peter C. Hubbard, Adelino V.M. Canário, Luis Conceição, Pavlos Makridis, Maria T. Dinis.
Total budget: 100.000 Euro; **Funding for CCMAR**: 100.000 Euro

Title: "The underlying mechanisms of the effect of microalgae on the early life stages of fishes – **MICROALGAE**"
Summary and Objectives: The benefical role of microlagae on the development of marine fish larval is widely reported, however the mechanisms are still poorly understood. This project aims to understand a bit further how microlage affect marine fish larvae early stages. Two species of microalgae (Tetraselmis chui and Isocrhysis galbana), a commercial microalgae concentrate (Fitobloom®, Necton) and clear water, will be used as treatments. Sea bream and sole were the marine fish species used in this study since they are commonly used for aquaculture in the southern Europe. This project intends to analyse: 1) the effect of microalgae on larval ontogeny, growth and survival, biochemical composition and larval condition; 2) the effect of microalgae on the activity of digestive enzymes and some key enzymes of intermediary metabolism; 3) the effect of microalgae on food intake; and 4) the effect of microalgae on the modulation of the intestinal microflora. The project expects to increase the knowledge on the effect of microalgae on fish larval development, which will contribute to obtain higher quality aquaculture products.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/BSE/37378/2001
Duration: Out 2002- Set 2005
Research team: CCMAR: Maria Teresa Dinis, Laura Ribeiro, Luis Conceição, Pavlos Makridis, Rui Rocha, Pedro Cação, João Sendão; ICETA: Emídio Gomes.
Total budget: 67,000 Euro; **Funding for CCMAR**: 59518.02 Euro

Title: "The use of RMBC's for bacterial management in marine larval fish – **RMBC**"
Reference and funding entity: European Community - CRAFT Project Q5CR-2002-72221
Duration: Jan 2003 – Dec 2004
Summary and Objectives: The challenge of this study consists in screening mixtures of bacteria at specific stages of the larval rearing where bacterial interference may be critical. These bacterial communities, referred to as RMBC's (Revolving Multifunction Bacterial Communities), will be derived form good performing live food cultures, healthy farmed animals, and organically rich environment. The bacterial mixtures will be tested on live food (rotifers and *Artemia*) and fish eggs

and larvae. The benefit for the seabream hatcheries sector will be, thanks to using RMBC's, the development of a sustainable, more predictable method of production of live food and larval fish and the exclusion of antibiotics.
Research team: CCMAR: Maria Teresa Dinis, Pavlos Makridis
Total budget for CCMAR: 50.000 Euros

Completed in 2003

Title: "Characterization of the endemic infection of the clam *Ruditapes decussatus* by the parasite *Perkinsus atlanticus* (protista, apicomplexa) in the Portuguese coast"
Summary and Objectives:
To evaluate the status of bivalves towards infection by *Perkinsus* sp. in the Portuguese coast. To initiate an epidemiological study.
Reference and funding entity: PDCTM/P/mar/15308/1999
Duration: October 2000-2003
Research team: L. Cancela: Project Coordinator: C. Azevedo (CIMAR-ICBAS- Univ Porto) CCMAR team:M. T. Dinis, R. Leite, Ricardo Afonso, Laurence Elandalloussi.
Total budget: 125.000 Euro; **Funding for CCMAR**: Euro

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, P.M. Rodrigues
Funding: 98,000 Euro **Funding for CCMAR**: Euro

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: “Determination of the ideal dietary amino acid profile for larval and post-larval marine fish – **IDEAAL**”

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- Jul 2003

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

Total budget: 104.398 Euro; **Funding for CCMAR**: 95.240 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: 2.450.118 Euro; **Funding for CCMAR**: 116.192.40 Euro
Web site: http://www.abdn.ac.uk/aqcess/

Division of Living Resources

New and Ongoing beyond 2003

Title: "**ALIENS** - Algal Introductions to European Shores"
Summary and Objectives: 1.- To explain the underlying ecological causes of the introduction, establishment and development of seaweed invasions on European shores
2.- To generate a baseline dataset on the present status of seaweed introductions to European shores, and of future susceptibility to further introductions/invasions
3.- To elucidate the genetic structure of various populations of selected invasive seaweeds in Atlantic and Mediterranean Europe, with a view to determining whether there have been multiple cryptic European introductions
4.- To evaluate the economic impact of existing seaweed invasions on a European scale, comparing losses with costs associated with prevention and eradication
5.- To carry out risk assessment and propose a screening protocol for invasive macroalgae to be used in coastal zone management
Reference and funding entity: EVK3-2001-0008
Duration: 3 years
Research team: Rui Santos (coordinator), Ester Serrão, Gareth Pearson, Aswin Engelen
Total budget: **Funding for CCMAR**: 177 000 Euro
Web site:

Title: "**CEPHSTOCK** -Cephalopod stocks in European waters: Review, Analysis, Assessment and Sustainable Management"
Summary and Objectives: This project aims to review current knowledge and issues in cephalopod fisheries science, to assemble, organise, analyse and synthesise data from ongoing national projects, previous and new EC-funded R&D projects, and to recommend future actions for scientists and cephalopod fishery managers in European waters. This will be achieved through (a) co-ordinated reviews and (b) development of common databases and associated GIS analysis capability, leading to (c) a series of co-ordination meetings and workshops at which the status of fished European cephalopod stocks will be reviewed, stock assessments carried out and management recommendations proposed.
Reference and funding entity: (QOL-2001-5.1.2) EC- DGXII
Duration: 01-10-2002 to 30-09-2005
Research team: University of Aberdeen (Coordinator) and 20 institutions from all Europe; from CCMAR/UAlg Teresa Cerveira Borges (coordinator) and Pedro Andrade.
Total budget: **Funding for CCMAR:** 27 600 Euro

Title: "Chloroplast gene expression in intertidal macroalgae (**CHLORGEN**)"
Funding institution: FCT (POCTI / 38863 / BSE / 2001.)
Duration: Sept 2002-Aug 2005
Objectives/Summary: The aim is to evaluate whether the variability in photosynthetic parameters and gene expression in *Fucus vesiculosus*, is related to circadian and/or circatidal signals and/or endogenous rhythms. The work involves determining gene sequences from macroalgal chloroplasts (plastids) to study their regulation and control of expression in photosynthetic organisms living in intertidal environments.

Research team: G Pearson, E Serrão, A Lago-Leston, M Valente
Funding: 61 632 Euro

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: 2000-2004
Research team: R Santos, R. Carmona
Total budget: **Funding for CCMAR**: 120 000 Euro

Title: "Evaluation of the impact of dredging for beach nourishment on the marine communities off the Central Algarve"
Summary and Objectives: The impacts of dredging on the aquatic environment are generally considered to be temporary, short term, high intensity perturbations that can result in a temporal changes in the populations. Nevertheless, there are in general few in depth studies concerning the type and evolution of the changes in populations affected by the extraction of sand and gravel from the marine sub-tidal zone, with none from Portugal. Thus, the study of the impacts on biological communities of dredging for the purposes of beach nourishment is in certain aspects pioneering at the national level and may be of considerable use as a reference for future projects in similar areas. The objective of this project is to characterise, in baseline terms, the biological component of the underwater zone between Albufeira and Vale do Lobo-Quarteira (Central Algarve) as well as to evaluate the impacts resulting from the extraction of sand and gravel by means of systematic monitoring of the impacted marine communities.
Reference and funding entity: DRAOTA
Duration: 2003-2006
Research team: CCMAR: Jorge M.S. Gonçalves and Karim Erzini
Total budget: 202791 Euro; **Funding for CCMAR**: 202791 Euro
Web site: http://www.ualg.pt/fcma/cfrg/

Title: "Experimental By-catch Reducing Devices (BRD) in the demersal purse-seine fishery and evaluation of survivorship"
Summary and Objectives: Preliminary studies have shown that mean discard rates in Algarve (southern Portugal) purse seine fisheries vary between 0.20 and 0.30; with between 5,000 and 10,000 mt of discards per year (Borges et al., 1997, 2000, Erzini et al., 2001). The objective of the proposed project is to test simple by-catch reducing devices (BRDs) for the demersal purse seine in order to reduce the by-catch and discarding of under-sized and/or non-commercial species. In particular, we will evaluate the use of both larger mesh sizes and square meshes in part of the net. These modifications should increase the escapement of juveniles and non-commercial species. Quantification of the effectiveness of BRDs will be carried out by means of experimental fishing trials using small-mesh covers over the BRD sections. In addition, the condition of fish that have escaped will be evaluated and post-escapement survivorship monitored in tanks. The relationships between condition (scale loss and other signs of external damage), stress (monitored by cortisol radioimmunoassay) and long-term survivorship will be evaluated experimentally. The results of these experiments will be transmitted to the fishing community.
Reference and funding entity: FCT/POCTI/BSE/43113/2001

Duration: 2002-2004
Research team: CCMAR: Jorge M.S. Gonçalves, Karim Erzini and Adelino Canário
Total budget: 73000 Euro; **Funding for CCMAR**: 73000 Euro
Web site: http://www.ualg.pt/fcma/cfrg/

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

Title: "Global related changes in the Portuguese marine flora over a long time scale"
Summary and Objectives: The main aim of this proposal is to describe the long-term changes in the benthic marine flora of the continental coast of Portugal by comparing the actual situation with the only available description of the Portuguese marine flora, which was done in the 1960´s by Ardré (1970, 1971).
Reference and funding entity: POCTI/BSE/48918/2002
Duration: 2003-2006
Research team: R Santos. E. Barecibar, J. Silva
Total budget: **Funding for CCMAR**: 92 000 Euro

Title: "Instrumentos promotores de formação e de participação dos cidadãos: EqEA - Equipamentos para a Educação Ambiental em Portugal"
Summary and Objectives: We aim to assess the environmental equipments in Portugal, their implementation and development. Criteria will be established to evaluate them.
Reference and funding entity: Protocolo de colaboração entre o Instituto do Ambiente e o CCMAR
Duration: 2003-2005
Research team: R Santos. H. Barracosa
Total budget: **Funding for CCMAR**:

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 Serrão, G Pearson, C Daguin, C Engel, *et al*.
Funding: 150 000 Euro

Title: "Monitoring and Management of European Seagrass Beds (**M&MS**)".
Summary and Objectives: 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
Funding institution: EU- EnvSD (EVK3-CT-2000-00044)
Duration: Feb 2001-Aug 2005
Research team: At CCMAR-UAlg: E. Serrão, S. Arnaud-Haond, F. Alberto, O. Diekmann, M. Billingham, R. Santos, S. Cabaço, R. Machás, A. Cunha, *et al*.
Funding for CCMAR: 330 000 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

Title:"Recruitment of Fish Species of Commercial Interest in the Arade River Estuary"
Summary and Objectives: Knowledge concerning estuarine fish communities is particularly important for the sustainable management and exploitation of our natural resources. Given that the estuary of the Arade river is one of the largest in the south of Portugal, there is a pressing need to better understand the ichthyofauna and how the different fish species use the estuary. Information concerning the type of recruitment, migrations and habitat use (spawning, shelter and feeding), especially with regards to juveniles of commercially important species, is of primary importance for coastal fishing activity and the conservation of these resources. On the other hand the existence of protected or threatened species and habitats should be evaluated in a way that sustainability can be ensured by means of appropriate measures. The dissemination of this knowledge is urgent given the utility for the public in general and for the various public and private economic entities involved. It is not enough to merely know it is also necessary to inform so that a sustainable relationship between man and the natural resources can be established and perpetuated. The objectives of this project are twofold: 1- The characterisation of the structure of the fish community and the distribution of the different species in the estuary of the Arade river, with particular emphasis on the juvenile stages of commercial and threatened species and the interactions between their distribution and abundance and estuarine environmental parameters. 2 - The dissemination of scientific knowledge to the public in general, to public and private entities and to the scientific community.
Reference and funding entity: MARE Programme - P.O. Pescas (Cód. 22-05-01-FDR-00017)
Duration: 2003-2006
Research team: CCMAR: Jorge M.S. Gonçalves and Karim Erzini
Total budget: 139170 Euro; **Funding for CCMAR**: 139170 Euro
Web site: http://www.ualg.pt/fcma/cfrg/

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 Serrão, R Santos, M Billingham, F Alberto, *et al*.
Funding: 75 000 Euro

Title: "Scientific bases for the management of fisheries resources of common interest (Bases científicas para a gestão de recursos pesqueiros de interesse comum)"
Summary and Objectives: This INTERREG III project, co-ordinated by the Centro Regional de Investigação Pesqueira do Sul of IPIMAR, involves the CFRG (CCMAR), and the Centros de Investigación y Cultivo de Especies Marinas de Agua del Pino (Huelva) y "El Toruño" (Cádiz) of the Consejería de Agricultura y Pesca (Junta de Andalucía, Spain). The CFRG is responsible for studies focusing on understanding the spatial and temporal scale of fish movements and habitat use, with emphasis on several species of sea breams (Diplodus sargus, Sparus aurata) and the artificial reefs located on the Algarve continental shelf. Long-term automated monitoring of fish

tagged with pingers will be carried out using Ultrasonic Receiver/monitors fixed on the bottom. Short-term (daily movements) will be studied by tracking tagged fish from a small vessel with a directional hydrophone and ultrasonic receiver. The acoustic telemetry data will provide important information on the spatial and temporal dynamics of these species. In particular, it will be possible to obtain data on habitat preferences, habitat use, daily activity patterns, home ranges and long-term movement patterns. The results of this project will contribute to better conservation and management of the fisheries of the Algarve, where sea breams and bass are an important component of the demersal fish community and of the fisheries landings and where artificial reefs and stock enhancement (sea breams and other species) are important elements of the longterm management strategy.
Reference and funding entity: INTERREG III (Projecto GESTPESCA)
Duration: 2002-2004
Research team: CCMAR: Karim Erzini and Jorge M.S. Gonçalves, IPIMAR and Junta de Andalucia
Total budget: 52500 Euro; **Funding for CCMAR**: 612100 Euro
Web site: http://www.ualg.pt/fcma/cfrg/

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: 2001-2004
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: "**SEMAPP** - Science, Education and Marine Archaelogy Programme in Portugal "
Summary and Objectives: Co-operation to study the marine ecosystem at selected locations in Portuguese territorial waters. The aim of this programme is to develop the knowledge and research of oceanography related to fisheries, an important and desirable area of research in Portugal. The University of Connecticut has access to, and expertise in utilising underwater systems and technologies that allow for detailed *in-situ* observations and research in water depths up to 2,000 meters. The initial proposed study site is Portimão Submarine Canyon and adjacent Continental Slope and Shelf region, off Portugal's Algarve coast. The plan is to spend the first 2 years conducting detailed bathymetric surveys and sub bottom profiling in order to map the ocean floor in those regions. In 2004, it is planned using manned submersibles and robot technology to assist with studies in Fisheries Biology, Benthic Ecology and Environmental Sciences.
Duration: 2000 …
Funded by: OTF, USA & others
Research team: University of Connecticut: Prof. Richard Cooper (co-ordinator of programme); CCMAR: Teresa Cerveira Borges (Marine Biology); Autonomous University: Dr. Adolfo Silveira Martins (Marine Archaeology); Hidrographic Institute.
Total budget: variable **Funding for CCMAR:**

Title: "Signals for gamete release by intertidal species with external fertilization (**GAMETE**)"
Funding institution: FCT (POCTI / 39431 / BSE / 2001.)
Duration: Mar 2002-Feb 2005
Objectives/Summary: Study of factors that affect the reproductive success of externally fertilizing fucoid algae, such as signals for synchronous gamete release, timing for gamete release during tidal cycles and lunar cycles, consequences for dispersal and recruitment.
Research team: E Serrão, G Pearson, C. Faustino, C. Monteiro.
Funding: 45 000 Euro

Title: “Study of the environmental conditions of the Guadina River estuary and adjacent areas”
Summary and Objectives: The study was initiated from the necessity of the portuguese and spanish administrations to characterize the environment at the lower reaches of the Guadiana, in particular the estuary and the coastal area, defining desirable scenarios of environmental quality for the area. It should identify management strategies for the dams being built that will lead to ecosystem sustainability.
Reference and funding entity: Funded by Instituto da Água
Duration: 05/2003 (delayed by INAG)
Research team: Karim Erzini (Ictiofauna e Cadeia Trófica), José Calvário e Martin Sprung (Macrofauna Bentónica), Maria Alexandra Chícharo e Luís Chícharo (Produtividade Primária – Fitoplâncton e Zooplâncton), Rui O. Santos (Cadeia Trófica e Produtividade Primária - Spartina)
Total budget: 402 280 Euro; **Funding for CCMAR**: 402 280 Euro

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 Serrão, G Pearson, C Daguin, L Ladah, C Engel, et al.
Funding: 225 000 Euro

Completed in 2003

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: “**CORRAM** - Cephalopod octopodid: relation between de resource and the marine environment)”
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.
Reference and funding entity: POCTI/1999/ 2/2.1/MAR/1707/95 FCT
Duration: 01.02.1999 to 30.06.2003
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: "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:

International and Interinstitutional Cooperations

Division of Aquaculture and Biotechnology

Title: "Identification of Sex Pheromones from the Anal Gland of Male Blennies, *Salaria pavo* and *S. fluviatilis* (Pisces: Blenniidae)."
Summary and Objectives: *Salaria pavo* is small bottom living fish in the shallow littoral zone of the Mediterranean and adjacent Atlantic coast. The closely related freshwater species, *S. fluviatilis*, inhabits rivers and lakes in the vicinity of the Mediterranean. In both species, the mating system is promiscuous. Males occupy holes or crevices in the hard substrate where females come to spawn and the males subsequently guard the eggs. These are a good model fish species to investigate specialization in sex pheromone production. The males develop anal glands from the epidermis of the first two rays of the anal fin concurrent with development of the gonads. The Portuguese team has shown that the anal gland of *S. pavo* is a source of substances that attract pre-ovulatory females which promotes male reproductive success. This suggests that male blennies are "active signallers" in contrast with known pheromone systems in teleosts, where receivers are "chemical spies" detecting gonadal steroids or prostaglandins passively excreted by females into the water *via* the urine or gills.
The proposed work aims to identify the chemical structures of putative sex pheromones in the two species. Although strictly a freshwater species, the marine origin of *S. fluviatilis* is well established and, as with *S. pavo*, it is plausible that the anal gland has a pheromonal function in female attraction. If so, one would expect the pheromonal components of the two species to have similar or closely related chemical structures. In addition, the inclusion of *S. fluviatilis* in the project will simplify the chemical identification, since recording of electro-olfactograms is technically easier in freshwater, and can be combined with chromatographic separation of anal gland-produced substances.
Reference and funding entity: British Council/CRUP (Acções Integradas Luso-Britânicas nº B-14/03)
Duration: January 2003 - December 2004

Research team: CCMAR: Eduardo N. Barata, Peter C. Hubbard, Ana Freitas, Rui Serrano, Adelino V.M. Canário. Rothamsted Research – Biological & Ecological Chemistry Division, UK: Mike Birkett, Lester Wadhams & John Pickett.
Total budget: 6.000 Euro; **Funding for CCMAR**: 3000 Euro

Title: Identification of food-related attractants for the Senegal sole, *Solea senegalensis.*
Summary and Objectives: Fishes rely upon information received by all their senses for food detection, recognition and selection. Feeding behaviour shows a stereotyped sequence of behavioural components, including arousal, searching, food uptake and ingestion. The arousal phase is mostly mediated by olfaction, whereas in the searching phase the relative importance of different sensory modalities varies depending upon the feeding strategy and ecological niche of the species. Feeding behaviour is completed by food uptake and ingestion phase, which is triggered by chemical stimuli.
In different species, different chemical substances stimulate feeding behaviour. Some of these may act as attractants via olfaction, and others may act as promoters or enhancers of food consumption via both olfaction and gustation. Amino acids, nucleotides, nucleosides and quaternary ammonium bases have been identified as feeding stimulants from experiments with juveniles of different species. For example, glycil betaine, trimethylglycine and dimethylthetin have been reported as feeding stimulants for juvenile Dover sole, *Solea solea*. Some species specificity for feeding stimulants seems to exist, and in general mixtures are more effective than single compounds.
The Senegal sole (*Solea senegalensis*) is a good model species to investigate olfactory mechanisms underlying feeding behaviour, due to its feeding strategy, and a well-developed olfactory system accessible to electrophysiological recordings. The central aim of this project is to identify substances released by natural food sources (polychaets) that act as olfactory cues involved in food-search behaviour. This objective will be achieved by an integration of electrophysiological and behavioural measurements in response to candidate olfactory stimuli. Ultimately, the project will identify the chemical cues that are important in the initiation of food-search behaviour. This may be the ground for future technological development of new inert food diets that enhance the farming of this commercially important species.
Reference and funding entity: British Council/CRUP (Acções Integradas Luso-Britânicas nº B-71/04)
Duration: January 2004 - December 2004
Research team: CCMAR: Eduardo N. Barata, Peter C. Hubbard, Zélia Velez, Adelino V.M. Canário. University of Hull – Department of Biological Sciences, UK: Thomas Breithaupt, Helga Bartels-Hardege, Ralf Bublitz, Victor Sweetez & Joerg Detlef Hardege.
Total budget: 2.900 Euro; **Funding for CCMAR**: 1.450 Euro

Title: "Ontogeny of the endocrine system in *Solea senegalensis* and its application to the improved rearing of larvae"
Summary and Objectives: The investigation of new species for aquaculture, such as the sole (*Solea senegalensis*) contributes significantly to the development of marine fish culture in Spain and Portugal. However, one major bottleneck to the expansion of the industry is the rearing of larval fish. The industry relies on the production of a large number of high quality fry, which in turn relies on the successful first feeding, development and growth of the fish larvae. Embryonic development of marine fish is rapid and larvae hatch at a relatively early stage of development, resulting in an increased vulnerability to external factors. As hormones play an important role in regulating feeding, growth and metamorphosis, it is important to examine the structural and functional development of the endocrine system during early ontogeny of marine fish. This project aims to 1) investigate the development of the endocrine system in *Solea senegalensis* 2) determine how fish larvae respond to stressful situations and 3) study the possible beneficial effects of exogenously added hormones on growth, metamorphosis and pigmentation.
Reference and funding entity: CRUP (Acções Integradas Luso-Espanholas)
Duration: Jan 2004 – Dec 2005

Research team: CCMAR: Maria Teresa Dinis, Neil Ruane; Instituto de Ciencias Marinas de Andalucia, Spain - Maria del Carmen Sarasquete.
Total budget: 2.200 €; **Funding for CCMAR**: 2.200 €
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 focuses 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

Division of Living Resources

Mercado, Jesús - Instituto Español de Oceanografía, Málaga. Determinación del índice $\delta^{13}C$ y su relación con la actividad da anidrasa carbónica en productores primarios de la costa sur de la Península Ibérica Acordo de Cooperação GRICES/CSIC (contact person in CCMAR: Rui Santos)

Duarte, Carlos - IMEDEA, Islas Baleares. DOM production from contrasting communities in the Ria Formosa (S. Portugal), and export to the Atlantic Sea. Acordo de Cooperação ICCTI/CSIC (contact person in CCMAR: Rui Santos).

CCMAR Seminar programme

Brenig, Bertram. *Deciphering complex genomes.* March 28th, 2003

Collins, Michael. Land-ocean interaction: the influence of dams. July 3rd, 2003

Ekker, Mark. Characterization of the zebrafish genome with a collection of radiation hybrids. September 19th, 2003.

Gliesche, Christian. *Diversity and activity of methylotrophic bacteria in activated sludge.* February 10, 2003 (Seminar organized together with CDCTPV, Centro de Desenvolvimento de Ciências e Tecnologias de Produção Vegetal.)

Haond, Christophe. *Osmoregulation in the lobster* Homarus gammarus. May 26th, 2003.

Harris, Robert. *Survival and stress in trawled Norway lobster* Nephrops norvegicus – *is there life after the cod-end?* June 16th, 2003.

Kolkovski, Sagiv - Effects of commercially available bacterial retardant and nutrient enrichment treatments on microbial levels associated with Artemia nauplii and fish larvae cultures.

Kolkovski, Sagiv. *Food attractants for fish larvae.* July 22nd, 2003.

Naylor, Gavin. *Better models, better data, better trees, more impact. Some challenges and opportunities for the future of molecular systematics.* December 2nd, 2003.

Pickett, John. *Plants and animals under stress 'cry out' for help.* June 12th, 2003.

Weir, Bruce. *Characterizing the genetic structure of populations.* November 20th, 2003.Santos, R. Quantitative life cycle of seaweeds. Universidade de Barcelona, Jubilação do Prof. Seoane-Camba.

Seminars given by CCMAR members in other institutions

Cancela M.L., 2003. "Vitamina K e proteínas Gla: coagulação mas não só...!". *1º Encontro Nacional de Bioquímicos* (conferencista convidada). Universidade de Coimbra, 27 de Abril.

Cancela, M.L., 2003. "Matrix Gla protein: contribution from non-mammalian models to elucidate its mode of action and regulation of gene expression". *Instituto de Biologia Molecular e Celular* (IBMC) da Universidade do Porto.

Conceição, L.E.C. and Yufera, M. (2003), Aspectos Fisiológicos Básicos y Aplicados en Alimentación y Nutrición de Larvas y Juveniles de Peces. VIII Curso "Avances en acuicultura y calidad ambiental", Organization: Unidad Asociada de "Calidad Ambiental y Patología". Instituto de Ciencias Marinas de Andalucía (CSIC) y Facultad de Ciencias del Mar da Universidad de Cadiz, Puerto Real, Cadiz, Spain.

Dinis (2003), M. T.. A Aquacultura em Portugal. Forum IBEROEKA, Santiago do Chile, Outubro.

Dinis, M. T., L. Ribeiro, F. Soares, S. Engrola, C. Aragão and L. Conceição (2003), Recent advances on the cultivation of Solea senegalensis: brood stock management and larval rearing. VIII Curso "Avances en acuicultura y calidad ambiental", Organization: Unidad Asociada de "Calidad Ambiental y Patología". Instituto de Ciencias Marinas de Andalucía (CSIC) y Facultad de Ciencias del Mar da Universidad de Cadiz, Puerto Real, Cadiz, Spain.

Erzini, K. Curso de Doctorado,. 12-16 of May, 2003. 20 hours of teaching (Gear Selectivity and Fisheries Modelling) in the PhD Programme of the Universidad de Cádiz, Spain.

Erzini, K. Master of Science Programme "Chef de Projet Halieutique" 26-28 of March, 2003. CREUFOP-Université Montpellier2-France.

Gavaia, P.J., 2003. Aplicacion de tecnicas bioquímicas y moleculares en acuicultura y ecotoxicologia – Tecnicas de hibridacion in situ y immunohistoquimica. *VIII Course in Advances in Aquaculture and Environmental Quality*. ICMAN-CSIC , Cadiz Dec 1-5.

Santos, R. Quantitative life cycle of seaweeds. Universidade de Barcelona, Jubilação do Prof. Seoane-Camba.

Dissemination of scientific culture

Barata, E.N. Programa de Ocupação Científica de Jovens nas Férias promovido pela Agência Ciência Viva. Estágio, "Atracção química de fêmeas para machos numa espécie de peixe da Ria Formosa", frequentado pela aluna Teresa Vera de Moura Jerónimo da Escola Secundária João de Deus – Faro (Julho de 2003).

Coelho, R., Bentes, L., Gonçalves, J.M.S., Lino, P.G., Ribeiro, J. & Erzini, K. Reduction of deep-water sharks by-catch in the bottom longline fishery off the Algarve. Oral communication presented at the "Shark

Research in Portugal Workshop", during the "2nd shark catch and release sports fishing tournament", 13 June in Sesimbra 2003, Portugal.

"Pescar e Conservar- Desafios para o futuro" no âmbito do programa do CCMAR-CCVA "Os cientistas voltam à escola", proferida numa dezena de escolas do Algarve em que Intervieram Teresa Borges, Margarida Castro, Margarida Cristo, Karim Erzini, Jorge Gonçalves e Luís Chícharo

"Aquaculture, the blue revolution". no âmbito do programa do CCMAR-CCVA "Os cientistas voltam à escola", proferida numa dezena de escolas do Algarve Rui Rocha e ouyros membros do grupo de aquacultura

Gonçalves, J.M.S., Afonso, C., Ribeiro, M. and Monteiro, P. Participation in the "Gorringe 2003 Expedition" (July/August 2003) in close cooperation with the Atlantic Wildlife Association. This activity included a scientific dissemination program through newspaper articles, television documentaries and multimedia exposition.

Gonçalves, J.M.S. Mesas de Sinalização da Vida Marinha. Parque Natural do Sudoeste Alentejano e Costa Vicentina. Janeiro, 2003.

Visiting scientists

Hemmingsen, Willy - University of Tromsø, Norway. Visit for one week to discuss future collaboration in the area of marine fish parasitology. With a view to making a start on different lines of research, It was proposed the participation of Isabel Afonso-Dias in a research vessel survey along part of the coast of North Norway from 17 October to 3 November 2003 to learn different techniques of collecting, processing, preserving and identifying different groups of marine parasites. (hosted by Isabel Afonso-Dias, Centro de Ciências do Mar do Algarve).

Kolkovski, Sagiv - (Department of Fisheries, Western Australia).

Mackenzie, Ken – Zoology Department, University of Aberdeen, Scotland. Visit for one week to discuss several aspects regardings ichthyoparasitology, such as an exploratory survey of sardine parasites, including a discussion about the use of sardine parasites as biological markers. But the main focus will be on the survey of a coccidian protozoan parasite found in the testes of sardines, by chance, a year ago. Although this parasite has been reported before, there has been no recent study of its occurrence in Portuguese coastal waters. The last such study was published almost half a century ago. Since Dr. Mackenzie has worked previously with this coccidian in herring his expertise is highly important for this particular piece of work. (hosted by Isabel Afonso-Dias, Centro de Ciências do Mar do Algarve).

Pickett, J.A. & Birkett, M. Rothamsted Research. Collaboration within bilateral cooperation with E.N. Barata, P.C. Hubbard & A.V.M. Canário (Treaty of Windsor). June 2003.

Roland Schule. University of Frieburg. Germany. Research collaboration within a bilateral cooperation project with L. Cancela. FCT-BCC/22217/99. July 2003.

Sarasquete, M.Carmen. CSIC.Cadiz/ICMAN, Spain. Research collaboration within a bilateral cooperation with L. Cancela, project CSIC/ICCTI/GRICES, Proc 423/Espanha. 2003.

Organization of Conferences, Workshops, Courses

Advanced Statistics Course: Analysing Biological and Environmental Field Data. 13-15 January, 2003. Taught by Dr. Alain Zuur, Highland Statistics Ltd., hosted by K. Erzini.

Advanced Statistics Course: Analysing Biological and Environmental Field Data. 14-18 July, 2003. Taught by Dr. Alain Zuur, Highland Statistics Ltd., hosted by K. Erzini.

Basic histologic course: Practical course on how to process, embed, cut, stain and analyse fish gonads (species used was *Trachurus trecae*). 8 May to June 2003. Training given as an extension of the process started on board the R/V 'Dr. Fridtjof Nansen' during the summer of 2001. Taught by Isabel Afonso-Dias.

Experimental Design Course; by A.J. Underwood and G. Chapman. 16-28 July 2003, Univ. Algarve, Faro.

First CCMAR Radionuclide Safety Course. Supervised by J. Varela, L. Deloffre and S. Coesel.

Workshop on Production in Seaweeds Purifying Effluents from Marine Animal Holding Units. Universidade do Algarve, Faro, Portugal.

V Congresso Nacional de Etologia. 18-19 September 2003, Universidade do Algarve, Faro (E.N. Barata, P.C. Hubbard, A.V.M. Canário (members of the Scientific Committee and organization)