Centro de Ciências do Mar do Algarve
Algarve Centre of Marine Sciences
Annual Scientific Report
2005

Publications List

Division Aquaculture and Biotecnology

Books, edited books, chapters of books

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Patents

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FCT (POCTI / 38863 / BSE / 2001) - "Chloroplast gene expression in intertidal macroalgae" (CHLORGEN). Sept 2002-Aug 2005.

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Prizes and Honours

"Best Poster award" in the ICES 2005 Annual Science Conference, September 20-24th, Aberdeen, Scotland, U.K. - Gonçalves, J. M.S., Araujo, J., Bentes, L., Monteiro, P. Coelho, R., Corado, M. and Erzini, K. 2005. Evaluation of survivorship of fish that have escaped through a demersal purse seine BRD.

List of thesis supervised by members of the research unit

Division of Aquaculture and Biotechnology

Theses PhD

Completed

Gavaia, Paulo J. (2005) Functional analysis of osteocalcin (Bone Gla protein, BGP) from bony fish during skeletal development. University of Algarve (Supervisors: M. Leonor Cancela and Carmen Sarasquete from the CSIC, Cadiz, Spain).

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Ongoing

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.

Campinho, Marco. The molecular and endocrine basis of finfish embryo development and metamorphosis. (Supervisors: Deborah M. Power and Dr Glen Sweeney (University of Cardiff, UK). Completion expected 2006

Carvalho, Inês. Abundance, site fidelity and population structure of humpbacks whales (*Megaptera novaeangliae*) in S. Tomé e Príncipe. University of Algarve (Supervisors: Howard Rosenbaum from the American Museum of Natural History and the Wildlife Conservation Society, NY, USA, and M. Leonor Cancela). Completion expected in 2008.

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.

Estêvão, Dulce. Endocrine regulation of extracellular matrix proteins in calcified tissue in teleost fish. (Supervisor: Deborah M. Power) Examination January 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 2006

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). (Fellowship temporarily suspended).

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 2005.

Henriques, Nuno. Regulation of the gene expression associated with the carotenoid biosynthesis in the microalga *Dunaliella salina*. University of Algarve (Supervisors: M. Leonor Cancela and João Varela). Completion expected in 2006.

Kolmakov, Nikolai. Pheromone olfactory receptors in fish: isolation and functional characterization. (supervisors: Adelino Canário, Peter Hubbard and João Coimbra). Completion expected in 2007.

Leite, Ricardo Mário Bastos. Characterization of metabolic pathways in the protozoan parasite *Perkinsus atlanticus/olseni*: Identification of potential targets for therapeutic drugs. University of Algarve (Supervisors: M. Leonor Cancela). Completion expected in 2008.

Martins, Rute Sofia. Dax-1, a sex determination gene in fish? Universidade do Porto (supervisors: Adelino Canário and João Coimbra). Completion expected in 2007.

Mesquita, Sandra Maria Sengo. Isolamento e caracterização de cianofagos de *Microcystis* sp. Implicação no desenvolvimento de um método de diagnóstico molecular associado á detecção da produção/libertação de microcistinas. University of Algarve (Supervisors: Rachel Noble from the Insitute of Marine Sciences, University of North Caroline, Chapel Hill, USA, and M. Leonor Cancela) Completion expected in 2008.

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 2006.

Morais, Sofia. Digestive and assimilation capacity of marine fish larvae with respect to dietary lipid/protein ratios and lipid quality. Universidade do Algarve (Supervisors Maria Teresa Dinis, Luis E.C. Conceição and Ivar Rønnestad). Completion in 2006.

Morgado, Isabel (2002) Transthyretin: its role in the transporte of thyroid hormone in fish and the influence of endocrine disruptors. (Supervisor: Deborah M Power). Completion expected 2006.

Pais, Miguel Caldeira. Seleção e utilização do habitat pela população não reprodutora de Aguia de Bonelli (Hieraaetus fasciatus) no Sul de Portugal. (Supervisors: M. Leonor Cancela and Pedro Beja (Erena]) Completion expected in 2006.

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 2006.

Pombinho, António R. Matrix Gla protein signalling pathways in fish: from extracellular regulators to promoter responsive elements and binding factors. University of Algarve (Supervisors: M. Leonor Cancela and Vincent Laizé) Completion expected in 2008.

Rafael, Marta Isabel da Silva. Role of the transcriptional regulator FHL2 in bone formation: Identification of fish bone-specific target genes and regulatory mechanisms. University of Algarve (Supervisors: M. Leonor Cancela and Vincent Laizé, and Roland Schüle from the University of Freiburg, Germany) Completion expected in 2008.

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.

Saavedra, Margarida. Requisitos em aminoácidos de larvas e pós-larvas de sargo (Diplodus spp.): efeitos na performance e na qualidade. Universidade do Algarve (Supervisors Maria Teresa Dinis, Luis E.C. Conceição and Dr Pedro Pousão- Ferreira). Completion expected in 2008.

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 2007.

Silva, P.A.S. Estados vibracionais excitados e o funcionamento das proteínas (supervisor: L.eonor Cruzeiro). Completion expected in 2006.

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.

Teodósio, Rita. Fish parathyroid hormone-like proteins: in search for functions. Universidade do Porto (supervisors: Adelino Canário, Deborah Power and João Coimbra). Completion expected in 2009.

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.

Viegas, Carla Alexandra São Bento. Molecular cloning and regulation of the genes encoding matrix and bone Gla proteins from sturgeon, an archaic teleost fish. Studies towards understanding the evolution of their molecular mechanisms of action. University of Algarve (Supervisors: M. Leonor Cancela and Dina Simes). Completion expected in 2008.

Theses Master of Science

Completed

Fonseca Vera (2005) Identification of genes differentially expressed during the mineralization of fish bone-derived cell lines. MSc of Biotecnology, University of Algarve (Supervisors: M. Leonor Cancela and Vincent Laizé).

Pombinho, António Ribeiro (2005) Effect of extracellular calcium on MGP gene expression. MSc in Biotecnology, University of Algarve (Supervisors: M. Leonor Cancela and Vincent Laizé).

Weber, Robilson António (2005). Determinação do efeito de condição de stress agudo e crónico no cultivo de pós-larvas de *Solea senegalensis*. Diploma de Estudos Avançados, Institute of Aquaculture, University of Santiago de Compostela, Spain.

Ongoing

Cepo, Susana Isabel Coelho dos Santos. Contributo para a implementação de um sistema de gestão integrado de recolha e valorização de óleos alimentares usados. MSc in Gestão e Conservação da Natureza, University of Algarve (Supervisors: Raul Costa, António Portugal and M. Leonor Cancela). Completion expected in 2006

Costas, Benjamim. "The effects of stressfull conditions on amino acids metabolism of Senegalese sole (Solea senegalensis, Kaup 1858)". Universidade do Algarve (Supervisors: Luís Conceição and Cláudia Aragão). Completion in 2006.

Roberto, Vania. Characterization of the skeletal development of *Pagrus auriga* and *Scophthalmus maximus* through histological methods. International Masters in Aquaculture, University of Algarve (Supervisors: M. Leonor Cancela and Paulo J. Gavaia). Completion in 2006.

Santos, Erika Silva. Potencial de utilização do *Cistus ladanifer* L. na vegetalização de áreas mineiras. MSc in Gestão e Conservação da Natureza, University of Algarve (Supervisors: Mª Manuela Abreu, Cristina Nabais and M. Leonor Cancela). Completion expected in 2006.

Silva, Conceição. Desenvolvimento de ferramentas interactivas para divulgação das aplicações da biotecnologia ambiental em biorremediação. MSc in Biotechnology, University of Algarve (Supervisors: Carlos Rocha and M. Leonor Cancela). Completion expected in 2006,

Graduation Honours thesis (Estágio de licenciatura)

Completed

Brito, Anabela Ben'Simon (2005) Clonagem e estudos de regulação da expressão do gene da HPH (prolina hidroxilase do HIF) do parasita *Perkinsus olseni* (Supervisors: M. Leonor Cancela and Ricardo Leite).

Carneiro, João Tiago Estêvão Tomé (2005) Contribuição para a optimização de uma metodologia de detecção de cistos de *Cryptosporidium spp* em amostras de águas (Supervisors: M. Leonor Cancela and Ricardo Leite).

Coelho, Nelson Alexandre Castilho (2005) Estudo da transdiferenciação adipocítica de linhas celulares do tipo osteoblástico derivadas de *Sparus aurata* (Supervisors: M. Leonor Cancela and Vincent Laizé).

Pereira, Susana Cristina Oliveira (2005) Caracterização dos níveis e padrões de diversidade genética associados ao gene humano da proteína gama-carboxilada da matriz (Supervisors: M. Leonor Cancela, and Jorge Rocha from IPATIMUP).

Ongoing

Lopes, Rui. Cloning and characterization of *Sparus aurata* tissue-non-specific alkaline phosphatase and role in tissue mineralization (Supervisors: M. Leonor Cancela and Vincent Laizé)

Pinto, Bruno. The role of matrix Gla protein during *in vitro* biomineralization: Phosphorylation as a key mechanism for protein function (Supervisors: M. Leonor Cancela e Dina Simes)

Division of Living Resources

Theses PhD

Completed

Alberto, Filipe. Dispersal,sex and clonality in the marine environment: population genetic structure of the seagrass Cymodocea nodosa on Mediterranean and Atlantic coasts.. Univ. Algarve (supervisor E. Serrão and C. Duarte, CSIC, Spain). Sept 2005.

Stobberup, Kim Araujo. 2005. Study of community structure, trophic interactions and exploitation pattern in the Cape Verde coastal ecosystem. PhD. Dissertation, Universidade do Algarve. (supervisor: Karim Erzini).

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Ongoing

Alexandre, Ana. Metabolismo do azoto na angiospérmica marinha *Zostera noltii* (Hornem.) na Ria Formosa Universidade do Algarve. (supervisor Rui Santos, CCMar)

Bartilotti, Cátia Alexandra Vieira: "Processos bio-ecológicos dos estádios larvares de crustáceos decápodes na região adjacente à Ria de Aveiro: morfologia e ecologia larvar". (Supervisor A. Dos Santos and M. Castro). Completion expected in 2009.

Beldade, Manuel Ricardo do Ó de Oliveira. Patterns of recruitment and stability in rocky coast cryptic fish communities. (Supervisors: Karim Erzini and Emanuel Gonçalves, ISPA). Completion expected in 2006.

Berecibar, Estibaliz. "Global related changes in the Portuguese marine flora". Universidade do Algarve (Supervisor: Rui Santos, CCMar).

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.

Blomqvist, Inês de Melo Fernandes da Silva. Acoustic communication and social dynamics of a stable group of bottlenose dolphins (Tursiops truncatus) in human care. (supervisors: Karim Erzini and Mats Amudin, Linkoping University, Sweden. Defense scheduled for February 17, 2006.

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

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, Rui Pedro Andrade. Biology, spatio-temporal dynamics and conservation and management of deep water sharks. (supervisor: Karim Erzini) Completion expected in 2007.

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

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, Paulo Jorge Menano Ribeiro da. Selectivity of trawl and gill nets on the Portuguese continental coast. (supervisor: Karim Erzini) Completion expected in 2006.

Garrido, S. Ecologia Alimentar Da Sardinha (*Sardina Pilchardus)* Ao Largo Da Costa Continental Portuguesa Universidade do Algarve (supervisors: Maria Alexandra Chícharo (CCMar) Carl van der Lingen (MCM – África do Sul), Emilia Cunha (IPIMAR)

Godinho, Cecile. "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 Maria João Bebianno).

Hazin, Humberto Gomes. Influence of oceanographic parameters on the population dynamics of swordfish, Xiphias gladius (Linnaeus, 1758), caught in the Atlantic ocean. (supervisor: Karim Erzini) Completion expected in 2006.

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, Francisco Miguel de Sousa. Contribution of the artificial reefs of the Algarve coast to the trophic ecology of Sparidae. (Supervisors: Karim Erzini and Miguel Neves dos Santos, IPIMAR CRIP-Sul). Completion expected in 2007.

Lino, Pedro Gil. Potential of restocking with cultivated fish on the south coast of the Algarve. (Supervisors: Karim Erzini, CCMAR and Miguel Neves dos Santos, IPIMAR, Olhão).

Marçalo, Ana Luisa Barreto. Evaluation of stress in the sardine (Sardina pilchardus) during seining. (Supervisors: Karim Erzini, CCMAR and Yorgos Stratoudakis, IPIMAR, Lisboa). Completion expected in 2007.

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, CCMar and Bruce Peterson, Marine Biological Laboratory, Wood´s Hole, USA).

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).

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

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, Joaquim. Ecology and dynamics of ichthyofauna of the Ria Formosa. (supervisor: Karim Erzini) Completion expected in 2007.

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).

Serafim, Maria Paula. Universidade do Algarve (supervisor Margarida Castro). Completion expected in 2008.

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 Mui, Universidade de Stirling).

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).

Vieira, Vasco. Modelo ecológico da Ria formosa, , Universidade do Algarve. (supervisor Rui Santos, CCMar and Ramiro Neves, Instituto Superior Técnico, Universidade de Lisboa)

Vasconcelos, Paulo da Conceição Silva (supervisors Miguel Gaspar (IPIMAR) and Margarida Castro). Completion expected in 2006.

Theses Master of Science

Completed

Bartilotti, Cátia Alexandra Vieira (2005) "Dinâmica larvar de *Pisidia longicornis* (Linnaeus, 1767) na região adjacente à Ria de Aveiro. Mesters in Estudos Marinhos e Costeiros, Universidade do Algarve. (Supervisors A. Dos Santos and M. Castro).

Ferreira, RL. 2005. Caracterização das capturas de tartaruga careta (Caretta caretta) e influência de parâmetros ambientais e pesqueiros, na pesca dirigida ao espadarte (Xiphias gladius) nos Açores. Tese de Mestrado em Estudos Marinhos e Costeiros, Universidade do Algarve. (Supervisors: Karim Erzini and Helen Martins, D.O.P., Açores).

Ongoing

Araújo, João. Análise de capturas de aparelho de anzol em função de variáveis ambientais e de pesca. Completion expected in 2006. (Supervisors: Karim Erzini and Jorge Gonçalves).

Conduto, Telma. Utilização de indicadores para a avaliação do impacte de diferentes artes de pesca. Mestrado em Aquacultura e Pescas. (Supervisor: Karim Erzini) Completion expected in 2006.

Rosa, Ana. Guia de Campo das algas do intertidal da Praia da Vigia. Mestrado de Biologia e Geologia para o Ensino, Universidade do Algarve (supervisor Rui Santos and João Silva, CCMar) Completion expected in 2006.

Machado, Daniel Miranda. Distribution patterns of flatfishes in 3 marine habitats: Coastal area, Coastal lagoon and Estuary. Mestrado em Ecologia, Faculdade de Ciências e Tecnologia da Universidade de Coimbra.(Supervisor: Jorge Gonçalves e Miguel Pardal) Completion expected in 2006.

Nhanca, Florentino José Lopes. Avaliação das capturas na ZEE da Guiné-Bissau e desenvolvimento de um plano de amostragem e monitorização. Mestrado em Gestão e Conservação da Natureza (Supervisor: Karim Erzini) Completion expected in 2006.

Monteiro, Carla. Reproductive Ecology of Sargassum muticum (Yendo) Fensholt in Viana do Castelo (Northern Portugal). Mestrado em Biologia e Ecologia do Litoral Marinho. Universidade de Évora. (supervisor Rui Santos and Aschwin Engelen, CCMar)Completion expected in 2006.

Xavier, B. Indicatores de eutrofização em comunidades bentónicas na Ria Formosa (Supervisors Luis Chícharo e Alexandra Chícharo)

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Graduation Honours thesis (Estágio de licenciatura)

Completed

Abreu, Sérgio 2005. Caracterização da ictiofauna associada a substratos móveis costeiros ao largo do Algarve Central. Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve (Supervisors: Jorge Gonçalves and Karim Erzini).

Barradas, Vânia (2005). Morfologia e morfometria dos otólitos sagitta de 11 espécies de peixe da costa do Algarve. Relatório de Estágio da Licenciatura em Biologia Marinha e Pescas. Faculdade de Ciências do Mar e do Ambiente da Universidade do Algarve. (Supervisors: José Xavier + Teresa Cerveira Borges)

Candeias, Rui. Identificação de marcadores moleculares para a determinação do sexo em *Gelidium sesquipedale* (Rhodophyta) via "RAPD" (Random Amplified Polymorphic DNA). (supervisor Rui Santos, CCMar)

Coelho, Daniela Claro (2005). Diagnóstico sócio-económico da comunidade de pesadores artesanais de Zimbros (Bomminhas, Santa Catarina - Basil). (supervisors Roberto Wahrlich [U. do Vale do Itajaí, Brasil] and M. Castro.

Ferreira, Ana Margarida Marques (2005). A dimensão bioética das pescas. (Supervisors Humberto Rosa [FCL-UCL] and M. Castro).

Freitas, Rui Patrício (2005). Fecundity of the Spiny lobster Panulirus regius in the northwest islands of the Cabo Verde archipelago. (supervisor M. Castro).

Jumpe, Raul Joaquim (2005). A pesca de arrasto de crustáceos no Algarve. (supervisor M. Castro).

Massa, Sónia. 2005. Estudo da diversidade genética de Cymodocea nodosa. Univ. Algarve (supervisors: F. Alberto and E. Serrão).

Mesquita, Carlos Alexandre (2005). Análise de dados de capturas comerciais e de campanhas acústicas para o estudo da distribuição e movimentos da sardinha (Sardina pilchardusWalbaum, 1792) na Península Ibérica. (supervisors Yorgos Stratoudakis [IPIMAR] and M. Castro.

Monteiro, Joana. (2005) Descrição do desenvolvimento larvar de Gobius xanthocephalus (Pisces: Gobidae). Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve. 54 pp (Supervisors: Maria Alexandra Chícharo e Emanuel Gonçalves ISPA)

Primo, Lígia. (2005). Caracterização da fauna bentónica associada a recifes naturais no Algarve Central. Estágio de Licenciatura em Biologia Marinha e Pescas, FCMA, Universidade do Algarve, pp. 50. (Supervisors: Jorge Gonçalves, Luís Fonseca e Carlos Afonso)

Rousselot, Jaques Emmanuel (2005). Caracterização sócio-económica da comunidade peicatória da ilha de Quirimba, Norte de Moçambique (Supervisor M. Castro).

Segurado, Susana Piper Bivar (2005). Statistical Power analysis of candidate ecosystem state indicators. (Supervisor M. Castro).

Sousa, Inês 2005. Aspectos da variabilidade na abundância de juvenis de Mero (*Epinephelus marginatus*) (Lowe, 1834) (Pisces, Serranidae) na Costa Sul da Ilha Terceira, Açores. Licenciatura em Biologia Marinha e Pescas, Faculdade de Ciências do Mar e do Ambiente da Universidade do Algarve. (Supervisors: Jorge Gonçalves e Luís Fonseca).

Tavares, Diogo M.R. 2005. Comparação do método do quadrato de fotografia subaquática com o método do transecto através de censos visuais. Licenciatura em Biologia Marinha e Pescas, Faculdade de Ciências do Mar e do Ambiente da Universidade do Algarve. (Supervisors: Karim Erzini, Jorge Gonçalves and Carlos Afonso).

Ongoing

Assis, Jorge. Caracterização Espacial e Ecológica do Sítio de Interesse Comunitário "Franja de Mogan" da Ilha de Gran Canária (Espanha) para proposta a AMP (Área Marinha Protegida). (Supervisors: Margarida Castro e Jorge Gonçalves). Completion expected in 2006.

Carvalho, Joana Fernandez. Aplicação de técnicas de vídeo subaquático na caracterização de biocenoses marinhas. (Supervisors: Jorge Gonçalves and Karim Erzini). Completion expected in 2006.

Guerra, Luís. Modelação da Produção Primária Bentónica no Ormonde (supervisors Rui Santos, CCMar; Henrique Coelho, Hidromod)

Pedro, P. Padrões de Crescimento em Otólitos de Larvas de *Pomatoschistus pictus* (Pisces, Gobiidae) Licenciatura em Biologia Marinha e Pescas, Universidade do Algarve. (Supervisors: Maria Alexandra Chícharo e Emanuel Gonçalves ISPA)

Pires, Francisco. Levantamento taxonómico e distribuição de esponjas (Filo Porífera) na costa do Algarve. (Supervisors: Jorge Gonçalves, Luís Fonseca, Carlos Afonso). Completion expected in 2006.

Possante, André. Algas do intertidal da ilha de São Vicente, Cabo Verde. Estágio de licenciatura em Biologia Marinha e Pescas, Universidade do Algarve (supervisors Rui Santos and João Silva, CCMar) Completion expected in 2006.

Vieira, P. Levantamento taxonómico e distribuição de gorgónias (Anthozoa: Gorgonacea) na costa do Algarve. (Supervisors: Jorge Gonçalves, Luís Fonseca, Carlos Afonso). Completion expected in 2006.

Description of the Research activities

Division of Aquaculture and Biotechnology

Research team

Leader: M. Leonor Cancela
Visiting Scientist: Ivar Ronnestad
Researchers and post docs: Vincent Laizé, Laurence Elandalloussi, Juan Ortiz Delgado, Pedro Rodrigues, Natércia Conceição, Dina Simes, Sandra Marques, Vanesa Robles, Paulo Gavaia
PhD students:, Nuno Henriques, Sara Mira, Daniel Tiago, Rita Ascenso, António Pombinho, Marta Rafael, Carla Viegas, Ricardo Leite
MSc students:, Vania Roberto
Technicians/ research assistants: Ricardo Afonso, Daniel Braga, Marta Valente, Cátia Marques Brigite Simões, Sofia Cavaco
Undergraduate students: Anabela Brito, Nelson Coelho, Susana Pereira, João Carneiro, Márcio Simão, Bruno Pinto, Rui Lopes, Rita Rainha

Summary of activities and progress during 2005 and Plans for 2006

1. Molecular determinants of extracellular matrix calcification

Main purposes:
Research focus is primarily on: **1**) understanding molecular pathways of tissue mineralization and its regulation in adult life and during development, using teleost and cartilaginous fish and amphibians as model organisms; **2**) the role of vitamin K-dependent proteins (matrix and bone Gla proteins), hormones and growth factors in this process. Additional goals include: **3**) 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; **4**) molecular adaptations of mechanisms that control extracellular matrix mineralization; v) evolution of mineralization-related gene organization and protein function; **5**) mechanisms of mineralization in mollusc bivalves.
Achieved in 2005:
Major results included: **1**) Studies on the comparison of sites of BGP/MGP gene expression and protein accumulation in adult teleost fish and during larval development; **2**) Functional analysis of mineralization-related gene promoters in amphibians and fish; **3**) The elucidation of the 3D structure of osteocalcin from a teleost fish with emphasis on its functional implications; **4**) Identification of signal transduction pathways involved in the mechanisms of regulation of tissue mineralization; **5**) Continuous effort on development and characterization of bone- and cartilage-derived cell lines from fish and amphibian; **6**) Studies on environmental factors affecting bone biology; **7**) Evolution of mineralization-related genes and functional implications.
Plan for 2006:
Major goals include: **1**) integrated multidisciplinary approaches for *in vivo* and *in vitro* functional analysis of bone- and cartilage-related genes through techniques of overexpression and RNA

interference; **2**) Evolutionary studies on specific gene function; **3**) Effect of environmental parameters (pollutants) on bone biology; **4**) Development of cell lines from fish with different types of calcified tissues: agnathe (lamprey), cartilaginous fish (shark), marine and freshwater teleost fish (seabream, solea and zebrafish); **5**) Development of transgenic zebrafish lines expressing BGP/GFP and MGP/GFP fusion proteins and a line expressing antifreeze proteins; **6**) Identification of polymorphisms in specific genes related to environmental adaptations and in populations at risc of developing specific phenotypes; **7**) Identification of mineralization-related proteins from mollusc bivalves.

2. Studies on the biology of clam (R. decussatus) infection by the parasite Perkinsus olseni and development of new drug therapies

Main purposes:

Research focus is primarily on: **1**) *in vivo* and **2**) *in vitro* analysis of parasite-host interactions. Additional goals include: **3**) molecular characterization of specific parasite genes involved in host infection; **4**) identification of parasite metabolic pathways in an effort to develop new drug therapies; **5**) Identification of clam marker genes responsive to environmental iron stress.

Achieved in 2005:

Major results included: **1**) Effects on environmental factors on the infection of Portuguese clam by *Perkinsus*; **2**) Identification of structural components of *Perkinsus*; **3**) Identification of genes involved in possible drug target pathways; **4**) *in vitro* screening of various drugs for therapy of perkinsiosis using as model system a clonal culture of *P. atlanticus*; **5**) Identification of regulatory mechanisms for purine salvage and shikimate pathways in *Perkinsus*; **6**) Development of subtractive cDNA libraries focussing on identification of genes involved in host-parasite interactions; **7**) Effect of sediment iron levels on clam infection; **8**) Cloning and expression analysis of iron stress marker genes from clam.

Plan for 2006:

Major goals include: **1**) Studies towards the identification of metabolites produced *in vitro* by *Perkinsus* cell lines with biotechnological applications; **2**) Identification and expression studies of genes involved in host-parasite interaction through an integrated genome/proteome approach; **3**) Identification of environmental parameters capable of modulating parasite growth *in vivo* and *in vitro*; **4**) Gene expression analysis of environment stress-responsible genes from *P. Atlanticus*; **5**) Development of a transfection method for *P. atlanticus* cells; **6**) Search for new therapeutic agents capable of modulating in vivo clam infection.

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

Main purposes:

Research has focused on population genetic studies of endangered or special interest species through **1**) development of suitable molecular markers for studies on the Bonelli's eagle and the coastal otter and **2**) genotyping and analysis of population structures.

Achieved in 2005:

For the Bonelli's eagle: **1**) genotyping of the southern Portugal population from 1994 to present and **2**) comparison with older populations from Iberic peninsula and outside locations through the use of museum specimens. For otters, non-invasive methods developed allowed continuation of the monitorization of individuals from coastal area of Southern Portugal and allowed the implementation of a service for outside users.

Plan for 2006:

1) Continue analysis of museum collections (including New York Science Museum and UK Museums) to pursue comparison of Bonelli's eagle population from Southern Portugal with those from populations found in different parts of the world. **2**) Extend our offer of external services for sexing and paternity analysis. **3**) Return to work on population genetics and paternity of crustaceans (nephrops) in collaboration with other researchers from CCMAR and IPIMAR.

Group: Comparative and Molecular Endocrinology

Research team

Leaders: Adelino V. M. Canário and Deborah Power
Visiting scientist: Karin Pittman
Principal investigators and Post-docs: Eduardo N. P. Barata, Juan Fuentes, Ana Lúcia S.de Passos, Peter C. Hubbard, Ana Freitas, Laurence Deloffre, Begoña Redruelo, Pedro Miguel Guerreiro da Costa Guerreiro, Teresa Isabel Mendonça Modesto, João Carlos dos Reis Cardoso, Cristophe Haond, Lília Brinca, Susana Sangiao Alvarellos, .
PhD students: Dulce Estevão, Patrícia Pinto, Isabel Morgado, Rui Manuel Lanceiro Serrano, Natalia Moncaut, Zélia Cristina Pereira Velez, Marco António Nascimento Sequeira Jesus Campinho, Helena Rita Teodósio, Nikolai Kolmakov.
Technicians/ research assistants: Liliana Isabel Tomé dos Anjos, Elsa Maria Anjinho do Couto, Bruno Louro, François Noel Hubert, Olinda Gomes de Almeida, Antonio Luis Miranda, Patrícia Guerreiro, Adriana Silva.
Undergraduate students: Rita Isabel G. Soares, Vânia F. Martins, Aurore Coppieters.

Summary of activities and progress during 2005

This year has seen a shift in the intergroup organisation with the establishment of a steering committee for research and training. The research activites of the group are integrated in key areas: 1. Health and Welfare, 2. Biotechnology and 3. Environment. 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 and chemical communication (with emphasis on sex determination and pheromones), endocrine disruption, calcium regulation focussing on hypercalcaemic and hypocalcaemic hormones, and the stress response to normal physiological challenges (with emphasis on ion regulation). An integrated systems approach is being taken and genomics, molecular biology, proteomics, 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, tissue specific proteome and changes in response to hormones, post-translational and post-secretory processing, receptor binding, signal transduction and finally the response at a cellular and whole animal level.

Calciotropic hormones

The work carried out in this area has progressed on several different fronts which include, molecular, biochemical, and physiological approaches. The target species being studied have been extended to take advantage of the diversity of fishes and also their divergent life strategies. A key question with regards to calcium homeostasis is to establish the role of internal deposits and external sources of calcium on this process.
Molecular studies using in silico analysis of the genome of model organisms has led to the identification of a new family of genes in teleosts, the PTH/PTHrP family. All the PTH/PTHrP-like genes are expressed but there appears to be a differential expression of the different forms. The biological activity of the N-terminal “calcitropic” regions of the protein product encoded by the genes, also reveals siginificant differences in activity and raises exciting new possibilities about the role of this gene family in fish. A number of biochemical assays have been developed and used to study the action of the N-terminal region of PTHrP on a number of different tissues. Receptor type, affinity (ligand.binding, secondary messenger activation) and tissue responsiveness (gene targets and gene expression) have all been studied in a number of different tissue.
In vivo studies in the sea bream and tilapia confirmed the calcitropic nature of PTHrP in teleosts. The importance of different epithelia involved in calcium movements were studied and revealed that the intestine appears to play an important role, and preliminary studies suggest other osmoregulatory organs are important and that PTHrP has an important role in regulating calcium

movements. The euryhaline killifish (*Fundulus heteroclitus*) has been developed as a model system for studies of ion regulation and during 2005 bioelectrical characterisation of the response of opercular membranes to PTHrP was initiated. This year analysis was extended to the Chondrostean i.e. sturgeon. Calcium regulation in sturgeon is of special interest because they have a primarily cartilaginous skeleton and calcium demand is supposed to be low due to the lack of endoskeleton calcification. It was possible to demonstrate that sturgeon regulate calcium balance very tightly. Moreover, piscine PTHrP is able to regulate net calcium uptake showing the hypercalcemic nature of PTHrP. In sturgeon PTHrP has a calcitropic (putatively hypercalcemic) action, opening the door to a range of different studies.

Hormonal control of development and growth of fish eggs and larvae

The development of the musculo-skeletal system of the atlantic halibut has been studied and specific tools have been developed (genes, histological methods), and used to characterize the developmental expression of skeletal and muscle specific genes before and during metamorphosis. These studes have been extended to the intestinal development, endocrine system ontogeny and in particular the role of the thyroid hormone axis in the halibut. Studies have continued into the ontogeny of the musculo-skeletal system in sea bream with a detailed consideration of tissue ontogeny and gene expression studies.

Sea bream genome mapping

At the beginning of 2005 a seabream whole-genome radiation hybrid (RH) panel was generated (Senger et al. 2006) and the quality ascertained by the construction of a 2 Mb resolution RH map. The map emcompasses 440 markers – microsatellites as well as gene-based markers - suitable for linkage analysis and comparative mapping studies. During the year over 1500 annotated genes and ESTs have been mapped on the radiation hybrid panel along with as 300 microsatellites markers. These microsatellites will be used to produce a linkage map and also to merge the two maps. A microarray was generated from different sea bream embryonic stages and it was used to assess large scale gene expression in key embryonic stages (Sarroupolou et al., 2005). The sea bream genome mapping has been a collaborative effort with Greek, Italian and French collegues.

Molecular evolution of hormones and receptors

Family 2 GPCRs is one of the major hormone and neuropeptide receptor families present in vertebrate genomes, although relatively little is known about their evolution in metazoan.

a) Evolution of family 2 GPCRs in metazoans

A comprehensive description of the evolution of family 2 GPCRs in teleost genomes was carried out using *in silico*. Teleost fish were found to contain extra gene copies of the human homologue family 2 GPCRs and only in the diploid genome of the model specie Takifugu rubripes a total of 21 members have been identified. This was the first time that the duplication of this family was reported in a vertebrate and it is assumed to be a consequence of a teleost specific gene or genome duplication. Moreover, a detailed study of the evolution of this family was also carried out in invertebrate genomes. Using sequence comparative approaches (sequence, gene organisation and gene environment) and expression studies combined with phylogenetic studies a hypothesis about the evolution of family 2 members was proposed.

b) Duplication and functional divergence of teleost family 2 GPCRs members

The role of teleost duplicated family 2 GPCRs was investigated. PAC1 receptors (a family 2 member) were isolated in sea bream and were functionally characterised using mammalian cell lines. Duplicated PAC1 (1A and 1B) receptors and an alternative splice form were characterised and were found to have differential tissue distributions and activation profiles. It appears that functional divergence may explain the persistence of duplicate copies of the GPCRs in teleost genomes.

c) Teleost ligands are their receptors

The secretin family gene precursors, the ligands of family 2GPCRs were isolated and characterised in several teleost genomes and their expression investigated. The overall aim will be

to address questions about coevolution of ligands and receptors. According to the results obtained so far for this family it appears that the ligand may have arisen prior to the receptors.

Steroid receptor expression and function

Two estrogen receptor subtypes (ERα_and ERβ) mediate most estrogen actions in vertebrates, including fishes. In sea bream, three ER have been cloned, ERα and two ERβ (a and b). The three sbER genes have a partially overlapping but differential distribution in male and female sea bream tissues and produce multiple transcripts. Two exon2 deleted sbERα variants were also identified with different tissue distribution and hormonal regulation. Estradiol-17β (E_2) up-regulated the expression of sbERα and down-regulated both sbERβs in liver, suggesting a major role for ERα in vitellogenesis. Agonistic effects were identified for the "pure antiestrogen" ICI 182,780 in several estrogenic responses, probably mediated by sbERα up regulation in liver. The immunolocalization of sbER proteins sea bream scales suggested that the calcium mobilising actions of E_2 in scales are via a direct action on osteoclasts. Subtractive hybridization followed by RT-PCR demonstrated for the first time in fish testis the E2 up regulation of some typical liver E2-induced genes (e.g. vitellogenins and choriogenins).

Control of sexual determination and differentiation

Fish have a variety of sex determining mechanisms, including environmental sex determination. DAX-1 (NR0B1), a member of the nuclear receptors super family, has been shown to be involved in the genetic sex determination and in gonadal differentiation in several vertebrate species. We have isolated the DAX-1 full-length cDNA from testis of the European sea bass. DAX-1 is present in both female and males and is not restricted to the gonads, as it is detected in the digestive tract, heart, gills, muscle and kidney. Although DAX-1 appears to be important for the development and differentiation of gonadal tissue in fish, it is not apparently sex determining.

PIF1 is the member of a helicase subfamily that is conserved from yeasts to humans. A PIF homologue was cloned, by differential display expression, from the ovaries of two teleosts, the Mozambique tilapia, *Oreochromis mossambicus*, and the European sea bass. PIF-1 transcripts were detected in pituitary, head kidney and gonads of adult fish. The level of expression was much higher in ovary compared to testis. These results and the patterns of expression of PIF1 during fish development suggest that a potential role in relation to ovarian physiology.

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

The understanding of olfaction in fish is fundamental to investigations to understand the role of olfaction in the biology of fish, particularly where this relates to chemical communication, feeding and environmental sensing. During the last year the role(s) of chemical communication between the sexes of the mouth-brooding cichlid *Oreochromis mossambicus* (the Mozambique tilapia), the eel (*Anguilla anguilla*) and the peacock blenny (*Salaria pavo*) have been studied using a combination of behavioural assays, chromatography and electrophysiology in order to establish the chemical identities and biological roles of the odorants involved. A related project has focused on identifying odours released by natural prey species of the Senegalese sole (*Solea senegalensis*). This has obvious applications for the aquaculture industry, but also allows assessment of olfactory processing of sensory information in that the two epithelia of this fish are in contact with different environments, and show a degree of functional asymmetry; the two olfactory systems (upper and lower) may have slightly different biological roles. Related studies involve investigating the olfactory sensitivity of fish to inorganic ions, principally calcium (Ca^{2+}) and sodium (Na^{+}) in both marine (seabream *Sparus aurata* and bass *Dicentrarchus labrax*) and freshwater species (goldfish *Carassius auratus*). This work has aimed firstly to identify both the receptor neurons and cellular mechanisms involved but future work will address the biological role(s) of this sensitivity.

<u>Plan for 2006</u>

Calciotropic hormones

Physiological and molecular studies will be continued in order to elucidate the role of PTH-like molecules in reproduction, skeletal development, calcium balance and immune response in sea bream and other teleosts. The role of other calcium regulating hormones in these processes will also be studied as will their interaction with PTH/PTHrP. The approaches include 1) the mechanisms through which PTHrP regulates calcium balance in fish, the importance of PTHrP in the regulation of ion balance in the opercular membrane and kidney will be established using a range of bioassays and cell culture (enterocyte and gill mitochondria rich cells); 2) receptor characterization and identification in a tissue specific manner of PTHrP/PTH responsive genes using a range of approaches; 3) identification of PTHrP responsive tissues using bioassays and biochemical assays; 4) bioactivity and post-translational and post-secretary processing of the diverse PTH and PTHrP proteins identified in fish; 5) in situ hybridisation to establish cellular location and ontogeny of PTH/PTHrP and responsive genes in fish; 5) Knock-down models to establish the importance of PTHrP responsive genes.

Hormonal control of development and growth of fish eggs and larvae

Studies will continue to relate the major morphological transformations of round and flat fish to genes and proteins (in particular endocrine factors) during metamorphosis, using external morphology and internal morphology (whole mount and sections). 2. Development and application of molecular and biochemical markers of key physiological systems, nervous system, digestive system, musculo-skeletal system, endocrine system in round fish and flat fish. Comparison of marker ontogeny in round and flat fish. 3. Experiments to establish the effect of absence of excess of hormone on morphological markers of metamorphosis and molecular/biochemical markers. Correlation of markers with metamorphic progression. 4. The endocrine, nutritional and other abiotic factors underlying the development of abnormal skeletons in fish will be studied and efforts will be made to integrate producers in these studies.

Molecular evolution of hormones and receptors

The presence in teleosts of extra family 2 GPCRs and peptide genes opens extensive areas of research in the study of gene evolution, gene function and gene regulation in vertebrates. The existence and persistence of duplicated gene within vertebrate genomes is a major subject in science and several theories have been proposed and this work will greatly contribute for a better understanding of their origin using teleost fish as models. The study of the evolution and function of family 2 GPCR receptor genes and their ligand peptide will be carried on during 2006. Functional characterisation of teleost family 2 GPCRs will proceed for other family members (previously isolated) and compared with the available data from mammals. Moreover, studies of gene regulation will be initiated using the duplicate receptor genes in order to identify conserved non-coding motifs involved in their regulation. The putative ancestral receptor genes previously identified *in silico* will be investigated *in vivo* in invertebrate model species such as the mosquito using fluorescent in situ hybridisation techniques. Emphasis will be given to the evolution and function of family 2GPCRs and their ligands with known importance in vertebrate calcium regulation. Their evolution and function in invertebrates will be investigated.

Bioprospecting

Evolutionary studies are highlighing the potential which exists to exploit biomolecules produced in one class of organisms to vertebrate health and wellbeing. Studies are being initiated to identify compounds from less complex organisms which are active in more complex organisms. A number of different technologies and collaborations will be established this year in order to start developing this area.

Sea bream genome mapping

Studies will be continued in the context of the results obtained from BRIDGEMAP/Aquafirst this will entail: 1. Implementation of genotyping and phenotype analysis in a commercial aquaculture company in order to characterise the broodstock. 2. Physiological studies will be initiated in the context of a European project to establish the heretability of stress and disease resistance. Experiments will be conducted and sample analysis initiated. 3. Mapping of genes on the sea bream radiation hybrid will be continued in order to place further markers on the map. 4. Linkage analysis will be conducted with candidate genes to establish their weight in QTL. In depth analysis of QTL regions will be carried out using BACs in order to identify potential candidate genes.

Control of sexual determination and differentiation

Work will concentrate on developing a medaka transgenic model overexpressing DAX-1, the analysis of gene promoter regulation and the comparative analysis of sex determination gene expression in different species..

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

In addition to continuing the work initiated in 2005, the role of olfactory sensitivity to bile acids in fish will be studied. This phenomenon is widespread amongst different groups of fishes but its biological significance is poorly understood. In particular, the existence of sex-related differences in the types of bile acids produced and detected by conspecifics will be studied and the possibility that bile acids may be involved in inter-specific chemical communication will be explored. Preliminary results strongly suggest that fish, in general, are able to detect bile acids released by other, unrelated, species.

Group: Biophysics

Leader - Leonor Cruzeiro
Paulo Silva, PhD Student

Summary of activities and progress during 2005

The Davydov model for energy transfer was applied to the calculation of the amide I absorption band of protein α-helices and comparison with the experimentally measured one allowed for the determination of an important parameter of the model. Work was also done on an extension of the Davydov model, to include the many kinds of interactions present in proteins and also the interaction of amide I excitations with the bending mode of water, which was then applied to the human prion. One result was a suggestion for one reason why prions, and in general, proteins with greater amounts of glutamine and asparagine, are more unstable than other proteins. Finally, work on a model for protein conformational changes also continued, with the study of the different dynamical regimes that can arise, as a function of the parameters of the model

Plan for 2006

The plan for 2006 is to study the influence of protein secondary structure on the absorption lineshape of amide I and compare the results with experimentally measured absorption bands,
as well as to proceed with the investigation of extended models to describe protein conformational changes. Also, more general research on non-linear systems will be carried out in collaboration with Prof. Chris Eilbeck.

Group: Aquaculture

Research team

Researchers: Maria Teresa Dinis

CIMAR-LA Researchers: Luis Conceição
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PhD students: Sofia Morais
MSc students: Benjamin Costas
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Research assistants: Sofia Engrola, Marc Lacuisse, Rui Rocha, Ana Couto.
Aquaculture technicians: Helena Damásio, Helena Teixeira

Summary of activities and progress during 2005

The central aim of the group is to contribute to the sustainable development of the aquaculture industry, through basic and applied research aiming at optimisation of rearing techniques and identification of bottlenecks in the cultivation of new fish species. Ongoing projects involve nutrition physiology, digestive physiology, stress physiology in particular during the early stages of marine fish, broodstock management, as well as the development and optimisation of production systems and feeding regimes for fish. One of the main areas of work in recent years is the optimisation of the farming techniques of Senegalese sole, *Solea senegalensis*. Several advances in broodstock management, spawning in captivity, and husbandry in larval and post-larvae rearing have been accomplished, and research continues on these issues. Recently, the group started similar work with red porgy (*Pagrus pagrus*) and dusky grouper (*Epinephelus marginatus*), where the main objective is to establish the zootechnical basis for juveniles production using mesocosms systems. The Aquaculture research group members are also involved in research projects studying: (1) nutritional requirements, in particular amino acids, of fish larvae and juveniles and the basis for the understanding of the relation between nutrition, metabolism, stress and abnormalities; (2) ontogeny of larval digestive functionality and control, in relation to feeding plans and weaning; and (3) identification of the effects of "green water" technique on larvae performance, feeding, nutrition, and microbial ecology. The main progress accomplished in 2005 was:

Effect of dietary neutral lipid level and source on food intake, digestion and growth in marine fish larvae

The requirements for essential fatty acids (EFA) have been extensively studied in marine fish larvae but few studies have examined quantitative lipid needs in larval diets. The attempt to meet larval requirements using poor sources of EFA (predominantly neutral lipids) may result in excessive lipid content and a number of authors have reported poor larval growth and performance associated with a high lipid content of the diet. Studies were carried out on commercially valuable farmed species (Solea senegalensis, Sparus aurata and Dicentrarchus labrax), to investigate the effects of total neutral lipid level and lipid source (i.e., FA composition) on key factors involved in larval growth. The results seem to collectively indicate that lipid transport from the enterocytes into the body may be more problematic in larval stages dealing with high lipid diets than lipid digestion, although both factors are likely to intervene. Food intake did not appear to be strictly regulated by total lipid content of the diet and lipid source may have an important role in controlling ingestion. Therefore, lipid level in diets for marine fish larvae may have a significant impact in several factors influencing growth but clearly it cannot be dissociated of its fatty acid composition, which appears to play a central role on the nutritional and physiological effects of dietary lipid, at the ingestion, digestion and absorption levels. This study was concluded in 2005.

The effect of green water on larval development

The use of microalgae in larval rearing tanks is a common procedure in marine aquaculture. The aim of this project is to analyse the influence of different microalgae species on fish larvae development. *Tetraselmis chuii* was used at different periods of sole and sea bream development to evaluate which stage of development would be more influenced by the presence of microalgae. Sea bream larvae showed to be more dependent on the presence of microalgae than sole, but both species preferred to have microalgae at mouth opening. No significant differences were observed by the end of the experiment, although a compensatory growth could be observed for both species after adding microalgae to the rearing tanks. Other experiments, intended to analyse sole and seabream larvae condition when reared with different microalgae species by measuring RNA/DNA ratio, however no significant differences were observed among treatments. The influence of different microalgae species was also inferred by measuring lipogenic and amino acid metabolic enzymes in Sparus aurata, as observed previously for sole no clear differences were observed among microalgae treatments; however fish species exhibited slightly different patterns of variation. The presence of microalgae (*Tetraselmis chuii*, *Isochrysis galbana*, Phytobloom®) in the rearing water did not increase the nutritional value of previously enriched live food (Protein Selco and Reach, respectively), since after 3 hours live food (rotifers and Artemia metanauplii) protein and lipid content were similar among treatments including the control treatment. So, this observation indicates that the presence of microalgae do not maintain the nutritional quality of the live prey.

Understanding the regulation of the digestive function on marine fish larvae

The use of microdiets from mouth opening will be one of the most important achievements in marine aquaculture. Although marine fish larvae do ingest microdiets and exhibit digestive capacity after hatching, growth and survival rates are still lower when compared with fish larvae fed live food. The aim of this project is to understand how fish larvae regulates the digestive process. Studies were runned using sea bream and sole larvae, reared under different feeding regimes. Samples were taken and fixed for imunohistochemical analysis, that will analysed during 2006. Since chemical and mechanical stimulus are important factors in the digestive process, seabream larvae were submitted to dfferent types of stimulus and after an hour samples were collected for digestive enzymes analysis. Preliminary results indicated that chemical and mechanical stimulus increased amylase specific activity; this pattern was not observed for trypsin and alkaline phosphatase enzyme specific activity.

Modelling the utilisation of dietary amino acids in fish larvae

Further improvement of growth performance in fish larviculture is closely linked to a better understanding of the dietary amino acid (AA) requirements, and therefore of the processes involved in AA metabolism. In recent years major advances in the understanding of fish larvae amino acid metabolism have been accomplished, in particular through the use of tracer studies. However, interpretation of information from tracer studies is usually limited to the comparison of a number of body compartments in a few time points. Modelling is a holistic approach to integrate knowledge on growth and metabolism and identify gaps .A dynamic mechanistic model that simulates AA metabolism of fish larvae was developed using the POWERSIM package. Its objective is to improve the understanding of larval digestion and absorption of dietary AA, and the postprandial AA metabolism and growth. The model should also assist in the interpretation of results obtained using tracer studies. The model is driven by amino acid intake, with the absorbed dietary AA being used for energy production or for biosynthetic processes. The first version of the model was so far tested with Senegalese sole (Solea senegalensis) fed Artemia, and was parameterized using literature data. The model allows to integrate the results obtained after feeding a single meal with tracer AA, and following these tracer AA in the free AA and protein pools of larval gut and larval body at different time points after the meal. Model simulations permit to study the dynamics of the changes in the larval free AA and protein pools. Calibration of this dynamic model using tracer studies data also allow to calculate the instantaneous rates of different processes involved in AA metabolism: gut AA catabolism, gut protein synthesis, gut AA evacuation, transfer of AA from the gut to the body, body protein synthesis and body AA catabolism. Due to its mechanistic nature, the present model can be used with different AA tracers, and also for other fish species. Further work was performed at the level of model refinement and calibration during 2005. (Work in collaboration with Prof. Ivar Ronnestad, Univ. Bergen, Norway)

Stressfull husbandry conditions and dietary amino acid requirements in sole

Stressful conditions are known to cause growth suppression in cultured fish, either by impacts on appetite reduction, a stimulated catabolism, or a combination of both. As growth is essentially protein deposition, its optimisation depends on the understanding of protein and amino acid (AA) metabolism. The relative balance of the different metabolic pathways involved in AA metabolism is affected by the physiological condition of the animal. Thereby, stressful husbandry conditions do affect AA requirements. The central objective of this study is to contribute to a better understanding of the metabolic processes impinging on amino acid requirements of animals when they are exposed to stress situations. Post-larval and juvenile Senegalese sole (*Solea senegalensis*) will be used as model species, because it is a species resistant to stress in terms of survival and also because it is a species of importance to the Portuguese marine aquaculture industry. It is intended to verify to what extent the amino acid metabolism of fish change when fish are under stress situations, and also whether the metabolic and growth depression effects of stress can be reduced by AA supplementation. So far, the effects of selected stressful husbandry conditions on growth and AA metabolism are being assessed in post-larvae and juvenile sole. AA utilisation was studied using tracer studies for sole post-larvae under density and ammonia chronical stress. Results show a tendency for higher feeding incidence in post-larvae cultured at low densities. The results regarding the ammonia experiments show a tendency for a higher feeding incidence as the ammonia concentrations in water increases. This result suggests that these post-larvae may increase their feed consumption to compensate for their higher energy demands as a result of stress. Experiments were also performed with juveniles under different chronic and acute stressors. Stress levels were assessed through plasma cortisol, glucose and lactate levels. Amino acid requirements were studied using plasma free amino acid levels. Results suggest that cortisol may not be the best indicator to monitorize stress in fish chronically exposed to incresead ammonia levels in water. Growth and feed utilisation are lower as the ammonia concentration in water increases. Due to the benthonic and passive behaviour of sole, lactate levels do not seem to be good indicators of stress response in this species. Amino acid analysis suggests that sole under chronic ammonia stress may have a higher requirement of glucogenic amino acids and also of some amino acid related with inflammatory processes. Acute stress tests show also that some amino acid involved in the hormonal regulation of stress responses are also affected.

Broodstock management

The objectives for 2005 were the identification of the parameters responsible for sole maturation and reproduction, one important bottleneck on sole cultivation. Four broodstocks were maintained in a open system at the Experimental Station of Ramalhete. Two groups were feeding with squid and worms and the remaining groups were fed with squid and mussels. Spawnings with good quality eggs were obtained from four groups, kept under natural photoperiod and temperature. 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. Blood was collected to quantify amino acids in plasma and identify differences related with the feed regime used.

In October a new male broodstock was established. All fish were tagged and sex determined. Fluent and non-fluent males were identified based on their fluency and sperm quality was analysed fortnightly during all year. Sperm volume and production, seminal plasma, osmolarity, pH, spermatozoa motility and cell concentration were the parameters choosen to characterize sperm quality. Results demonstrated that fluent males can produce motile sperm all year with several peaks of spermeation with high cell density and sperm volume. Some males did not spermeate or produced sperm with bad quality, indicating that a previous selection of males is required.

The dusky grouper broodstock is being kept in collaboration with IPIMAR/CripSul, at their EPPO facilitity, was monitored through canulation in spring-summer 2005, and hormonal induction of spawning in grouper using GnRHa slow-releasing devices was attempted. The first successful spawnings in this broodstock were obtained, and were fertilised with good results. Fertilisation was

only possible because precocious induction of male groupers with methyl-testosterone was attempted with sucess.

Zootechnical improvements in the larval rearing of new species for Aquaculture

Studies were conducted in the optimization of the larval rearing conditions and feeding regimes of sole (*Solea senegalensis*), red porgy (*Pagrus pagrus)* and grouper (*Epinephelus marginatus*). Weaning is one of the traditional bottlenecks in sole culture. In continuation of studies from the previous years at CCMAR. In order to determine the impact of feeding regime on weaning performance by sole, three feeding regimes were studied during the pelagic phase and four feeding regimes during the benthic phase. Feeding regime had an impact on growth and survival at the end of the pelagic phase. The treatment that had less fish produces also the bigger post-larvae. Feeding regime had an impact on growth but not on survival. At the end of the weaning period the bigger post-larvae were the ones that eat live and inert diet since mouth opening. The larval rearing of red porgy (*Pagrus pagrus)* and grouper (*Epinephelus marginatus*) is still difficult and with unpredictable results. The main problems, often reflected in massive mortalities, seems to be the identification of suitable feeding regimes for these species and to provide conditions of minimal stress. In order to tackle these problems, a large scale semi-intensive system (mesocosms) for rearing these species continued to be tested. Results were fair for red porgy, with the production of a significant number of juveniles, and a few grouper fry were also produced using this system.

Plan for 2006

The research during 2006 will continue to focus on various aspects of fish nutritional and digestive physiology, but also 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*).

The effect of green water on larval development

Determine the influence of different microalgae on:
a) sole larvae protein turnover, using an energetic balance approach.

Understanding the regulation of the digestive function on marine fish larvae

The importance of different stimulus on fish larvae digestive activity will be further studied. The development of digestive neuropeptides under different feeding regimes will also be evaluated. Another purpose of this project will be to determine cholecystokinin content using radioimmunoassay.

Physiological importance and metabolism of aromatic and sulphur AA during fish ontogeny

The major fate of amino acids (AA) is towards protein synthesis, but some AA are involved in the synthesis of other compounds of physiological importance. Among them, sulphur and aromatic AA may seem to have a special importance during the fish ontogenesis. The main objective of this study is to acquire a better knowledge on the physiological importance and metabolism of these AA during the early life stages of fish, focusing especially on taurine and tyrosine. Senegalese sole (Solea senegalensis), gilthead seabream (Sparus aurata), and toadfish (*Halobatrachus didactylus*) will be used as model species. The free amino acid levels along egg development and in fed and starved larvae will be monitorised. This will allow comparing the amino acid metabolism in species with demersal and pelagic eggs and in species with marked metamorphosis. Some tube-fedding experiments will also be done, in order to analyse the utilisation and metabolism of aromatic and sulphur amino acids along the fish development.

Modelling the utilisation of dietary amino acids in fish larvae

The existing dynamic mechanistic model that simulates AA metabolism of fish larvae will be further refined. It will be used with different AA tracers, and it will be attempted to run it with more than one

tracer amino acid simultaneously. The model will also be used to study the changes in AA metabolism during development of different larval species. (Work in collaboration with Prof. Ivar Ronnestad, Univ. Bergen, Norway)

Stressfull husbandry conditions and dietary amino acid requirements in sole

The relation between stressfull conditions and amino acid requirements on sole postlarvae and juveniles will be further studied. Stressfull conditions for sole will be further characterized and amino acid requirements will be studied using plasma free amino acid levels, tracer studies, and intermediary metabolic enzyme activities as indicators. The effect of amino acid supplementation on performance under stress conditions will also be assessed. It will be verified to what extent the negative effects of stressful husbandry conditions on amino acid metabolism and retention can be minimized through supplementation of the diets with individual amino acids.

Nutritional effects on fish proteome expression

Work will be performed on the effect of dietary nitrogen on fish proteome expression. White bream and zebrafish will be used as models. Two-dimension gel electrophoresis will be followed by proteome comparision and identification of differentially expressed proteins.

Broodstock management

a) Identify the effect of using worms in the broodstock feeding on reproduction success and quality of eggs and larvae of sole.
b) Study the effect of sole female/male interaction on sperm quality
c) Determine the influence of water salinity/osmolarity in sole sperm activation and fertilization
d) Determine the influence of stress on sole male and female gamete quality and reproduction
e) Attempt to increase reproducibility of hormonal induction of spawning in dusky grouper using GnRHa slow-releasing devices, and induction of precocious males using hormonal treatment. Cryopreseervation of grouper semen will also be attempted.

Zootechnical aspects of larval and juvenile rearing

a) Improve the success and reproducibility of grouper (*Epinephelus marginatus*) larval rearing in mesocosms systems.
b) Determine if weaning success and quality of sole post-larvae can be further improved through co-feeding with different relative amounts of live food and inert microdiets.

Division of Living Resources

Group: Marine Ecology and Evolution - MAREE

Research team

Researchers: Ester Serrão (L)
CIMAR Researchers: Gareth Pearson (L)
Post-docs: Sophie Arnaud, Onno Diekmann, Elena Varela, Filipe Alberto
PhD students: Asuncion Lago-Leston, Emmanuelle Billard
(L= team leaders)

Summary of activities and progress during 2005

Genetic Structure of Marine 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.

Achieved in 2005:

SPATIAL GENETIC STRUCTURE, NEIGHBOURHOOD SIZE AND CLONAL SUBRANGE
The extent of clonality within populations strongly influences their spatial genetic structure (SGS), yet this is hardly ever thoroughly analysed. We employed spatial autocorrelation analysis to study effects of sexual and clonal reproduction on dispersal of the dioecious seagrass Cymodocea nodosa. Analyses were performed both at genet level, (i.e. excluding clonal repeats) and at ramet level. Clonal structure was characterized by the clonal subrange, which we defined as a spatial measure of the linear limits where clonality still affects SGS. We showed that the clonal subrange is equivalent to the distance where the probability of clonal identity approaches zero. This combined approach was applied to two meadows with different levels of disturbance, Cadiz (stable) and Alfacs (disturbed). Genotypic richness, the proportion of the sample representing distinct genotypes, was moderate (0.38 Cadiz, 0.46 Alfacs) mostly due to dominance of a few clones. Expected heterozygosities were comparable to those found in other clonal plants. SGS analyses at the genet level revealed extremely restricted gene dispersal in Cadiz (Sp=0.052, a statistic reflecting the decrease of pairwise kinship with distance), the strongest SGS found for seagrass species, comparable only to values for selfing herbaceous land plants. At Cadiz the clonal subrange extended across shorter distances (20-25m) than in Alfacs (30-35m). Comparisons of sexual and vegetative components of gene dispersal suggest that, within meadows, clonal spread is at least as equally important a dispersal vector as sexual reproduction. The restricted dispersal and SGS pattern in both meadows indicates that the species follows a repeated seedling recruitment strategy.

THE WEST IBERIAN COAST: CONSEQUENCES OF SMALL POPULATION SIZES, HABITAT DISCONTINUITY AND NEARSHORE CURRENTS

The effects of oceanographic patterns on marine genetic biodiversity along the SW Iberian Peninsula are poorly understood. We addressed the question of whether gene flow in this region depends solely on geographic distance between isolated patches of suitable habitat or if there are superimposed effects correlated with other factors such as current patterns. Zostera noltii, the dwarf eelgrass, is the keystone habitat-structuring seagrass species on intertidal mudflats along the Iberian west coast. We used nine microsatellite loci to analyze population genetic diversity and differentiation for all existing 8 populations from NW Spain (Ria de Vigo) to SW Spain (Puerto Real, Cadiz). Populations are highly genetically differentiated as shown by high significant FST (0.08-0.26) values. A neighbor-joining tree based on Reynold´s distances computed from allele frequencies revealed a split between northern and southern populations (bootstrap support of 84%). This pattern of differentiation can be explained by 1) coastal surface current patterns present during Z. noltii reproductive season causing a barrier to dispersal between the northern and southern populations of this region, 2) habitat isolation, due to large geographic distances between suitable habitats, preventing frequent gene flow, 3) small effective population sizes, causing high drift and thus faster differentiation rates.

MEDITERRANEAN-ATLANTIC TRANSITION ZONE:

A central question in evolutionary ecology is the nature of environmental barriers susceptible to limit gene flow and to induce population genetic divergence, a first step toward speciation. In order to address this question we are studying the geographical barrier formed by the transition zone between the Atlantic Ocean and the Mediterranean Sea, using as model *Cymodocea nodosa and Zostera noltii. In 2005 we have completed the study on* Cymodocea nodosa*,* a seagrass distributed throughout the Mediterranean basin and in the Atlantic, from central Portugal to Banc d'Arguin in Mauritania. We used eight microsatellite markers to compare 18 populations in the Atlantic and 27 in the Mediterranean Sea, focusing on their transition zone. Populations from these two regions form coherent groups containing several unique high frequency alleles for the Atlantic and for the Mediterranean, with some admixture on the Atlantic side and west of the Almeria-Oran Front, a pattern typical of a secondary contact zone. This coincides with populations where only one or a few genotypes were found for all but Cadiz (Portugal, SW Spain and Morocco), but remarkably still show the footprint of a contact zone.

The most significantly divergent groups however are the two groups at the range limits: particularly the southernmost Atlantic populations (Mauritania, Madeira and Canary Islands), probably due to founder effects and geographic isolation, but also the easternmost Mediterranean, also a potential footprint of vicariance. Significantly higher allelic richness ($\hat{A}$) was found on the Mediterranean than in the Atlantic; this and the extremely high value found for Cyprus, the easternmost sample, suggest that colonization of the Atlantic proceeded from the Mediterranean.

VICARIANCE IN THE MEDITERRANEAN SEA: EAST-WEST CLEAVAGE AND LOW DISPERSAL

The seagrass Posidonia oceanica is endemic to the Mediterranean Sea, where previous studies suggested that it exhibit predominant clonal growth leading to low level of intra-meadow clonal diversity. We used dinucleotide microsatellites with higher resolution that allowed the efficient identification of genetic individuals (genets), rending possible the reliable estimation of intra and inter population genetic parameters. At the scale of the whole Mediterranean Sea, a high East-West cleavage (22% of the variance is due to the genetic divergence between Eastern and Western groups) was highlighted by AMOVA. This split is likely to represent the footprint of vicariance due to the last Pleistocene ice age, maintained by a presently low gene flow, which is supported by the careful analysis of samples from a putative secondary contact zone, the Siculo-Tunisian Strait. The screening for factors responsible for the maintenance of presently limited gene flow was then performed at different spatial scale. Values of F-statistics (from 0.09 to 0.71) revealed an inter-meadow high genetic structure, both at small scale (about 2 to 200 kilometers) and at medium scale within Eastern and Western basin, without correlation with genetic distance. At the intra-meadows scale, spatial autocorrelation revealed in six out of fifteen locations showed a significant limitation of dispersal at the scale of several tens of meters. A highly stochastic pattern of effective migration due to very low population size, turnover and seed survival is likely to explain this pattern of high restriction to gene flow, despite an a priori important dispersal potential of the

seeds. These results emphasize the diversity of evolutionary processes leading to genetic structure at different spatial scales

Population genetic structure of mangrove forests of Avicennia sp. in Vietnam

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 commercially important species. 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.

Achieved in 2005:

LOW DIVERSITY AND HIGH INBREEDING IN SE ASIA MANGROVE (AVICENNIA MARINA) POPULATIONS
Understanding the genetic composition and mating systems of edge populations provides important insights into the environmental and demographic factors shaping species distribution ranges. We analyzed samples of the mangrove Avicennia marina from Vietnam, Northern Philippines and Australia, with microsatellite markers. We compared genetic diversity and structure in edge (South-Eastern Asia, and Southern Australia) and core (North and Eastern Australia) populations, and also compared our results with previously published data from core and Southern edge populations. Comparisons highlighted significantly reduced gene diversity and higher genetic structure in both margins compared to core populations, which can be attributed to very low effective population size, pollinator scarcity and high environmental pressure at distribution margins. The estimated level of inbreeding was significantly higher in North Eastern populations compared to core and Southern populations. This suggests that despite the high genetic load usually associated with inbreeding, inbreeding or even selfing may be advantageous in margin habitats due to the possible advantages of reproductive assurance, or local adaptation. The very high level of genetic structure and inbreeding show that populations of Avicennia marina are functioning as independent evolutionary units more than as components of a metapopulation system connected by gene flow. The combinations of those characteristics make these peripheral populations likely to develop local adaptations and therefore to be of particular interest for conservation strategies as well as for adaptation to possible future environmental changes.

MANGROVE GENETIC DIVERSITY STILL INCREASING 30 YEARS AFTER AGENT ORANGE
Widespread use of Agent Orange over Southern Vietnam by United States Forces1 led to the decimation of mangrove forests in the Mekong Delta2. 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 showed that genetic recovery of the mangrove Avicennia alba population is still increasing in the Mekong Delta three decades following the end of the war, but is reaching an asymptotic level. We sampled three A. alba stands generated during natural recovery in each of two areas in the Mekong Delta, Vietnam, a region that was totally deforested by Agent Orange. Rhizophora mangroves were replanted whereas recolonization by other mangrove species, such as A. alba was not assisted. Leaf samples were collected from trees (N =232) ranging from seedlings to the largest size found, for genetic analyses with six microsatellite markers. Tree age was determined from the number of internodes the plant produced throughout their life span, or from linear regression analysis between plant girth and total number of internodes for the oldest trees. In addition we thoroughly searched the potential mangrove habitat of the impacted area to sample the oldest, scattered Avicennia alba trees (N=18) to characterize the genetic diversity of the population immediately following the disturbance. Age determinations indicated that only four of these trees were present before the disturbance and fourteen immediately after. The recovery of genetic variability in the population was analysed as

the average (± SE) number of alleles present in the A. alba population at five-year intervals since the disturbance, in trees recruited before 1978, 1983, 1988, 1993, 1998. In order to account for the decreasing sample size with time since present, allelic richness was computed by resampling (1000 iterations) from each combined age class to maintain a homogeneous sample size (N=18, the smallest observed sample size, of the oldest age classes). We found allelic richness to show a non linear, significant, increase since the end of the war. Hence, allele richness increased by 14% over 25 years, but the rate of increase in allelic diversity declined from a maximum of 0.05 alleles locus-1 year-1 in the mid 1980's (1 % year-1) to a marginal increase of 0.0069 alleles loci-1 year-1 (0.14 % year-1) a decade later. These results show that the genetic recovery is still progressing, although the slow rate of increase in genetic diversity in the mid 1990's suggests that genetic diversity was reaching an asymptotic level three decades following the end of the radical disturbance experienced. However, it is impossible to say whether genetic recovery is complete due to the absence of records prior to disturbance or of comparable undisturbed populations. The destruction of the Mekong Delta mangrove forests by Agent Orange is possibly the largest human disturbance event experienced by any ecosystem. Recent assessments have revised upwards the impact of Agent Orange on human health in Vietnam, the results presented here demonstrate similarly pervasive effects on the ecosystem.

Evolution of reproductive strategies in fucoid algae.

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.

Achieved in 2005:

GENETIC ENTITIES AND MATING SYSTEM IN HERMAPHRODITIC FUCUS SPIRALIS AND ITS CLOSE DIOECIOUS RELATIVE F. VESICULOSUS (FUCACEAE, PHAEOPHYCEAE)

To date, molecular markers have not settled the question of the specific status of and/or natural hybridization between the closely related, but phylogenetically unresolved, brown seaweeds, hermaphroditic Fucus spiralis and dioecious F. vesiculosus. To test the degree of species integrity and to assess effect of the mating system on the population genetic structure, 288 individuals coming from parapatric (discontinuous) and sympatric (contiguous) spatial configurations at two sites were genotyped with five microsatellite loci. Using a Bayesian admixture analysis, our results show that F. spiralis and F. vesiculosus comprise clearly distinct genetic entities (clusters) generally characterized by cosexual and unisexual individuals, respectively. Genetic diversity within each entity suggests that F. spiralis reproduces primarily through selfing while F. vesiculosus is characterized by an endogamous breeding regime. Nevertheless, aberrant sexual phenotypes were observed in each cluster, no diagnostic alleles were revealed and 10% of study individuals were intermediate between the two genetic entities. This pattern can be explained by recent divergence of two taxa with retention of ancestral polymorphism or asymmetrical, introgressive hybridization. However, given (1) coincident monomorphism at three loci in spiralis clusters and (2) that significantly more intermediates were observed in sympatric stations than in parapatric stations, we argue that interspecific gene flow has occurred after divergence of the two taxa. Finally, we show that whether recently separated or recently introgressive, the divergent breeding systems probably contribute to species integrity in these two taxa.

HYBRIDIZATION IN THE FUCUS SPIRALIS / F. VESICULOSUS SPECIES COMPLEX: SEXUAL PHENOTYPE AND PREZYGOTIC FERTILITY

In the genus Fucus the character dioecy/hermaphroditism has undergone multiple state changes and hybridization is possible between taxa with contrasting mating systems, e.g., between the dioecious Fucus vesiculosus and the hermaphroditic F. spiralis. In the context of mating system evolution, we evaluated the potential consequences of hybridization by studying the variation in sexual phenotype and in prezygotic fertility. First, as a result of hybridization between the two sexual systems, gender variation may arise depending on the relative importance of genes with large versus small phenotypic effects. We thus qualitatively examined the extent of gender variation within and among individual hybrids in comparison with both parental species. Second, if hybridization breaks up co-adapted gene complexes, hybrid fertility may be reduced in comparison with both parental species. We therefore also quantified male and female prezygotic fertility in parental species and their hybrids in order to test for reduction in hybrid fitness. A total of 89 sexually mature individuals (20 F. spiralis, 40 F. vesiculosus 10 hermaphroditic hybrids and 19 dioecious hybrids) were sampled in two geographically distant regions (France and Portugal) and six conceptacles (i.e. cavities containing the sex organs) per individual were observed. Within-individual variation was very restricted qualitatively—only one hybrid carried a conceptacle with a different sexual phenotype from the five others—as well as quantitatively. This suggests a simple genetic system for sex determination involving a few genes with major effects. In addition, analyses showed no significant decrease in hybrid fertility compared with parental species. Moreover, hybrids exhibited all sexual phenotypes, suggesting several generations of hybridization and backcrossing and, therefore, that hybrids are reproductively successful. Finally, the occurrence of sterile paraphyses in female and hermaphroditic individuals was interpreted as a relic of male function and suggests that, as in higher plants, evolution from hermaphroditism to dioecy may be the most parsimonious pathway.

GENETIC ISOLATION BETWEEN THE THREE CLOSELY RELATED TAXA: FUCUS VESICULOSUS, F. SPIRALIS AND F. CERANOIDES

The phylogenetic relationships within the Fucaceae, particularly within the genus Fucus are still controversial despite the great interest this taxon has long been subject to. As all traditional markers, phenotypic or phylogenetic, have failed to discriminate the taxa composing the 'F. vesiculosus / F. spiralis / F. ceranoides' species complex, we used five microsatellite markers to compare the allelic frequencies between populations of the three taxa. The aim of this study was to assess whether the different populations were grouped according to their geographical location, to their habitat (open coast vs. estuary) or according to their a priori taxonomic assignment. Our results clearly supported the separation of F. vesiculosus, F. spiralis and F. ceranoides into distinct species, independently of geography. In F. vesiculosus, we also identified a secondary level of genetic variation according to ecotypic differentiation

POPULATION GENETIC STRUCTURE OF DIOECIOUS VERSUS HERMAPHRODITE *FUCUS* SPECIES, HYBRIDISATION ALONG SYMPATRIC AND ALLOPATRIC ZONES

Sister species with contrasting reproductive modes provide unique opportunities for understanding the effects of mating system and hybridisation on the evolution of populations. The morphologically similar and phylogenetically closely related algae F. spiralis (hermaphroditic) and F. vesiculosus (dioecious) have broad areas of sympatry along their distribution, but at their southern distributional limits, the two species are only found in allopatry. In this study, genetic markers were used to assess the consequences of mating system and hybridisation for population genetic structure in sympatric and allopatric zones along their distribution. Complete reproductive isolation between the two species was observed in allopatry whereas the occurrence of hybridisation and introgression was confirmed when the distributions of the two species overlap. Three main genetically distinct regions, possibly related to ice age refugia, present day marginal habitats and potential contact zone between differentiated populations, were defined. They were common to both species suggesting similar evolutionary history and barriers to gene flow independent of mating system. In contrast, mating system was important for genetic diversity and likely determined

the contrasting distribution of the two species at their geographical limits. Finally, very strong genetic differentiation among F. vesiculosus populations indicates reproductive isolation within this species.

ASEXUAL LIFE IN MARGINAL POPULATIONS OF THE BROWN SEAWEED FUCUS VESICULOSUS

Reproduction of attached large brown algae is known to occur only by sexual zygotes. Using microsatellites we show evolution of asexual reproduction in the bladder wrack promoting population persistence in the brackish water Baltic Sea (<6 psu). Here a dwarf morph of Fucus vesiculosus is dominated by a single clone but clonal reproduction is also present in the common form of the species. We describe a possible mechanism for vegetative reproduction of attached algae, and conclude that clonality plays an important role in persistence and dispersal of these marginal populations, in which sexual reproduction is impaired by low salinity.

The molecular basis for stress-tolerance in brown algal species.

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 2005:

POPULATION-LEVEL VARIATION IN DESICCATION-RESPONSIVE GENE EXPRESSION

Fucus vesiculosus has an arctic to warm temperate distribution, occupying a broad range of habitats, from rocky and estuarine intertidal shores, to the low-salinity and atidal Baltic Sea. The latter are constantly submerged populations, and we have previously shown, based on physiological data, that they have evolved a reduced tolerance to emersion stress during the last 3-7 K yrs (age of the Baltic Sea). We are interested in population-level divergence in stress responses at the gene and transcript levels in this species, in order to identify molecular mechanisms involved in local adaptation. We therefore produced an EST sequence database of partial cDNAs obtained from subtractive libraries of algae undergoing desiccation stress, and screened the expression of these genes in several populations in common-garden stress experiments. Results show that transcriptional responses to desiccation vary among populations, particularly within the Baltic. Comparison of desiccation-responsive gene expression among populations using radiolabeled (32P) mRNA hybridization probes prepared from desiccated and control tissue for Brittany, North Sea and the Baltic, showed constitutive expression of these genes in both intertidal populations (Brittany and North Sea) but the subtidal population from the Baltic revealed upregulation of several genes when subjected to emersion stress. These results are consistent with the different levels of emersion stress with tidal variations and supports the idea that populations that experience regular stress maintain their resistance permanently, but this may be a costly investment, since in recently diverged subtidal populations, several stress responsive genes were not being expressed but could be induced when exposed to the stress condition that they would never experience in the natural habitat..

SIMPLE AND RAPID RNA EXTRACTION FROM LYOPHILIZED TISSUE

A cost-effective and rapid RNA extraction protocol for brown algae and seagrasses was developed, based on homogenization in a simple CTAB buffer and selective precipitation of RNA with lithium chloride. The protocol avoids the use of toxic chaotropic agents and phenol; high concentrations of dithiothreitol are used to inhibit RNase activity and to prevent oxidative cross-linking of nucleic acids by phenolics. A relatively high throughput of ca. 100 samples in 24 h can be achieved for, e.g., Northern analysis. Yields of 50 – 200 µg/gFW-1 are comparable with those obtained for higher plants using commercially-available kits. Tests of the extraction procedure demonstrate that high quality, intact RNA can be obtained from a variety of lyophized brown algal tissues, even after prolonged storage at room temperature. Lyophilization is therefore suggested as an alternative to freezing tissue at –70 to –80 C. The RNA obtained was used directly in several downstream applications to detect Fucus plastid-encoded transcripts; RNA labeling, RT-PCR and Northern analysis. Northern analysis of RUBISCO large subunit (rbcL) expression in Fucus serratus indicated that transcripts are rapidly down-regulated by high temperature (30 C), independently of light over a 10-fold range of irradiances between 50-600 µmol m-2 s-1.

Reproductive ecology of marine 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 species with external fertilization can achieve high reproductive success despite the gamete diluting effects of high water motion and what mechanisms improve their reproductive success. We also aim at understanding whether for these intertidal populations gamete dispersal is restricted and where is the population bottleneck between the large amounts of gametes that are released and the few juveniles that are found.

Achieved in 2005:

GAMETE DISPERSAL AND REPRODUCTIVE SUCCESS OF SUBTIDAL FUCUS VESICULOSUS

Short-range dispersal patterns of eggs from F. vesiculosus were determined in 2 subtidal areas in the Baltic Sea. Under the calm conditions in which eggs are released, most eggs fall in the immediate vicinity of the source. The effective sperm dispersal shadow was estimated from the proportion of eggs that were fertilized at various distances (up to 2 m) and directions from a point source. The sperm dispersal shadow is broader than the egg shadow, suggesting that male-mediated gene flow is larger than female-mediated, although dispersal by thallus fragments and sperm competition may also have significant effects. The natural success of fertilization (i.e., the proportion of eggs that were fertilized) was high at the whole range of male-female distances that occur in these natural stands. Thus, recruitment in these populations is not limited by fertilization success. However, the period between egg settlement and establishment of zygotes as new recruits represents a bottleneck for F. vesiculosus in the Baltic Sea. Daily monitoring of egg settlement revealed that large numbers of eggs (up to 1000 cm-2) settled on the sea bed over an entire reproductive season, but the number of young Fucus embryos counted in the same locations at the end of the two-month long reproductive season was at least an order of magnitude lower. Thus, surviving at pre-recruitment stages may be the most limiting phase for reproductive success.

TIMING AND SUCCESS OF EXTERNAL FERTILIZATION IN INTERTIDAL ZONES.

Timing of gamete release (at the lunar month scale) was described during the reproductive season for two species: *Fucus vesiculosus* (dioecious) and *Fucus spiralis (hermaphroditic)*; although reproductive structures were present throughout the year, release occurred synchronously on only a few days of the year following approximate semi-lunar cycles. Long-term patterns of gamete release, studied in estuarine and open coast populations were highly synchronous. Many replicated measurements have been performed to estimate the timing of gamete release during tidal cycles for both Fucus vesiculosus and Fucus spiralis, during periods when maximal release was expected. All these many measurements have been showing so far that gamete release can

occur not only at slack high tide and as the tide receeds but also at low tide in both the hermaphroditic and the dioecious species. The timing of low tide during the circadian cycle appears to be implicated in the release patterns. Levels of fertilization success (proportion of the eggs released that become fertilized) were estimated in natural intertidal populations of two fucoid species with external fertilization. Our finding of high overall levels of external fertilization success for both F. spiralis and F. vesiculosus showed that dioecious species of fucoids can achieve as high fertilization success as hermaphroditic species even in exposed environments. Studies of fertilization success in intertidal (non-tide pool) fucoid populations on exposed shores are rare. Our discovery of high fertilization success in exposed shore intertidal populations of fucoids with contrasting reproductive modes shows an important role of gamete release mechanisms in synchronizing release with ideal conditions for fertilization. Settlement and recruitment were monitored in Viana do Castelo (both species are sympatric) and at Alcochete and Ericeira (where the species are allopatric). High settlement and low recruitment observed so far suggest that failure to recruit and/or high post-recruitment mortality appear to be important bottlenecks for the establishment and dynamics of populations of these fucoid algae

GAMETE DISPERSAL
The dispersal of gametes was studied by quantifying recruitment at several distances away from a source area with several reproductive individuals. This was done both in an estuarine environment (Alcochete) and in an open coast environment (Viana do Castelo). The results suggest that dispersal is nearly null on some days (all recruits appear under the source) and predominantly downstream, which correlates well with the results described above, gamete release both at low and high tide. The only sampling date when gametes were found beyond distance zero was when unattached drift fragments of Fucus were found to have fallen on top of the disks as the tide receded, again demonstrating an important role for unattached drift algae in promoting dispersal. Low dispersal (or nearly null dispersal) of the gametes themselves is consistent with gamete release occurring at low tide or at slack high tide on days with very calm water conditions. Our genetic approaches based on assessing the variation in genetic distance with geographic distance on the shore, showed that there is no limitation to gene flow within a 10 m distance zone, which in conjunction with the previous results suggests an important role for sperm-mediated gene dispersal and/or dispersal by unattached algae.

Colonization strategies and population 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 2005:
Development of microsatellite markers for *Caulerpa prolifera* has continued. We obtained in total 14 candidate microsatellites (9 perfect dinucleotide repeats and 5 compound dinucleotide repeats) for Caulerpa prolifera and 6 candidates (2 perfect dinucleotide and 4 compounds) for C. taxifolia. We have selected 10 microsatellites with at least 8 repeats (from 8 to 18 repeats) for C. prolifera

to test for polymorphism. Primers have been designed and are being tested in 40 individuals for the two species. For C. taxifolia, we are still screening the libraries for more repeats.
We have sampled 14 Caulerpa sp. meadows around the Balearic Islands and samples ready to be genotyped.
A new method for isolating high quality DNA was developed (now accepted for publication) for the green algae Caulerpa sp (C. racemosa, C. prolifera, and C. taxifolia) and the brown alga Sargassum muticum. These are introduced and invasive species in Europe except for the native C. prolifera. Previous methods of extraction, like CTAB or different commercial kits were attempted to isolate genomic DNA but either no DNA or DNA of very low quality was obtained. Genomic libraries were attempted in three occasions and either the restriction enzyme, the Taq polymerase or the T4 ligase was inhibited, probably by the large amount of polysaccharides in these algae. This method consists in rapid isolation of stable nuclei, followed by DNA extraction. Yields of 10-20 µg genomic DNA from 0.1 g fresh blades was obtained. The quality of the DNA is suitable for PCR, cloning and hybridization probes. Four microsatellite libraries were successfully constructed (one per sp). This technique is inexpensive and appropriate for the isolation of multiple samples of DNA from a small amount of material.

New plans for 2006:

The research lines indicated above will be continued, particularly through the publication of the above mentioned results for the various completed objectives, some of which were already published during 2005. We therefore outline below only the new research lines, distinct from those listed above, that will be developed during 2006.

ADAPTIVE POPULATION DIVERGENCE AND COMPARATIVE POPULATION STRUCTURE
We will investigate adaptive evolution of abiotic stress tolerance within and between species of Fucus, a genus containing closely related species differing in stress tolerance. The gene polymorphism database that is being generated, particularly as EST databases, will be used to test the neutral theory of evolution for genes involved in abiotic responses. Polymorphic loci will be used to compare patterns of genetic structure ate neutral (microsatellites) versus selected loci. Genome wide variation in stress-responsive gene expression between species occurring in sympatry and between isolated populations across species' ranges will be studied by the use of macroarrays of desiccation-responsive cDNAs. Finally we will compare patterns of population structure using molecular markers with analogous estimates of quantitative trait variation in a common garden experimental design. Half-sib (for dioecious *Fucus* species) or full sib (for hermaphroditic *Fucus* species) families will be produced by in vitro fertilization and outplanted at suitable field sites. Estimated quantitative trait variation at several response variables will be compared with genetic structure for neutral markers and with that for candidate stress genes if sufficiently polymorphic sites will be available.

GENETIC DIVERSITY AND STABILITY
We will use an experimental approach to test for the existence of a relationship between the genetic diversity of ecosystem-structuring intertidal seagrass meadows (*Zostera noltii*) and demographic stability (resistance and resilience). This will be performed by controlling the genotypic diversity at microsatellite markers in experimental plots and following their demographic evolution as well as ecosystem properties. We will also screen polymorphic stress-responsive genes from a congeneric species in order to look for this relationship based on neutral and non-neutral loci.

NETWORK ANALYSES: UNDERSTANDING PROCESSES BEHIND THE SPECTRUM OF GENETIC DIVERSITY (developed in collaboration with the Physics Department and the Natural Resouces Department, at IMEDEA, CSIC, Spain)

The methods used in population genetics to estimate parameters such as departure from panmixia or population structure rely on theoretical and mathematical models with a narrow range of underlying parameters and demographic models, which strongly limit the scope of demographic situations that can be accurately explored. Among others, two examples in which classical assumptions are violated are the cases of endangered and invasive species, indeed studied precisely because they exhibit population dynamics that strongly depart from equilibrium. These constraints to the application of conventional methods of population genetic structure are even more evident for clonal organisms, for which the notion of effective population size or generation time are challenged, and a clear concept of the unit (genetic individual) on which evolutionary forces are acting is lacking.
We will develop a novel approach, based on the examination of the spectrum of genetic diversity, to examine the population genetic structure of clonal organisms, and for the depiction of inter-individual genetic distances by a network. Contrary to classical genetic summary statistics, we will not impose pre-defined hierarchical models constraining the range of temporal scale and evolutionary processes that can be accurately screened, but, rather, take advantage of all the information contained in the dataset to let the data define their own topology, and we go one step further from graph illustration by detailing the network properties using the statistical tools specific to network analyses classically used in physics, in order to extract key information as to the hierarchical genetic structure in the population studied. We will use this approach to characterize the spectrum of genetic diversity within P. oceanica populations, and identify biological processes determining intrapopulation structure by means of numerical simulations. This approach will later be extended in order to explore the genetic structure of virtually any populations.

REVISING METHODS OF ANALYSIS FOR POPULATION GENETICS OF CLONAL PLANTS
Although clonal plants are dominant in many habitats, as are a large number of clonal species, from unicellular organisms to marine invertebrates, ecological and particularly evolutionary studies on clonal organisms have been strongly limited due to the difficulty in assessing the number and extent of individuals in a population. The development of more polymorphic markers has allowed some progress in this area, however, many population genetics studies on clonal plants still typically pay little attention to clonality, or use classical populations genetic data analyses without accounting for the potential bias due to the possible replication of the same individuals in the sampling. Moreover, such studies use a variety of indices to describe clonal structure that are not homogeneous, precluding comparison among studies reporting results described with those distinct indices. We will review the indices and statistics used so far to estimate genotypic and clonal diversity and describe clonal structure in plants, by examining their advantages and weaknesses as well as various conceptual issues associated with statistical analyses of population genetics data on clonal plants. We will test them on two empirical datasets of microsatellites of the seagrasses Posidonia oceanica and Cymodocea nodosa. We will also propose a selection of new methods to estimate clonal diversity and describe clonal structure in a way that allows the comparison of future studies on clonal plants, some of which may be of interest for clonal organisms in general.

Group: ALGAE - Marine Plant Ecology

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Summary of activities and progress during 2005

1. METABOLISM OF COASTAL SYSTEMS

This research line is being developed in Ria Formosa lagoon and aims the role of marine plants in the organic matter and nutrient fluxes in the ecosystem so that important processes, such as the regulation of the N and C cycles of the ecosystem, its human utilization and global change scenarios can be addressed.

This research line is under strong development with the start of the project "Carbon uptake by Ria Formosa intertidal communities", POCI/MAR/58172/2004. This project aims to assess the seasonal variation in the metabolic processes of communities' production and respiration. A special methodology was developed which allows, for the first time, the assessment of the communities' production and respiration when submerged, measuring CO2 fluxes (Silva et al. 2005). This methodology is being further developed for technical optimization and will be extensively used to collect field data. Within this projects' framework, the photosynthetic responses of key species to changes in light, temperature and water stress during tidal cycles are also being investigated. Preliminary lab essays for methodological tuning and protocol refinement have also started, concerning antioxidant enzymes activity determinations. A first field sampling was conducted and tests are now running for the enzymes ascorbate peroxidase (APx), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), catalase (Cat), superoxide dismutase (SOD) and glutathione reductase (GR).

Research on the nitrogen metabolism of the seagrass *Zostera noltii* in Ria Formosa lagoon was also developed at a molecular level using an in vitro method, to determine the activity of two important enzymes (nitrate reductase and glutamine synthetase) in the nitrogen assimilation process. Several methodological difficulties related with the use of in vitro techniques for enzymatic determination in this species are being assessed for the first time. Further investigations on the relationship between the acquisition of the nitrogen forms (nitrate and ammonium) and the activity of these two enzymes, as well as the effects of the environmental factors on the enzymes activities will follow when methodological problems are solved.

The development of an ecological model of the Ria Formosa lagoon started in collaboration with Ramiro Neves of the Instituto Superior Técnico, University of Lisbon. An ecological module will be integrated in the hydrological model MOHID, developed in that institute. The ecological model will integrate the benthic processes, particularly those related to the marine plants, with the planktonic and transport processes. It will be a powerful tool to assess the whole metabolism and C and N budgets of the ecosystem and to simulate the anthropogenic impacts in the Ria Formosa lagoon.

2. HUMAN IMPACTS ON COASTAL SYSTEMS

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 southern Portugal. Our aim is to contribute with sound scientific inputs to the conservation and management of these systems.
A major threat to the coastal ecosystems results from the increasing nutrient load with antrophogenic origin. Eutrophication of coastal systems such as Ria Formosa lagoon can lead to drastic qualitative changes of the ecosystem where the key seagrass species are substituted by opportunistic green algae. Episodes of green algae blooms occur within Ria Formosa in winter and along the coastal beaches during summer. Two projects were initiated in 2005 that address the research being carried out on this item. The project "CLONMACMORPH" is financed by the European Commission (Marie Curie European Reintegration Grant to Erik-jan Malta) started in April 2005 and focuses on the effects of increased nutrient load on the architecture of clonal marine macrophytes and seagrasses and its consequence for the competition with ephemeral green macroalgae. In this project the group cooperates with the University of Cádiz (Spain). One paper was published (Malta et al. 2005) which provides proof for the clonal behaviour of the green

macroalga *Caulerpa prolifera* in response to nitrogen limitation. Furthermore a monitoring program has been set-up, with the voluntary help of third parties operating along the beaches of the Ria Formosa (salvavidas, firemen, concesionarios, general public) to monitor the occurrence of green algal blooms. It appears that the peak of these bloom occurs in May and June. Other elements of the monitoring program includes the analyses of abiotical variables (climate, nutrient concentrations in the algae and in the water). This approach is taken further in the project "Green macroalgal blooms in Ria Formosa and adjacent coastal beaches", financed by the FCT (POCI/MAR/58427/2004). This project, started in December 2005 and involves a cooperation with the geohydrology group of the IMAR. An aerial survey and field visits revealed that first green algae activity started by the end of December. Nutrients and freshwater might trigger algal proliferation. Although we are awaiting the results of the analyses of water samples, it is already clear that the streams and rivers discharging in the Ria Formosa form an important nutrient source to the system. One single, small stream, was estimated to be responsible for a daily input of 25 kg of dissolved nitrogen, even when rainfall was absent. A geophysical survey indicated the presence groundwater inflow in the lagoon, a source which is difficult to characterize but probably very important in terms of nutrient input.
One other project that has started in 2005 was the characterization of the impact of the urban wastewater treatment works (ETARs) in the key macrophyte communities of three coastal systems, the estuary of Arade, the Ria Formosa lagoon and the estuary of Guadiana. The aim of the project was to assess the base line situation of macrophyte communities under the influence of the ETARs and to develop vegetation indexes for monitoring the effect if urban effluents on the system.

3. ECONOMIC VALORIZATION OF SEAWEEDS

The research on the economic valorisation of seaweeds focused on the integrated aquaculture of seaweeds in order to biofiltrate their effluents and to produce economically valuable biomass. Following the findings of the EU project "SEAPURA - species diversification and improvement of aquatic production in seaweeds purifying effluents from integrated fish farms", QLRT - 1999 – 31334, where the integrated cultivation of the red seaweed *Falkenbergia rufolanosa* in fish farm effluents was revealed to be successful, a comparison between the performance of this species and the most commonly used green seaweeds of the genera *Ulva* was done. The high performance of both *F. rufolanosa* production and biofiltration was revealed.
A new research project "Cultivation and halogen compounds yields of Bonnemaisoniaceae red seaweeds", POCTI/MAR/56956/2004, started in October 2005. This project aims the study of the ecophysiological controls of biomass and halogen production of the seaweed *Falkenbergia rufolanosa*. The photosynthetic mechanisms of inorganic carbon acquisition by *F. rufolanosa* were studied in order to understand if the species is limited by carbon under cultivation conditions and how this limitation can be attenuated.

4. EFFECTS OF GLOBAL CHANGE ON THE PORTUGUESE MARINE FLORA

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. We are assessing the long-term changes in the benthic marine flora by comparing the marine flora of the 23 sites along the Portuguese coast that were described by Ardré in the 1960´s with the present situation. The relationships of the patterns of change, particularly the changes in the distribution limits and the origin of the new introduced species, with the water temperature increase will be assessed. The analysis of the samples that was done during 2005 revealed about 40 new species for the Portuguese flora, which will be the subject of publications during the following years.
The wide geographical transplant of aquaculture species, mainly oysters, have increased the number of introduced species from Japan. The brown seaweed *Sargassum muticum* has invaded the European coasts from Norway to Portugal in about 30 years. In sheltered habitats, the species develops extensive masses, dominating the system and being a nuisance to local users such as tourists and fishermen. The species has been observed this year in southern Portugal for the first time. This may have disastrous, biological and economical consequences for areas like sheltered

touristy bays and marinas and particularly for the National Park of Ria Formosa. Following the final results of the EU project "ALIENS - ALgal Introductions to EuropeaN Shores", EVK3-2001-0008A, a manuscript was prepared on the demographic matrix model analysis of the invasion dynamics of *Sargassum muticum* in the southwest coast of Portugal, including simulations of various harvest strategies to control the invasion. During 2005 we developed a micro-satellite bank and identified of micro-satellites for *Sargassum muticum* to quantify genetic differences on several spatial scales throughout the species distribution and examine key processes of invasion. Continuing this research line, a national funded project started this year, "The invasive theory of the pest seaweed *Sargassum muticum* in Southern Portugal", POCI/MAR/55377/2004 and funded was granted by the European Science Foundation for the organization of a workshop on *Sargassum muticum* biology on European shores, to be held in 2006.
Another invasive species started to be investigated in 2005, the saltmarsh species, *Spartina densiflora*, which was introduced in southern Spain from South America and now was observed in Ria Formosa lagoon. Collaboration was developed with the research group of Henrique Figueroa from the University of Seville, whom have been studying this species in southern Spain. A preliminary assessment of the species distribution in Ria Formosa was done. As well, an experiment was conducted to assess the photosynthetic performance of the invader and the native species, *Spartina densiflora* under various degrees of submergence.

5. ENVIRONMENTAL EDUCATION

This research line focuses on the characterization of the Portuguese Environmental Education Centres (EEC), which correspond to all the initiatives, including appropriate installations and specialised educational teams that offer environmental education programmes and activities. The research aims to evaluate how established are the EECs in Portugal as well as the limitations and problems encountered in their educational action. This line of research is unique in our country where the environmental education area is still little developed.
Based upon questionnaire responses from 103 of the 126 Portugal environmental education centres, interviews and focus group methodologies we described these centres in terms of the infrastructures and installations, the human resources and the environmental educational program offered, the employment opportunities (internships and volunteer positions), funding sources, problems faced etc. A qualitative approach was conducted to determinate quality criteria for such facilities. These were selected through numerous group interviews that were conducted in several regions of Portugal. The results of this participatory approach will be presented in January 2006 on the "II Seminário Nacional Equipamentos para a Educação Ambiental: Qualidade e Inovação" held at the University of Algarve.

Plan for 2006

1. METABOLISM OF COASTAL SYSTEMS

This research line will be continued to assess the seasonal variation in the metabolic processes of community production and respiration of the biological communities of Ria Formosa lagoon. The prototype system based on the approach of using incubation chambers fitted to the sediment, and to route the air into a non-dispersive infrared gas analyser (IRGA) that measures CO_2 concentrations, will be finished. This methodology will be adapted to deeper underwater operation (> 2m) enabling carbon uptake measurements in submerged communities, under natural conditions. The plants responses to the over-saturating irradiances and the desiccation stress experienced during low tide will be determined. In situ responses to low tide stress will be assessed by chlorophyll fluorescence. These field measurements will be complemented with sampling for determination of leaf compounds known to play a significant protective role in response to stresses. Amongst these are the antioxidant enzymes ascorbate peroxidase (APx), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), catalase (Cat), superoxide dismutase (SOD) and glutathione reductase (GR).
The circadian and seasonal patterns of inorganic nitrogen fluxes between the sediment and the water column in different seagrass communities (*Zostera noltii* and *Cymodocea nodosa* both in Ria

Formosa and in Cabrera, Mallorca) will be determined using incubation chambers developed by the group, which allows assessing the community production and respiration when submerged.

Further investigation on the effects of the environmental factors on *Zostera noltii* NR activity, using the *in vitro* method, will be developed to understand the determinants of the nitrate uptake by this species.

2. HUMAN IMPACTS ON COASTAL SYSTEMS

The CLONMACMORPH project ends on 1 April 2006, while the FCT project will face its first full year of execution. Research efforts of both projects will be combined. We plan to continue the work focusing on three levels: a system level, a community level and an organism level. An annual nitrogen budget of the whole lagoon will be assessed by monthly monitoring the discharges of the rivers and streams and the oceanic exchange through the two most important inlets (Armona inlet and Faro channel). Other sources and sinks, such as groundwater inflow and the role of the sediments will also be addressed. Occurrence of green algal blooms will be assessed by aerial surveys combined with ground truthing in which samples will be taken for biomass determination and tissue nutrient composition. The specific ecophysiological characteristics of the dominant algal species will be studies in experiments, focusing on bloom initiation and die-off and the processes which trigger these. The origin of the blooms will be studied with the help of molecular tools (comparison of ITS ribosomal DNA sequences, population identification using microsatelites).

3. ECONOMIC VALORIZATION OF SEAWEEDS

A project submitted for national funding from Agência de Inovação to install a seaweed-based biofiltration system at Zoomarine a marine theme park of Algarve, was financed and the contract will be signed in the beginning of 2006. This will allow us to determine the feasibility of seaweeds to biofiltrate the water in recirculation cultivation systems, where nitrates are the most abundant nutrient. Concerning the study of the ecophysiological controls of biomass and halogen production of the seaweed *Falkenbergia rufolanosa*, this year will focus on the determinants of the halogenated compounds metabolism, responsible for the biological activity against pathogens. This research will be conducted in cooperation with the “Fundação da Faculdade de Ciências de Lisboa” and the “Instituto Nacional de Engenharia e Tecnologia Industrial”.

4. EFFECTS OF GLOBAL CHANGE ON THE PORTUGUESE MARINE FLORA

The analysis of the 23 sites along the Portuguese coast that were described by Ardré in the 1960´s will continue. We expect to reveal biogeographic zones along the Portuguese coast based on the multivariate analysis on the quantitative data on the abundance of the prominent seaweed species of the locations sampled. Manuscripts will be submitted on the new additions to the marine flora of the continental coast of Portugal.
On what respects the research of the invasive seaweed, *Sargassum muticum*, the following goals are planned:
- To test the Sargassum muticum micro-satellites for their genetic resolution and cross amplification on other Sargassum species.
- To obtain Sargassum muticum samples from locations all over the world and start genotyping them.
- To organize and conduct the ESF workshop on Sargassum muticum in November 2006.
- To perform several feeding experiments and analysis of anti-herbivore compounds in native and invasive seaweed species.
- To identify differences in the herbivore community comparing tide pools dominated by native algae versus pools dominated by the invader Sargassum muticum.
- To test in the field whether gastropods present in tide pools feed selectively on propagules of native seaweeds and not on propagules of Sargassum muticum.
- To submit papers on: (1) The timing of release and settlement of propagules of Sargassum muticum. (2) Are life history traits the ecological basis of invasiveness? (3) Reproductive dynamics of Sargassum polyceratium.
The investigation on the alien saltmarsh species *Spartina densiflora* in Ria Formosa lagoon, in cooperation with the University of Seville, will continue. The development of this research line will

depend on the available funding. The available micro-satellite markers for the genus *Spartina* will be tested to develop a molecular tool to assess the invasive ecology of the species.

An awareness program of the users and local entities of the Ria Formosa lagoon will be developed to reveal the potential negative impacts of the two recent invaders, *Sargassum muticum* and *Spartina densiflora*.

5. ENVIRONMENTAL EDUCATION

We will promote and organize the "II Seminário Nacional equipamentos para a Educação Ambiental. Qualidade e Inovação", at the University of Algarve in January. This seminar is essential to discuss and establish the quality criteria with the national environmental education stakeholders based on a participatory approach. We will conclude and submit to Instituto do Ambiente, a final proposal of Quality Criteria to Environmental Education Centres in Portugal. That proposal will regulate the activities of the EEC´s in Portugal and will establish, for the first time, the rules that may regulate the activities of these centres. A publication on the Directory of Nature Centers, Outdoor Study Areas and Environmental Education Centers in Portugal will be compiled from survey responses and published during 2006. This will constitutes a guide to established outdoor and environmental education centers in Portugal. Organized by region, approximately 100 entries will be included.
We also plan to finish the analysis of the significant life experiences (SLEs) study. This will address the life factors that influence the development of environmental knowledge and concern to the environment. We plan to publish the results of this long term study (8 years of data) in 2006.

Group: Ecohidrologia e Recursos de Estuários e Zonas Costeiras (Ecorecursos)

Research team

Leader: Luis Chícharo, Alexandra Chícharo
Visiting scientist: Eric Wolanski
Post-docs: Marina Delgado, Radhouane Ben Hamadou
PhD students: Pedro Morais, Joaquim Teodósio, Ana Amaral, Rita Borges, Vanessa Moschino, Adriana Candeias
Honours thesis students: Tania Pedro, David Pilo
Research Assistant: Ana Amaral, Ana Faria

Summary of activities and progress during 2005

-Implementation of subroutines in the Guadiana Estuary Ecohydrology model in collaboration with the visiting scientist Dr. Eric Wolskanki, during setember 2005, To predict flow condition that avoid eutrofications conditions
-Participation in UNESCO Scientific Advisory council on Ecohydrology in Bali during November 2005
Approval in November 2005 of UNESCO Demosite: How to Manage Biodiversity in Guadiana Estuary (Portugal) using Ecohydrology & Phytotechnology Approaches
- Supervising of two undergraduate students under the programme "Ciencia Viva" during July – August 2005
- Organisation of the annual meeting of the Scientific comitte of the UNESCO Working Group about Ecohidrology of Estuaries and Coastal zones, Institute of Oceanography in Split-Croatia, apresentado a comunicação: Modelling the use of the alien bivalve Corbicula fluminae to control algal blooms in the Guadiana River (South Portugal): an ecohydrologic approach. September 2005
- Approval in May 2005 of the project GUADIRIA, FCT: Nutritional condition of fish larvae in two marine protected area of the South of Portugal (Ria Formosa and Guadiana estuary) POCTI/BIA-BDE/59200/2004

5- Edition of a spcial issue of the Journal Estuarine Coastal Shelf Science ECSS about: “Managing the Guadiana Estuary – the Ecohydrology and Phytotechnology approaches”

6- Submition to FCT of the projecto (CliVOcean): (Climatic Variability and Ocean Action and Reaction coordinated pelo CIIMAR

Plans for 2006

To continue research lines started, with particulary emphasis in:

- Remediation and mitigation studies of the impacts of anthropogenic activities on the ecosystems, with emphasis in the Guadiana demo Site in Ecohydrology
- Ecophysiological and biochemical studies of exotic versus indigenous species
- Condition and retention mechanisms of estuarine and coastal fish larval
- Organising an international Congress on Ecohydrology, under the support of UNESCO.

Group: Fisheries Biology and Hydrobiology

Research team

Leader: José Pedro Andrade
Principal researchers and post docs: Pedro Domingues, Jorge Palma
PhD students: Eduardo Esteves, António Sykes
MSc student: Miguel Correia

Summary of activities and progress during 2005

Cuttlefish culture:
During 2005, research was focused on the nutrition of cuttlefish.
A study on the embryonic development of both wild and culture eggs was conducted.
Determinations of protein, lipid, free and protein amino acids, fatty acids; were conducted in order to determine differences between both type of eggs. Also, a characterization of non-viable eggs was made in terms of hatching percentage and lipid content.
Nutritional characterization of wild-caught cuttlefish and its degradation during ice-storage was also studied and a QIM (Quality Index Method) was developed in a joint study with the IPIMAR’s DITVPP (Departamento de Inovação Tecnológica e Valorização dos Produtos da Pesca).

Crustacean research
Throughout 2005, this work group developed investigation on the production of live diets for aquaculture, namely, the shrimp species *Palaemonetes varians* e *Palaemon elegans*. The use of natural diets, diet binders, effect of plant protein sources and feed utilization, effect of dietary protein on growth of juveniles and the quantitative dietary requirement for the amino acids, lysine, methionine and arginine on the growth of these species were analysed. Beside that, other experiments have been performed with spider crab, *Maja squinado* and those were mainly related with the breeding techniques of this species.
review on the cuttlefish potential as a new species for aquaculture was also published.

Plan for 2005

Cuttlefish culture
During 2006, the research on the nutrition of cuttlefish will carry on.
A study on fed and starved cuttlefish hatchling will help to determine nutrition requirements of hatchlings after internal yolk exhaustion (3-5 DAH).
The influence of bottom areas on the reproduction of cuttlefish, as a way to increase egg number by female will be determined. Also, nutritional aspects of spawner reproduction will be studied.

Also and, as a complement of the nutritional study on the embryonic phase, a photographical and histological follow of the embryonic stages will be conducted in order to provide a crossing of this information with the nutritional one.

Crustacean research
During 2006, it is planed to continue the research on these species, in issues like; induced spawning outside breeding season, optimal tank characteristics and rearing density for maximum larval growth and survival, protein utilisation and diets digestibility with different protein sources and effect of nutrient leaching on growth performance.

Group: Coastal Fisheries Research

Research Team

Leader: Karim Erzini
CCMAR-CIMAR Researcher: Jorge M.S. Gonçalves.
MSc Resercher Pedro Monteiro.
PhD student: Joaquim Ribeiro, Rui Coelho, Humberto Hazin.
MSc student: Luis Bentes, Daniel Machado, João Araújo.
Researchers Carlos Afonso, Pedro Veiga, David Abecasis, Frederico Oliveira, Cheila Almeida.

Summary of activities and progress during 2005

Telemetry and tagging studies:

During 2005, preliminary field work was carried withing the project "Scientific bases for the management of fisheries resources of common interest" (GESTPESCA-INTERREG III). Common sea breams (*Diplodus sargus*) implanted with pingers and released in the vicinity of artificial reefs located on the Algarve continental shelf were tracked with an array of hydrophones on the bottom and manually from a boat. The results showed that the cultured common sea breams did not remain on the artificial reefs for more than a few hours. Within 24 hours, all the released fish had passed the array of hydrophones located around the artificial reefs. Manual tracking by researchers on board boats failed to locate the fish within the Ria Formosa and the adjacent coast. This suggests that the fish may have moved further along the coast, beyond the search area or had been caught by fishermen.

Within the framework of the FCT funded project “Sea bream spatio-temporal dynamics and habitat use in the Ria Formosa lagoon”, 2229 fish of several species were tagged with external T-tags (Floy) during 6 sampling trips at eleven locations in the Ria Formosa lagoon. A total of 67 fish have been recaptured to date. Posters for informing fishermen and offering rewards were distributed in fishing tackle and bait shops as well as other locations such as cafes and bars frequented by commercial and sport fishermen. Press releases were also disseminated in order to inform the general public and fishermen in particular.

Monitoring programmes:

Studies of the Ria Formosa fish community continued with the annual monitoring program. A series of locations were sampled with a beach seine in the Spring of 2005. This continued the time series of sampling initiated in 2000 and considered of great importance for evaluating possible changes resulting from climatic or human-induced changes.

The importance of lagoon and estuarine systems as nurseries and essential habitat for fish was also studied in the Arade river, where a monitoring programme was also initiated.

Population dynamics:

Work on different aspects of the fisheries biology and population dynamics of a number of species was carried out, with emphasis on age and growth, reproductive biology and feeding ecology of several deep water shark species and species of sea breams (Sparidae). The biology and ecology of non-commercial species such as pipefishes were also studied.

Modelling and stock assessment:

Landings and catch-per-unit-effort multi-species time series were modelled using innovative time series techniques that incorporate explanatory variables (environmental and fisheries). A new approach to fisheries management based on reproductive value was completed. The spatial and temporal distribution of swordfish longline catches in the south Atlantic as a function of environmental variables was also modelled using GAMs and GLMs. Work is also continuing on the evaluation of indicators for evaluating the effects of fishing and for comparing the relative impacts of different gears.

Essential fish habitat:

Studies on the identification of essential fish habitat were carried out, based on data from experimental fishing trials and data characterising the bottom type and associated fauna and flora off the Algarve coast. These data are being analysed with GAMs and GLMs and mapped with GIS.

Estuarine ichthyofauna

The field work on the Arade estuary ichthyofauna was accomplished and data analysis on the species composition (abundance, biomass, spatial and monthly variation) and on the habitat use (recruitment, trophic levels, abiotic gradients) with particular focus on the juveniles of commercial important species was carried out. The use of 6 sampling techniques (beam trawl, beach seine, pushnet, fish traps, trammel net, visual census) proved to be wise as the number of species observed, 99, was the highest ever achieved for a Portuguese estuary in just one study. The first results showed that the fish families more represented were Sparidae (16 species), followed by Gobiidae (13), Soleidae (8), Syngnathidae (7) and Labridae (7). Of the 64 commercial species caught, some has high economic value such as the red mullet (Mullus surmuletus), the soles (Solea spp.), (Scophthalmus rhombus), the seabass (Dicentrarchus labrax), the white seabreams (Diplodus spp.), the porgies (Dentex spp.) and the guilthead seabream (Sparus aurata). Most of these species were represented almost exclusively by juveniles, which confirms the importance of estuaries, and in particular the Arade, as an important nursery and feeding grounds for fish species. It seems that the spatial distribution of these species is specially conditioned by food availability and that there is a mechanism for the reduction of inter competition that determine the different times and places of recruitment. The intermediate areas of the estuary showed the highest values of abundance and diversity and in the Spring-Summer seasons there is a massive immigration of marine species (eg Sardina pilchardus and Diplodus vulgaris), mainly juveniles, while in Winter months the fish communities are dominated by resident species (eg Halobatrachus didactylus).

Sublittoral communities mapping

The second stage of RENSUB project began with the epibenthic communities mapping of the seafloor from the Central Algarve (Ancão-Galé). The biological characterization is being made at a 1:25000 scale and it includes density maps, several biodiversity, vulnerability and ecological sensibility indexes. The sampling procedure includes three main methods: underwater visual census for ichthyofauna and macrofauna on rocky bottoms; quadrat method for algae; beam trawl and video transects for sandy bottoms.

All this information is being analysed through geostatistics and integrated in Geographic Information Systems in order to produce maps usable for coastal management purposes.

Plan for 2006

The group will focus on the biological impact assessment of coastal dredging; the seabed mapping and sensibility/vulnerability analysis; recruitment of commercially important species in the Arade estuary (Algarve) and the monitoring of the ichthyofauna of the Ria Formosa. Studies on fisheries biology and population dynamics of swordfish and deep water sharks will continue. Another important line of research will be habitat use and spatio-temporal distribution of fish based on telemetry.

The evaluation of the impact of coastal dredging will involve sampling with a variety of gears and methodologies, including underwater transects by video and divers, beam trawling and bottom grabs and cores. Species will be identified, measured and counted in the laboratory. The data will be input into a GIS, allowing mapping of species distributions, composition and abundance as a function of bottom type and depth. This information, together with various indicators (e.g. diversity indices) and the results of multivariate analysis will allow the identification of particularly important or sensitive areas. Special emphasis will be placed on the identification of essential fish habitats (EFH).

For the Arade estuary, work will be focus on dissemination of scientific knowledge through talks, multimedia exhibitions and publication of papers.

GESTPESCA II project:

Fieldwork on the use of artificial reefs by sea breams will be finished in the Spring of 2006. Common sea bream (*Diplodus sargus*), both wild and cultured, will be implanted with pingers (Vemco Ltd.) and released on the reefs. Their daily movements will be recorded using hydrophones and receivers. A network of receivers around the reefs will record data on a long-term basis while a hydrophone on board a research vessel will be used periodically to study short-term movements.

Ria Formosa telemetry project:

For this project FCT project approved in 2005, more fish (approximately 2500) will be caught and tagged with external Floy T-tags in the Ria Formsa lagoon in order to study habitat use and movements. In addition, fish will be tagged with small pingers and their movements monitored by active telemetry from a boat over 24 hour periods, while long-term movements will be tracked using a number of receivers attached to navigation buoys throughout the Ria and in the adjacent coastal waters.

Monitoring of ichthyofauna:

Studies of the Ria Formosa and Arade fish communities will continue with the annual monitoring programmes. In the case of the Ria Formosa, a series of locations will be sampled with a beach seine in the Spring of 2006. This will continue the time series of sampling initiated in 2000 and considered of great importance for evaluating possible changes resulting from climatic or human-induced changes. The importance of estuarine systems as nurseries and essential habitat for fish will also be studied in the Arade river. Here, a variety of sampling gears will be used to study the fish community along a salinity gradient and over tidal, daily and seasonal bases.

Population dynamics, modelling and stock assessment:

.Work on the population dynamics and stock assessment of sea breams and of deep water sharks will continue. In the latter case, age structured models will be developed and life table constructed.

A new approach based on Fisher's reproductive value will be applied to evaluate the state of the populations and their vulnerability to exploitation. Work on essential fish habitat and indicators for fisheries management and for comparing the impacts of different fishing gear, taking place within the framework of thesis research will be finished.

Group: Biodiversity and Biology in Fisheries (BIOPESCAS)

Research team

Leader: Teresa Cerveira Borges
Post Doc: José Xavier
PhD students: Maria Esmeralda Costa, Sónia Olim, João Sendão
Technicians/ research assistants: Paulo Morais

Summary of activities and progress during 2005

Ongoing projects

1. Project "Science, Education and Marine Archaelogy Programme in Portugal" (SEMAPP)

All video documentation was analysed to estimate species diversity and relative abundance, as well as specific faunal behaviour. Analysis of trawl fisheries impact was also documented and discussed. All results were presented to the 40th European Marine Biological Symposium (EMBS) and submitted in the meeting proceedings.

Paulo Morais was at the University of Connecticut, National Under Research Center (NURC), to learn to process the information collected on video during the campaign SEMAPP2004.

The visit of 5 students and two teachers from the University of Connecticut and one technician from the Ocean Technology Foundation (OTF) took place for sea/land work at Boca-do-Rio, Budens. This group also visited the faculty and a communication was presented about CCMAR and its research.

2. Project "Biodiversity in fisheries off the South coast of Portugal (Algarve) (BIOFISH)" (Ref: MARE-22-05-01-FEDER-00031).

This project was initiated in February 2005, and the main objectives of this study are:

- Identification of all species caught by the most important fishing gears off the south coast of Portugal (Algarve);
- Compilation of all information on the biology, ecology, fisheries and socio-economic importance of each of these species;
- Production of a book with all the above information;
- To make a reference collection of all species existent in the fisheries, to be available to all community (academic, schools, etc.).

During 2005, three campaigns were made, in which around 20 trips on fishing vessels were made, around 420 species were identified and 250 were fotographed. The compilation of information on the biology and ecology of around 200 species has been done. The graphic design and layout of the book has been already decided.

Finished Projects

1. Project "Cephalopod stocks in European waters: Review, Analysis, Assessment and Sustainable Management" (QOL-2001-5.1.2) (CEPHSTOCK)"
2. Project "Fishery of the common octopus in Algarve: Improvement of the Artisanal fishing gears (POLVARTE)" (Ref: 22-05-01-FEDER-00018)

Other studies

Post-doc José Xavier study projects (from Oct. 2004). The start of Post-doc at CCMAR focused on reviewing the importance of cephalopods in European waters, particularly South of Iberian Peninsula. The quality of datasets, collected in previous research projects, was also assessed and integrated into a framework of publishing papers. Further work related to the Antarctic was carried in two projects already initiated at British Antartic Survey (BAS), focusing on the predator-prey interactions in relation to environmental variables and fisheries. These two pos-doctoral research projects allowed the development of acoustic techniques, combined with trawling and biological sampling techniques, in order to understand the Antarctic fish fauna dynamics.

Plan for 2006

1. Project "Science, Education and Marine Archaelogy Programme in Portugal" (SEMAPP)

A visit of several students and teachers from the University of Connecticut is expected to take place in June. This time the area will be the Ria Formosa, Olhão.

2. Project "Biodiversity in fisheries off the South coast of Portugal (Algarve) (BIOFISH)" (Ref: 22-05-01-FEDER-00031)

It is expected to continue with the onboard sampling collection and photography of species until May 2006. After the uniformisation of text and necessary corrections, all material will be send to be printed. The distribution of the book to technicians and professionals from the fishery industry will be at the end of the year 2006 (November/December)

3. Trip onboard the old fishing sailing boat "Creoula".

A proposal was delivered for a trip onboard an old cod fish fishing boat to the Portuguese Navy, entity responsible for this boat. This trip will be for university students, pre-university students, university teachers and researchers. The main objectives will be the experience of life on board an old fishing sailing boat, sampling and data collection (molluscs) and observation of mammals. During the trip, several seminars will be presented by specialists. The areas involved will be Biology, Fisheries and History.

Other studies

Post-Doc study I (Portugal). Work will focus on a wide range of aspects of cephalopod ecology in European waters, including the South Coast of Portugal: 1) Characterisation of the cephalopod fisheries in South Portugal, 2) Evaluation of trends of cephalopod landings in Portugal and in Northeastern European waters, and 3) Preliminary assessment of cephalopod consumption by top predators.

Post-Doc study II (Antarctic). The research will focus on publishing data on the characterization of the vertical and horizontal distribution of the mackerel icefish *Champsocephalus gunnari*, and other Antarctic fish, in relation to the physical (temperature, currents, depth) and biological (e.g. Antarctic krill *Euphausia superba* distribution) characteristics in the Southern Ocean using a combination of trawling and acoustics.

Post-Doc study III (Antarctic). The research will focus on predator-prey interactions between top predators and cephalopods in Antarctic waters through the analysis of the diet of albatrosses, possibly king penguins, and collection of biological data of Antarctic squid.

Group: Biodiversity and Conservation (BioCon)

Research team

Researchers: Rita Castilho, Margarida Castro, Margarida Cristo and Margarida Machado.
PhD students: Regina Cunha and Paula Serafim.

Other colaboraros: Dora Jesus (M. Sc.)
Honors thesis students: Miguel Soares

Summary of activities and progress during 2005

Molecular Evolution, Phylogeny/Molecular systematics and Phylogeography

Organization of the third "*Conus* Cape Verde Expedition III" to collect species of *Conus* (Gastropoda) and continue the work on molecular systematics and phylogeography of this marine gastropod genus which is the topic of a PhD thesis taking place at the Museo Nacional de Ciencias Naturales in Madrid, Spain.

Collection of *Galeus atlanticus* samples from the MNHN, Paris, to compare with material collected in Portugal. A paper will be submitted next year with a morphometric and genetic analysis of two sympatric species (*Galeus atlanticus* and *Galeus melastomus*).

Genetic work on a Azorean endemic bird species (*Pyrrhuyla pyrrhula*) started with a relevant number of samples processed.

Ecology and Fisheries of Decapods

Completion of first essays to collect larvae of spiny lobster resulting in the modification of the trap design. New sampling sites chosen and collaborators identified.

Continuation of work in progress related with the reproductive biology of *Nephrops norvegicus* involving mechanisms of sperm competition and mating systems (in collaboration with the "Molecular Biology of Marine Organisms".

Non-cladoceran branchipods in temporary ponds

Continuation of the inventory of species present in ponds form the South of Portugal and studies related with the biology and population dynamics of the most important species

Achieved in 2005:

1. Publication/acceptance/submission of papers from last year research activities on:
    - *Mauremys leprosa* population genetics;
    - Population structure of anchovy (*Engraulis encrasicolus*) in the Mediterranean and the Atlantic: revealing strong unexpected subdivision(s);
    - Patterns of Cladogenesis in the Venomous Marine Gastropod Genus *Conus* from the Cape Verde Islands;
    - Population genetics of *Scomber scombrus* and *Scomber japonicus* in the Mediterranean and the adjacent Atlantic Ocean;
    - Population genetics of *Dicentrarchus labrax* from Turkey and Greece.
    - Fisheries ecology of *Nephrops nrvegicus* (2 papers)
    - Fisheries ecology of Spiny lobster species (3 papers)
2. Organization of the "*II Molecular Evolution Workshop*" with the presence of David Swofford, Gavin Naylor and Mark Holder from Florida State University, USA.
3. New projects:
    - Population genetics of *Conger conger* - POCI/MAR/58837/2004 "Study of the life history of the European conger eel (*Conger conger*) as revealed by DNA analysis and chemistry - elemental and isotopic composition of fish otoliths"
    - Larvar recruitment mechanisms and stock identifiaction in lobsters - POCTI/BIA-BDE/ 59426/2004 "LOBASSESS - Norway lobster stocks in Portugal. Basis for assessment using infORmation on larval production and ecology".

Plan for 2006

Molecular Evolution, Phylogeny/Molecular systematics and Phylogeography

Two deep-water species will be assessed from the population genetics point of view (*Hoplostethus atlanticus* and *Aphanopus carbo*). This is a particularly interesting area, as commercial exploitation continues with no existent evaluation of the sink-source population effects. Work on the Azorean endemic bird species (*Pyrrhuyla pyrrhula*) will be finished and submitted for publication, this will constitute the very first genetic approach to this unique bird. Evaluation of the *Conus* species complex (*C. venulatus* and *C.nifiver*) will be accomplished giving interesting insights as to the island evolution processes.

Ecology and Fisheries of Decapods

Continuation of work in progress related with the reproductive biology of *Nephrops norvegicus* involving mechanisms of sperm competition and mating systems (in collaboration with the "Molecular Biology of Marine Organisms".

Setting up of larvae traps on the ropes for oyster aquaculture inside the harbor of Sagres for identification of settlement periods and associated oceanographic conditions.

Compared selectivity of traps and tangling nets for capturing spiny lobster and proposals for management option for the artisanal lobster fishery on the SW coast of Portugal.

Advances in the collection of data for modeling larval recruitment processes in lobsters.

Non-cladoceran branchipods in temporary ponds

Continuation of the inventory of species present in ponds form the South of Portugal and studies related with the biology and population dynamics of the most important species.

Group: Fish Parasitology and Reproduction

Research team

Post-doc: Isabel Afonso-Dias with Ken Mackenzie acting as a consultant regarding Ichthyoparasitology.

Summary of activities and progress during 2005

The research of this group is mainly related to fisheries biology, particularly fish reproduction and the use of parasites as tags for stock identification. The studies carried out during 2004 included the following subjects:

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

This study started during February 2004 and is progressing according to plan. So far different species of digeneans (*Aphanurus* sp., *Hemiurus* spp.) and nematodes (*Anisakis* sp., *Hysterothylacium* sp.) were found in the stomach of sardine collected off France, Portugal and Spain. Although not all samples (viscera collection was made by different technicians / scientists) had gall bladder and/or liver, smears of these structures were made whenever possible to try to find myxosporeans and microsporideans. Hitherto two different species of myxosporean were found in the gall bladder of sardine. These species were sent to an Indian Colleague, Prof. Kalavati (Andhra University) for identification to species level.

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

A summary of the current status of this work was provided by Dr Ken Mackenzie and it was 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.
Within the scope of this activity the paper entitled "A checklist of the protozoan and metazoan parasites reported from the anglerfishes *Lophius piscatorius* L. and *L. budegassa* Spinola" was published in the Bulletin of the European Association of Fish Pathologists (see list of publications).
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 Prof. C. Kalavati, they all proved to be new species. During 2004 a poster was prepared and presented in the IX EUROPEAN MULTICOLLOQUIUM OF PARASITOLOGY (EMOP IX), Valencia, Spain (Three new species of myxosporeans (Myxosporea: Bivalvulida) from the gall bladders of *Lophius* spp. (Pisces: Teleostei) in European waters), 18-23 July 2004. Detailed descriptions are now being prepared for publication.
Opportunistic samples of *L. piscatorius* from the West coast of Portugal were collected during 2004, whenever possible.

3. "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. During 2004, a manuscript entitled "Problems with assessing the ovaries of Sardine (*Sardina pilchardus*, WALBAUM) to the appropriate macroscopic maturity stage", was produced and will be submitted during 2005.

4. "Testicular infestation of *Sardine pilchardus* (Walb.) by the coccidia *Eimeria sardinae* (Thélohan)"
The activities in this field of research during 2004 included:
- Preparation and presentation of a poster in the IX EUROPEAN MULTICOLLOQUIUM OF PARASITOLOGY (EMOP IX), Valencia, Spain (Testicular infestation of sardine *Sardina pilchardus* (Walb.) by the coccidian parasite *Eimeria sardinae* (Thélohan, 1890)), 18-23 July 2004.
- Preparation and submission of a scientific project to FCT (Fundação para a Ciência e Tecnologia) in July, entitled Sardine (*Sardina pilchardus*, Walb.) castration due to the Coccidia Eimeria sardinae: exception or rule?" (POCTI/MAR/61732/2004).

5. "Comparing the parasite fauna of *Lophius* spp. with that of another top predatory fish, the halibut *Hippoglossus hippoglossus*"
This study that started in 2003, continued during 2004 with the participation of Isabel Afonso-Dias in a multidisciplinary research vessel survey along part of the coast of North Norway from 16 to 25 October 2004. The number of halibut sampled so far is still not sufficient to produce a coherent study on the parasitic fauna of these two top predators.

General Plan for 2006

1. "Gastrointestinal parasites of Sardine (*Sardina pilchardus, Walbaum 1792*): Study to assess the possibility of using sardines' parasites as biological tags"
This study will progress as planned to try to identify possible biological tags for stock identification. This study intends to complement the already in placement study of stock identification of this host species. During 2005 the laboratory work will progress as planned and it is also planned for the coming year to prepare written material for publication.

2. "Anglerfish (*Lophius piscatorius and L. budegassa*) Parasites: Study to assess the possibility of using anglerfish parasites as biological tags"
Like it was explained in the previous report, it is possible that the different species of the genus *Stephanostomum* could make useful biological tags. Since one of the main diagnostic features of these digeneans is the oral spines, that tend to disappear in frozen specimens, we are still

examining fresh samples of *Lophius* spp., whenever possible. This is a very time consuming task and it will proceed, sporadically, during 2005.

After encountering three different species of myxosporeans in the gall bladders of *Lophius piscatorius* and *L. budegassa* from west of Scotland and off the Algarve coast, work will continue to:

a. Publish detailed descriptions of the species found;
b. To assess the possibility of using these species as biological tags;

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

This activity will be successfully finished during 2005, after the publication of the paper under preparation.

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

The activities planned in this field of research for 2005 are stationary until further notice from FCT. If the proposal presented to FCT is approved, then this activity will resume in 2005, if adequate.

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

During 2005 it is intended to carry on collecting more samples of *H. hippoglossus* and assess the viability of further developing this investigation.

Externally funded Projects

Division of Aquaculture and Biotechnology

New and ongoing beyond 2006

Title: "Mecanismos da sensibilidade olfactiva aos catiões não orgânicos em teleósteos" (Mechanisms of olfactory sensitivity to inorganic cations in teleosts)

Summary: Without exception, teleosts are able to maintain extracellular concentrations of physiologically important ions, such as calcium and sodium, at levels dramatically different from those of the environment. Much work has focussed on the mechanisms responsible for maintaining this differential; however, little is known about how the environmental concentrations of these ions are sensed. This is particularly important for those species that habitually encounter rapidly changing concentrations of these ions such as estuarine or migratory fish. We have recently shown that a range of different teleosts have high olfactory sensitivity to calcium and, to a lesser extent, sodium. The aim of the proposed project is to establish whether teleosts have distinct olfactory receptor mechanisms for both calcium and sodium, rather than a single 'salinity' receptor mechanism. To do this we will investigate the effects of changes in environmental ions to the olfactory sensitivity to sodium and calcium in three model species; the marine gilthead seabream (Sparus auratus), the estuarine bass (Dicentrarchus labrax) and the freshwater goldfish (Carassius auratus). Although all three species can tolerate some change in salinity, only the bass is truly euryhaline. Furthermore, we will investigate the long-term effects of salinity changes on the olfactory sensitivity to sodium and calcium. This is important to understand whether this olfactory sensitivity is primarily linked to internal ionic homeostasis, or to inform the fish where exactly, in a fluctuating environment, it is. Lastly, we will begin to investigate where, within the CNS, this primary sensory information is relayed. This will be done by a combination of activity-dependent neuronal labelling and immunocytochemistry for the early response element c-fos. We expect that primary sensory input concerning food-related odorants to be processed differently from that concerning levels of inorganic cations. However, do calcium sensitive neurones project to different areas of the olfactory bulb from those sensitive to changes in sodium? Once these questions are answered, future studies can be directed as to how these ions are detected (at a cellular and molecular level) and exactly what use the fish makes of this sensory information.

Objectives: To assess how changes in concentration of one cation may influence the olfactory sensitivity to others in marine, estuarine and freshwater teleosts.

To assess how long-term adaptation to altered salinities may influence olfactory structure and sensitivity to cations in marine, estuarine and freshwater teleosts.

To determine the types of cells responsible for olfactory sensitivity to Ca2+ and Na+ and to where in the olfactory bulbs this information is relayed.

Reference and funding entity: POCI/BIA-BCM/55467/2004 (FCT)

Duration: 1st January 2005 - 31st December 2007 (3 years)

Research team:P.C. Hubbard (PI), C. Haond, E.N. Barata and A.V.M. Canário

Total budget: 45000 euro

Title: "Comunicação química na tilápia Moçambicana, Oreochromis mossambicus." (Chemical communication in the Mozambique tilapia, Oreochromis mossambicus.)

Summary: Chemical communication is believed to play diverse and important roles in the biology of fishes. However, the number of species that have been studied in detail remains very low. Given their distinctive reproductive strategies and complex social behaviour, the cichlids have received surprisingly little attention in this respect. Over the past three years, our laboratory has made significant inroads into the understanding of chemical communication in the Mozambique tilapia, an African mouth-brooding cichlid. It is clear that this fish uses chemical signals both during reproduction and in the maintenance of social hierarchies. Thus the aim of the proposed project is

to extend and embellish these initial findings, particularly with regard to the chemical cues that the females release, and answer some of the questions raised by previous research in both our and other laboratories. Firstly, the identification of putative pheromones released by pre-ovulatory females will be carried out in conjunction with IACR-Rothamsted (United Kingdom). We already know that pre-ovulatory females smell different from post-ovulatory to males, and that males behave differently towards them, depending on this olfactory cue. Our aim is to establish what these cues may be, and their likely source and routes of release. Once the likely site of pheromone synthesis is established - the ovaries - we can assess the endocrine factors responsible for the regulation of pheromone production in vitro. This may also prove to be a convenient way to collect relatively large amounts of pheromones for identification. Thirdly, the effects of the putative pheromones on male physiology and behaviour will be investigated; we already have good evidence that female pheromones induce an increase in the urination rate of males as part of their courtship 'display' (male urine is a potent odorant to females). Lastly, we will begin to investigate how this pheromonal information is processed by the CNS using a combination of neuronal activity-dependent labelling and immunocytochemistry for the early response element c-fos; to where in the olfactory bulbs (and possibly beyond) this information is relayed. In the future, this will allow us to define how the pheromonal message is translated into the appropriate behavioural and physiological responses. This species has a number of advantages for this type of study; the social behaviour is well-described, males and females are easily recognisable and are reproductively active throughout the year. It is also a resilient fish and ameniable to the type of experimental manipulations outlined in this study. As such, it is an ideal introductory model for young scientists to learn how to formulate, and test, hypotheses. We think that the proposed project will establish the Mozambique tilapia as the model species for studies in chemical communication in cichlids and provide an important addition to the studies of this phenomenon in teleosts as a whole.
Objectives: To identify chemicals released to the environment by pre-ovulatory females that are detected by males.
To identify the source and routes of release of these chemicals.
To investigate the effects of endocrine factors that govern the rate of synthesis of these putative pheromonal compounds by the ovaries (and/or other tissues) in vitro.
To identify the biological effects of these chemicals on male behaviour and physiology.
To trace the neural pathways of olfactory receptor neurones for these chemicals to the olfactory bulb.
Reference and funding entity: POCI/BIA-BDE/55463/2004 (FCT)
Duration: 1st June 2005 to 31st May 2008 (3 years)
Research team: Peter Hubbard (PI), Eduardo Barata, Christophe Haond, Adelino Canário, Olinda Almeida (BIC),.
Total budget: 82500 euro

Title: Diferenciação sexual do robalo (Dicentrarchus labrax): identificação de genes com expressão dimórfica de sexo e efeito ambiente
Summary and Objectives: Seabass, *Dicentrarchus labrax*, is a gonochoristic marine fish with a high market value. In farming conditions males grow 20-30% slower than females. Hatchery stocks often yield over 90% males, with an important loss of revenue. Gonadal differentiation occurs only 11 months post-hatching and coincides with a period of maximum sensitivity to the action of exogenous steroids . Rearing temperature has a strong influence on sex ratio in particular during the first two months after hatching . Steroid hormones (androgens and estrogens) are the ultimate mediators of phenotypic sex and temperature strongly influences aromatase activity required for ovary differentiation. Other genes in the sex differentiation cascade may also be influenced by temperature , some of which interact directly with steroidogenic P450 enzymes .
The main objectives of the project are to: 1) Identify and characterize sex specific genes or genes that are differentially expressed in males and females 2) Determine the expression profile in early development of identified genes and how their expression is modified by temperature.
Reference and funding entity: POCTI/CVT/47124/2002
Duration: April 2005-March 2009

Research team: Adelino Canário, Laurence Deloffre, Rute Martins
Total budget: 50,000 euros
URL:

Title: Molecular and cellular basis of teleost fin regeneration
Summary and Objectives: Inducing regeneration of damaged tissue is currently a major focus of biomedical research. Outstanding questions include: How can the adult tissue be reprogrammed during regeneration? How is organ morphogenesis and patterning achieved during regeneration? The overall objective of this project is to determine the timing and morphology of the different stages of teleost caudal fin regeneration after ablation as well as the dissection and control of the pivotal genes responsible for each stage. As model organism we will use the zebrafish (Danio rerio) due to the readily available molecular tools and approaches necessary to conduct complex research from a genetic point of view.
Reference and funding entity: FCT POCTI/MAR/61091/2004
Duration: 2006-2008
Research team: Begoña Redruello, Adelino Canário, Deborah Power
Total budget: 83 963 euro**.**
URL:

Title: Physiology of PTHrP in fish, relevance to calcium balance of an unique hypercalcemic factor
Summary and Objectives: The objective of this project is to determine the specific roles of PTHrP in fish tissues responsible for calcium handling The specific objectives are 1. Set up a radioimmunoassay for PTHrP measurement of circulating plasma levels across species with different osmoregulatory pattern 2. Identify the relative importance of calcium sources for the whole body calcium budget i.e. environmental vs. dietary. 3. Investigate the "ex vivo"/"in situ" specific role of PTHrP and its truncated forms in calcium transport in isolated tissues and cells i.e. gill epithelia, intestinal preparations, "in situ" kidney preparations 4. Analyse the cellular action of PTHrP in calcium transport and expose the mechanisms sensitive/insensitive to PTHrP.
Reference and funding entity: POCTI/CVT/48946/2002 (FCT)
Duration: 01/01/2005-31/12/2007
Research team: Juan Fuentes Diaz, Pedro Miguel Guerreiro, Deborah M. Power
Total budget: 50.000 euro

Title: Identification and function of crypt-type olfactory receptor neurons (CORN) in marine fish.
Summary and Objectives: The aim of the project is to study the crypt-type olfactory receptor neurons (CORNs) in the olfactory epithelium of marine fish. The fisrt objective is to perform a comparative structural study of the olfactory epithelium of marine fish using histology, immunohistochemistry and electron microscopy. The originality of the study will be the use of the immunolocalization of the Na+,K+-ATPase as a tool for the visualization of the CORNs. The second objective of the study will be to investigate the function of the CORNs by applying the agmatine staining procedure that labels activated neurons.The sea bass and the seabream will be the principal models.
Reference and funding entity: FCT; POCI/BIA-BCM/60554/2004
Duration: 2005-2008.
Research team: Scientist in charge Christophe Haond; Adelino Canario; Peter Hubbard (CCMAR); Alexandre Lobo Cunha (Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto).
Total budget: 50 000€

Title: Stanniocalcin in sea bream: interaction with hypercalcemic factors in calcium balance
Summary and Objectives:

The endocrine control of calcium availability to the body fluids in higher vertebrates is well studied and primarily controlled by the interplay of the parathyroid hormone and calcitonin. In contrast, the endocrine control of calcium balance in lower vertebrates remains little understood. Endocrine factors with species-specific actions like cortisol, prolactin, estradiol or vitamin D have been suggested to actively participate in calcium metabolism in fish. We aim at characterise the putative interplay between PTHrP and stanniocalcin (STC), in short term calcium balance, and to establish their relative importance in calcium regulation in fish.We also propose to establish the relationship between short term and long term endocrine regulation of calcium balance in fish. The overall aim of the proposal is to establish the physiological role of the most important short term calcium regulating hormones in fish, STC and PTHrP and to establish the feed-back mechanisms by which calcium homeostasis is maintained.
Reference and funding entity: POCI/CVT/55683/2004 (FCT)
Duration: 1/06/2005-31/12/2007.
Research team: Juan Fuentes Díaz, Lilia Brinca, Adelino Canário, Pedro Miguel Guerreiro, Deborah Power
Total budget: 74.820 Euro

Title: Aquafirst- Combined genetic and functional genomic approaches for stress and disease resistance marker assisted selection in fish and shellfish
Summary and Objectives: The overall aim of this project is to identify genes associated with stress and disease resitance in oyster, trout, sea bream, and sea bass in order to provide a physiological and genetic basis for marker-assisted selection.
These studies will be carried out for fish using stress and pathogen challenges directly relevant to aquaculture and for oyster using environmental conditions that are known to lead to significant summer mortalities. In Part 1 of the project the genes involved in the functional response to stress or pathogen exposure will be identified. This will be carried out in the four species by (i) constructing relevant EST collections using SSH cDNA libraries which will be spotted on microarrays, followed by (ii) analysis of gene expression profiles in various tissues of animals exposed to stressors or pathogens. This analysis will be also carried out in families that are divergent for stress response or disease resistance (fish) or for summer survival (oyster). In order to investigate relationship between potential candidate genes and QTL for these traits, Part 2 of the project will identify Single Nucleotide Polymorphisms (SNP) in these candidate genes in oyster and trout. This polymorphism will be analysed both in the EST sequences and in the promoter region. In Part 3 of the project QTL analysis will be used to identify genes that are associated with stress specific traits and disease resistance traits using previously characterized SNP and also microsatellites markers. We will also carry out mapping of these genes in linkage and gene maps. Part 4 is devoted to outline operational genetic protocols incorporating identified QTL and traditional breeding approaches in oyster, sea bream and sea bass. This knowledge will be transferred to the industry through organisation of workshops gathering scientists and RTD performers.
Reference and funding entity: sixth framework programme - contract n° 513692
Duration: 1/11/2004 – 31/08/2008
Research team: Adelino Canário, Deborah Power, Pedro Guerreiro.
Total budget: 3,799,954 EUR Euro; **Funding for CCMAR**: 63 493 Euro
URL: http://lotus5.vitamib.com/hnb/aquafirst/aquafirst.nsf/Web/

Title: Marine Genomics Europe - Implementation of high-throughput genomic approaches to investigate the functionning of marine ecosystems and the biology of marine organisms
Summary and Objectives: The overall aim of this project is to set up and develop a European Network of Excellence, referred to as "Marine Genomics", for the implementation of high-throughput genomic approaches in the biology of marine organisms. "Marine Genomics" will promote, develop, and spread throughout the European Union a broad range of genomic approaches, to investigate a wide range of questions related to the functioning of marine

ecosystems and to the biology of marine organisms. With this aim in view, we propose to group and network experts in genomics, proteomics, and bioinformatics from several Centres of Excellence in genomics in Europe with marine biologists who can make use of high-throughput genomics data. This will involve the dedication and the development of common research infrastructures, both in genomics and in marine biology. Joining together these distinct scientific communities will establish Europe's lead in marine genomics.

The J.E.R. of "Marine Genomics" is broken down into Comparative, Functional and Environmental Genomics, three sections which structure more traditional streamlines, leading to various microbial, algal, evolution development and diversity, and fish and shellfish nodes. This research can be applied to the management of marine ressources (prediction of global changes in marine populations, conservation of biodiversity, fisheries management and improvement of aquacultured species) and to gene mining for health and biotechnology.

The Integration activities of Marine Genomics are based on the following strategies: i) jointly develop enabling technologies; ii) sharing existing technical platforms iii) collectively gaining access to major Genomic centres; iv) regrouping under a common Bioinformatics Centre; and v), create and develop a Knowledge and Communication System, a permanent web-based interface for communications within and outside the network.

Spreading activities will include workshops and courses implemented by a Training & Education Council. Marine Genomics will also develop complementary dissemination strategies, targeting public, private and institutional communities with the purpose of enhancing the integration of marine biologists in the ERA.

Reference and funding entity: NoE sixth framework programme - contract n° 505403
Duration: 1/3/2004 – 28/02/2008
Research team: Adelino Canário, M. Leonor Cancela, Ester Serrão, Gareth Pearson, Deborah Power,Vincent Laizé, João Cardoso e outros.
Total budget: 10,000,000 EUR Euro; **Funding for CCMAR**: >100 000 euro
URL: http://www.marine-genomics-europe

Title: “Chemical Communication in the Tilapia, *Oreochromis mossambicus*”
Summary and Objectives: Chemical communication is believed to play diverse and important roles in the biology of fishes. However, the number of species that have been studied in detail remains very low. Given their distinctive reproductive strategies and complex social behaviour, the cichlids have received surprisingly little attention in this respect. Over the past three years, our laboratory has made significant inroads into the understanding of chemical communication in the Mozambique tilapia, an African mouth-brooding cichlid. It is clear that this fish uses chemical signals both during reproduction and in the maintenance of social hierarchies. Thus the aim of the proposed project is to extend and embellish these initial findings, particularly with regard to the chemical cues that the females release, and answer some of the questions raised by previous research in both our and other laboratories. Firstly, the identification of putative pheromones released by pre-ovulatory females will be carried out in conjunction with IACR-Rothamsted (United Kingdom). We already know that pre-ovulatory females smell different from post-ovulatory to males, and that males behave differently towards them, depending on this olfactory cue. Our aim is to establish what these cues may be, and their likely source and routes of release. Once the likely site of phermone synthesis is established - the ovaries - we can assess the endocrine factors responsible for the regulation of pheromone production *in vitro*. This may also prove to be a convenient way to collect relatively large amounts of pheromones for identification. Thirdly, the effects of the putative pheromones on male physiology and behaviour will be investigated; we already have good evidence that female pheromones induce an increase in the urination rate of males as part of their courtship 'display' (male urine is a potent odorant to females). Lastly, we will begin to investigate how this pheromonal information is processed by the CNS using a combination of

neuronal activity-dependent labelling and immunocytochemistry for the early response element *c-fos*; to where in the olfactory bulbs (and possibly beyond) this information is relayed. In the future, this will allow us to define how the pheromonal message is translated into the appropriate behavioural and physiological responses. This species has a number of advantages for this type of study; the social behaviour is well-described, males and females are easily recognisable and are reproductively active throughout the year. It is also a resilient fish and ameniable to the type of experimental manipulations outlined in this study. As such, it is an ideal introductory model for young scientists to learn how to formulate, and test, hypotheses. We think that the proposed project will establish the Mozambique tilapia as the model species for studies in chemical communication in cichlids and provide an important addition to the studies of this phenomenon in teleosts as a whole.

Reference and funding entity: POCTI/BIA-BDE/55463/2004, FCT

Duration: 1/06/2005 – 31/05/2008

Research team: Peter C. Hubbard (Coordinator), Eduardo N. Barata, Adelino V.M. Canário, Christophe Haond.

Total budget: 91 620 Euro; **Funding for CCMAR**: 91 620 Euro

Title: "Mechanisms of olfactory sensitivity to inorganic cations in teleosts"

Summary and Objectives: Without exception, teleosts are able to maintain extracellular concentrations of physiologically important ions, such as calcium and sodium, at levels dramatically different from those of the environment. Much work has focussed on the mechanisms responsible for maintaining this differential; however, little is known about how the environmental concentrations of these ions is sensed. This is particularly important for those species that habitually encounter rapidly changing concentrations of these ions such as estuarine or migratory fish. We have recently shown that a range of different teleosts have high olfactory sensitivity to calcium and, to a lesser extent, sodium. The aim of the proposed project is to establish whether teleosts have distinct olfactory receptor mechanisms for both calcium and sodium, rather than a single 'salinity' receptor mechanism. To do this we will investigate the effects of changes in environmental ions to the olfactory sensitivity to sodium and calcium in three model species; the marine gilthead seabream (*Sparus auratus*), the esturine bass (*Dicentrarchus labrax*) and the freshwater goldfish (*Carassius auratus*). Although all three species can tollerate some change in salinity, only the bass is truly euryhaline. Furthermore, we will investigate the long-term effects of salinity changes on the olfactory sensitivity to sodium and calcium. This is important to understand whether this olfactory sensitivity is primarily linked to internal ionic homeostasis, or to inform the fish where exactly, in a fluctuating environment, it is. Lastly, we will begin to investigate where, within the CNS, this primary sensory information is relayed. This will be done by a combination of activity-dependent neuronal labelling and immunocytochemistry for the early response element *c-fos*. We expect that primary sensory input concerning food-related odorants to be processed differently from that concerning levels of inorganic cations. However, do calcium sensitive neurones project to different areas of the olfactory bulb from those sensitivie to changes in sodium? Once these questions are answered, future studies can be directed as to how these ions are detected (at a cellular and molecular level) and exactly what use the fish makes of this sensory information.

Reference and funding entity: POCTI/BIA-BCM/55467/2004, FCT

Duration: 1/01/2005 – 31/12/2007

Research team: Peter C. Hubbard (Coordinator), Eduardo N. Barata, Adelino V.M. Canário, Christophe Haond.

Total budget: 45 000 Euro; **Funding for CCMAR**: 45 000 Euro

Title: Characterization of folate and purine metabolism in the protozoan parasite *Perkinsus atlanticus*: Identification of genes involved in these pathways and potential therapeutic targets - **PTarget**
Summary and Objectives: The majors objectives are the identification of genes/pathways that are known to differ between protozoan parasites and their hosts such as folate and purine metabolism whose pathways appear to vary significantly between parasites and their hosts and therefore offer excellent opportunities for chemotherapeutic exploitation.
Reference and funding entity: POCTI/CVT/57982/2004, FCT
Duration: 06/2005 - 2008
Research team: R. Leite, R. Afonso, R. Ascenso, A. Brito, M.L. Cancela (coordinator)
Total budget: 84,552 euros

Title: Spacial-temporal pattern of expression and regulation of matrix Gla protein (MGP) during early development in *X. laevis* - **GLARE**
Summary and Objectives: *Xenopus laevis* will be used as model system to investigate the function of matrix Gla protein (MGP) in early vertebrate development building upon our results in this model system. (1) By injecting either distal (IA) or proximal (IB) promoter constructs of xMGP gene in fertilized eggs and detecting lacZ and GFP activity after midblastula transition we expect to clearly identify where and when each promoter is functional during early development. (2) Using the one hybrid screen technology, the already available promoter deletion mutants and our recently developed *Xenopu*s X1 cell line we expect to identify and clone the regulatory nuclear factors involved in the regulation of MGP gene transcription after MBT and confirm binding by cotransfection and co-microinjection of wild type and mutant factors with the responsive DNA binding elements from MGP promoter. (3) Through maternal versus zygotic knockdown of the xMGP messengers using morpholino oligos we expect to unravel the functional relevance of these two transcripts and access the phenotype of xMGP loss of function.
Reference and funding entity: POCTI/BIA-BCM/58677/2004, FCT
Duration: 04/2005 - 2008
Research team: N. Conceição (Coordinator), M. L. Cancela, D. Simes, A.C. Silva and J. Belo
Participant institutions: CCMAR/UALG and CBME/UALG
Total budget: 90,000 euros

Title: MGP functional role regarding local regulation of extracellular matrix mineralization: Identification of the structural motifs responsible for its function - **Sparusprot**
Summary and Objectives: The major objectives are to determine which MGP protein domains and/or aminoacids are essential for the maintenance of its physiological function as an inhibitor of mineralization, using MGP point and deletion mutants which will be transfected into a homologous cell system capable of mineralization and recently developed and characterized in our laboratory.
Reference and funding entity: POCI/MAR/57921/2004, FCT
Duration: 09/2005 - 02/2008
Research team: D. Simes (coordinator), S. Marques, M.L. Cancela, V. Laizé, S. Cavaco.
Participant institutions: CCMAR/UALG
Total budget: 86,270 euros

Title: Identification and characterization of *C. gigas* nacre proteins inducing bone mineralization
Summary and Objectives: The main objective of this project is to identify and to characterize the water soluble matrix proteins involved in mollusc mineralization and to test their activity on vertebrate and invertebrate cells.
Reference and funding entity: F-47/06 Actions integrées Luso-Francaises
Duration: 2005-2006
Research team: M.L. Cancela (coordinator), D. Simes, S. Cavaco, C. Delsert
Participant institutions: CCMAR/UALG - CRBM/Univ Monpellier II

Total budget: 2,000 euros

Title: "Efeito dos factores ambientais na infecção originada por *Perkinsus atlanticus* nas populações da amêijoa *Ruditapes decussatus* – **Ambiperk**"
Summary and Objectives: The major objectives are to investigate the importance of the environmental factors on the infection of the Portuguese clam *Ruditapes decussatus* by *Perkinsus atlanticus* and the effect of this infection on the reproduction of the clam populations.
Reference and funding entity: FEDER - MARE - Programa para o Desenvolvimento Sustentável do Sector da Pesca Fundação para a Ciência e Tecnologia, 22-05-01-FDR-00020
Duration: 07/2004 - 06/2007
Research team: R. Leite, R.Afonso, P.Dias, D. Matias, S. Joaquim, M.L. Cancela (coordinator),
Total budget: 191313 euros

Title: Skeletal development, alterations and malformations during larval ontogeny of species with interest for aquaculture. Molecular, cellular and biochemical approaches - **SPARUGENES**
Objectives: To perform an integrated study to evaluate the onset of skeletogenesis and development of skeletal structures during larval ontogeny of commercially important species in the southern Peninsula and Mediterranean area (Senegal sole, Solea senegalensis; Gilthead sea bream, Sparus aurata; white sea bream, Diplodus sargo and hurta, Pagrus auriga). Expression of specific genes and accumulation of these proteins will be compared between standard rearing conditions (live food and/or equilibrated diets) and by supplying microencapsulated diets, deficient or enriched in essential micronutrients.
Reference and funding entity: Spanish funding agency CICYT
Duration: 12/2003-11/2006
Research team: Coordinator: Carmen Sarasquete CSIC/Cadiz: Participants from UALG-CCMAR, M. Leonor Cancela , P. Gavaia, JB Ortiz-Delgado, Carla Viegas
Total budget: 150.000 Euro;

Title: "Mineralization processes in marine and fresh water teleosts: function of Gla proteins (Matrix Gla and osteocalcin) – **FishDev"**
Summary and Objectives: The main objectives are to investigate the mineralization processes during development and the involvement of BGP and MGP in these processes using zebra fish and solea as fresh water and marine model organisms.
Reference and funding entity: POCTI/CVT/42098/2002 - FCT
Duration: 06/2003-2006
Research team: Coordinator: L. Cancela. Participants: P, Gavaia, D.Simes, Susana domingues, MT Dinis (consultant)
Total budget: 65.000 Euro

Title: "Effect of extracellular calcium on MGP gene expression – **SaMGP**"
Summary and Objectives: The major objectives are to investigate the function of matrix Gla protein (MGP) in the regulation of the extracellular matrix mineralization and in cell differentiation through 1) characterization of the extracellular calcium effect on the regulation of MGP gene expression by identifying calcium sending mechanisms, transcription regulatory elements and signal transduction pathways; and 2) evaluate the effect of altered MGP levels on ECM mineralization and specific gene expression.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/BCI/48748/2002
Duration: 03/2003 - 02/2006
Research team: V. Laizé (coordinator), M.L. Cancela, N. Conceição, A.R. Pombinho, D. Simes, S.M.P. Marques, V. Fonseca, D. Tiago
Total budget: 99.620 euros

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. 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.

Duration: 1/10/2002 – 31/03/2006
Research team: D. M. Power
Total budget: Euro; **Funding for CCMAR**: 217 536 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: "Minimization of the effects of stress in senegal sole through amino acid supplementation - **STRESSAA**"
Summary and Objectives: Stressful conditions are known to cause growth suppression in cultured fish, either by impacts on appetite reduction, a stimulated catabolism, or a combination of both. As growth is essentially protein deposition, its optimisation depends on the understanding of protein and amino acid (AA) metabolism. The relative balance of the different metabolic pathways involved in AA metabolism is affected by the physiological condition of the animal. Thereby, stressful husbandry conditions do affect AA requirements. The central objective of this study is to contribute to a better understanding of the metabolic processes impinging on amino acid requirements of animals when they are exposed to stress situations. Post-larval and juvenile Senegal sole (Solea senegalensis) will be used as model species, because it is a species resistant to stress in terms of survival and also because it is a species of importance to the Portuguese marine aquaculture industry. It is intended to verify to what extent the amino acid metabolism of fish change when fish are under stress situations, and also whether the metabolic and growth depression effects of stress can be reduced by AA supplementation. The project will involve a first part were the effects of selected stressful husbandry conditions on growth and AA metabolism will be assessed in post-larvae and juvenile sole. This will involve the study of AA metabolism through different angles and methodologies: AA utilisation will be studied using tracer studies for individual AA, nitrogen balances and plasma free AA levels; food consumption will be determined using 14C as tracer for post-larvae and feed with glass beads plus x-rays for juvenile fish; stress condition will be ascertained by measuring plasma levels of cortisol, lactate and glucose; and the relative activity of the different intermediary metabolism pathways will be assessed through the determination of the activities of different enzymes. The second part of the project will verify to what extent the negative effects of stressful husbandry conditions on amino acid metabolism and retention can be minimized through supplementation of the diets with individual amino acids. Post-larvae and juvenile fish will be reared under selected acute and chronic stressful conditions based on the results of the first part of the project. Diets will be supplemented with individual key AA depending also on the results of the first part of the project. The same methodologies as before will be used to evaluate the results. Ultimately, this project expects to contribute to: 1) clarify the relation between stressful husbandry conditions and AA metabolism; 2) understand to what extent stress can affect animal growth and AA requirements; and 3) ascertain whether stress effects on growth and susceptibility to disease can be minimized through AA supplementation.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/CVT/49324/2002
Duration: Fev 2005- Jan 2007
Research team: Luis Conceição, Cláudia Aragão, Maria Teresa Dinis.
Total budget: 50,000 Euro; **Funding for CCMAR**: 50,000 Euro

Title: "Understanding the regulation of the digestive function on marine fish larvae - **DIGFISH**"
Summary and Objectives: Scarce information exists concerning the digestive function of marine fish larvae. However, understanding the digestive function of fish larvae and the mechanisms they use to regulate this function at different stages of development is extremely important. This information will allow to stimulate food intake, to adequate diet composition to a specific stage of development and related them with food assimilation rates. Therefore, the knowledge of these mechanisms will contribute to enable marine fish larvae fed with microdiets to achieve growth and survival rates identical to live food.

To achieve this goal it is important to study the ontogeny of the neuro-endocrine system associated to marine fish larvae digestive tract, which in resemblance with other larval systems is poorly developed at first feeding although functionally adapted to this stage of development. According to existing bibliography, different periods can be identified on the neuro-endocrine system ontogeny, reflecting developmental differences in the regulation mechanisms of larval digestive function. The immunohistochemical methods are essential in this type of studies, since they allow the identification and location of nervous fibres and neuropeptides, although the latter only at a semi-quantitative level.
Some studies reported the importance of visual and chemical stimuli on the increase of food intake, either with live food or microdiets. Although an increase in pancreatic enzymes was reported, no relation was established with the regulation of the digestive function. The small dimensions of fish larvae difficult the use of standard methodologies for this kind of studies. The use of a new methodology will allow the quantification of cholecystokinin (CCK) secretion on individual larvae. With this method the influence of different stimuli on digestive function will be assessed, especially the pancreatic function that is essential during the larval stages of marine fishes. In parallel, the use of labelled food, based in another new methodology, will allow to quantity the effect of different stimuli on food ingestion and assimilation by fish larvae that will allow the evaluation of digestive function efficiency at different stages of development.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/CVT/58790/2004
Duration: 2005- 2008
Research team: CCMAR: Laura Ribeiro, Maria Teresa Dinis, Deborah Power, Cláudia Aragão.
Total budget: 73056 Euros

Title: "Physiological importance and metabolism of aromatic and sulphur AA during fish ontogeny - **SULFAAR**"
Summary and Objectives: The major fate of amino acids (AA) is towards protein synthesis, but studies showed that the determination of the AA requirements should consider not only the AA profile of the proteins being synthesised, but also which AA are used for energy or for other metabolic purposes. Some AA are involved in the synthesis of other compounds of physiological importance, therefore a better understanding of its physiological role and metabolism deserves special attention. Among them, sulphur and aromatic AA may seem to have a special importance during the fish ontogenesis. The main objective of this study is to acquire a better knowledge on the physiological importance and metabolism of these AA during the early life stages of fish, focusing especially on taurine and tyrosine. Several questions regarding these AA need to be clarified and intend to be study within this project. Three model species will be used in this project: toadfish, which is a marine species with demersal eggs, seabream, and sole, which are marine species with pelagic eggs. Furthermore, sole and seabream are species with or without a marked metamorphosis, respectively. The first part of this project intends to analyse the aromatic and sulphur AA metabolism along development and to compare this metabolism in species with dermersal and pelagic eggs. This will involve the analysis of the free AA profile in eggs and larvae of the three model species. In this first part, the larvae will also be tube-fed with radio-labelled precursors of taurine and tyrosine and is intended to verify if the pathways for the biosynthesis of these AA are available in young fish stages. The second part of this project intends to better understand the effects of dietary taurine or tyrosine supplementation in growth, metamorphosis, and AA metabolism of fish larvae. This will be done by conciliating traditional studies on AA metabolism, involving the rearing of the species and the analysis of several paramenters, with more recent techniques, such as the tube-feeding of radio-labelled AA, in order to analyse differences in AA utilisation. Larvae will be reared according to standard procedures and using diets supplemented or not with taurine or tyrosine. The first trials will analyse the effects of taurine supplementation on growth performance of the three model species. The second trials will analyse the effects of tyrosine supplementation on metamorphosis of fish species with and without a marked metamorphosis process, using sole and seabream as model species. For both experimental trials, the effects of AA supplementation on AA utilisation will be analysed. Fish receiving or not a dietary taurine or tyrosine supplementation will be tube-feed 35S- or 14C-labelled AA and the fate of this AA will be followed in the fish. This project will ultimately result in a

better understanding of the AA requirements during fish ontogenesis, which will have an impact in the aquaculture industry.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/CVT/60176/2004
Duration: 2005 - 2008
Research team: CCMAR: Cláudia Aragão, Laura Ribeiro, Luís Conceição, Maria Teresa Dinis.
Total budget: 91,500 Euro; **Funding for CCMAR**: 91,500 Euro

Title: "Optimização da reprodução do linguado (*Solea senegalensis*)- **REPROSOL**"
Summary and Objectives: The investigation of new species for aquaculture, such as the sole (*Solea senegalensis*) could offers potential development for many coastal regions along the Mediterranean belt (Dinis et al, 1999). However the mass production of this species has not yet been achieved. One of the major problems in the culture of this species is the control of reproduction in captivity and the production of regular high quality spawns. It is well known that environmental factors such as temperature and photoperiod as well as nutrition, play an important role in fish reproduction. However, little is known about how those factors can be controlled and evaluated, in order to produce high quality gametes in sole fish. Egg quality is a specific requirement for the production of healthy larvae. The aim of the present project is evaluate the parameters (zootecnical and feeding plan) that can control reproduction in sole maintained in captivity. Several sperm and egg parameters (biochemical and physiological) will be determined and analysed for correlations with fertility and hatching rates.
It is proposed to achieve these objectives:

a. Parameters optimization on the broodstock reproduction
b. The importance of controlled husbandry conditions on the maturation and emission of gametes, as well as in the production of high quality spawns.
c. Characterize the influence of husbandry conditions, such as temperature, photoperiod and nutrition on gametes and egg quality.
d. Effect of the hormonal induction in the broodstock reproduction
e. Identification and caracterization of the feeding plan for sole
f. Characterize and identify quality in gametes and spawns

Reference and funding entity: MARE- 22-05-01-FDR-00026
Duration: 2004 - 2006
Research team: CCMAR: Florbela Soares, Maria Teresa Dinis, Elsa Cabrita.
Total budget: 224.507 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- Feb 2006
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: “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 – Mar 2006

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: “Minimization of the effects of stress in senegal sole through amino acid supplementation - **STRESSAA**”

Summary and Objectives: Stressful conditions are known to cause growth suppression in cultured fish, either by impacts on appetite reduction, a stimulated catabolism, or a combination of both. As growth is essentially protein deposition, its optimisation depends on the understanding of protein and amino acid (AA) metabolism. The relative balance of the different metabolic pathways involved in AA metabolism is affected by the physiological condition of the animal. Thereby, stressful husbandry conditions do affect AA requirements. The central objective of this study is to contribute to a better understanding of the metabolic processes impinging on amino acid requirements of animals when they are exposed to stress situations. Post-larval and juvenile Senegal sole (Solea senegalensis) will be used as model species, because it is a species resistant to stress in terms of survival and also because it is a species of importance to the Portuguese marine aquaculture industry. It is intended to verify to what extent the amino acid metabolism of fish change when fish are under stress situations, and also whether the metabolic and growth depression effects of stress can be reduced by AA supplementation. The project will involve a first part were the effects of selected stressful husbandry conditions on growth and AA metabolism will be assessed in post-larvae and juvenile sole. This will involve the study of AA metabolism through different angles and methodologies: AA utilisation will be studied using tracer studies for individual AA, nitrogen balances and plasma free AA levels; food consumption will be determined using 14C as tracer for post-larvae and feed with glass beads plus x-rays for juvenile fish; stress condition will be ascertained by measuring plasma levels of cortisol, lactate and glucose; and the relative activity of the different intermediary metabolism pathways will be assessed through the determination of the activities of different enzymes. The second part of the project will verify to what extent the negative effects of stressful husbandry conditions on amino acid metabolism and retention can be minimized through supplementation of the diets with individual amino acids. Post-larvae and

juvenile fish will be reared under selected acute and chronic stressful conditions based on the results of the first part of the project. Diets will be supplemented with individual key AA depending also on the results of the first part of the project. The same methodologies as before will be used to evaluate the results. Ultimately, this project expects to contribute to: 1) clarify the relation between stressful husbandry conditions and AA metabolism; 2) understand to what extent stress can affect animal growth and AA requirements; and 3) ascertain whether stress effects on growth and susceptibility to disease can be minimized through AA supplementation.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/CVT/49324/2002
Duration: Fev 2005- Jan 2007
Research team: Luis Conceição, Cláudia Aragão, Maria Teresa Dinis.
Total budget: 50,000 Euro; **Funding for CCMAR**: 50,000 Euro

Title: "Understanding the regulation of the digestive function on marine fish larvae - **DIGFISH**"
Summary and Objectives: Scarce information exists concerning the digestive function of marine fish larvae. However, understanding the digestive function of fish larvae and the mechanisms they use to regulate this function at different stages of development is extremely important. This information will allow to stimulate food intake, to adequate diet composition to a specific stage of development and related them with food assimilation rates. Therefore, the knowledge of these mechanisms will contribute to enable marine fish larvae fed with microdiets to achieve growth and survival rates identical to live food.
To achieve this goal it is important to study the ontogeny of the neuro-endocrine system associated to marine fish larvae digestive tract, which in resemblance with other larval systems is poorly developed at first feeding although functionally adapted to this stage of development. According to existing bibliography, different periods can be identified on the neuro-endocrine system ontogeny, reflecting developmental differences in the regulation mechanisms of larval digestive function. The immunohistochemical methods are essential in this type of studies, since they allow the identification and location of nervous fibres and neuropeptides, although the latter only at a semi-quantitative level.
Some studies reported the importance of visual and chemical stimuli on the increase of food intake, either with live food or microdiets. Although an increase in pancreatic enzymes was reported, no relation was established with the regulation of the digestive function. The small dimensions of fish larvae difficult the use of standard methodologies for this kind of studies. The use of a new methodology will allow the quantification of cholecystokinin (CCK) secretion on individual larvae. With this method the influence of different stimuli on digestive function will be assessed, especially the pancreatic function that is essential during the larval stages of marine fishes. In parallel, the use of labelled food, based in another new methodology, will allow to quantity the effect of different stimuli on food ingestion and assimilation by fish larvae that will allow the evaluation of digestive function efficiency at different stages of development.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/CVT/58790/2004
Duration: 2005- 2008
Research team: CCMAR: Laura Ribeiro, Maria Teresa Dinis, Deborah Power, Cláudia Aragão.
Total budget: 73056 Euros

Title: "Physiological importance and metabolism of aromatic and sulphur AA during fish ontogeny - **SULFAAR**"
Summary and Objectives: The major fate of amino acids (AA) is towards protein synthesis, but studies showed that the determination of the AA requirements should consider not only the AA profile of the proteins being synthesised, but also which AA are used for energy or for other metabolic purposes. Some AA are involved in the synthesis of other compounds of physiological importance, therefore a better understanding of its physiological role and metabolism deserves special attention. Among them, sulphur and aromatic AA may seem to have a special importance during the fish ontogenesis. The main objective of this study is to acquire a better knowledge on the physiological importance and metabolism of these AA during the early life stages of fish, focusing especially on taurine and tyrosine. Several questions regarding these AA need to be

clarified and intend to be study within this project. Three model species will be used in this project: toadfish, which is a marine species with demersal eggs, seabream, and sole, which are marine species with pelagic eggs. Furthermore, sole and seabream are species with or without a marked metamorphosis, respectively. The first part of this project intends to analyse the aromatic and sulphur AA metabolism along development and to compare this metabolism in species with dermersal and pelagic eggs. This will involve the analysis of the free AA profile in eggs and larvae of the three model species. In this first part, the larvae will also be tube-fed with radio-labelled precursors of taurine and tyrosine and is intended to verify if the pathways for the biosynthesis of these AA are available in young fish stages. The second part of this project intends to better understand the effects of dietary taurine or tyrosine supplementation in growth, metamorphosis, and AA metabolism of fish larvae. This will be done by conciliating traditional studies on AA metabolism, involving the rearing of the species and the analysis of several paramenters, with more recent techniques, such as the tube-feeding of radio-labelled AA, in order to analyse differences in AA utilisation. Larvae will be reared according to standard procedures and using diets supplemented or not with taurine or tyrosine. The first trials will analyse the effects of taurine supplementation on growth performance of the three model species. The second trials will analyse the effects of tyrosine supplementation on metamorphosis of fish species with and without a marked metamorphosis process, using sole and seabream as model species. For both experimental trials, the effects of AA supplementation on AA utilisation will be analysed. Fish receiving or not a dietary taurine or tyrosine supplementation will be tube-feed 35S- or 14C-labelled AA and the fate of this AA will be followed in the fish. This project will ultimately result in a better understanding of the AA requirements during fish ontogenesis, which will have an impact in the aquaculture industry.
Reference and funding entity: Fundação para a Ciência e Tecnologia, POCTI/CVT/60176/2004
Duration: 2005 - 2008
Research team: CCMAR: Cláudia Aragão, Laura Ribeiro, Luís Conceição, Maria Teresa Dinis.
Total budget: 91,500 Euro; **Funding for CCMAR**: 91,500 Euro

Title: "Optimização da reprodução do linguado (*Solea senegalensis*)- **REPROSOL**"
Summary and Objectives: The investigation of new species for aquaculture, such as the sole (*Solea senegalensis*) could offers potential development for many coastal regions along the Mediterranean belt (Dinis et al, 1999). However the mass production of this species has not yet been achieved. One of the major problems in the culture of this species is the control of reproduction in captivity and the production of regular high quality spawns. It is well known that environmental factors such as temperature and photoperiod as well as nutrition, play an important role in fish reproduction. However, little is known about how those factors can be controlled and evaluated, in order to produce high quality gametes in sole fish. Egg quality is a specific requirement for the production of healthy larvae. The aim of the present project is evaluate the parameters (zootecnical and feeding plan) that can control reproduction in sole maintained in captivity. Several sperm and egg parameters (biochemical and physiological) will be determined and analysed for correlations with fertility and hatching rates.

It is proposed to achieve these objectives:

a. Parameters optimization on the broodstock reproduction
b. The importance of controlled husbandry conditions on the maturation and emission of gametes, as well as in the production of high quality spawns.
c. Characterize the influence of husbandry conditions, such as temperature, photoperiod and nutrition on gametes and egg quality.
d. Effect of the hormonal induction in the broodstock reproduction
e. Identification and caracterization of the feeding plan for sole
f. Characterize and identify quality in gametes and spawns

Reference and funding entity: MARE- 22-05-01-FDR-00026
Duration: 2004 - 2006
Research team: CCMAR: Florbela Soares, Maria Teresa Dinis, Elsa Cabrita.
Total budget: 224.507 Euro

Title: "Dietary amino acids and skeletal development in white bream (*Diplodus spp.*) - SAARGO"

Summary and Objectives: Although high larval survival rates are commonly observed in aquaculture production of white sea bream (Diplodus spp.), skeleton deformities are one of the main constrains. Diets with poor protein content and amino acid deficiencies have been related to development of skeletal deformities. The central objective of this study is to evaluate the possibility of minimizing the skeletal deformity problems commonly found when Diplodus spp. are cultured, through the use of amino acid supplements or increasing the quantity of available dietary nitrogen. It is also intended to verify how the expression of key proteins involved in skeletal development are affected, in order to better understand the mechanisms involved in skeletal development. Graded levels of protein hydrolisates will be supplied in the diet in order to verify to what extent skeletal deformities can be reduced through improvement of the quantity of available nitrogen in the diet of larval Diplodus spp. The efficiency of supplementation of a diet with different indispensable amino acids on larval Diplodus spp. will be determined. The effect of a diet well balanced in the different indispensable amino acids, or supplemented with amino acids involved in skeletal formation, on performance and skeletal deformities of larval Diplodus spp. will also be studied. In addition, the expression of selected skeletal proteins will be analyzed in normal and deformed fish obtained from the different dietary treatments. It is expected that the study of the skeletal proteome will allow to identify modulated protein clusters in skeletal tissue in response to dietary stimuli. The mechanisms through which dietary nitrogen influences skeleton formation should then be better understood.

Reference and funding entity: Fundação para a Ciência e Tecnologia, POCI/MAR/61623/2004

Duration: Nov 2005 - Oct 2008

Research team: CCMAR: Luis Conceição, Paulo Gavaia, Dina Simes, Pedro Rodrigues; IPIMAR: Pedro Pousão Ferreira, Margarida Saavedra.

Total budget: 86373 Euro; **Funding for CCMAR**: 55404 Euro

Title: "Algarve-Andalucía cooperation for promotion of the marine aquaculture resources in the South-Atlantic coast - PROMAR"

Summary and Objectives: To establish an inter-regional scientific and technical cooperation on rearing of new aquaculture species in order to promote marine aquaculture, through:

- Improvement of broodstock of new aquaculture species of common interest
- Improvement of experimental pilot aquaculture facilities
- To improve the rearing techniques of new aquaculture species such as fish, bivalves, gastropods and cephalopods of common interest.
- To establish a monitoring programme of the most common pathologies of farmed species
- Evaluate the genetic diversification of broodstock and identify genetic markers for the main aquaculture fish species.

Reference and funding entity: Programme **INTERREG IIIA, SP5.P117/03**

Duration: Jan 2006 - Dec 2007

Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Florbela Soares, Laura Ribeiro, Cláudia Aragão, Sofia Engrola, Leonor Cancela, Paulo Gavaia, Sara Mira; IPIMAR: Pedro Pousão Ferreira, Margarida Saavedra; Junta da Andalucia

Total budget: 995000 Euro; **Funding for CCMAR**: 150000 Euro

Completed in 2005

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 WPI-5 will be used to screen sea bream (Sparus aurata) generated in a breeding program on a SME fish farm. This will result in the transfer of molecular biotechnological methodologies to conventional genetics and the implementation of technology transfer from science to industry, 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: 11/2001-4/2005
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 – 31/08/2005
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: "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: "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:" 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: "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: "Cooperation Algarve-Andalucia for Diversification in Marine Aquaculture in the South-Atlantic Coast – DIVERAQUA"
Summary and Objectives: To establish an inter-regional scientific and technical cooperation on rearing of new aquaculture species in order to promote marine aquaculture, through:

- Improvement of broodstock of new aquaculture species of common interest
- Improvement of experimental pilot aquaculture facilities
- To improve the rearing techniques of new aquaculture species such as fish, bivalves, gastropods and cephalopods of common interest.

Reference and funding entity: Programme **INTERREG IIIA, SP5.E36**
Duration: Jan 2003 - Dec 2005
Research team: CCMAR: Maria Teresa Dinis, Luis Conceição, Florbela Soares, Laura Ribeiro, Cláudia Aragão, Sofia Engrola, Marc Lacuisse; IPIMAR: Pedro Pousão Ferreira, Margarida Saavedra; Junta da Andalucia
Total budget: 885000 Euro; **Funding for CCMAR**: 180000 Euro

Division of Living Resources

New and Ongoing beyond 2006

Title: Decreasing fisheries resources: are non-professional fishermen to blame?
Summary and Objectives: The main objective of the proposed study is to quantify the impact of rod and line sport fishing in the south and south-west coast of Portugal, from Sines on the west coast to the mouth of the Guadiana river on the border with Spain. This will be done by: 1) quantifying the number of fishermen and mapping the distribution of their fishing effort over the year and area, 2) characterising and quantifying the sport fishing catches over a one year period and 3) evaluating the impact of sport fishing by comparison with the landings of professional small-scale fisheries in this area. A secondary objective is to characterize the sport fishermen from the demographic and socio-economic perspective through questionnaire surveys and to evaluate the economic importance of sport fishing at the regional scale by obtaining data on costs and expenditures associated with the sport fishing activity. The study will provide valuable information that can be used to provide guidelines for sustainable management and conservation of inshore fisheries.
Reference and funding entity: FCT - (POCI/MAR/58157/2004)
Duration: Nov 2005- Oct 2007
Research team: Karim Erzini, Jorge Gonçalves, Pedro Veiga, David Abecasis, Luís Bentes, Pedro Monteiro
Total budget: 72500 Euro; **Funding for CCMAR**: 72500 Euro

Title: Sea bream spatio-temporal dynamics and habitat use in the Ria Formosa lagoon
Summary and Objectives: The main objective of this project is to study habitat use within the Ria Formosa lagoon. We will be using tagging studies (telemetry and external T-tags) to obtain information on sea bream movements within the lagoon. We will be able to answer questions such as: how important are the sea grass beds for sea breams? do sea breams use the small creeks and the areas that are flooded at high tide? are there daily migratory patterns? The information obtained will be useful for conservation and management plans for the Ria Formosa lagoon, which is a protected area of international importance.
Reference and funding entity: FCT - (POCTI/BIA-BDE/61949/2004)
Duration: Apr 2005- Mar 2007
Research team: Karim Erzini, Jorge Gonçalves, Pedro Veiga, David Abecasis, Luís Bentes, Pedro Monteiro
Total budget: 86,500 Euro; **Funding for CCMAR**: 86,500 Euro

Title: "**LOBASSESS** – Norway lobster stocks in Portugal. Basis for assessment using information on larval production and ecology"
Summary and Objectives: 1.- Estimate abundance and propose management options based on the understanding of larval recruitement processes.
2- To investigate larval eschanges among the different adult populations along the Portuguese coast.
Reference and funding entity: POCTI/BIA-BDE/59426/2004
Duration: 2005-2006
Research team: Margarida Castro (coordinator of the U. Algarve participation), Sara Mira.
Total budget: 89 946 **Funding for CCMAR**: 9 300.
Web site:

Title: "Impacto ambiental de artes de pesca fixas na costa sudoeste de Portugal. Conciliar a pesca e a conservação do ecossistema marinho"
Summary and Objectives: 1.- Compared selectivity of traps and fixed nets targeting spiny lobsters. 2- To propose management measures for the spiny lobster fishery in the SW coast of Portugal.
Reference and funding entity: POCTI/CTA/549248/2002
Duration: 2006-2008.
Research team: Margarida Castro, Dora Jesus + bolseiro.
Total budget: 50 000 **Funding for CCMAR**: 50 000.
Web site:

Title: "The molecular basis for differential stress-tolerance in sympatric, ecologically similar algal species – STRESSREG"
Summary and Objectives:
In order to address the poorly-understood role of physical stress in driving evolution in marine ecosystems, fucoid algae with contrasting stress-tolerances will be used as models to investigate the molecular basis of their evolutionary divergence with respect to desiccation-stress responses. The models are three sympatric species of the genus Fucus (F. serratus, F. vesiculosus, and F. spiralis) with distinct vertical distributions in the intertidal zone, correlated with distinct physiological tolerances to desiccation stress. The recent evolution of the genus suggests that small molecular changes, in genes or regulatory regions, may account for differences in stress-tolerance.
This project will compare homologous desiccation-responsive genes from the 3 species, and their cis-regulatory regions. Results from current work in isolating desiccation- and rehydration-specific transcripts using suppression subtractive hybridization will be used to 1) Screen cDNA libraries for 3 species to obtain full sequences of homologous genes; 2) Screen for species-specific variations in gene expression in response to desiccation using Northern analysis. Candidate genes will be selected for further analysis. Since important species differences may lie at the level of gene

regulation, promoter regions will be sequenced, either using a PCR-based strategy, or by screening genomic libraries. Online tools and sequence comparisons will be used to search for conserved DNA-binding elements and species variations. Major novel scientific advances: 1) Understanding the role of physical stress in driving molecular evolution in marine ecosystems, 2) Identify which evolutionary changes at the molecular level are responsible for divergence in stress-resistance in closely-related species, 3) Improve the limited knowledge of stress-responsive genes and regulatory pathways in non-green photosynthetic organisms. In summary, the project will provide insight into the evolution of stress-responsive genes in sympatric species in marine environments.
Reference and funding entity: FCT (POCTI / BSE / 48317 / 2002)
Duration: Apr 2004-Mar2007
Research team: G. Pearson (coordinator), E Serrão, A. Lago-Leston
Total budget: 105490 Euro **Funding for CCMAR**: 105490 Euro

Title: Conservation of marine prairies: causes of regression and effects on ecosystem function
Summary and Objectives:
The goal of the proposed research is to assess the causes of the decline of seagrass meadows, the development of early warning techniques to detect stress to seagrass meadows and the evaluation of the health of seagrass meadows and their functions. Finally, the project aims at the design of strategies for the effective conservation of seagrass meadows in the Mediterranean, Caribbean and Gulf of Mexico. The project will provide the scientistic basis necessary to propose a conservation plan for the sustainable management of the meadows in protected areas.
Reference and funding entity: Fundación BBVA, Spain.
Duration: 1/09/2005 - 31/08/2008
Research team: list of PI's: IMEDEA, CSIC-UIB (Carlos M. Duarte, coordinator), CEAB, CSIC (Esperança Gacia, ecology), IVIA Valencia (Ester Marco, bacterial infections), CCMAR, Portugal (Ester Serrao, genetic markers), ICML, Univ. Aut. Mexico (Susana Enriquez, physiology), IDO, Cuba (Mercedes Cano, biodiversity), CIEC, Cuba (Adán Zúñiga, coastal dynamics), Dauphin Island, U. Alabama (DISL), USA (Just Cebrián, competition eutrofication).
Total budget: 170 000 Euro; **Funding for CCMAR**: CCMAR does not receive a fixed amount, but will receive reimbursement for travel costs and for expenses associated to the field and laboratory work, as needed.

Title:
Genetic diversity and differentiation in the seagrass species *Zostera noltii* and *Cymodocea nodosa* across the Atlantic-Mediterranean divide.
Summary and objectives:
The southern Atlantic and Mediterranean coasts of the Iberian Peninsula are biogeographic transition zones where many marine species encounter their distribution limits. The Atlantic-Mediterranean junction is an important region because it may function as a barrier to gene flow affecting population structure and connectivity. Only few studies have addressed questions related to this putative biogeographic barrier to gene flow and none of these involved seagrasses. Two possible explanations for maintaining genetic discontinuities are the sea surface currents associated with the Strait of Gibraltar and the existence of a hydrogeographic surface water boundary between Almeria and Oran, the Almeria-Oran ocean front. Two seagrass species, *Zostera noltii* and *Cymodocea nodosa* are dominant in this region often growing in the same locations though in different habitats. In the proposed project intra-specific phylogenetic relationships, genetic diversity, genetic structure and connectivity will be studied between populations of two important seagrass species with different life-histories, *Zostera noltii* and *Cymodocea nodosa*, along the Southern Iberian coast across the Strait of Gibraltar. Intra specific phylogenetic relationships will be studied using ITS rDNA and matK cpDNA sequences. Genetic

diversity, population genetic structure and gene flow will be investigated using high resolution microsatellite markers specifically designed for the *Z. noltii* and *C.nodosa*.
The proposed project will lead to the following novel scientific contributions:
1) The importance of the Atlantic/Mediterranean divide as a biogeographic barrier to gene flow.
2) Understanding of population dynamics as to where are sources or sinks for gene flow. This information will be of great importance for conservation and restoration of seagrass meadows and their habitat.
Reference and funding entity : POCI/MAR/60044/2004, FCT
Duration of the project : 01/11/2005 – 31/10/2008
Research team : O. Diekmann (coordinator), F. Alberto, E. Serrao, S. Arnaud-Haond
Total budget : 41400€ **Funding for CCMAR**: 41400€

Title: Genetic Networks and Evolution: from individuals to populations
Summary and objectives:
A network is a system composed of several interacting nodes related among them by links. A variety of networks have been defined in physics, all exhibiting specific properties as to the pattern of dominance of certain nodes or the resistance to perturbations of the whole system. During the last decades, the principles of networks have been shown to apply to a wide variety of biological phenomena, from ecosystems to food webs, or gene expression. In this project, we apply network analysis to the structure of the genetic relationship between different levels of organization, from individuals to populations, in clonal marine plants and algae with contrasting life spans and life histories: Posidonia oceanica (high longevity), Cymodocea nodosa (younger but stable meadows), and Caulerpa prolifera (short life-span, colonizing species). At the intra population level, a network organization will help to describe and analyze the genetic relationship, and genealogy, between individuals. This task will have a particular importance and will be specifically developed to deal with those populations of clonal organisms in which some clones are susceptible to being highly dominant, both in terms of space occupation and in terms of contribution to the next generation. At the inter population level, it will provide a description of the metapopulation systems in terms of migration links, or preferential colonization pathways between populations. This will allow to test for the possible existence of some migration pathways or dominant populations acting as "central nodes" relaying gene flow, and then for the possible consequences of local extinctions. Such information are essential to understand population dynamics, and the impact of population fragmentation or local extinctions on a metapopulation system. Finally, this project will allow to explore the network properties of metapopulation systems and will open new prospects as for the possible applications of network analysis in population genetics.
Reference and funding entity : POCI/MAR/57342/2004, FCT
Duration of the project : 1 Jul 2005 – 31 Jun 2007
Research team : S Arnaud (coordinator), E. Serrao, F. Alberto, E. Varela (with the teams of E. Hernandez and C. Duarte, from CSIC, IMEDEA)
Total budget : 45000 € **Funding for CCMAR**: 45000 €

Title: Population dynamics, geographical distribution and genetic diversity of macroalgal species at their southern distributional limits (LIMITS)
Summary and objectives:
The Portuguese coast constitutes the southernmost distribution limit of nearly 40 species of macroalgae. Populations living at their southern distribution limits experience adverse environmental conditions that, magnified by climatic changes, may lead to changes in population dynamics and geographical distribution of these species. These populations, isolated near their limits of distribution can became genetically differentiated as a consequence of limited interpopulational gene flow and local adaptation to their environment. This project aims at understanding whether southern limit populations on the portuguese coast have diverged from

central range populations, particularly aiming at answering the following questions: 1: are growth, reproductive rate and population dynamics different at the southern limits? 2: is intra-population genetic diversity lower and are populations more differentiated at this distributional limit? 3: have these species undertaken changes in their southern distributional limits in the last 40 years? 4: what is the capacity of resistance and recovery of these populations to environmental disturbance? In order to address these questions the current distributional limits of the nearly 40 species with southern distribution limits in the Portuguese coast will be established and compared with the data available in previous studies. To address the other questions 4 species of brown algae will be selected (Fucus serratus, Ascophyllum nodosum, Pelvetia canaliculata e Himanthalia elongata) as study models. These species have different distributional heights in the intertidal zone (2 in the midlittoral zone and 2 in the low littoral zone) thus representing different conditions of environmental stress. In conclusion, the objective of this project is to understand whether these populations are adapted to the higher stress conditions that they have to face at their distributional limits and in case the marginal populations should disappear what is the uniqueness of the genetical heritage that will be lost.
Reference and funding entity : POCI/MAR/56149/2004, FCT
Duration of the project : 1 Sept 2005-31 Aug 2008
Research team : I. Sousa-Pinto (coordinator, CIIMAR), R. Araújo (CIIMAR), E. Serrão, G. Pearson, F. Alberto
Total budget : 54000 € **Funding for CCMAR**: 25020 €

"Recrutamento de Espécies Piscícolas de Interesse Comercial no Estuário do Rio Arade" (DGPA – MARE P.O. Pescas: 22-05-01-FDR-00017). Jorge Gonçalves.

Title: "**LOBASSESS** – Norway lobster stocks in Portugal. Basis for assessment using information on larval production and ecology"
Summary and Objectives: 1.- Estimate abundance and propose management options based on the understanding of larval recruitement processes.
2- To investigate larval eschanges among the different adult populations along the Portuguese coast.
Reference and funding entity: POCTI/BIA-BDE/59426/2004
Duration: 2005-2006
Research team: Margarida Castro (coordinator of the U. Algarve participation), Sara Mira.
Total budget: **Funding for CCMAR**:
Web site:

Title "Biodiversity in fisheries off the South coast of Portugal (Algarve) (BIOFISH)" (Ref: 22-05-01-FEDER-00031)

Summary and Objectives: The main objective of this project is the compilation, characterization and photografic registration of all faunal species caught by commercial fisheries off the south coast of Portugal (Algarve), not only known commercial species, but also non-commercial species, which are generally discarded due to their inexistent commercial value in Portugal. All species caught by the most important fishing gears (trawl, purse seine, trammel net, longline, etc) will be identified, photographed, and all biological, ecological and fisheries information as well as their socio-economic importance, will be compiled. The result will be the production of a book with all species caught by fishing gears used off the coast of Algarve, to divulge in the fishing community (fishermen, researchers, managers) and public in general. A reference collection will also be criated, open to all community.
Duration: 2005-2007
Funded by: Programme MARE, DGPA
Research team: Teresa Cerveira Borges (coordinator), Sónia Olim, Paulo Morais, Luís Fonseca, Margarida Cristo, Margarida Machado, Jeff Wallace, João Sendão, José Xavier, António Malaquias, Carlos Afonso, Esmeralda Costa, João Gomes, David Francisco (photographer).
Total budget: 246 052 € **Funding for CCMAR:** 246 052 €

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:”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, Pedro Veiga, Daniel Machado, Luis Bentes, Pedro Monteiro, Rui Coelho and Karim Erzini
Total budget: 139170 Euro; **Funding for CCMAR**: 139170 Euro
Web site: http://www.ualg.pt/fcma/cfrg/

Title:”Condição nutricional das larvas de peixes nas principais áreas marinhas protegidas do sul de Portugal (Ria Formosa e Estuário do Guadiana)” **GUADIRIA**
Summary and Objectives
In the south of Portugal there are two important and contrasted marine protected areas: the Ria Formosa and the Guadiana estuary. The Ria Formosa is a tidal coastal lagoon, with low depth and high rate of water exchange with the sea, and the Guadiana river has is a medium estuarine area, with an irregular hydrological regime, with severe droughts and floods, and its under increasing pressure for exploitation of water resources, being the construction of Alqueva dam the most recent example. Both systems are highly productive and provide ideal conditions for initial phases of fish’s life cycle.
Besides previous studies, that have show that these coastal systems are important nurseries areas for juveniles, especially the salt-marshes areas, for many commercially important fish species, several issues are still not understood. They are: Is the nutritional condition of fish larvae higher inside these systems compared with the same species captured in coastal zone? Is this due to higher food availability or to higher predation inside the systems that remove weak larvae quickly or to absence or retention strategies? Are there, from typical fish species, some more sensitive to

inanition then others? Are larval species from benthonic eggs more resistant to adverse environmental condition than that from pelagic egg? Our main objective will be an in depth investigation of the nutritional condition of larval phase of the fishes inside the Ria Formosa and the Guadiana estuary. Specific objections are the answers to the above questions, through the use of nucleic acids derived indices, such as RNA/DNA, RNA residuals and DNA per mg of larva dry weight. Those indices have been used successfully in several larval fish species in different phase of its development to access their nutritional condition. This project will tstudy the relative importance of ontogenic (benthonic or pelagic eggs) or ecohydrologic factors (currents, winds, food availability) in the nutritional condition of fish larvae in the selected systems. It will be selected fish species residents in these systems such as Gobidae with benthonic eggs and larvae from planktonic eggs, usually temporary species, such as Soleidae or Sparidae for the Ria Formosa, and Engraulis encrasicolus for the Guadiana estuary. The sampling of this larval species will be done inside the Ria Formosa and the Guadiana estuary and in both adjacent coastal areas. Light trap will be use to capture the fish larvae, in order to minimize the physiologic stress cause by net tow and to increase the size of fish larvae caught by the traditional icthyoplanktonic gears. The results of the present study obtained in different marine protected coastal areas, will help in the support or refute an important hypothesis in larval ecology and recruitment field, "the member vagrant or retention hypothesis", that link high larval condition (and future recruitment) with successful strategies of retention during the larval stage in adequate areas.
Reference and funding entity: **POCTI/BIA-BDE/59200/2004 , FCT**
Duration: 2005-2007
Research team: CCMAR: Maria Alexandra Chícharo, Luis Chícharo, Ana Faria, Ana Amaral, Pedro Morais
Total budget: 42620 Euro Euro; **Funding for CCMAR**: 42620 Euro
Web site: http://www.ualg.pt/fcma/

Completed in 2005

Title: "Fishery of the common octopus in Algarve: Improvement of the Artisanal fishing gears (POLVARTE)" (Ref: 22-05-01-FEDER-00018)
Summary and Objectives: Main objectives of this project are: 1) comparison of two of the most important fishing gears to catch octopus – pots and iron traps ("covos"), in relation to their catches; 2) introduction of a new type of plastic pot to catch octopus; 3) to study the behaviour of octopus towards pots (clay and plastic) and iron traps ("covos"); 4) to study possible environmental impacts of the use of plastic pots in comparison to clay pots; 5) study of the economic cost-benefit on the use of each of the octopus fishing gears.
Duration: 2003-2005
Funded by: Programme MARE, DGPA
Research team: Teresa Cerveira Borges (coordinator), Anxo Conde, Sónia Olim
Total budget: 105 053.15 € **Funding for CCMAR:** 105 053.15 €

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: "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: "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: "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: "Impacto da pesca com ganchorra no ecossistema. Áreas de interdição da pesca: instrumento de gestão ambiental e pesqueira".(*Fishing impacts. Use of control areas*)
Aims: Análise da utilização de áreas de interdição de pesca com ganchorra como medida de protecção ambiental e dos mananciais, e forma de assegurar a sustentabilidade da actividade da pesca no futuro.
Duration: 01-012-2002 a 01-012-2004
Funding: FCT Programa SAPIENS (POCTI/MGS/42319/2001)
***Team*:** Luís Chícharo (**Responsávelno CCMAR**), Alexandra Chícharo (Universidade do Algarve), Carlos Costa Monteiro (IPIMAR CRIP/Sul); Miguel Gaspar (Coordinator, IPIMAR CRIP/Sul).
***Budget*: 30.247,5** EURO (Universidade do Algarve)

Title (*Ecological and socioeconomic impacts of river inflow changes in three Portuguese rivers on the estuaries and coastal areas* " (ERIC)
Aims: analisar as consequência ecológicas e impacto socioeconómico das modificações dos caudais dos rios Minho, Tejo e Guadiana nas zonas estuarinas e costeiras.)
Duration: 01-011-2000 a 01-11-2004
Funding: FCT Programa FCT/MAR/15263/99
Team: Maria José Costa (Instituto de Oceanografia da Faculdade de Ciências (Coordenadora), Alexandra Chícharo (**CCMAR**), Luís Chícharo (
Budget: 24000 EURO (Universidade do Algarve)

International and Inter-Institutional Cooperations

Division of Aquaculture and Biotechnology

Title: "Development of a seabream pre-adipocyte cell line and its application to the study of adipocyte differentiation and functional regulation"
Summary and Objectives: The main objective of this proposal is to study the physiological basis of adipocyte differentiation in fish. In fish, contrary to the situation in mammals, the cellular and molecular mechanisms which govern the process of differentiation of adipocytes are largely unknown. In addition, the nature or origin of precursor cells which are recruited to differentiate into mature adipocytes in fish adipose tissue is not known. In order to identify factors which are responsible for the acquisition of an adipocytic phenotype in fish cells, an in vitro cell culture system will be developed and validated. For this purpose, we propose to use a seabream (Sparus aurata) pre-osteoblast cell line which can acquire a pre-adipocytic phenotype and determine the factors and culture conditions required for its differentiation into mature adipocytes. Therefore, a stable pre-adipocyte cell culture system will be established and will be used to study changes in gene expression associated with acquisition of an adipocytic phenotype as well as the regulation of lipid metabolism.
Reference and funding entity: Conselho de Reitores das Universidades Portuguesas, AI-E-104/05
Duration: 2005-2006
Research team: M.L. Cancela (coordinator), V. Laizé, A.R. Pombinho
Total budget: 2.500 euros

New instruments for the detection of immunosupression in aquaculture. Acção integrada Luso-Espanhola E-135/2004 com Lluis Tort (Univ. Autónoma de Barcelona). Adelino V.M. Canário, Rita Teodósio

Identification of atractants related to preys of sole, *Solea senegalensis*. Acções Integradas Luso-Britânicas nº B-71/2004 com Joerg Hardge (Universidade de Hull, Reino Unido). Eduardo Barata, Zélia Velez.

Induced-spawning and non intrusive assessment of reproductive and welfare status of sturgeons. Collaborative Linkage Grant NATO LST.CLG.979493. Adelino V.M. Canário, Tatiana Semenkova (Central Fish Laboratory, St Petersburg, Rússia) e Constantinos Mylonas (IMBC, Creta, Grécia).

Division of Living Resources

Research Coordination Network funded by N.S.F., USA. The CORONA Project: Historical Ecology of the trans-Atlantic Marine Biota. 2002-2006. Coordinator: C. Cunningham, Duke University, USA. CCMAR participants: E. Serrão, G. Pearson.

Bilateral cooperation CRUP/Univ. Pierre et Marie Curie. "Evolution of reproductive systems in four species of Fucus." 2004/2005. (E. Serrão, M. Valero, Station Biologique de Roscoff, France).

Bilateral cooperation PESSOA- GRICES/CNRS. "Evolution of reproductive systems in algae"2004/2005. (E. Serrão, M. Valero, Station Biologique de Roscoff, France).

Bilateral cooperation GRICES/CSIC. "Clonal structure of populations of marine macrophytes". 2004/2005. (E. Serrão, C. Duarte, IMEDEA, CSIC, Spain)

Silvert, W. Meeting with minister of Fisheries, Dili, East Timor 2005.

Seminars given by CCMAR members in other institutions

Borges, T.C. 2005. "Fisheries Impact in the South coast of Portugal (Algarve)". Smithsonian Tropical Research Intitute (STRI), Panamá. March 2005.

Canario, A.V.M. Ecophysiology and genomes in Antarctica: global importance. I Workshop Portugal e a Antárctida. 18 de Novembro de 2005. Centro de Estudos Geográficos, Universidade de Lisboa

Canario, A.V.M. Rothera- Uma estação de Investigação na Antártida. Conferência na Sociedade de Geografia de Lisboa. 3 Junho 2005.

Canario, A.V.M. As 'Ómicas' em Biologia Marinha. Congresso da Ordem dos Biólogos. 3 de Março, Porto.

Canario, A.V.M. Universidade do Algarve: teaching and research. VI Forum of Portuguese Association of Post Graduate Students. Chicago, 23-24 April.

Coelho, R. Teaching during a workshop for West African scientists focusing on elasmobranch identification, biological sampling, and conservation, as part of the "Sub-Regional Plan of Action for Sharks". This training workshop was held at the University Cheikh Anta Diop at Dakar, Senegal, on 13-19 December 2005.

Gonçalves, J.M.S. Taugh a course on "Taxonomy of marine commercial species from Mozambique" in the Instituto Nacional de Investigação Pesqueira de Moçambique, Maputo, 25/7 to 5/8/2005.

Silvert, W. 2005. Three seminars and worked on problems related to aquaculture impacts at the University of Haifa, Israel.

Conceição, L.E.C. (April 2005). "Nutritional physiology of Senegalese sole (*Solea senegalensis*) larvae and juveniles" at the National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway.

Cancela, M. Leonor (April 21, 2005) Proteínas Gla do osso e cartilagem: importância dos anfíbios e peixes como modelos biológicos para elucidação da sua função e evolução. 1º Encontro "O Mar e as Ciências da Saúde", Cooperativa Egas Moniz, Caparica. (conferencista convidada).

Conceição, L.E.C. (Jun 2005). "Acondicionamento de reprodutores y cria larvaria del mero (*Epinephelus* sp.)" Jornadas de Divulgação e transferência de resultados do programa INTERREG IIIA de cooperação transfronteiriça Algarve-Andalucía na área da investigação pesqueira e aquícola, 28 and 29 of June, Ayamonte.

Lacuisse, M. (Jun 2005). "Cria larvaria de sargos (*Diplodus* sp.)" Jornadas de Divulgação e transferência de resultados do programa INTERREG IIIA de cooperação transfronteiriça Algarve-Andalucía na área da investigação pesqueira e aquícola, 28 and 29 of June, Ayamonte.

Ribeiro, L. (Jun 2005). “Cultivo larvae y alevinaje del lenguado (*Solea senegalensis*)” Jornadas de Divulgação e transferência de resultados do programa INTERREG IIIA de cooperação transfronteiriça Algarve-Andalucía na área da investigação pesqueira e aquícola, 28 and 29 of June, Ayamonte.

Soares, F. (Jun 2005). “Reproducción del lenguado (*Solea senegalensis*)” Jornadas de Divulgação e transferência de resultados do programa INTERREG IIIA de cooperação transfronteiriça Algarve-Andalucía na área da investigação pesqueira e aquícola, 28 and 29 of June, Ayamonte.

Cancela, M. Leonor (June 8, 2005) Tissue mineralization: Old thoughts and new insights on mechanisms affecting spatial restriction. Instituto de Biologia Molecular e Celular (IBMC) da Universidade do Porto.

Cancela, M. Leonor (June 8, 2005) Gla proteins and bone formation. Universidade do Minho, Dept de engenharia biológica.

Cancela, M. Leonor (June 9, 2005) Gla proteins and bone development in fish, effect of environmental stress. Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto.

Cancela, M. Leonor (August 24, 2005) Gla proteins, tissue mineralization and skeletal development. Matredal, Norway.

Cancela, M. Leonor (October 28, 2005) A Bioquímica em Portugal: uma porta aberta para a investigação de excelência. 1º Jornadas de Bioquímica da UALG, Universidade do Algarve.

Cancela, M. Leonor (November 30, 2005) Osteocalcin: Evolution and function. Faculdade de Ciências da Universidade de Lisboa.

Conceição, L.E.C. (Dec 2005). “Crecimiento, Bioenergética y Metabolismo de Larvas de Peces” at the “X Curso Avances en Acuicultura y Calidad Ambiental”, Instituto de Ciencias Marinas de Andalucía (CSIC) y Facultad de Ciencias del Mar da Universidad de Cadiz, Puerto Real, Cadiz, Spain.

Conceição, L.E.C. (Dec 2005). “Crecimiento, Bioenergética y Metabolismo de Larvas de Peces” at the “X Curso Avances en Acuicultura y Calidad Ambiental”, Instituto de Ciencias Marinas de Andalucía (CSIC) y Facultad de Ciencias del Mar da Universidad de Cadiz, Puerto Real, Cadiz, Spain.

Ribeiro, L. (Dec 2005). “A Aquacultura em portugal”, seminar presented at Escola Superior de Tecnologia da Universidade do Algarve.

Dissemination of scientific culture

Afonso, C. 2005. Colecção de moluscos gastrópodes da costa algarvia na Exposição de fotografia “Mar Imenso, Mar intenso” organizada pelo CCMAR para a comemoração do dia internacional do Mar e que decorreu na biblioteca central da UALG de 15 a 30 de Novembro.

Afonso-Dias, I. “A vida microscópica num tanque de água doce”. Actividade desenvolvida na escola EB1 de Alto Rodes de Faro, no âmbito da semana da Ciência em Novembro de 2004.

Almeida, C., Gonçalves, J.M.S., 2005. Workshop no IX Encontro Nacional de Estudantes de Biologia, com o tema “Macrofauna Associada a substratos móveis”, dia 21 de Março de 2005, na Universidade do Algarve, Faro.

Castro, M., 2005. A pesca de arrasto no Algarve. Encontro Nacional de Estudantes de Biologia. Faro, 19 de Março 2005.

Coelho, R. 2005. Biologia e conservação de tubarões & seus parentes. Invited lecture presented during the IX ENEB (9th National Meeting of Biology Students). 19 March. University of the Algarve, Portugal. Erzini, K., Ribeiro, J., Bentes, L., Lino, P., Monteiro, P., Coelho, R, Correia, C., Gonçalves, J.M.S “Ghost fishing in European Waters”, a video produced within the framework of the FANTARED II project.

Conceição, L.E.C. Orientação de estágio integrado no programa Ocupação Científica de Jovens nas férias, promovido pela Agência Ciência Viva, dos alunos do ensino secundário António Luís Ferreira Rodrigues e Manuel Lisboa Brandão de Melo, com o tema “Cultivo de Peixes Marinhos”, no Centro de Ciências do Mar do Algarve (CCMAR), de 4 a 15 de Julho de 2005.

Conceição, L.E.C. Orientação de estágio integrado no programa Ocupação Científica de Jovens nas férias, promovido pela Agência Ciência Viva, das alunas do ensino secundário Marli Patricia Barata Ventura e Rosana Martins Afonso, com o tema “Cultivo de Peixes Marinhos”, no Centro de Ciências do Mar do Algarve (CCMAR), de 18 a 29 de Julho de 2005.

Gonçalves, J.M.S, Bentes, L., Ribeiro, J., Machado, D., Veiga, P., Monteiro, P., Oliveira., F., Almeida, C. and Erzini, K. “Bombordo” RTP television programme on sea horses of the Ria Formosa lagoon.

Gonçalves, J.M.S., Monteiro, P., Ribeiro, J., Afonso, C., Coelho, R., Almeida, C., Ramires, T., Veiga, P.,Machado, D., Machado, M., Berecibar, E., Oliveira, F. & Bentes, L. “Biocenoses Marinhas no Algarve – Exposição de fotografia subaquática”. Conferência “Portugal Cultura Oceânica” organizado pela Cooperativa de Comunicação e Cultura em Torres Vedras a 4 de Novembro, 2005.

Gonçalves, J.M.S., Monteiro, P., Ribeiro, J., Afonso, C., Coelho, R., Almeida, C., Ramires, T., Veiga, P.,Machado, D., Machado, M., Berecibar, E., Oliveira, F. & Bentes, L. “REN Submarina”. Exposição de fotografia subaquática que decorreu durante o mês de Janeiro 2005 na CCDR Algarve, Faro.

Gonçalves, J.M.S., Monteiro, P., Bentes, L., Coelho, R., Corado, M., Araújo, J., Canário, A. & Erzini, K. 2005. "Dispositivos para a redução das rejeições no cerco demersal". Comunicação oral no IV Encontro das Pescas, que decorreu na Universidade do Algarve a 4 de Junho.

Gonçalves, J.M.S., P. Veiga, D. Machado, F. Oliveira, L. Bentes, P. Monteiro, R. Coelho, M. Ruano, J. Ribeiro & K. Erzini. 2005. Comunidades piscícolas de interesse comercial do estuário do Arade. Poster no IV Encontro das Pescas, que decorreu na Universidade do Algarve a 4 de Junho.

Machado, D., Veiga, P., Gonçalves, J.M.S. 2005. Workshop no IX Encontro Nacional de Estudantes de Biologia, com o tema "Caracterização de comunidades estuarinas do rio Arade", dia 21 de Março de 2005, na Universidade do Algarve, Faro.

Ribeiro, J. 2005. Os Peixes da Ria Formosa. Escola Secundária de Albufeira, 24 de Novembro. Semana da Ciência e Tecnologia – Programa "Os cientistas vão à escola" CCVIVA-CCMAR.

Ribeiro, J., L. Bentes, D. Machado, F. Oliveira, R. Coelho, D. Abecasis, Veiga, P., Afonso, C., Monteiro, P. & J.M.S. Gonçalves 2005. Fotografias subaquáticas do projecto RENSUB na Exposição de fotografia "Mar Imenso, Mar intenso" organizada pelo CCMAR para a comemoração do dia internacional do Mar e que decorreu na biblioteca central da UALG de 15 a 30 de Novembro.

Soares, F. Orientação de um estágio de formação Profissional integrado no curso de técnico de Aquacultura do Forpescas, da aluna Vânia David, no Centro de Ciências do Mar do Algarve (CCMAR), de 20 de Maio 12 de Agosto de 2004.

Veiga, P. 2005. Participação numa peça de divulgação científica sobre o projecto ARADE (Programa MARE) no programa de rádio "Na Corda Bamba", rubrica "Ecoplaneta" que passou na Radio Costa d'Ouro no dia 15 de Janeiro de 2005.

Visiting scientists

Carlos Duarte, IMEDEA, CSIC, Spain. (host: team MAREE)

Thorsten Reusch, Max-Planck Institute for Limonology, Germany (host: team MAREE)

Doutora Helena Fortunato do Smithsonian Tropical Research Intitute (STRI), Panamá. April 2005

Professor Richard A. Cooper University of Connecticut (guest of Teresa Cerveira Borges)

Organization of Conferences, Workshops, Courses

M. Leonor Cancela (May 5-13, 2005). University of Algarve, CIMA. Curso de especialização "Detecção e quantificação de enterovirus por métodops rápidos de filtração e por técnicas de biologia molecular". Leccionado em colaboração com a Doutora Rachel Nobel, University of North Carolina, USA.

Workshop on Marine Biodiversity and Ecosystem Stability, in the framework of the Network of Excellence MARBEF, Marine Biodiversity and Ecosystem Function. Tavira, March 15-19, 2005 (Organized by the team MAREE)

IV Encontro das Pescas (IV Fisheries Meeting)**.** 4 June 2005. Anfiteatro Azul, University of Algarve, Campus de Gambelas – A total of 110 people were present, **where** 10 oral comunications **and 12 posters were presented.**

Silvert, W. 2005. Participated in a planning session for a course for aquaculture managers in Spain.

Participation in scientific and advisory committees

M Leonor Cancela (July 2005) Annual Meeting of the Scientific Council of the Federation of European Biochemistry Societies, Budapest, Hungary

Karim Erzini participated in Sixth Framework Programme project evaluations (DG XIV, Brussels, Belgium (7 – 11 of March, 2005).

Karim Erzini was involved in the preparation of the work programme for Priority 8 "Policy oriented research" of the Sixth Framework (DG XIV, Brussels, Belgium, 25 – 26 of April, 2005).

Dinis, M.T. Jornadas de Divulgação e transferência de resultados do projecto DIVERAQUA - cooperação transfronteiriça Algarve-Andalucía na área da investigação pesqueira e aquícola, financiado pelo programa INTERREG IIIA, 28 and 29 of June, Ayamonte, Spain.

Dinis, M.T. LARVAR 06 - Workshop in Fish Larval Research. 9 January 2006, Centre of Marine Sciences (CCMAR), Faro. Organizers: Maria Teresa Dinis (CCMAR), Karin Pittman (Univ. Bergen, Norway) and Luis Conceição (CCMAR)

Member of Scientific Advisory Council on Ecohydrology under UNESCO IHP Programme