

WELCOME

It is a great pleasure to welcome you to the ASSEMBLE conference.

The aim of this conference is to highlight scientific outcomes resulting from research carried out in ASSEMBLE Marine Stations through on-site and remote access to marine resources.

The needs of users and improvements on this Transnational Access scheme will be discussed, especially in relation to the development of ASSEMBLE into the extended and long-term ESFRI marine infrastructure EMBRC (European Marine Biological Resource Centre; www.embrc.eu). Your contributions are paramount to improve what the marine stations can offer.

VENUE AND REGISTRATION

The meeting takes place at the Royal Marina Hotel at the fishing village of Olhão, close to Faro (<http://www.realhotelsgroup>).

Registration will be open on Monday between 17.30-19:00, and on Tuesday between 8.30-11:00. RUG, USP and SSC can register before their meetings.

LOCAL ORGANIZATION (Centre of Marine Sciences, CCMAR)

Adelino Canário

Patrícia Pinto

Andreia Pinto

INFORMATIONS TO SPEAKERS

Oral communications must be accommodated within 15 min + 5 min for questions. Computer projection will be available in the lecture room. Please hand in your presentation in the room well in advance of your session.

POSTER SESSION

The poster session will take place on Tuesday 23th October. Posters should be in place on Monday at the end of the day or Tuesday morning. They should be removed Thursday morning. The registration desk can help.

SOCIAL PROGRAM

Monday, 22nd October:

19:00-19:30- **Conference ice breaker**

The welcome drink will be held at the Real Marine Hotel terrace.

Wednesday, 24nd October:

20:00-23:00- **Conference dinner**

The conference dinner will be held at the restaurant "O Lagar", in Pechão (5 km from Olhão). Two buses will be at the entrance of the hotel to take the participants leaving at 19:30, and will bring the participants back to the hotel at 23:00.

Thursday, 25nd October:

14:30-19:00- **Visit to the sand bar islands**

A boat tour through the Ria Formosa" to the "Farol Island" is planned pending on meteorological conditions. Swimming is possible for the full hardy.

The boat will leave the pier in front of the hotel at 14:30 and will start the journey back to Olhão at 18:10 (30 min trip).

PROGRAMME

Monday, 22nd October:

09:00-17:00- **ASSEMBLE user selection panel (USP) meeting**

Coffee breaks will be served at the conference hall at 11:00 and 15:30, lunch will be served at the hotel restaurant at 13:00.

10:00-17:00- **EMBRC reference user group (RUG) meeting**

Coffee breaks will be served at the conference hall at 11:00 and 15:30, lunch will be served at the hotel restaurant at 13:00.

13:30-19:00- **EMBRC steering committee (SSC) meeting**

Lunch will be served at the hotel restaurant at 13:00, one coffee break will be served at the conference hall at 15:30.

19:00-20:30- **Conference ice breaker**

The welcome drink will be held at the Real Marine Hotel terrace.

Tuesday, 23rd October:

ASSEMBLE conference, Real Marina Hotel

09:00		Welcome notes; ASSEMBLE Management Team (Ulrika Hjelm, Niklas Andersson and Malin Karlsson): "Assemble: an overview of progress"
09:20	ERA	Adelino Canário (Faro, PT) . "EMBRC - A Marine Infrastructure for the future"
09:40		Mike Thorndyke (Gothenburg, SE) – Euromarine - Integration of Marine Research Networks of Excellence
10:00		O1- Wojciech Wawrzynski (Copenhagen, DK). "Towards an integrated marine and maritime science and technology community"
10:30		Break
11:00	S1	O2- Isabelle Carre (Warwick, UK). "Probing transcriptional regulatory networks in <i>Ostreococcus</i> "
11:20		O3- Isabel Rodríguez-Moldes (S. Compostela, ES). "Contributions of developmental studies in <i>S. canicula</i> to the functional and evolutionary knowledge of the brain of vertebrates"
11:40		O4- Paul Andrews (London, UK). "Assessment of general and local anaesthesia in <i>Octopus vulgaris</i> "
12:00		O5- Andreas Hejnol (Bergen, NO). "Deuterostomy in the protostome <i>Priapulid caudatus</i> (Priapulida)"
12:20		LUNCH
14:00	S2	O6- Katerina Moutou (Thessaly, GR). "Age-related response to feeding levels of gene expression profiles in gilthead sea bream (<i>S aurata</i> L.) white muscle"
14:20		O7- Oleg Simakov (Heidelberg, DE). "Population genomics of the polychaete worm <i>Platynereis</i> , an interesting model to study evo-devo since the Cambrian"
14:40		O8- Christos Chinopoulos (Budapest, HU). "Generation of mitochondrial proteome compendiums from marine species in search of the proteins comprising the permeability transition"
15:00		O9- Konstantinos Kormas (Thessaly, GR). "Cosmopolitan, common and rare marine <i>Archaea</i> : an experimental approach"
15:20		O10- Frithjof Kuepper (Newburgh, UK). "New insight into the bioinorganic chemistry of <i>Ectocarpus</i> "
15:40		Break
16:10	S3	O11- Melody Clark (Cambridge, UK). "Environmental stress and oysters "
16:30		O12- Frederico Batista (Tavira, PT). "Temporal and spatial variation of <i>Crassostrea angulata</i> and <i>C. gigas</i> in southern Portugal "
16:50		O13- Adrian Harrison (Copenhagen, DK). "An Investigation of oyster electrophysiology"
17:10		O14- Uwe Spremberg (Copenhagen, DK). "First observation of copulation by a barnacle dwarf male: implications for sexual strategy"
17:30		Poster session
19:00		

Wednesday, 24th October:

ASSEMBLE conference, Real Marina Hotel, conference area

09:00	S4 O15- Ulf Jondelius (Stockholm, SE). "Evolution and diversity of <i>Acoela</i> "
09:20	O16- Suncica Bosak (Zagreb, CRO). "Biodiversity of marine diatoms: morphological and molecular characterization"
09:40	O17- Peter Kroth (Konstanz, DE). "Studying fresh water and marine biofilms"
10:00	O18- Bente Edvardsen (Oslo, NO). "Diversity and dynamics of marine haptophytes"
10:30	Break
11:00	USER GROUP FEEDBACK SESSION
12:20	LUNCH
14:00	S5 O19- Nicholas Kamenos (Glasgow, UK). "Can we use coralline algae to reconstruct ENSO and associated biotic changes?"
14:20	O20- Heidi Burdett (Glasgow, UK). "The potential impact of coralline algae habitats on local climate"
14:40	O21- Magnus Johnson (Scarborough, UK). "Swimming behaviour in pelagic crustaceans: sleepy krill and climate change"
15:00	O22- Covadonga Orejas (Santander, ES). "Capture rates of the Cold Water Coral <i>Lophelia pertusa</i> on living phyto and zooplankton under different current speed regimes"
15:20	O23- Christina Pavloudi (Crete, GR). "Effects of stressful environmental factors in lagoonal ecosystems on marine microbial and invertebrate populations"
15:40	Break
16:10	S6 O24- Natalia Niedźwiecka (Gdansk, PL). "Biological effects of xenobiotics on marine organism"
16:30	O25- Marco Campinho (Faro, PT). "Developing hypothalamus-pituitary-thyroid axis disruption in zebrafish by common aquatic contaminants"
16:50	O26- Ivana Bošnjak (Zagreb, CRO). "The impacts of BPA on response genes involved in chemical defense and cell-cycle regulation during rocky (<i>P. lividus</i>) sea urchin embryonic development"
17:10	O27- María Segovia-Viadero (Alicante, ES). "Assessing the genetic impact of sea urchin restocking "
17:30	
20:00	Conference dinner

Thursday, 25th October:

ASSEMBLE conference, Real Marina Hotel, conference area

09:00	S7 O28- Fulvia Bradassi (Trieste, IT). "Impacts of ocean acidification on early stages of the crustose coralline alga <i>Phymatolithon lenormandii</i> "
09:20	O29- Rael Horwitz (Eilat, IL). "Ocean acidification impacts regeneration of Red Sea corals"
09:40	O30- Dalit Meron (Ramat Gan, IL). "Changes in microbial communities associated with Cnidarian in a natural pH gradient"
10:00	O31- Elisa Schaum (Edinburgh, UK). "Plastic Fantastic: plastic responses to ocean acidification in 16 ecotypes of a globally distributed picoplankton, <i>O. tauri</i> "
10:30	Break
11:00	O32- Astrid Mejia (Rome, IT). "Effect of different CO2 and N regimes on the seagrass <i>Cymodocea nodosa</i> associated microbial community "
11:20	O33- Jean-Michel Claverie (Marseille, FR). "Exploring the frontier of microbiology thanks to ASSEMBLE"
11:40	S8 O34- Esther Isorna (Madrid, ES). "Peripheral oscillators in fish. The sea bass pituitary as a clock and its regulation by melatonin and cortisol"
12:00	O35- Elia Benito-Gutierrez (Heidelberg, DE). "Laboratory cephalochordates: the beginning of a new experimental era in amphioxus research"
12:20	LUNCH
14:00	Social activities
19:00	

Friday, 26th October:

09:00-15:00- **ASSEMBLE project implementation committee (PIC) meeting**

This meeting will take place in one room at the hotel conference area.

One coffee break will be served at the conference hall at 11:00, lunch will be served at the hotel restaurant at 13:00.

ORAL PRESENTATIONS

EMBRC - A Marine Infrastructure for the future

Adelino Canário

Centre of Marine Sciences (CCMAR), Faro, Portugal

The European Marine Biological Resource Centre (EMBRC) is a planned ESFRI road map infrastructure which will comprise a consortium of key European marine biological and molecular biology laboratories, together providing: (1) access to a wide range of European coastal marine biota and their ecosystems; (2) an integrated supply of marine organisms for interdisciplinary research, including existing and new models; (3) coordinated services including state-of-the-art biobanks and dedicated platforms for genomics, structural and functional biology, microscopy and bioinformatics; (4) interdisciplinary training in marine biological sciences and genomics; and (5) outreach to stakeholders, users and the public at large. New and improved services will be provided for research, training, education and innovation, and access to resources will be provided both on site (to resident and non-resident users) and remotely (e.g. sending samples, e-resources). End users are expected to comprise not only marine biologists and in-house staff, but also researchers from other scientific institutes, universities, governmental and NGO agencies, SMEs and industry, who wish to apply cutting-edge approaches to study marine model organisms across the biological, biomedical and environmental sciences. The presentation will address how this can be achieved and current status.

EuroMarine - Integration of European Marine Research Networks of Excellence

Mike Thorndike

University of Gothenburg, Sweden

EuroMarine is a FP7 coordination and support action designed to bring together the three FP6 marine Networks of Excellence (NoE) communities; EUR-OCEANS, MarBEF and Marine Genomics Europe. In doing so, EuroMarine will provide the best expertise and innovation available in European Marine Research, that can respond rapidly to societal needs, environmental demands, well-being and sustainability.

The Challenge

Many questions in marine research can only be answered using multidisciplinary methodologies, from the molecular level with genomics and other new emerging technologies integrated together with an ecological, physical and biogeochemical ecosystem approach. This allows us to address novel questions in marine research, paving the way to new and more integrated knowledge systems that impact the way human society deals with the oceans. The challenge of EuroMarine will be to bring marine sciences into the multidisciplinary perspectives of the 21st century. The EuroMarine consortium will build and strengthen a community of marine scientists from marine laboratories in Europe, building the academic foundation for marine research that is the base for innovation.

O1- Towards an integrated marine and maritime science and technology community

Wawrzynski, W.

International Council for the Exploration of the Sea, Copenhagen, Denmark

The International Council for the Exploration of the Sea (ICES) is leading a group of 12 major science networks from the marine and maritime sectors that joined forces in order to create a new, integrated research governance and consultancy model - The European Marine and Maritime Science and Technology Forum. The Forum follows a cooperation building process under the FP7 'MARCOM+' (Towards an integrated marine and maritime science community) project and serves as a central 'hub' of experts.

The MARCOM process contributes to developing interdisciplinary science dialogue and interactions between the research community and other partners. This dialogue includes research infrastructure development and involves the various stakeholders (e.g. the ESFRI community).

The goal of this presentation is to present the idea of the Marine/Maritime Forum and provide the Association of European Marine Biological Laboratories with updated information about the initiative's action plan.

O2- Probing transcriptional regulatory networks in *Ostreococcus*

Carre, I.A.; Gorton, K.G.; Bouget, F.Y.

University of Warwick, Coventry, United Kingdom

The prediction of gene expression patterns from DNA sequence blueprints requires an understanding of how genes interact with each other as part of a network. Genes are regulated through complex interactions between their promoter region and a number of transcription factors that bind short DNA sequence motifs and either work synergistically or antagonistically to regulate transcription. Predictions of these interactions from sequence data has limited value because binding specificity is only known for a small subset of transcription factors and therefore prediction of regulatory connections on the basis of promoter sequence is unreliable. The development of the ChIP-Seq technique has recently enabled the experimental determination of genome-wide binding sites for transcription factors. We propose to use this technique to investigate transcriptional regulatory networks in the unicellular marine alga, *Ostreococcus tauri*. *Ostreococcus* is an ideal system for such analyses because of its compact promoter structure, its minimal set of transcription factors (approximately 100) and its very low level of gene redundancy. This simple eukaryote is transformable, therefore amenable to reporter studies and classical reverse genetic approaches such as overexpression and gene silencing technologies. In order to test the regulatory function of binding interactions identified in ChIP-seq

experiments, individual transcription factors will be expressed from an inducible transgene subsequent changes in expression of putative target genes will be monitored. Genes that are identified as binding targets in ChIP experiments and that show differential expression following induction of the transcription factor will be identified as direct regulatory targets. We want in the longer term to extend these analyses to the full set of transcription factors in *Ostreococcus*. This will provide a comprehensive map of binding and regulatory interactions and will be the first resource of this kind in a photosynthetic organism. This will provide the framework required to develop an understanding of the gene network dynamics that mediate responses to the environment.

O3- Contributions of developmental studies in *Scyliorhinus canicula* to the functional and evolutionary knowledge of the brain of vertebrates

Candal, E.; Pose-Méndez, S.; Quintana-Urzaínqui, I.; Sánchez-Farías, N.; Santos-Durán, G.; Rodríguez-Moldes, I.

Universiity of Santiago de Compostela, Santiago de Compostela, Spain

Neuroanatomy aims to understand the anatomical organization of the nervous system which results from morphological processes including cell growth, differentiation and organization of cells into functionally different structures. These processes mostly depend on the thight coordination of where and when developmental genes are expressed. Most of these genes are conserved throughout evolution, though each of them is expressed in particular regions and so they have particular roles over time. We thus approached the study of the regionalization of the brain from an evolutionary and a functional point of view. The lesser spotted dogfish *Scyliorhinus canicula* has been increasingly used as a model organism to study the development of the nervous system. Particularly, we have analysed the expression pattern of regulatory genes involved in the regionalization of the brain, retina and olfactory system of this species and we followed their derivatives across morphogenesis in relation to the distribution of various neurochemical markers. This model organism offers great advantages including the slow growth and big size of their brains, which is important to make detailed studies of particular encephalic regions. From and evolutionary point of view, cartilaginous fishes represent an ancient radiation of gnathostome vertebrates, so they are essential to investigate the ancestral condition and the evolution of the development of the nervous system. Similarities or novelties observed with respect to other vertebrates may serve to support homologies or as a cue to decipher the influence of particular genes in the evolution and development of the nervous system.

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O4- Assessment of general and local anaesthesia in *Octopus vulgaris*.

Andrews, P.^{1,2}; Valentino, M-G.^{1,3}; Ponte, G.¹; Afuso, A.^{1,3}; Fiorito, G.¹

¹Stazione Zoological Villa Comunale, Naples, Italy, ²St George's University of London, UK, ³BIOGEM, Ariano Irpino, Italy; ²Division of Biomedical Sciences, St George's University of London, London, United Kingdom

Assessment of both local and general anaesthesia in cephalopods will be essential for humane conduct of surgical procedures under Directive 2010/63/EU. A particular issue is to ensure that general anaesthetic agents such as the commonly used MgCl₂ do not act as neuromuscular junction (NMJ) blockers. Complete immersion of *Octopus vulgaris* in gassed MgCl₂ (3.5%) induced surgical anaesthesia (in ~15min) defined by skin paling (reduced neural drive to chromatophores), reduced arm muscle tone and sucker adhesion, suppression of respiration, loss of righting reflex, immobility and loss of responses to noxious stimuli (mechanical and chemical [1% acetic acid on arm tip]). Electrical stimulation of motor nerves (pallial nerve or arm nerve cord) under MgCl₂ anaesthesia resulted in mantle contraction, arm extension, skin darkening and papilla formation (mantle) indicating a lack of functional NMJ block. Topical application of mepivacaine (3%) to the arm nerve cord blocked (onset ~5min) motor responses (chromatophores/ arm extension) distal to the point of nerve stimulation. This demonstrates that mepivacaine can be used as a local anaesthetic in Octopus.

O5- Deuterostomy in the protostome *Priapulid caudatus (Priapulida)*

Martín-Durán, J.M.¹; Janssen, R²; Wennberg, S²; Hejnol, A.¹; Budd, G.²

¹ Sars International Centre for Marine Molecular Biology, University of Bergen, Thormøhlensgate 55, 5008 Bergen, Norway; ² Department of Earth Sciences, Palaeobiology, Uppsala University, Villavägen 16, Uppsala 75236, Sweden.

Despite representing more than the 80% of the known animal species, our understanding of the origin and diversification of the Ecdysozoa and its major groups, such as arthropods and nematodes, is still poor. The Priapulida is a small group of vermiform marine invertebrates, with only 19 described species. Recent phylogenomic studies consistently place priapulids within the Ecdysozoa in a lineage separate from the well-studied Nematoda and Arthropoda, presenting the strong possibility that the genomic and developmental program of priapulids may have diverged from nematodes and arthropods prior to the striking specializations that occurred in each of these lineages. As such, functional genomic analyses in priapulids are important for a better understanding of the genomic and ontogenic nature of the ancestral ecdysozoan. Unfortunately, very little is known about their development. *Priapulid caudatus* is the most accessible species for the study of priapulid development, with a few recent papers describing their cleavage, early gastrulation,

and larval forms. In this study, we characterized the embryonic development of *Priapulid caudatus*, from gastrulation to hatching using immunohistochemistry and confocal microscopy. We show that the site of gastrulation is at the vegetal pole, which gives rise posterior structures (the trunk) and the anus, while the animal pole gives rise to anterior structures (the introvert), and the mouth is formed anew after gastrulation is completed. We further extended our studies to investigate the expression of evolutionarily conserved anteroposterior gut markers, by means of whole-mount in situ hybridization. The expression of brachyury, foxA, orthodenticle, gooseoid and GATA456, among others, support our previous morphological observations, solidifying the conclusion that *P. caudatus*, an early branching ecdysozoan, exhibits a deuterostomic development. These results challenge the dogma that the ecdysozoan ancestor was protostomic and could impact our understanding of the last common bilaterian ancestor.

O6- Age-related response to feeding levels of gene expression profiles in gilthead sea bream (*S aurata L.*) white muscle

Moutou, K.A.; Georgiou, S.; Power, D.M.; Mamuris, Z.
University of Thessaly, Larissa, Greece

Growth as increase in body size, is an age-dependent phenomenon that is further influenced by nutritional, physiological and environmental factors. In fish, white muscle is the largest growing tissue and often at periods of starvation muscle exhibits signs of wasting. In higher vertebrates a close interaction between muscle and adipose tissue has been shown, mediated locally by several signaling molecules.

In the present study juvenile sea bream of three age/size groups were subjected to a starvation-refeeding regime and the expression patterns of genes involved in myogenesis, growth signaling and lipid metabolism were determined by qPCR.

Overall, SGR decreased with age. Notably, SGR during fasting was similar in all age groups. Metabolic shift to starvation-refeeding was age-related. Hierarchical clustering analysis also differentiated with age revealing significant correlations at younger age.

O7- Population genomics of the polychaete worm *Platynereis*, an interesting model to study evo-devo since the Cambrian

Simakov, O. Arendt, D.
EMBL Heidelberg, Heidelberg, Germany

We are developing an integrative 'eco-evo-devo' approach to aid the reconstruction of the functional cell type repertoire of the bilaterian ancestor and the study of its subsequent diversification. Most of the previous work was based primarily on developmental, expression and morphological comparisons lacking information from

small-scale (environmental and population) and long-scale (genomic) variation. Here I discuss a new integrative approach that combines comparative genomics studies of bilaterian genomes with eco-transcriptomics of the natural populations and a molecular study of cell-type development of the cosmopolitan polychaete *Platynereis dumerilii*. I will summarize the datasets we were able to acquire in the last years and describe an integrative framework for cell type evolution, its implication for hypothesis testing and possible extension to other species.

O8- Generation of mitochondrial proteome compendiums from marine species in search of the proteins comprising the permeability transition

Chinopoulos, C

Semmelweis University, Budapest, Hungary

Subjecting mammalian mitochondria to a pathological stress leads to a transition in their permeability, resulting in their demise, substantiated by the assembly of proteins with unknown identity. Mitochondria obtained from crustaceans, however, do not exhibit this phenomenon. Using organelle proteomic techniques we catalogue the mitochondrial proteins of species belonging to phylogenetic groups that exhibit this permeability transition, and from other marine species that do not display this phenomenon. Organelle proteomes are subsequently compared using phylogenetic profiling algorithms. A beneficial by-product of this work is the generation of compendiums of mitochondrial proteins from a variety of marine species. The availability of the emerging mitochondrial proteomes may improve the consensus of the phylogenetic tree for marine organisms.

O9- Cosmopolitan, common and rare marine Archaea: an experimental approach

Karayanni, H.; Kormas, K.A.; Genitsaris, S.; Moustaka-Gouni, M.

University of Thessaly, Greece, Volos, Greece

We investigated the dynamics of the archaeoplankton during a mixing experiment. Three coastal sea water samples, from two mediterranean sites (Banyuls [B], France and Pagasitikos [P], Greece, Gulfs) and from the Atlantic Ocean (Woods Hole, [W], USA) were inoculated in the same growth medium consisting of unamended particle free sterile water the coast of Banyuls. Triplicate 20l batch cultures were run for each of the three inocula, incubated at 180C in darkness until no further prokaryotic cell growth was observed (max. 21 days). 16S rRNA gene diversity was assessed by pyrosequencing analysis at days 0, 5 and 17. We analysed 2691 – 32114 tags per replicate per sampling point. Archaeal tags (97% similarity) in B, P and *W inocula* were 19, 24 and 43, respectively, with five being common. A total of 6, 5 and 1 tags from the inocula also occurred at d17 in the B, P and W treatment, respectively. Species richness increased 3-10 times at d0 compared to the inocula but decreased

up to 10 times at d5 and d17, but shared tags increased up to 36.5% at d5 and d17. We showed that marine *Archaea* from distant sites grow under the same environmental conditions, rendering them true cosmopolitans.

O10- New insight into the bioinorganic chemistry of *Ectocarpus*

Kuepper, F.C.; Böttger, L.H.; Miller, E.P.; Meyer-Klaucke, W.; Feiters, M.C.; Wellenreuther, G.; Matzanke, B.F.; Carrano, C.J.

Oceanlab, University of Aberdeen, Newburgh, United Kingdom

As heterokont organisms with a distinct evolutionary history, brown algae have a number of unique adaptations which are also reflected at the bioinorganic level. We would like to present new insights about the accumulation of trace metals and halogens in *Ectocarpus*. Iron is essential for all living organisms due to its ubiquitous role in redox and other enzymes, especially in respiration and photosynthesis. Genomic data suggests that *Ectocarpus* uses a ferrireductase-based uptake mechanism, but that it lacks ferritin for Fe storage. Short term radio-iron uptake studies verified that iron is taken up by *Ectocarpus* in a time and concentration dependent manner. Upon long term exposure two ⁵⁷Fe metabolites have been identified using Mössbauer and XAS spectroscopies. These include an iron-sulfur cluster accounting for approx. 26% of the total intracellular iron pool and a second component with spectra typical of a (Fe³⁺O₆) system with parameters similar to the amorphous phosphate-rich mineral core of bacterial and plant ferritins (around 74% of the cellular Fe pool). Therefore, we suggest that *Ectocarpus* contains a mineral-based, non-ferritin Fe storage pool.

O11- Environmental stress and oysters

Clark, M.S.¹; Amaral, A.²; Thorne, M.A.S.¹; Reis, J.²; Batista, F.³; Power, D.M.²

¹ British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, UK; ² Center of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; ³ Instituto Nacional de Recursos Biológicos, IPIMAR, Estação Experimental de Moluscicultura de Tavira, Vale Caranguejo, 8800 Tavira, Portugal

Evaluating organism responses to environmental stress provides us with important information in several areas: it enables us to understand their persistence in the natural habitat and provides us with measures of physiological flexibility and tipping points, which allow us to predict how they may respond under changing environmental conditions. This is vital given the current climate issues with changing sea water temperatures and the acidification of our oceans.

In terms of aquaculture species, which are maintained in open marine systems (such as oysters) this should allow us to predict potential culture problems, but also to forecast the best geographic areas for culture facilities over the next few decades.

This may lead to the development of new aquaculture facilities and industries in countries other than those traditionally associated with this type of industry.

In this project we studied the response of oysters (*Crassostrea gigas*) to 3 months culture in varied environmental conditions: summer temperatures (23°C), normal pH (pH 8); summer temperatures (23°C), low pH (pH 7.6); winter temperatures (18°C), normal pH (pH 8) and winter temperatures (18°), low pH (pH 7.6). We conducted both physiological measurements and high throughput transcriptomics using SOLiD sequencing in a combined study, the results of which will be presented in this talk.

O12- Temporal and spatial variation of *Crassostrea angulata* and *C. gigas* in southern Portugal

Batista, F.M.; Grade, A.; Power, D.M.; Lapegue, S.; Boudry, P.; Leitão, A.
Instituto Nacional de Recursos Biológicos, IPIMAR, Tavira, Portugal

F1 hybrids of the Portuguese oyster *C. angulata* and the Pacific oyster *C. gigas* occur under controlled conditions. This has raised questions about the risk of invasion and hybridization of imported *C. gigas* with the resident Portuguese oyster in pure populations in the Mira and Sado rivers. Samples were collected in the Sado river and the Guadiana river in Southern Portugal. DNA was extracted and individuals scored using mitochondrial and nuclear markers. No evidence of invasion by *C. gigas* of the natural *C. angulata* beds in the Sado river was found. A reduction in the *C. gigas* haplotype frequency between 2002 and 2011 in the Guadiana river, was associated with a high percentage of *C. gigas* and *C. angulata* hybrids. No tidal preference was detected between oysters carrying *C. angulata* and *C. gigas* mtDNA.

Acknowledgements: INTERREG Atlantic European Projects SEAFARE and ShareBiotech

O13- An Investigation of oyster electrophysiology

Harrison, A.P.¹; Batista, F.M.²; Power, D.M.³

¹ IVKH, Faculty of Health Sciences, Copenhagen University, Denmark; ² Instituto Nacional de Recursos Biológicos, IPIMAR, Estação Experimental de Moluscicultura de Tavira, Vale Caranguejo, 8800 Tavira, Portugal; ³CCMAR, Universidade do Algarve, Campus de Gambelas, Faro, Portugal

The adductor muscle, the mantle and the gills all play an active role in the regulation of water flow in the Oyster. In the current study, an ADInstruments PowerLab (4/25T) recording at 40,000 samples per second, in conjunction with 24 carat gold electrodes, as well as sterling silver electrodes, was used to record electrical signals from the adductor muscles, the mantle, the gills and the pericardium of cupped oysters *Crassostrea gigas* and *C. angulata*. Recorded signals were also assessed and confirmed using hand made hydrophones positioned close to open oysters. We found no evidence of an ECG in the intact, exposed or isolated hearts, although

neural signals preceding a muscular depolarization were evident, and these muscular depolarization signals related closely to the contracted state of the oyster heart.

Acknowledgements: Funded by the Seventh Framework Programme (FP7/2007-2013 grant agreement n° 227799- ASSEMBLE

O14- First observation of copulation by a barnacle dwarf male: implications for sexual strategy

Spremberg, U. University of Copenhagen, Copenhagen, Denmark

Barnacles are common and important marine animals because they dominate many communities such as the rocky intertidal and because of their impact on human society by being the most detrimental fouling organisms on man-made objects in the sea.

Here we use video microscopy on live specimens of the androdioecic barnacle *Scalpellum scalpellum* to document for the first time how a cirripede dwarf male extends a long cuticular penis (also documented by SEM) into the brood chamber of the partner, where it interacts with the cirri of the latter.

We have also observed how several males co-located on the same hermaphrodite can simultaneously attempt to copulate, highlighting male-male competition. The androdioecic status of *S. scalpellum* puts this species on stage as a possible intermediate in the evolution from hermaphroditism to dioecy or the reverse and therefore highlights its value for understanding the evolution of sexual strategies in general.

O15- Evolution and diversity of Acoela

Jondelius, U. Swedish Museum of Natural History, Stockholm, Sweden

Acoela are microscopic marine worms that are part of the meiofauna with many species living interstitially between sand grains. Acoels are of particular interest in zoology since they have been hypothesized to be the oldest bilaterian lineage. Except for the North sea area, the global acoel fauna is poorly known. Here I will present some new acoel species discovered with support from the ASSEMBLE project. I will also present an estimate of the total (largely unknown) global acoel diversity, i.e. total number of species, and show how the results from the fieldwork carried out with ASSEMBLE support influences this estimate.

O16- Biodiversity of marine diatoms: morphological and molecular characterization

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Diatoms are important primary producers that play a crucial role in global marine ecosystems. The aim of the ASSEMBLE project “Biodiversity of marine diatoms:

morphological and molecular characterization (BIOMARDI)“ was to investigate the planktonic species from the Adriatic Sea belonging to the genera *Pseudo-nitzschia*, *Chaetoceros* and *Bacteriastrium*. The general morphology and ultrastructure of these diatoms were studied with light and electron microscopy and compared with the information from databases, field samples and culture collection available in the host institution (SZN). The results revealed the existence of 9 *Pseudo-nitzschia* species of which 6 are considered to be potential producers of toxin domoic acid. In addition to the morphological analyses the *Bacteriastrium* taxa were also analysed in terms of molecular phylogeny. In one of the investigated species, *B. jadrantum*, we discovered and described a novel strategy of colony formation apparently unique among marine planktonic diatoms.

O17- Studying fresh water and marine biofilms

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Benthic surfaces in marine and fresh water habitats can be covered with biofilms, which are often dominated by diatoms and bacteria. Within the framework of ASSEMBLE we have performed two projects focusing on biofilms. We have isolated diatom strains from the Red Sea at the IUI Station in Eilat that were taken from stones at different depths. We wanted to find out whether diatom/bacteria interactions are stronger in oligotrophic waters. In a second project scheduled in September 2012 cryopreservation of diatoms will be studied at the SAMS marine station in Oban. Molecular projects with diatoms strongly depend on the availability of the respective strains. Although many diatoms can be kept in culture over longer periods of time, some strains may become smaller and smaller. Therefore it is essential to develop protocols to maintain and revive frozen diatom cells.

O18- Diversity and dynamics of marine haptophytes

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Haptophytes are microalgae and a major component of marine plankton communities. Our aim is to provide new knowledge on the diversity and distribution of haptophytes below the genus-level, and how this is affected by environmental factors. We have developed and tested methods for analysing diversity using 454-pyrosequencing, We designed haptophyte-specific rDNA primers and constructed haptophyte databases and phylogenies for taxonomic assignation. We sequenced an artificial haptophyte community and compared the diversity and relative abundances from DNA and RNA/cDNA using various rDNA primers, and different programs to

remove errors from sequence data. We applied 454-pyrosequencing to samples from six European coastal sites and seasonal samples from Oslofjorden, and combined this with electron microscopy. We propose a strategy to more accurately depict the haptophyte diversity using 454-pyrosequencing and present results using this strategy.

O19- Can we use coralline algae to reconstruct ENSO and associated biotic changes?

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Red coralline algae have been used to show biotic-climate relationships in the north Atlantic and provide an ideal tool for investigating historic ENSO associated upwelling events and their relationships to biotic change. ENSO cycles can be associated with significant rainfall which reduces coastal water salinity over several days. Thus, before reconstructing upwelling events using coralline algae we investigated if changes in salinity affected the accuracy of palaeoclimatic signals recorded by the algae. Intertidal and subtidal coralline algae from Chile were exposed to falls in salinity simulating ENSO associated runoff events. Stress levels in the algae were monitored by assessing intracellular dimethylsulphoniopropionate (DMSP) concentrations and recording algal photosynthetic yield. Salinity did not significantly alter intracellular DMSP concentrations or photosynthetic yield suggesting no substantial stress responses by the algae. Absence of freshwater induced stress suggests red coralline algae faithfully record palaeoclimatic signals and sets the scene for understanding ENSO associated upwelling and associated biotic changes.

O20- The Potential Impact of Coralline Algae Habitats on Local Climate

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Algae produce dimethylsulphoniopropionate (DMSP) and its derivative dimethylsulphide (DMS). Atmospheric DMS promotes cloud formation, impacting local climate. Until recently, dinoflagellates in corals were considered the main benthic DMSP producers. We have discovered that coralline algae are another major benthic producer of DMSP so their influence on DMS-driven climatic effects may be considerable. We investigated concentrations of DMS/P over coralline algae (coralligene biotopes), sea grass and sand from 3 to 65m near Banyuls-Sur-Mer, France. Water above sandy areas contained variable DMS/P concentrations (13-25 $\mu\text{mol /L}$), while water above sea grass contained low DMS/P concentrations ($\sim 12 \mu\text{mol /L}$) possibly behaving as a sink. Water above coralligene biotopes contained consistently high DMS/P concentrations at all depths. DMS/P concentrations were all higher than those found in pelagic waters during phytoplanktonic blooms ($< 3 \mu\text{mol /L}$).

/L). Coastal waters, and in particular waters above coralligene habitats, may be important sources of atmospheric DMS/P fluxes with potential influences on local cloud formation and thus local climate.

O21- Swimming behaviour in pelagic crustaceans: sleepy krill and climate change.

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Developing an understanding of the behaviour of pelagic crustaceans can be challenging. They are difficult to observe in the wild without them reacting to the observer and they are generally not comfortable in aquaria. During a summer campaign in Kristineberg Marine Station we developed a new method that allows the researcher to quantify and analyse swimming activities of the Nordic krill (*Meganyctiphanes norvegica*). We demonstrated that there were sex-related and moult stage related differences in behaviour. Subsequent to this we were able to take the technique to sea and apply it to Antarctic krill (*Euphausia superba*). Here we demonstrated that krill suffer from post-prandial narcosis and may sequester more carbon than we previously thought. In our most recent trip to the waters around South Georgia we have developed a new piece of software that allows detailed analysis of swimming behaviour at sea using short term Fourier transform.

O22- Capture rates of the Cold Water Coral *Lophelia pertusa* on living phyto and zooplankton under different current speed regimes

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To determine prey capture rates by the cold water coral (CWC) *Lophelia pertusa*, under conditions designed to simulate the natural environment, feeding experiments have been conducted with colonies of *L. pertusa* from the Mingulay reef complex (Sea of Hebrides, UK). Potential prey items used were a culture of the marine diatom *Skeletonema marinoi* and freshly captured zooplankton. This is the first time feeding trials with *L. pertusa* have been carried out using living algae and naturally-occurring zooplankton as opposed to aquarium-grown prey (e.g. *Artemia salina*) or frozen food material. The experimental apparatus consisted of four circular tanks (three experimental tanks and a control); in each tank current speed was regulated, and in order to test the live prey capture efficiencies, three different current speeds (2 cm.s⁻¹, 5 cm.s⁻¹ and 10 cm.s⁻¹) were used for each experiment. Our results shows clear

differences between the three current speeds. *Lophelia* successfully captured algae under 5 cm s⁻¹ and zooplankton between 2 and 5 cm s⁻¹ whereas at the higher current speeds both algae and zooplankton capture could not be significantly detected. These results will be discussed in context to the natural environmental conditions of this CWC species.

O23- Effects of stressful environmental factors in lagoonal ecosystems on marine microbial and invertebrate populations

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Lagoons are extreme and dynamic ecosystems characterized by vigorously changing temporal and spatial conditions. Lagoons host a number of important human activities inside and around their limits, and as a result such fragile environments may be subjected to intense stress. Stress responses of benthic populations and bacterial communities were investigated on three different levels: a) Imposex implications of gastropod species were examined, b) Genetic structure of polychaete communities was investigated through process of COI gene and c) Bacterial DNA was analyzed with cultivation-independent approaches to depict the bacterial community composition. The aim of the present study was to elucidate the role of stress factors on the benthic communities of lagoons and to compare responses between lagoonal communities of different geographical areas.

O24- Biological effects of xenobiotics on marine organism

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It has been well established that many environmental contaminations can act as toxicants and marine organism appears to be sensitive to the effects of xenobiotics. Toxicant can compete with metabolism for their nuclear and cellular bindings sites. The molecular and cellular actions of these toxicants have been tested in our laboratory. Tributyltin (TBT) is known to exert toxic effect on herring spermatozoa viability, lactate dehydrogenase activity and cause creatine kinase release from damaged spermatozoa. Cadmium is one of the most harmful heavy metals to animals and is an environmental contaminant in sea water. Cadmium shows strong inhibition of the malic enzyme in herring spermatozoa and could influence viability and motility of spermatozoa in the water of Gdansk Bay, region where herring spawning occurs. Mutagenic effect of some xenobiotics can be tested on marine bacteria *Vibrio harveyi* A16 strain using Ame's mutagenicity test.

O25- Developing hypothalamus-pituitary-thyroid axis disruption in zebrafish by common aquatic contaminants

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loxynil (IOX) and diethylstilbestrol (DES) are common contaminants in aquatic systems. Previous studies indicate IOX and DES disrupt the hypothalamic-pituitary-thyroid (HPT) axis in teleosts but via a non-goitrogenic effect. We hypothesize that the effect of both IOX and DES is through their action on the differentiation state of tissues and cells of the HPT axis rather than through disruption of central thyroid hormone regulation. Experiments revealed that neither IOX nor DES disrupted ZF hypothalamic development but both modified thyrocyte development. The inhibitory effect of DES on ZF thyroid development only occurred at low concentrations. The inhibition of thyrocyte development by IOX (1 and 0.1 μ M) and DES (0.1 μ M) was correlated with altered heart morphology and may indicate an indirect action via altered cardiac development.

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O26- The impacts of bisphenol A (BPA) on response genes involved in chemical defensome and cell-cycle regulation during rocky (*Paracentrotus lividus*) sea urchin embryonic development

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Global usage of bisphenol A (BPA) in production of polycarbonate plastic resulted with high concentrations and negative effect of this synthesized chemical in the environment. BPA is severe endocrine disrupting compound and it has high toxic impact on aquatic organisms. Our research focused on determination of toxic effects of BPA on embryonic stages (2-cell, pluteus) of rocky sea urchin. The main goals were: real time quantitative PCR (qPCR) measurement of target genes expressions involved in chemical defensome: multixenobiotic resistance (MXR) mechanism (P-glycoprotein/P-gp, Multidrug Resistance-associated Protein/MRP); endocrine disruption (orphan Steroid Hormone Receptor/SHR2); and cell-cycle regulation (Cyclin B, CDK); insight of ultramorphological changes by transmission electron microscopy (TEM). qPCR results indicate the importance of Pgp as the main member of MXR mechanism in defense against BPA while upregulation of SHR2 gene expression clearly indicate endocrine disruption. TEM results strongly support the hypothesis that higher sublethal concentration of BPA induces disorder in karyokinesis and developmental retardation.

O27- Assessing the genetic impact of sea urchin restocking

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Human exploitation has drastically reduced the abundance and distribution of several invertebrate populations through overfishing and habitat destruction. Restocking can potentially mitigate these impacts and help to reconstitute depleted stocks but genetic repercussions must be considered. In the present study, the degree of genetic similarity between sea urchin (*Paracentrotus lividus*, Lamarck 1816) individuals reared for restocking purposes and the receiving populations in North of Spain was assessed using 9 microsatellites.

The farmed populations (CIMA & CEP) showed important reduction in the number of alleles, which could indicate an incipient inbreeding. Both the farmed populations and the target ones for restocking (Finisterra, Punta de la Cruz and Laredo), showed significant values of genetic differentiation due to different allele frequencies, number of private alleles and total number of alleles. Also, lower values of observed and expected heterozygosity were found in reared populations specially in CEP.

These findings indicate a low degree of genetic similarity between reared and sink populations, therefore this restocking initiative is not advisable. Thus it is recommended to utilize broods from the same population for restocking purposes to provide a better genetic match to the wild populations.

O28- Impacts of Ocean Acidification on early stages of the crustose coralline alga *Phymatolithon lenormandii*

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Coralline algae (Corallinales, Rhodophyta) are predicted to be negatively impacted by near-future ocean acidification. The effect of low pH / high pCO₂ on early life stages of *Phymatolithon lenormandii* (Areschoug) Adey was studied in a perturbation experiment, carried out by the Sven Lovén Centre for Marine Sciences, Kristineberg, Sweden. Several parameters including mortality, fecundity (spore production and germination), calcification (calcein staining) and development (growth and abnormalities) have been followed for a month under three acidification scenarios: control pH (8.1), -0.1, -0.2 and -0.4 pH units. Our results demonstrate that survival and growth of *P. lenormandii* is impacted by small pH changes (Δ = -0.1 pH unit). A negative impact of decreasing pH was observed on all tested parameters including a decreased growth, an increased mortality and a higher rate of morphological

abnormalities. A positive growth and calcification was observed at the lowest pH ($\Delta = -0.4$ pH unit) but the maintenance of the skeleton under low pH was only possible through a dynamic dissolution/calcification process, an energetically costly mechanism potentially draining resources from other vital processes.

O29- Ocean acidification impacts regeneration of Red Sea corals

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Ocean acidification (OA) is threatening the future of coral reef ecosystems. Mounting experimental evidence suggests that OA negatively impacts fundamental life functions of scleractinian corals, including growth and sexual reproduction. Although regeneration is regarded as a chief life function in scleractinian corals, the effect of OA on regeneration processes has not yet been investigated. Regeneration is critical to the persistence and resilience of coral reefs and is essential to coral survival. The rapidity of this process is vital, since fast lesion healing prevents settlement and subsequent overgrowth by competing organisms or total disruption of the physiological integration capacity of the colony. To evaluate the effects of OA on regeneration, the common Indo-Pacific corals *Favia fava*, *Porites sp.*, *Acropora eurystoma* and *-Stylophora pistillata* were inflicted with lesions and placed in three pCO₂ levels: ambient seawater, medium and high pCO₂ conditions. The long-term study examined physiological, histological and photosynthetic parameters. Results revealed all coral species with significantly higher tissue regeneration in ambient conditions as compared to medium and high pCO₂ conditions. Specifically, regeneration of *Porites sp.* decreased by 42% and 54% in medium and high pCO₂ treatments, respectively, compared to ambient conditions. Additionally, the dark-adapted maximal quantum yield (F_v/F_m) measured in *Porites sp.* was reduced by 14% and 10% in medium and high pCO₂ treatments, respectively, compared to ambient conditions. This study demonstrates that OA could have a compounding influence on coral regeneration following injury infliction, potentially affecting the capacity of a reef to recover following environmental disturbance.

O30- Changes in microbial communities associated with Cnidarian in a natural pH gradient

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Ocean acidification, resulting from rising atmospheric carbon dioxide concentrations, is a pervasive stressor that can affect many marine organisms including their symbionts. Studies which examine the host physiology and microbial communities

have shown a variety of responses to the ocean acidification process. Recently, several studies were conducted based on field experiments, which take place in natural CO₂ vents, exposing the host to natural environmental conditions of varying pH.

Two Mediterranean coral species, *Balanophyllia europaea* and *Cladocora caespitosa*, were transplanted and exposed to the natural pH gradient. In addition the study examines the sea anemone *Anemonia viridis* which is naturally found along the pH gradient in Ischia (Italy). The physiological parameters of *A. viridis* and its microbial community were monitored.

O31- Plastic Fantastic: Plastic Responses to Ocean Acidification in 16 Ecotypes of a Globally Distributed Picoplankton, *Ostreococcus tauri*

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Phytoplankton are the basis of marine food webs, and affect biogeochemical cycles. As CO₂ levels rise, shifts in the frequencies and physiology of ecotypes within phytoplankton groups will affect their nutritional value and biogeochemical function. Here, we measure changes in cellular function and growth rate at atmospheric CO₂ concentrations predicted for the year 2100 in 16 ecotypes of the marine picoplankton *Ostreococcus*. We find that variation in responses among ecotypes is on par with published between-genera variation, indicating that single or few ecotypes cannot be used to estimate the future physiology or composition of a species, and thus its response to CO₂ enrichment. We show that ecotypes best at taking advantage of CO₂ enrichment by changing their photosynthesis rates most should increase in frequency in a high-CO₂ environment. Finally, information on sampling environment, and not phylogenetic relatedness, is a good predictor of ecotypes likely to increase in frequency.

O32- Effect of different CO₂ and N regimes on the seagrass *Cymodocea nodosa* associated microbial community

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The importance of seagrasses in the coastal environment is well known, but the response of the meadows to global change is still poorly understood. The analysis of microbial communities under different CO₂ and N regimes can provide important information about the contribution of the microbial component to the carbon/nitrogen cycles under global warming or organic pollution conditions. For this purpose, the modified SSCP molecular technique and the subsequent identification of the associated microbial community 16S rDNA to the seagrass *Cymodocea nodosa*

have been utilized in a case control study. The first phase of this work has been performed under the ASSEMBLE framework in the Ramalhete Marine Station in Faro (Portugal), where mesocosms are maintained at controlled CO₂ and N regimes. The molecular analysis has been performed in the Tor Vergata laboratory, Rome (Italy). Samples of *Cymodocea nodosa* (leaves, roots, rhizomes) were collected from both the mesocosms and in the field, in summer and winter. The metagenome from the microbial communities associated with the different plant compartments has been extracted and analyzed. SSCP and band sequencing have provided information about the community structure. This study highlights the role of microbes in seagrass ecosystems under different environmental conditions, as global warming and/or organic pollution.

O33- Exploring the frontier of microbiology thanks to ASSEMBLE.

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Historically, the term "virus" characterizes the smallest parasitic microorganisms. It is only recently that the concept of giant virus (an oxymoron) was introduced after the discovery of *Mimivirus*, a DNA virus possessing genome of more than 1.2 Million nucleotides enclosed in a micron-sized particle. Few years later, it is now clear that the largest viruses are found in aquatic environments where they infect protists of all major eukaryotic clades. We recently identified the largest of all giant virus, called *Megavirus chilensis*, off the coast of Chili near the Las Cruces marine station. The detailed genomic analysis of this new virus confirmed our previous hypothesis that there was a link between the origin of these viruses and the emergence of eukaryotes. Even more intriguing marine microorganisms were isolated during the same campaign, indicating that totally unexpected life forms are still in the sea to be found.

O34- Peripheral oscillators in fish. The sea bass pituitary as a clock and its regulation by melatonin and cortisol

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The *Fish Neuroendocrinology Research Group* of the Complutense University of Madrid investigates the cross-talking between fish circadian and endocrine systems, with emphasis in hormones related with feeding and stress. Currently we study the interaction between cortisol, orexin and ghrelin, with different central and peripheral oscillators in the goldfish (*Carassius auratus*). In order to extend this basic research to aquaculture applications, it is important the use of species with interest in aquaculture, as the sea bass (*Dicentrarchus labrax*). The aim of our Assemble Access

Projects in the *Laboratoire Aragó* was to study the putative role of the pituitary as a part of the sea bass circadian system. We have performed two approaches: cell cultures and organotypic cultures. The optimal conditions to perform the culture of pituitary cells were not found. The pituitary organotypic cultures revealed that melatonin and cortisol could regulate the expression of some clock genes and pituitary hormones in the sea bass.

O35- Laboratory cephalochordates: the beginning of a new experimental era in amphioxus research

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The chordate amphioxus is a key model system to understand vertebrate evolution. It has been long used in comparative anatomy and developmental biology and more recently in molecular genetics. This long-standing interest in amphioxus has generated a demand over the last 200 years to keep amphioxus in the lab, and currently, to implement functional assays in such a remarkable model. In response to this demand, we have designed, constructed and experimentally validated the first standardised facility for amphioxus husbandry. The facility solves all major drawbacks associated to the practical use of amphioxus as a model system in molecular biology, which are mainly characterised by a limited access to amphioxus material due to the natural constraints of wild populations. Our inland marine facility brings unprecedented possibilities of generating amphioxus lab-based colonies, which are able to breed for significantly longer periods than in nature, providing a regular and controlled source of embryos through the year to implement functional assays in this model. Funding from Assemble was key to understand and reproduce a fairly natural marine ecosystem inland, and to introduce amphioxus from disparate sites in order to develop a normalised system for long-term culturing of virtually any known cephalochordate.

POSTERS

P1- EuroMarine - Integration of European Marine Research Networks of Excellence

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EuroMarine is a FP7 coordination and support action designed to bring together the three FP6 marine Networks of Excellence (NoE) communities; EUR-OCEANS, MarBEF and Marine Genomics Europe. In doing so, EuroMarine will provide the best expertise and innovation available in European Marine Research, that can respond rapidly to societal needs, environmental demands, well-being and sustainability.

The Challenge

Many questions in marine research can only be answered using multidisciplinary methodologies, from the molecular level with genomics and other new emerging technologies integrated together with an ecological, physical and biogeochemical ecosystem approach. This allows us to address novel questions in marine research, paving the way to new and more integrated knowledge systems that impact the way human society deals with the oceans. The challenge of EuroMarine will be to bring marine sciences into the multidisciplinary perspectives of the 21st century. The EuroMarine consortium will build and strengthen a community of marine scientists from marine laboratories in Europe, building the academic foundation for marine research that is the base for innovation.

P2- Prokaryotic phospholipase A2 structural domain is conserved in Cnidaria

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Phospholipases A2 (PLA2) are intracellular or secreted enzymes involved in many physiological and pathological functions, such as digestion of dietary phospholipids, inflammatory reaction and antimicrobial defence. PLA2 members were recently classified according to their structural domains in a novel classification system, and the conserved domain pfam09056 Phospholip_A2_3: Prokaryotic PLA2 was identified in secreted PLA2s in bacteria and fungi and also in representatives of the basal animal Phylum Cnidaria including sea anemone *Nematostella vectensis*, hydroid *Hydra magnipapillata* and coral *Acropora millepora* and subsequently lost during evolution. This raises questions about their functional roles and the evolutionary pressures that lead to gene elimination from protostome and deuterostome genomes.

P3- OSR but not density affects the strength of sexual selection in a marine fish

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The operational sex ratio (OSR) and density are considered important factors affecting the strength of sexual selection. We manipulated the OSR and density in a total of 36 experimental breeding populations of the two-spotted goby (*Gobiusculus flavescens*) – a fish with exclusive paternal care. Our treatments consisted of three different operational sex ratios, each at two different densities. We measured mating competition behavior, the opportunity for selection, and selection on four phenotypic traits in males. As predicted from operational sex ratio theory, males became more competitive with increasing female scarcity. In line with this prediction, both the opportunity for selection and realized selection on phenotypic traits increased in males with increasing female scarcity. By contrast, density did not significantly affect the strength of sexual selection. We conclude that the operational sex ratio but not density matters for the strength of sexual selection in this species.

P4- Marine eukaryotic metagenetics: methodological insights

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Eukaryotic diversity in environmental samples is often assessed via PCR-based amplification of nSSU genes. However, estimates of diversity derived from pyrosequencing environmental data sets are often inflated, mainly because of the formation of chimeric sequences during PCR amplification. We have analyzed the effect of sample richness, evenness and phylogenetic diversity on the formation of chimeras using an nSSU data set derived from 454 Roche pyrosequencing of replicated, large control pools of closely and distantly related nematode mock communities, of known intragenomic identity and richness. Chimera formation in eukaryotes was higher in both richer and more genetically diverse samples, and compared with prokaryote datasets gives a different perspective of chimera formation in pyrosequenced environmental data sets. Further to this, methods to prepare and analyse massive pyrosequencing datasets in this case from European coast samples were tested using different pipelines and in silico approaches. The study contributes to a better understanding of the impact of sampling strategies, inherent PCR-amplification errors and downstream in silico analysis, and gives insight into the pitfalls that can lead to misinterpretation of diversity levels.

P5- Quantification and in situ localization of *ABCB1* and *ABCC9* genes in toxicant-exposed rocky sea urchin (*Paracentrotus lividus*) embryos

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In sea urchin embryos ABC binding cassette (ABC) transport proteins present an efficient defense mechanisms. Still little is known on the expression of these genes during different embryonic stages. Our research focused on real time quantitative PCR (qPCR) measurement and gene localization of two ABC transporters – P-glycoprotein (*ABCB1*/P-gp) and sulfonylurea receptor like protein (*ABCC9*/SUR-like), during embryonic development (first 10.5 hours) of *P. lividus*. Developmental stages (egg, zygote, 2-cell, blastula) were incubated in sub-lethal concentrations of three environmentally relevant pollutants: oxybenzone (50 μ M OXI), mercuric chloride (300 nM HgCl₂) and tributyltin (100 pM TBT). In general, we evidenced an increase in mRNA transcript levels of both ABC transporters. Highest increase level was observed in 2-cell stage (*ABCB1* - 5.97-fold increase with 50 μ M OXI; *ABCC9* - 12.81-fold increase with 300 nM HgCl₂) and overall decrease in blastulae stage. *In situ* hybridization depicted ubiquitous localization of both transcripts within embryo cells, supporting qPCR data and fortifying their protective role in early embryonic development of *P. lividus*.

P6- Hormones, reproductive behaviour and secondary sexual traits in black gobies

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Males compete for female access. This commonly results in the emergence of dominants and sub-ordinants. Typically, dominants are larger and show pronounced secondary sexual traits, while subdominants partly compensate for their behavioural inferiority by enhanced sperm traits. Dominants commonly have elevated plasma androgen levels. It is unclear whether hormonal levels are raised due to dominance or whether dominance causes increased hormonal levels. To study this question wild black goby (*Gobius niger*) males were caught nearby Kristineberg Research station, Sweden. Post-capture fish were distributed into experimental tanks in groups of three; one large, 'naturally' dominant male and two medium-sized males. In each tank one of the latter males received a hormonal implant. Fish were then observed for behaviour for one week. After termination of the experiment secondary sexual traits were examined. Implanted males were outcompeted by the large natural dominants and behaved similarly to the non-implanted males, but their gonad characteristics resembled that of dominants.

P7- Mapping the viscerocranium during sole metamorphosis

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Flatfish metamorphosis is the most dramatic post-natal event in vertebrates. Driven by thyroid hormones (TH) the symmetric pelagic larva becomes an asymmetric benthic juvenile. During this process one eye migrates to the future dorsal side of the animal leaving the ventral side “blind”. The major tissues of the trunk, muscle and skin, appear to retain symmetry as assessed by histological and molecular markers. This led us to hypothesise that metamorphosis drives asymmetry in the head during flatfish metamorphosis. To test this hypothesis fluorescent light microscopy and immunohistochemistry is being used to map changes in the head during metamorphosis. The results suggest that asymmetry in the cranium is restricted to the eyes and accessory structures and that other muscle and skeletal structures remain symmetric.

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P8- Provision of access to biological materials – CCAP providing Remote Access and Transnational Access

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Biological materials provide the fundamental building-blocks on which science is developed. A key facet of the ASSEMBLE project has been the provision of access to marine biological materials. The Culture Collection of Algae and Protozoa (CCAP) at the ASSEMBLE partner institute of SAMS, has to date provided 156 cultures of algal, cyanobacterial and protozoan strains for 15 remote access (RA) projects. The cultures provided encompassed a broad taxonomic diversity including: cyanobacteria, brown seaweeds, diatoms, dinoflagellates, green microalgae, fungal seaweed pathogens, and colourless heterotrophic flagellates. Additionally, CCAP has hosted, or been responsible for providing biological materials for 18 out of the 35 transnational access (TA) proposals accepted for SAMS. The aims of these projects covered a wide spectrum of scientific topics including projects as diverse as: investigating effect of iron concentrations on cultures, cryopreserving algae, looking for biotech potential products in cyanobacteria, characterising polar cyanobacteria, feeding trials for cold water coral and more. During the lifetime of the project support protocols for visitors were developed and refined to provide a fit-for-purpose flexible system.

P9- Cryopreservation of *Phaeocystis antarctica*

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The prymnesiophyte genus *Phaeocystis* plays a crucial role in the ecology and biogeochemistry of almost all marine ecosystems. The two cold water colony-forming species of this genus, *P. pouchetii* in the Arctic and *P. antarctica* in the Southern Ocean, are known to be key species within their habitats. To address questions on the genetic diversity and ecological response patterns of these important taxa large numbers of clonal isolates have been established in a unique culture collection at the Alfred Wegener Institute in Bremerhaven, Germany. However, at present the wider scientific community cannot access these strains and their long-term conservation, after the project funding ends, cannot be guaranteed. Cryopreservation (ultra-low temperature storage) could be the answer to these problems as it could guarantee the long-term security of this genetically and ecologically interesting collection. Thus ensuring their availability, and accessible, to the wider scientific community. Preliminary results and the success of different cryopreservation methods including the application of novel non-penetrating cryoprotective strategies will be presented.

P10- Ocean acidification and gene expression in echinoderms

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Recent research indicates that ocean acidification (OA) will impact many marine organisms. OA is an anthropogenic global stressor and, despite intensive study, its short and long term impacts on marine biota are unclear and many open questions remain. Most OA research has focused on the effects of decreased pH on calcification rates of calcifying organisms. Relatively few studies were done at the genetic level and predictions about possible changes in gene expression under projected OA conditions are very limited. The proposed research will examine the effects of OA on echinoderm genetics as OA most likely has a species specific impact on echinoderms (Dupont et al., 2010). Echinoderms are keystone species in the marine ecosystem as ecosystem engineers and bioturbators (grazing sea urchins and sea stars), and as part of the food chain (prey for carnivorous fish and crustaceans). Sea urchins are also exploited for food in many coastal areas. Thus, this study will enhance the basic knowledge of the effects of projected OA on gene expression in echinoderms

P11- Evaluation of the potential of different cryptophytes for aquaculture applications

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The high nutritional value of several species of the class *Cryptophyceae* such as *Rhodomonas salina*, *Rhodomonas lens* and *Rhinomonas reticulate* has been previously demonstrated in the culture of copepods, *Artemia* and mollusk larvae (Leonardos and Lucas, 2000; Dahl et al., 2009; Seixas et al., 2009; Guevara et al, 2011). The limitation in the use of these species of cryptophytes is mainly due to the difficulties in maintaining stable mass cultures with adequate productivities.

The first objective of this work was to compare the productivity and the biochemical composition of continuous cultures of three cryptophytes: *Chroomonas placoidea*, *Rhodomonas salina* and *Rhodomonas lens*. In a second part our aim was to compare the growth of the rotifer *Brachionus plicatilis* when fed with monoalgal diets of two of these cryptophytes *R. lens* and *C. placoidea* with the growth obtained with the Eusmagtophyceae *Nannochloropsis gaditana*, a species commonly used in aquaculture for this purpose.

Microalgae were cultured using Algal media (Fábregaset al., 1986) at a concentration of 8 mg atom N/L. All species were cultured semi-continuously in quadruplicates with a daily renewal rate of 30% of the volume of the cultures at 21.5±1.5°C.

B. plicatilis was cultured in flasks containing 450ml of autoclaved seawater, with an initial density of 50 rotifers mL⁻¹. Three replicates were set for each diet. The food ration was calculated as an equivalent of 400 ng of dry weight per rotifer per day, which is equivalent to 4000 cells rotifer⁻¹ for *C. placoidea*, 3700 cells rotifer⁻¹ for *R. lens* and 66700 cells rotifer⁻¹ for *N. gaditana*. The number of rotifer and eggs was counted daily during 5 days.

The highest productivity in steady-state was obtained by *R. lens*, followed by *C. placoidea* and *R. salina* (0.34; 0.31 and 0.28 g L⁻¹ day⁻¹ respectively). The highest protein content was obtained by *R. lens* with 56% of dry weight, followed by *R. salina* (48%) and *C. placoidea* (38%) The highest values of polyunsaturated fatty acids (PUFAs) were obtained with *R. lens* and *C. placoidea* (62 and 60%, respectively). *R. salina* presented the lowest PUFAs content (44%). The best growth of rotifers was obtained with *C. placoidea*, reaching 230 rotifers ml⁻¹ at the end of the experiment, which is almost twice the rotifer density achieved with *R. lens* and *N. gaditana*, both with 145 rotifers ml⁻¹. Both species of cryptophytes promoted a higher egg production than *N. gaditana*.

Results demonstrate the feasibility of mass production of cryptophytes and their potential, for feeding filter-feeders in aquaculture, pointing out *C. placoidea* as a specially interesting species.

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P12- Improving the provision of cell lines of marine animals

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As part of the joint research activity performed within the scope of ASSEMBLE, primary cell cultures and cell lines of various tissues of marine animals – e.g. ray-finned fishes, cephalochordates, urochordates and echinoderms – were developed. Protocols towards improved cell isolation, dissociation and propagation, efficient delivery of DNA into the cells for functional genomics and successful cryopreservation for long term storage were established and made available to the scientific community as part of ASSEMBLE virtual tool box. Following the development of cell lines/cultures from these marine animals, particular processes were investigated and marker genes specific for these processes were determined. An interactive form was created to catalogue cell cultures/lines available within the scope of ASSEMBLE WP14 and to provide a list of cell type-specific marker genes. Cell lines/primary cultures developed within the scope of WP14 have been used for ASSEMBLE transnational access (TA).

P13- Cephalopods and the law: Implications of Directive 2010/63 / EU for research

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From 1st January 2013 when Directive 2010/63 / EU comes into force “all living cephalopods (adult and larval forms)” will receive the same legal protection in research as has been in place for vertebrates for over 100 years in several European countries. The main practical implications for cephalopod researchers will be: implementation of 3Rs (Reduction, Refinement, Replacement) principles and formal project (“ethical”) review; animal supply (capture from the wild); housing and handling; humane anaesthesia & euthanasia; objective criteria for pain, suffering, distress and lasting harm. Training programmes to support Directive implementation will also need development. CephRes coordinating cephalopod researchers (<http://www.cephres.org/>) and a cephalopod working group of the Boyd Group (<http://boydgroup.wordpress.com/>) in the UK are working with FELASA to develop evidence based “Guidelines” covering all aspects of the “use” of cephalopods in the laboratory to provide researchers with examples of best practice to ensure optimal welfare and conduct of research studies. The initiative will involve input from researchers in several ASSEMBLE sites.

P14- Cold, dark and salty: How to successfully maintain cold water coral *Lophelia pertusa* in the laboratory

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Success in cold water coral husbandry at SAMS has been greatly facilitated through the ASSEMBLE programme. We now have a holding facility for *Lophelia pertusa* in excess of 500 coral nubbins and are able to provide animals year round to visiting scientists. Achievements include: 1) Improved collection success of *Lophelia* from the Mingulay Reef Complex using a modified video grab; 2) a dedicated new *Lophelia* holding facility with a semi-open recirculation system and salinity mixing system; 3) automated seawater parameter logging accessible by web with alarms at critical points in system to email or text; 4) coral feeding which has been optimised and consists of live algae *Skeletonema marina* and *Artemia* supplemented with frozen krill and red calanus and finally; 5) recreating simulated *Lophelia* field conditions of flow using coral Vortex Resuspension Tanks. Our improved facilities and understanding of the corals will enable us to now provide these animals well into the future.

P15- Measuring the environmental interactions of mussel farms and motile megafauna using fatty acid biomarkers

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Aquaculture is considered as one of the most promising approaches to counter the global problem of stagnating fishery yields and constantly rising demand for seafood. At the same time, the impact of aquaculture on associated ecosystems, in particular mussel aquaculture, is discussed controversially: As on the one hand side mussel culture can increase the benthic diversity below the culture ropes, the associated organic enrichment can - on the other hand side - have a negative impact due to organic overloading of the sediment.

This study aims at the detection of the direct nutritional influence of mussel culture (*Mytilus edulis*) on the starfish *Asterias rubens*. As originally benthic organisms, mussels on culture ropes are detached from their usual substrate. Nevertheless they still influence the benthic ecosystem in their vicinity through sedimentation and fall off (pseudofaeces as well as entire organisms) from culture ropes. Simultaneously *Asterias rubens*, one of the main predators of blue mussels occurs in high densities below mussel culture lines.

The impact of mussel culture on starfish exhibits not only effects in an ecological and trophic sense, but also affects species biochemistry. Therefore the tissue composition of

an affected benthic key species is of substantial interest. We use fatty acid biomarkers to examine changes in tissue biochemistry of the starfish *A. rubens* in the vicinity of and apart from mussel farms.

Due to the optimal food and oxygen condition in their artificial pelagic habitat, mussels have higher production rates and a better condition than wild mussels. Therefore we expect differences in fatty acid content and composition of the respective associated starfish. Differences in tissue composition and applicability for fatty acid biomarkers are identified. Furthermore, the effect of habitat on the fatty acid pattern of selected starfish tissues are shown.

We conclude that fatty acid biomarkers are valid tools to characterise the impact of aquaculture on associated ecosystems without long term monitoring studies.

P16- Effects of ocean acidification in a warming climate on species interactions at distribution boundaries: mechanisms and consequences at ecosystem

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Previous research on the effects of hypercapnia on marine fishes led to the general notion that due to their powerful mechanisms of ion regulation they are not particularly vulnerable to ocean acidification. However, several studies demonstrated chronic effects of environmental hypercapnia on juveniles, and a sensitivity of eggs and early life stages higher than in adults. For an integrative analysis of ocean acidification and warming (OAW), it is therefore important to consider the different sensitivities of the various life stages, as well as their interaction with their prey organisms and thus, their dependence on the foodweb. We describe here our approaches within the second phase of the German programme BIOACID (2012-2015) to investigate how the combined effects of OAW will affect different life stages and interactions between Atlantic and Polar cod and their prey.

Objectives include addressing the question whether OAW affects interacting species differently due to divergent physiological optima and ranges, expressed in thermal tolerance windows and associated performance capacities and phenologies of specific life stages. We aim to identify fundamental mechanisms by unravelling the connections between levels of biological organisation, from genomic, molecular to cellular, individual and population level. Scopes for acclimation (physiology and behaviour) and adaptation (evolution) that together define species resilience will be studied in various life stages (eggs, larvae, juveniles, adults) and the most sensitive one(s) identified. Functional determinants of individual fitness such as ion and acid-base regulation, mitochondrial energy metabolism and immune response will also be examined. Furthermore, we aim to address how all these determinants may be influenced by food quality and availability.