6th Bilateral Seminar Italy-Japan

Palermo, Italy
19-20 November 2014

http://BIMAT2014.azuleon.org

Acknowledgements

Organised and supported by

Consiglio Nazionale delle Ricerche

Istituto di Biomedicina e Immunologia Molecolare "Alberto Monroy" - CNR

Japan Society for the Promotion of Science - Founded 1932

With the patronage of

Stazione Zoologica Anton Dohrn - Napoli

Under patronage of UNESCO
United Nations Educational, Scientific and Cultural Organization
Intergovernmental Oceanographic Commission

Città di Palermo

Panormitanae Studiorum Universitatis Sigillum
Università degli Studi di Palermo

STeBiCeF

CoNISMa
Consorzio Nazionale Interuniversitario per le Scienze del Mare

With the support of

Regione Siciliana

PO FESR Sicilia 2007/2013

Ceformed

EC TOX LDS

Information Technology & Communication
I.T.C. di Calvaruso Francesca

La Piana delle Bontà

NBS S.r.l.
New Life Science Tecnology

Night Shuttle

National Experts

Italy

Valeria Matranga
CNR Scientific Referent

Ilaria Corsi
Marco Faimali
Francesca Garaventa
Adrianna Ianora
David Pellegrini
Francesco Regoli

Japan

Masato Kiyomoto
JSPS Scientific Referent

Makoto Kakinuma
Keita Kodama
Kei Nomiyama
Mari Ochiai
Hajime Watanabe
Toshihiro Horiguchi
Yukio Yokota

Local and technical support

Mauro Biondo
Alessandro Pensato

Organising secretariat

Azuleon srl
BIMAT.2014@azuleon.org

PROGRAMME

PROGRAMME

Thursday, 18 November

18:00-18:30 **Cocktail to welcome the 6th BSIJ National Experts**

20:30 ***Social dinner at "Casena dei Colli"***

Wednesday, 19 November

8:45 **Registration**

9:00-9:20 **Opening Remarks**

Welcome of the Organisers

Valeria Matranga & Masato Kiyomoto

Addresses of Authorities
Pier Luigi San Biagio, President of the Area della Ricerca CNR of Palermo
Giovanni Viegi, Director of the Institute of Biomedicine and Molecular Immunology "A. Monroy"
Representative of the Palermo University
Representative of Assessorato Regionale dell'Agricoltura,dello Sviluppo Rurale e della Pesca Mediterranea (Regione Sicilia)

9:20-10:00 **Keynote Lecture**

Maria Byrne (Sydney, NSW, Australia)

Change in the world's oceans: responses of echinoderm development to warming and acidification and analysis of the effects of multiple stressors on marine embryos and larvae

10:00-10:15 Valeria Matranga (Palermo, Italy)

Physical and Chemical Impacts on Marine Organisms: 10 years later the first Bilateral Seminar Italy-Japan

Session 1

Current impacts in natural environment, effects on marine organisms (Mediterranean Sea, Japanese Seas, field surveys and analysis)

Chairs: Francesca Garaventa & Yukio Yokota

10:15-10:30 Yukio Yokota (Aichi, Japan)

Valuable lessons of the past

10:30-10:50 Mari Ochiai (Matsuyama, Japan)

PCBs, PBDEs and their hydroxylated metabolites in the brain of free-ranging toothed and baleen whales

10:50-11:10 ***Coffee break***

11:10-11:30 Francesco Regoli (Ancona, Italy)

Environmental impact of the Costa Concordia wreck through an integrated, multidisciplinary Weight of Evidence approach

11:30-11:50 Francesca Garaventa (Venice, Italy)

Use of ecotoxicity tests for the evaluation of marine sediments toxicity: a case study

11:50-12:10 Paola Gianguzza (Palermo, Italy)

Exploring the impact of the invasive algae *Caulerpa taxifolia* var. *distichophylla* and *C. cylindracea* on the performance of the sea urchin *Paracentrotus lividus*

12:10-12:30 Chiara Bonaviri (Palermo, Italy)

Climate change potentially affect keystone predation in subtidal systems

12:30-12:50 Simone Cappello (Messina, Italy)

Biotechnological applications for potential recovery strategies: the "SYSTEMS BIOLOGY" in the study of xenobiotic effects on marine organisms for evaluation of the environmental health status

12:50-13:10 Giuseppe Mancini (Catania, Italy)

SEAPORT and STITAM: two Italian National Project for the recovery of polluted harbours and marine areas

13:15-14:30 ***Lunch***

Session 2

Response mechanisms of marine organisms to environmental impacts
(From biological communities to tissue, cell and molecular levels)

Chairs: Keita Kodama & Marco Faimali

14:30-14:50 Keita Kodama (Tsukuba, Japan)

Exploration of factors affecting abundance in early-life stages of marbled sole, *Pseudopleuronectes yokohamae* in Tokyo Bay, Japan

14:50-15:10 Marco Faimali (Genoa, Italy)

Old model organisms and new behavioral end-points: swimming alteration as an ecotoxicological response

15:10-15:30 Julian Blasco (Puerto Real, Spain)

Laboratory simulation of CO_2 leakages during injection and storage in sub-seabed geological formations: metal accumulation and toxicity in the model benthic organisms *Hediste diversicolor* and *Ruditapes philippinarum*

15:30-15:50 ***Coffee break***

15:50-16:10 Tiziana Cappello (Messina, Italy)

Effects of petrochemical contamination on caged marine mussels using a multi-biomarker approach: histopathology, metabolomics, neurotoxicity and DNA damage

16:10-16:30 Narimane Dorey (La Rochelle, France)

Combined effects of ocean acidification and cadmium on the development of the sea urchin *Paracentrotus lividus*

16:30-16:50 Marco Munari (Padua, Italy)

Oxidative stress-related parameters in adults and larvae of the clam *Ruditapes philippinarum* under different combinations of pH values and diclofenac concentrations

Thursday, 20 November

Session 3

Analysis of the effects on marine organism caused by environmental impacts
(Model organisms, biomarkers, gene expression analysis)

Chairs: Hajime Watanabe & Francesco Regoli

9:00-9:20 Hajime Watanabe (Suita, Japan)

Genetic engineering of *Daphnia magna* for making biomonitoring tool

9:20-9:40 Maria Costantini (Naples, Italy)

Molecular response to toxic diatom-derived aldehydes in the sea urchin, *Paracentrotus lividus*

9:40-10:00 Anna Palumbo (Naples, Italy)

The deleterious effects of *Ostreopsis* cf. *ovata* bloom on *Paracentrotus lividus* are mediated by nitric oxide

10:00-10:20 Rosa Bonaventura (Palermo, Italy)

Co-exposure to cadmium and UVB radiation impairs sea urchin embryo development and induces cell stress response at gene and protein levels

10:20-10:40 Carlo Giacomo Avio (Ancona, Italy)

Microplastics in the marine environment: a true ecotoxicological risk?

10:40-11:00 ***Coffee break***

11:00-11:20 Laura Canesi (Genoa, Italy)

Organic contaminants as potential obesogens in mammalian and marine invertebrate cells

11:20-11:40 Siti Jaafar (Cork, Ireland)

Acute toxicity from pro-oxidant copper in *Mytilus edulis* gills: a redox proteomic analysis

11:40-12:00 Oriana Migliaccio (Naples, Italy)

Nitric oxide mediates the response to metals in *Paracentrotus lividus* developing embryos

12:00-12:20 Chiara Martino (Palermo, Italy)

Asymmetric skeleton patterns induced by gadolinium ions in sea urchin embryos: focus on mechanisms regulating skeletogenesis and comparison among phylogenetically distant species

12:20-12:40 CNR IN ANTARCTICA: EMOTIONS FROM THE WHITE CONTINENT (movie, photo and project resume)
by Marco Faimali (ISMAR-CNR)

13:00-15:00 ***Lunch & Poster Session***

Session 4

Sustainable use of marine resources
(Macroalgae, microalgae, bioassay, new biomaterial)

Chairs: Adrianna Ianora & Makoto Kakinuma

15:00-15:20 Makoto Kakinuma (Tsu, Japan)

Disorders induced by environmental stress in *Pyropia yezoensis* (Rhodophyta)

15:20-15:40 Adrianna Ianora (Naples, Italy)
Marine microalgae as a sustainable source of bioactives for drug discovery

15:40-16:00 Michela Sugni (Milan, Italy)
Echinoderms as sustainable source of collagen for innovative applications in regenerative medicine

16:00-16:20 Masato Kiyomoto (Tokyo, Japan)
Long-term preservation of echinoderm sperm under non-cryo condition for ecotoxicological bioassay

16:20-17:00 **General Discussion:**
Environment and marine life in the future - for a sustainable society

Friday, 21 November

Excursion (to be announced)
Reservation and payment necessary
(except for invited national experts)

ABSTRACTS

Oral communications
in chronological order of presentation

ABSTRACTS

Change in the world's oceans: responses of echinoderm development to warming and acidification and analysis of the effects of multiple stressors on marine embryos and larvae

M. Byrne
Schools of Medical and Biological Sciences, Univ. of Sydney

Early life stages of marine invertebrates are vulnerable to the stressors associated with global change, but identifying general patterns across response variables is challenging. A meta-analysis of multi-stressor studies on the effects of temperature, salinity and acidification on invertebrate development indicated that: 1) Synergistic interactions were more common than additive or antagonistic interactions. 2) Larvae were generally more vulnerable than embryos. 3) Interaction types varied among stressors, stages, and biological responses, 4) Ocean acidification is a greater stressor for calcifying than non-calcifying larvae. The analysis identified taxa that may be more vulnerable (e.g. molluscs, echinoderms) or robust (e.g. arthropods) to these stressors. For sea urchins, the effect of ocean acidification on development was determined in a global synthesis of data from 15 species from tropical to polar environments and from intertidal to subtidal habitats. The arm growth response in echinoplutei was used as a proxy of larval calcification in response to increased seawater acidity/pCO2 and decreased carbonate mineral saturation. Phylogenetic relatedness did not influence the observed patterns. Regardless of habitat or latitude, acidification impedes larval growth with a negative relationship between arm length and increased acidity/pCO2 and decreased carbonate mineral saturation. In multiple linear regression models incorporating these highly correlated parameters, pCO2 exerted the greatest influence on decreased arm growth. For tropical species decreased carbonate mineral saturation was most important. No single parameter played a dominant role in arm size reduction in the temperate species. Levels of acidification causing a significant reduction in arm growth varied between species. In 13 species, reduction in length of skeletal rods, was evident in near future (pCO2 800+ μatm) conditions while greater acidification (pCO2 1000+ μatm), reduced growth in all species. Although multistressor studies are few, when temperature is added to the stressor mix, near future warming can reduce the negative effect of acidification on larval growth. Overall, larvae from across world regions showed similar trends despite disparate phylogeny, environments and ecology. Larval success may be the bottleneck for species success with flow on effects for sea urchin populations and marine ecosystems.

Physical and Chemical Impacts on Marine Organisms: 10 years later the first Bilateral Seminar Italy-Japan

V. Matranga
Istituto di Biomedicina e Immunologia Molecolare "A. Monroy", CNR, Palermo, Italy

Marine resources are very important to both Italy and Japan for several viewpoints, including for instance the large consumption of sea food, various types of aquaculture, fishery and transformed products, tourism and public health. Unfortunately, in the last centuries serious impacts on the marine environment, mostly caused by human activities, have caused concern. It is our obligation to preserve the sustainability of the marine resources and leave the oceans clean and productive for future generations. With this in mind my colleague and friend Prof. Yukio Yokota proposed the first Bilateral Seminar Italy-Japan (BSIJ) under the broad name of: 'Physical and Chemical Impacts on Marine Organisms'. It is now since 2004 that BSIJs are a consolidated meeting point of Italian and Japanese experts, gathering on a biannual basis in Japan or Italy, investigating the effects of classical and emerging pollutants on marine organisms. Aimed at exchange of information and knowledge, based on independent research projects, reports concerned a variety of marine experimental systems, approached at the molecular, cellular, individual and population levels. The last editions have been opened to senior and junior scientists from other EU and industrialized countries, as well as emerging countries. BSIJs have been co-organized and sponsored by CNR and JSPS in the frame of the Bilateral Agreement of Scientific and Technological Cooperation between Italy and Japan, have received the high patronage of the Intergovernamental Oceanographic Organization of UNESCO, patronage and partial support of many National and International leading Institutions in the field of marine biology and safeguard.

Valuable lessons of the past

Y. Yokota
Dept Information Science & Technology, Aichi Pref. Univ., Japan

Homo sapiens is a completely different species from any other organisms. We are able to extinguish other species and actually extinguished several species. We put ourselves beyond the biological boundaries of a species because of our intelligence. Looking back upon our history, we have extensively exploited the biota and seriously damaged the natural environment. We are dependent on the productivity of marine and terrestrial biota, therefore the conservation of natural environment is crucial for human society. Thomas Huxley had told "All the great fisheries are inexhaustible, consequently any attempt to regulate these fisheries seems to be useless." Nowadays, his speech is not acceptable, further, we recognize some anthropocentrism. In order to make our society sustainable, conversion of our view point is required.

PCBs, PBDEs and their hydroxylated metabolites in the brain of free-ranging toothed and baleen whales

M. Ochiai[1], K. Nomiyama[1], T. Isobe[2], T.K. Yamada[3], Y. Tajima[3], M. Makara[3], M. Amano[4], T. Matsuishi[5], H. Iwata[1], S. Tanabe[1]
[1]Center for Marine Environmental Studies (CMES), Ehime Univ., Matsuyama, Japan
[2]Center for Environmental Health Science, National Inst. for Environmental Studies (NIES), Tsukuba, Japan
[3]Dept of Zoology, National Museum of Nature and Science, Tsukuba, Japan
[4]Faculty of Fisheries, Nagasaki Univ., Nagasaki, Japan
[5]Faculty of Fisheries Sciences, Hokkaido Univ., Hakodate, Japan

Cetaceans are chronically exposed to persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), by food-chain. Although these compounds are stable in the environment, many animals have detoxification mechanisms for their elimination. PCBs and PBDEs are partly transformed to hydroxylated metabolites (OH-PCBs, OH-PBDEs) by cytochrome P450 enzymes (CYP), and excretion is promoted by conjugation enzymes. A part of these metabolites structurally resembles thyroid hormone (TH) that is indispensable to a brain development and is known to cause cognitive and neurodevelopmental toxicities. In this study, blood and the brain samples were collected from 7 cetacean species (n = 31) using stranded or bycaught animals in Japanese coasts, and the levels and accumulation patterns of OH-PCBs and OH-PBDEs were examined. Levels of the compounds in the blood and brain had positive correlations among each other (p<0.05), indicating the possible penetration of these compounds through the blood-brain barrier. OH-PCBs were detected from all the brain samples analyzed (6.5 - 3600 pg/g wet wt.), and several cetacean species (e.g. killer whales, Dall's porpoises, melon-headed whales) have exceeded the inhibitory level of TH-mediated gene transcription using human brain cell lines. These results suggest possible adverse effects of these compounds on cetaceans, and further studies are required for the risk assessment of wild marine mammals.

Environmental impact of the Costa Concordia wreck through an integrated, multidisciplinary Weight of Evidence approach

M. Benedetti[1], S. Gorbi[1], D. Fattorini[1], M. Di Carlo[1], C. G. Avio[1], M. Mezzelani[1], M.E. Giuliani[1], G. D'Errico[1], I. Lanzoni[1], A. Nardi[1], P. Guidi[2], V. Scarcelli[2], G. Frenzilli[2], A. Gaion[3], A. Scuderi[3], S. Giuliani[3], G. Romanelli[3], Daniela Berto[3], Benedetta Trabucco[3], M. Nigro[2], D. Pellegrini[3], A.M. Cicero[3], F. Regoli[1]
[1]Dept of Life and Environmental Sciences, Polytechnic Univ. of Marche, Ancona, Italy
[2]Dept of Clinical and Experimental Medicine, Univ. of Pisa, Italy
[3]ISPRA, Institute for Environmental Protection and Research, Italy

After the Costa Concordia shipwreck, a complex framework of chemical, biological and oceanographic activities was immediately activated, to assess possible contamination events and the environmental impact during both emergency and wreck removal operations. Chemical analyses on sediments and ecotoxicological bioassays were integrated with the measurement of bioaccumulation and biomarkers in several bioindicator organisms, like transplanted mussels, native fish species of different trophic levels and intertidal/subtidal invertebrates, sampled with an almost monthly frequency. Tissue concentrations were measured for polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, polychlorinated biphenyls and halogenated pesticides, organotin compounds, trace metals, brominated flame retardants and detergents. Biomarkers included metallothioneins, peroxisomal proliferation, acetylcholinesterase, CYP450 biotransformation pathway, oxidative stress biomarkers, lipid peroxidation processes, lysosomal membrane stability, and level of DNA integrity. Data were elaborated within a quantitative weight of evidence model which provided synthetic hazard indices for each typology of data (or line of evidence, LOE), before their overall integration in an environmental risk index. Results excluded serious contamination events although some episodic spills with reversible effects were detected; biomarkers were in general accordance but more sensitive compared to bioaccumulation data, suggesting the possibility of synergistic effects, and further supporting the importance of a multidisciplinary approach for an effective monitoring of environmental impact and risk assessment during the operations of wreck removal. The proposed WOE model was confirmed a useful tool to summarize large datasets of complex data in integrative indices, and to simplify the interpretation for stakeholders and decision makers, thus supporting a more comprehensive process of "site-oriented" management decisions.

Use of ecotoxicity tests for the evaluation of marine sediments toxicity: a case study

E. Costa[1], V. Piazza[2], C. Gambardella[2], R. Moresco[3], M. Faimali[2], F. Garaventa[1]
[1]National Research Council – Inst. of Marine Science (CNR-ISMAR), Venezia, Italy
[2]National Research Council – Inst. of Marine Science (CNR-ISMAR), Genova, Italy
[3]Costa Edutainment S.p.A., Acquario di Genova, Genova, Italy

Marine sediment contamination represents a significant environmental problem. The main tool for sediment risk assessment is represented by chemical analysis, able to record contaminants concentrations, but not to provide data about effects. The need to couple chemical analysis with biological methods is widely recognized. In this study ecotoxicity tests are used to characterize sediments from Mar Piccolo (Ionian Sea), a semi-enclosed and strongly polluted basin. For decades, it has been subjected to different anthropogenic impacts (steel and oil plant waste and harbour activities) that caused strong sediments contamination. To evaluate the toxic effect and to derive ecologically relevant conclusions a multi organisms and multi end-points approach has been applied. Organisms have been exposed to sediment elutriates. The results indicate that, despite a high sediment contamination, the effect on marine organisms is almost absent suggesting that toxic compounds may not be bioavailable. To verify this hypothesis sediments have been subjected to a strong extraction process using Dichloromethane as a solvent to force the release of polar contaminants. Organisms have been exposed to such extracts. Results indicate e strong toxic effect of polar extract revealed both at a sub-lethal and lethal level. The results indicate a complex contaminants dynamic in sediment suggesting the need of an approach more and more integrated (ecotoxicology/chemistry/ecology).

Exploring the impact of the invasive algae *Caulerpa taxifolia* var. *distichophylla* and *C. cylindracea* on the performance of the sea urchin *Paracentrotus lividus*

P. Gianguzza[1], L. Musco[2], D. Russo[3], C. Bonaviri[1], S. Meek[4], V. Arizza[3]
[1]Dip. di Scienze della Terra e del Mare (DiSTeM), Univ. di Palermo, Palermo
[2]CNR-IAMC, Castellammare del Golfo (TP), Italy
[3]Dip. di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Univ. degli Studi di Palermo, Italy
[4]Univ. of Portsmouth School of Biological Sciences, King Henry Building, Portsmouth, UK

Among the alien algae recorded in the Mediterranean Sea, *Caulerpales* are particularly known for their high capacity to produce secondary metabolites, acting as repellents or *toxins to herbivores.* The co-occurrence of the new exotic *Caulerpa taxifolia* var. *distichophylla* and *C. cylindracea* in Southern Sicily offered the possibility to investigate the stress response of the sea urchin *Paracentrotus lividus* induced by the consumption of these algae. In particular, it was evaluated the modulation of the total number of circulating coelomocytes and their composition in response to a diet consisting exclusively of *C. cylindracea*, *C. taxifolia* var. *distichophylla* and a mix of both algae, compared with control individuals foraged with *Dictiota dycotoma*. The levels of the expression of heat shock proteins (HSP) HSP27, 70 and 90 expressed by sea urchin coelomocytes were assessed by immunoblotting utilizing monoclonal antibodies specific for human HSP27, 70 and 90 within 24, 48 and 72 hours after treatment. Results showed a clear tendency for *P. lividus* to consume more *C. cylindracea* than C*. taxifolia* var. *distichophylla*, the mix of the two *Caulerpa* species and *D. dycotoma*. No evident cellular damage was recorded and no significant differences were found after 24 h both in total and differential counts of coelomocytes between the control group and the group fed with *C. cylindracea* or *C. taxifolia* var. *distichophylla*, whereas a strong expression of HSP70 was found in the coelomocyte lysate from sea urchins treated with the mix of both *Caulerpa* algae. The stress effects seem to be reduced after 48 and 72 h.

Climate change potentially affect keystone predation in subtidal systems

C. Bonaviri[1,2], N.Shears[3], P. Gianguzza[1], M.H. Graham[2]
[1]Dip. di Scienze della Terra e del Mare (DiSTeM), Univ. di Palermo, Palermo, Italy
[2]Labs, B272 Moss Landing Road, Moss Landing, California, USA
[3]Univ. of Auckland, Dept of Statistics, Auckland, New Zealand

Sea surface water temperature dramatically oscillates along seasons in temperate regions. Additionally, sudden temperature anomalies are more and more frequent, probably due to global warming. Temperature may affect predation rate by key predators, with repercussions in the ecosystem functioning and structure. Starfishes are often important predators of sea urchins, the principal consumers of algal forests. In the Channel Islands, densities of the starfish *Pycnopodia helianthoides* and the urchin *Strongylocentrotus purpuratus* were strongly inversely related, confirming that this starfish controls urchin populations. Moreover, we observed that in warmer areas urchin abundance increased, suggesting that temperature may affect interaction strength between the two species. Predation experiments were conducted in the laboratory to determine the effect of water temperature increase on the feeding rate on *S. purpuratus* by *P. heliantoides*. Predation rate clearly decreased in warmer water. Moreover, the effect of starfish and urchin sizes was considered. Large sea stars consumed urchins regardless of prey's size, whereas small sea stars consumed conspicuously only small urchins. Altogether, our results indicate that local abundance and predation rate of ecthoterm predators such as starfishes may change in relation to the climatic region, seasonal temperature variations or sudden temperature anomalies.

Biotechnological applications for potential recovery strategies: the "SYSTEMS BIOLOGY" in the study of xenobiotic effects on marine organisms for evaluation of the environmental health status

S. Fasulo[1], G. Guerriero[2], S. Cappello[3], M. Colasanti[4], T. Schettino[5], C. Leonzio[6], G. Mancini[7], R. Gornati[8]
[1]Dept of Biological and Environmental Sciences, Univ. of Messina, Messina, Italy
[2]Dept of Biology, Univ. of Naples "Federico II", Naples, Italy
[3]Inst. for Coastal Marine Environment, National Research Center, Messina, Italy
[4]Dept of Science, Univ. of Rome "Roma Tre", Rome, Italy
[5]Dept of Biological and Environmental Sciences and Technologies, Univ. of Salento, Lecce, Italy
[6]Dept of Environmental Sciences, Univ. of Siena, Siena, Italy
[7]Dept of Industrial Engineering, Univ. of Catania, Catania, Italy
[8]Dept of Biotechnologies and Life Sciences, Univ. of Insubria, Varese, Italy

Environmental monitoring and bioremediation of marine habitats are amongst the focus keys of the European strategies for territory management. The anthropogenic disturbance is acting on ecosystems with serious consequences for the environment and human health, driving strong social and economic impacts on the community. Often, such in the case of chemical and petrochemical productions, the areas subjected to the most intensive industrialization are located along the coastline, being a severe threat to the marine environment. "SYSTEMS BIOLOGY" is a Project funded, recently, by MIUR and itì' articulated into two main phases: *i*) a field monitoring program to evaluate the effects of hydrocarbons and heavy metals on "sea sentinels" purposely placed in different contaminated areas; *ii*) a series of controlled mesocosms-scale experiments to measure the effects, on the same sea sentinels, of different remediation actions on waters and sediments collected from the contaminated areas under inspection. The project is aimed to understand how the xenobiotic contamination induces or modifies the biological processes in aquatic species, and to evaluate the potential effectiveness of different biotechnological strategies in controlling and reducing the impact of marine pollution on the biological compartment. Each phase is accomplished through a synergic collaboration and multidisciplinary approach among eight research units and constitutes a pioneering advance in the international scenario.

SEAPORT and STITAM: two Italian National Project for the recovery of polluted harbours and marine areas

S. Cappello[1], A. Luciano[2], M. Panzica[3], R. Musumeci[3], L. Cavallaro[3], G. Mancini[3]
[1]Inst. for Coastal Marine Environment (IAMC) – CNR U.O.S. of Messina, Italy
[2]ENEA Italian National Agency for New Technologies, Energy and Sustainable Economic Development, Casaccia Research Centre, Roma, Italy
[3]Dept of Industrial and Mechanical Engineering, Univ. of Catania, Italy

Two Italian National Projects, SEAPORT (*Development of innovative technologies for energy saving and environmental sustainability of shipyards and harbour areas*) and STI-TAM (*Development of Innovative technologies for the treatment of fluid wastes from shipping activities and for marine environment protection*), different each other, having a common denominator: energy and environmental requalification of polluted harbour and marine area. The main activities and first results of the two Project will be presented, focusing on technologies for the environmental protection/remediation of marine environment. Results from SEAPORT will be presented including characterization of pollution levels (waters and sediments) and the analysis and application of different remediation techniques at lab-scale. Specifically the bioremediation and bio-augmentation through specialized bacteria consortium as well as the application of aeration through nano-bubbles production will be considered. The best combination of remediation technologies, as selected through the lab-scale will be applied, on a full scale, in the selected harbor area. Results from STITAM Project will focus on the design and optimization of an integrated treatment plant including physical, chemical and biological phases for the removal of contaminants from marine wastes (e.g. bilge waters, slops). The Industrial Research phase is devoted to the evaluation develop the prototypes in the subsequent Experimental development phase.

Exploration of factors affecting abundance in early-life stages of marbled sole *Pseudopleuronectes yokohamae* in Tokyo Bay, Japan

J.H. Lee[1,2], K. Kodama[1], H. Shiraishi[1], T. Horiguchi[1]
[1]Center for Environmental Risk Research, National Inst. for Environmental Studies, Tsukuba, Ibaraki, Japan
[2]Southeast Sea Fisheries Research Inst., National Fisheries Research and Development Inst., Sannyang, Tongyeong, Gyeongsangnam, Korea

We investigated factors that might have disturbed the stock recovery of marbled sole *Pseudopleuronectes yokohamae* in Tokyo Bay by focusing on the early life stages. Firstly, we investigated yearly changes in egg production, larval and juvenile densities in Tokyo Bay between 2006 and 2010. Egg production was high from 2006 to 2008, and decreased thereafter. Meanwhile larval and juvenile densities were high in 2006 and 2008, but low in 2007, 2009 and 2010. The discrepancy in yearly trend of these parameters suggests that mortality during life stages between spawning and early larval phases might affect abundance of subsequent life stages. Secondly, we examined relationship between larval density and water quality. Mean water temperature in January, in which most of spawning and hatching occurred, was low in 2006 (8.6°C) and 2008 (9.6°C), compared to those observed in other years (10.4–11.4°C). Significantly negative correlation between larval density and mean water temperature in January implies that mortality during life stages between spawning and early larval phases would be higher in warmer years (water temperature > 10°C in January). To examine effects of water temperature on mortality of larvae, we conducted 4-week survival experiments with five temperature regimes (5.6, 9.1, 11.5, 12.6 and 14.6°C) under controlled laboratory conditions. Mortality rates showed a linear increase as water temperature elevated (from 14.4% at 5.6°C to 96,5% at 14.6°C; $r = 0.98$, $p < 0.01$),). This experimental result supports evidence of the field observations that warmer reproductive season (water temperature >10°C) might enhance mortalities of larvae.

Old model organisms and new behavioral end-points: swimming alteration as an ecotoxicological response

M. Faimali[1], C. Gambardella[1], E. Costa[2], V. Piazza[1], S. Morgana[1], S. Lavorano[3], F. Garaventa[2]
[1]National Research Council – Inst. of Marine Science (CNR-ISMAR), Genova, Italy
[2]National Research Council – Inst. of Marine Science (CNR-ISMAR), Venezia, Italy
[3]Costa Edutainment S.p.A., Acquario di Genova, Genoa, Italy

In marine ecotoxicology there is an urgent need to identify new end-points for the environmental risk assessment. Among end-points, behavioral ones, as the alteration of the swimming activity, represent very sensitive and easily measurable responses.
A comparison of sensitivity level of the swimming behavior alteration (frequency of pulsations; swimming speed) and acute responses (mortality; immobility) of some conventionally (sea urchin and brine shrimp) and unconventionally (jellyfish) marine invertebrates has been performed.
Larvae of the jellyfish *Aurelia aurita*, the sea urchin *Paracentrotus lividus* and the brine shrimp *Artemia* sp. were exposed to reference toxic compounds, pesticides and emerging compounds (e.g. neurotoxic compounds, nanoparticles).
Results show that, for jellyfish, the frequency of pulsations, independently from toxic compounds, was more sensitive than mortality/immobility.
Similar results were obtained comparing swimming speed alteration and immobilization results for sea urchin embryos and larvae exposed to reference toxic compounds. Conversely, for brine shrimp, swimming alteration was more sensitive than mortality with pesticides and nanoparticles, but not with reference toxic compounds.
These findings indicate that the use of behavioral end-points is an important potentiality in ecotoxicology; for that they can be strongly suggested in environmental risk assessment, and research on new model organisms and end-points deserves to be continued.

Laboratory simulation of CO_2 leakages during injection and storage in sub-seabed geological formations: metal accumulation and toxicity in the model benthic organisms *Hediste diversicolor* and *Ruditapes philippinarum*

A. Rodríguez-Romero[1], N. Jiménez-Tenorio[2], I. Riba[2], J. Blasco[1]
[1]Dep. de Ecología y Gestión Costera. Inst. de Ciencias Marinas de Andalucía (CSIC), Avda, República Saharaui, Puerto Real (Cádiz), Spain
[2]Cátedra UNESCO/UNITWIN/WiCop, Facultad de Ciencias del Mar y Ambientales, Univ. de Cádiz, Polígono Río San Pedro, Puerto Real (Cádiz), Spain

The CO_2 capture and storage in sub-seabed geological formations is proposed as one of the potential options to decrease the atmospheric CO_2 concentrations. However, possible CO_2 leakages could occur during the injection and sequestration procedure, provoking significant repercussions on marine environment. We investigate, the effects of acidification derived from CO_2 leakage in the benthic model organisms *Hediste diversicolor* and *Ruditapes philippinarum*. To this end, to independent lab scale experiments involving direct release of CO_2 were conducted at possible expected scenarios of pH values between 8.0 and 6.1. Individuals of both species were independently exposed during 10 days to seawater with sediment samples collected in two different coastal areas, one relatively uncontaminated sediment as reference (RSP) and the other with known contaminated sediment (ML). Survival rate and metal accumulation (Fe, Al, Mn, Cu and Zn) on whole body were employed as endpoint. Furthermore, burrowing activity and histopathological damages were quantified for *R. philippinarum*. Highly significant associations were observed between pH and the biological effects measured in *R philippinarum*, except for metal concentrations in tissues. However, for *H. diversicolor*, mortality was only significant at the lowest pH level in the contaminated sediment and an increase in metal uptake by worms exposed to the contaminated sediment was observed in line with the reduction of pH, Those findings may indicate that bivalves are more vulnerable than polychaetes when they are subjected to possible acidification scenarios caused by CO_2 leakages.

Effects of petrochemical contamination on caged marine mussels using a multi-biomarker approach: histopathology, metabolomics, neurotoxicity and DNA damage

T. Cappello, M. Maisano, A. Natalotto, V. Parrino, A. Giannetto, A. Mauceri, S. Fasulo
Dept of Biological and Environmental Sciences, Univ. of Messina, Messina, Italy

The present study was designed to evaluate the biological effects of petrochemical contamination on marine mussels. *Mytilus galloprovincialis*, widely used as bioindicators in biomonitoring studies, were caged in the "Augusta-Melilli-Priolo" industrial site (eastern Sicily, Italy), as one of the largest and most complex petrochemical sites in Europe, and Brucoli, as reference site. Chemical analyses of the sediments revealed very high levels of Hg and PAHs, exceeding the national and international guidelines, in the polluted site. Morphological alteration with intense hemocytic infiltration was observed both in the hepatopancreas, main site for bioaccumulation and detoxification of pollutants, and gills, mainly involved in nutrient uptake and gas exchange. 1H NMR-based metabolomics associated with chemiometrics analyses highlighted changes in metabolites involved in different metabolic pathways, i.e. cellular defense, osmoregulation, energy metabolism, and neurotransmission. In gills an alteration of serotoninergic (i.e. 5-HT and its receptor) and cholinergic (i.e. AChE and ChAT) systems was observed, as supported by enzymatic analysis of AChE activity. Evidences of eco-genotoxic impact were observed using the Micronucleus Test. The application of a multi-biomarker panel results thus effective in assessing the environmental influences of pollutants on the health of aquatic organisms.

This work has been funded by a National Project (PRIN 2010-2011, prot. 2010ARBLT7_001/008).

Combined effects of ocean acidification and cadmium on the development of the sea urchin *Paracentrotus lividus*

N. Dorey, M. Nouhaud, L. Murillo, T. Lacoue-Labarthe
UMR 7266 Littoral Environnement et Sociétés (LIENSs), CNRS-Univ. de La Rochelle, La Rochelle, France

We live in a multi-stressor world where oceans are impacted by both global (e.g. CO_2-driven climate change and ocean acidification) and local pressures. Coastal marine organisms are indeed already challenged by anthropogenic stressors such as pollutants, that may magnify the impacts of future global changes. For instance, ocean acidification (i.e. decreasing pH) is likely to change metal speciation as well as bioaccumulation and, consequently, metal toxicity. There is very few information available on the effects of these interactions on marine biota. In this study, we provide insight on the combined effects of ocean acidification and cadmium on the development of *Paracentrotus lividus*. In laboratory culture, sea urchin larvae appeared quite robust to predicted pH changes as well as to high (but environmentally-relevant) levels of cadmium contamination, albeit some morphometric changes. However, the association of both stressors significantly increased larval mortality and anomalies. As of today, we still lack an understanding of how biological mechanisms respond to co-occurring factors such as metal contamination and ocean acidification, making accurate projections regarding the future of ecologically- and economically-important marine ecosystems difficult.

Oxidative stress-related parameters in adults and larvae of the clam *Ruditapes philippinarum* under different combinations of pH values and diclofenac concentrations

M. Munari[1], V. Matozzo[1], F. Gagnè[2], M.G. Marin[1]
[1]Dip. di Biologia, Univ. di Padova, Padova, Italia
[2]Environment Canada, Montreal, Quebec, Canada

Climate changes, such as seawater acidification, could alter marine organisms susceptibility to emerging contaminants. In this context, the combined effects of seawater acidification and diclofenac (a non-steroidal anti-inflammatory drug, NSAID) were investigated for the first time in adults and larvae of the clam *Ruditapes philippinarum*. By using two different flow-through systems, adults were exposed to three pH levels (8.1, 7.7 and 7.4) and three environmentally relevant concentrations of diclofenac (0, 0.05 and 0.5 μg/L) for 14 days, while larvae were exposed to two pH levels (8.1 and 7.7) and two concentrations of diclofenac (0 and 0.5 μg/L) for 96 hours. Oxidative stress related responses and damage were measured in gills and digestive glands from adult clams [superoxide dismutase (SOD), catalase (CAT) and cyclooxygenase (COX) activities, lipid peroxidation (LPO) and DNA strand-break formation] and in larvae (CAT activity and LPO). Despite COX is know to be a target of NSAIDs, it was significantly influenced only by reduced pH in both gills and digestive gland of adult clams, while SOD and CAT did not show significant variations. Changes in LPO and DNA strand-break formation due to significant pH/diclofenac interaction were also found in gills. In larvae, only reduced pH significantly increased CAT activity, while no significant variations in LPO were observed among treatments.

Genetic engineering of *Daphnia magna* for making biomonitoring tool

Y. Kato, T. Nakanishi, M. Asada, H. Watanabe
Dept of Biotechnology, Osaka Univ., Japan

The water flea, *Daphnia magna*, has been the subject of study in ecology, evolution, and environmental sciences for decades. Genetic information such as the draft genome sequence and expressed sequence tags (ESTs) (1) is now available and functional approaches of *Daphnia* show great progress. Previously we established overexpression and gene silencing method (2). In addition, we succeeded to make transgenic lines using microinjection of plasmid into *D. magna* eggs. The green fluorescent protein gene under the promoter region of the *elongation factor 1α-1* gene was successfully integrated into the genome and transgenerationally inherited (3). We also applied an emerging genome editing technique mediated by the CRISPR/Cas system for genome editing and succeeded to introduce heritable mutations into desired gene (4). These techniques are useful to modify *D. magna* suitable for biomonitoring. In order to detect chemicals having hormone like activities, the ecdysone response element was inserted in the upstream region of a reporter gene and the DNA construct was injected into eggs. When the eggs were exposed to ecdysone, there was enhanced expression of the reporter gene at detectable levels, while the presence of an antagonist led to its downregulation (5). These results suggested that this system could be developed for monitoring chemical activities in media.

(1) Genome. 2005 48:606-9. Watanabe H et al. (2) PLoS Genet. 2011 7:e1001345. Kato Y et al. (3) PLoS One. 2012;7:e45318. Kato Y et al. (4) PLoS One. 2014 30;9:e98363. Nakanishi T et al. (5) Mar Environ Res. 2014 93:118-22. Asada M et al.

Molecular response to toxic diatom-derived aldehydes in the sea urchin, *Paracentrotus lividus*

S. Varrella[1], G. Romano[1], A. Ianora[1], M.G. Bentley[2], N. Ruocco[1], M. Costantini[1]
[1]Stazione Zoologica Anton Dohrn, Napoli, Italy
[2]Dove Marine Laboratory, School of Marine Science and Technology, Newcastle Univ., Newcastle upon Tyne, UK

Diatoms are a highly productive class of microalgae, widespread in marine and freshwater habitats, which are widely fed upon by both planktonic and benthic invertebrates. However, their beneficial role in sustaining marine food webs has been challenged after the discovery that they produce secondary metabolites, such as polyunsaturated aldehydes (PUAs), which negatively affect the reproductive success of many invertebrates. Because the sea urchin *Paracentrotus lividus* is considered a good model system to study the ecotoxicological response to environmental pollutants, we decided to adopt it for our study and treated sea urchin embryos with increasing concentrations of two PUAs, heptadienal and octadienal, to analyze morphological changes induced by exposure and to define their mechanism of action. We show that both PUAs are able to induce teratogenesis, as already reported for decadienal, the better-studied PUA of this group. Moreover, post-recovery experiments show that embryos can recover after treatment with all three PUAs, indicating that negative effects depend both on PUA concentrations and the exposure time of the embryos to these metabolites. We also identify the time range during which PUAs exert the greatest effect on sea urchin embryogenesis. We also followed, by real-time qPCR, thirty one genes (having a key role in a broad range of functional responses, such as development, differentiation and detoxification processes) to identify potential target genesaffected by PUAs and their correlation with morphological abnormalities. This study opens new perspectives for understanding how marine organisms afford protection from environmental toxicants through an integrated network of genes.

The deleterious effects of *Ostreopsis* cf. *ovata* bloom on *Paracentrotus lividus* are mediated by nitric oxide

O. Migliaccio, I. Castellano, D. Di Cioccio, P. Cirino, G. Romano, A. Zingone, A. Palumbo
Stazione Zoologica Anton Dohrn, Napoli, Italy

The sea urchin *Paracentrotus lividus*, a keystone herbivore in the Mediterranean sea, is a good model system to study the response to environmental stress. Recently, it has been shown that the signaling molecule nitric oxide (NO), formed by L-arginine oxidation by NO synthase (NOS), is involved in the stress response induced by the diatom aldehyde decadienal. Indeed, NO protects sea urchin embryos from the toxic effects of the aldehyde, contributing to the activation of *hsp70* gene expression.
In this study we investigated the effect of *Ostreopsis* cf. *ovata*, a dinoflagellate producing a range of palytoxin-like molecules during bloom episodes, on the sea urchin *P. lividus* . Animals were collected in an area of the gulf of Naples during the main bloom and at different times after the bloom. Female sea urchins and offspring were examined by morphological, biochemical and molecular approaches. An increased NO production was revealed in the gonads with concomitant nitration of the major protein toposome. Moreover, abnormal development was observed in the offspring which does not develop properly even after some months from the bloom. Gene expression analyses revealed differences in the expression of several skeletogenic, multidrug efflux and stress genes, including NOS. Further experiments performed under different NO levels will allow the identification of genes whose expression induced by *Ostreopsis* bloom is directly or indirectly mediated by NO.

Co-exposure to cadmium and UVB radiation impairs sea urchin embryo development and induces cell stress response at gene and protein levels

R. Bonaventura, R. Russo, F. Zito, V. Matranga
Istituto di Biomedicina e Immunologia Molecolare "A. Monroy", CNR, Palermo, Italy

Due to environmental pollution many agents affect marine organisms concurrently and this is a field of growing interest. Cadmium (Cd) is a strong pollutant linked to humans activities and natural events. UVB radiation (UVB) is a physical agent of major concern in the marine ecosystems related to the ozone layer reduction. We have previously shown that continuous exposures of sea urchin embryos to Cd caused developmental abnormalities, increased levels of *Pl*-MT mRNA and HSPs proteins. Similarly, UVB radiation exposure affected embryonic skeleton, induced HSP70 and p38 activation.
Here, we used *P. lividus* embryos treated continuously with 10^{-4}M Cd and irradiated with 200J/m^2 UVB (Cd/UVB-exposure) at the cleavage stage, to estimate the combined effects on development, gene expression and MAPKs activation. We found that Cd/UVB exposure induced a marked decrease in the percentage of normal embryos, mainly affecting the skeleton patterning, as observed by IF using a McAb to the skeletogenic marker MSP130. We analyzed the expression of *Pl*-14-3-3e, *Pl*-MT and *Pl*-jun genes in Cd/UVB-embryos and found a remarkable up-regulation of their mRNA levels as assessed by QPCR, and their spatial delocalization by WMISH. By WB we also found that p38 is the only MAPK activated soon after Cd/UVB exposure.
Our results are in favour of a synergistic effect induced by Cd/UVB-exposure in sea urchin embryos. Besides, these results confirm the *P.lividus* embryo as a valuable tool in assessing the effects of environmental stressors. Moreover, the analysis of the molecular pathways involved in stress response might help in developing valuable stress biomarkers.

Microplastics in the marine environment: a true ecotoxicological risk?

C. Avio[1], S. Gorbi[1], M. Milan[2], M. Benedetti[1], D. Fattorini[1], F. Regoli[1]
[1]Dept of Live and Environmental Sciences, Polytechnic Univ. of Marche, Ancona, Italy
[2]Dept of Comparative Biomedicine and Food Science, Univ. of Padua, Italy

Microplastics represent a growing environmental concern for the oceans due to their potential capability to adsorb different classes of pollutants, thus representing a still unexplored source of exposure for aquatic organisms. In this study, different micro-polymers were shown to efficiently bind polycyclic aromatic hydrocarbons with a time and dose-dependent trend, and transfer these chemicals to filter feeding mussels *Mytilus galloprovincialis*. Tissue localization of microplastics revealed their presence in haemolymph, gills and especially digestive tissues where a marked accumulation of PAHs was also observed. A wide spectrum of cellular effects was assessed, including immunological responses, lysosomal detabilization, peroxisomal proliferation, antioxidant and neurotoxic effects, onset of genotoxicity. The use a new DNA microarray platform demonstrated significant changes in gene expression profile of mussels exposed to both virgin and contaminated microplastics. Accumulation and presence of microplastics was demonstrated also in different fish species sampled along the Adriatic coast, further emphasizing the need to consider the potential (eco)toxicological role of microplastics in the marine environment.

Organic contaminants as potential obesogens in mammalian and marine invertebrate cells

A. Smerilli, E. Grasselli, C. Ciacci, T. Balbi, L. Canesi
Dept. of Earth, Environment and Life Sciences, DISTAV, Univ. of Genoa, Italy

Certain Endocrine Disrupting Chemicals (EDCs), such as alkylphenols, organotins, perfluorinated compounds, have been shown to affect energy and lipid metabolism in animals and humans, acting as obesogens. These effects can be systemic, or result from direct interactions with target cells. In vitro models may help screening the effects of EDCs on lipid homeostasis and identifying their mechanisms of action, not only in mammals also in marine organisms.
In rat FaO hepatoma cells, both BPA (Bisphenol A) and its brominated derivative TBBPA (tetrabromobisphenol A) can effect lipid homeostasis through rapid, non genomic mechanisms of action involving activation of kinase cascades. Here evidence is provided for lipid accumulation induced by these chemicals in cells isolated from the digestive gland (hepatopancreas), a tissue that plays a key role in energy metabolism and lipid storage in the marine mussel *Mytilus*. The observed increases in lipid content were rapid, and were prevented by specific inhibitors of kinase-mediated cell signaling.
The results underline complex interactions between different EDCs and the pathways involved in lipid homeostasis. The utilization of bivalve isolated digestive gland cells proved as a sensitive in vitro model to investigate the obesogenic potential of organic contaminants in marine inverterbrates.

Acute toxicity from pro-oxidant copper in *Mytilus edulis* gills: a redox proteomic analysis

S.N. Jaafar[1,2], D. Sheehan[1]
[1]Proteomics Research Group, School of Biochemistry and Cell Biology, Univ. College Cork, Ireland
[2]Marine Biology Program, School of Marine Science and Environment, Univ. Malaysia Terengganu, Terengganu, Malaysia

The pro-oxidant metal copper is a common environmental pollutant which can induce oxidative stress in tissues of aquatic organisms. An excess of copper in the environment, especially from mining and other anthropogenic activities could contribute significant contamination to marine ecosystems. In this study a popular sentinel species the blue mussel, *Mytilus edulis*, was exposed under controlled conditions to different sub-lethal doses of copper chloride (50 ug/L, 150 ug/L and 300 ug/L) for one hour with 24 hours recovery time. Effects on the gill, the principal feeding organ of the animal, were investigated, followed by measurement of enzymatic activities, protein carbonyl and thiol levels. Oxidation of protein thiols was studied by means of one-dimensional electrophoresis (1DE) and two-dimension electrophoresis (2DE). It was noted that there were significant difference in gill glutathione transferase activities, while no effect was evident on gill glutathione reductase or catalase activities. Protein spots were detected by colloidal Coomassie staining and protein profiles compared by the Progenesis SameSpots software. Analysis of blue mussels' proteome facilitates detection of subtle changes at the level of individual proteins in response to environmental stressors. This could potentially lead to discovery of novel biomarkers of exposure to chemical pollutants and may generate insights into underlying mechanisms of toxicity.

Nitric oxide mediates the response to metals in *Paracentrotus lividus* developing embryos

O. Migliaccio, I. Castellano, G. Romano, A. Palumbo
Stazione Zoologica Anton Dohrn, Napoli, Italy

In invertebrates, the physiological messenger nitric oxide (NO), produced by L-arginine oxidation by NO synthase (NOS), is involved in many biological processes. In sea urchins NO is implicated in important processes occurring at fertilization and at later developmental stages. Recently, it has been shown that NO production is the first response of *Paracentrotus lividus* sea urchin embryos to the diatom-derived aldehyde decadienal and that this gas mediates the response to this bioactive agent. In this study, we investigated the involvement of NO in the response of sea urchin embryos to cadmium and manganese, two metals with different properties and known to affect their development. To this aim, embryos were treated with different metal concentrations under reduced endogenous NO levels, using the NOS inhibitor TRIM. An increase in the number of abnormal plutei at increasing TRIM concentrations was observed, suggesting a protective role of this messenger in the stress response induced by these agents. Quantitative expression of a panel of 19 genes involved in stress response, skeletogenesis, detoxification and multidrug efflux processes was followed at different developmental stages and under different conditions: metals alone, metals in the presence of NOS inhibitor, NO donor and NOS inhibitor alone. These data allowed the identification of different classes of genes whose metal-induced expression was directly or indirectly mediated by NO. These results open new perspectives on the role of NO as a sensor of different stress agents in sea urchin developing embryos.

This project has been partly funded by RITMARE

Asymmetric skeleton patterns induced by gadolinium ions in sea urchin embryos: focus on mechanisms regulating skeletogenesis and comparison among phylogenetically distant species

C. Martino[1,2], R. Chiarelli[1], C. Costa[2], D. Koop[3], M.C. Roccheri[1], M. Byrne[3], V. Matranga[2]

[1]Dip. Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche, Univ. di Palermo, Palermo, Italy

[2]Istituto di Biomedicina e Immunologia Molecolare "A. Monroy", CNR, Palermo, Italy

[3]Dept of Anatomy and Histology, F13, Univ. of Sydney, New South Wales, Australia

Gadolinium (Gd) is a metal of the lanthanide series of the elements whose chelates are commonly employed as contrast agents for magnetic resonance imaging, and subsequently released into the aquatic environment. We analysed the consequences of embryo exposure to sublethal concentrations of Gd on development, focusing on skeletogenesis and developmental symmetry. We observed a strong inhibition of skeleton growth, frequently displayed by an asymmetrical pattern. Removal of Gd after 24 hours cause partial recovery of embryo development 48 hours post fertilization, demonstrating a reversible effect. Autophagy was investigated by Western blot analysis, showing an increase of the LC3 protein marker at 24h and 48h. Confocal microscopy studies confirmed the increased number of autophagosomes and autophagolysosomes, suggesting that the autophagic process is acting as a cell survival strategy. RT-PCR gene expression analysis showed the misregulation of several genes implicated in different functional levels of both the skeletogenic and the left-right axis specification networks. These included early expressed genes and signaling molecules acting upstream, as well as downstream skeletal structural genes. The comparison of the effects on phylogenetically distant sea urchin species confirmed that Gd highly perturbs skeletogenesis, with species-specific threshold levels of sensitivity. These results pose serious questions on the hazard of Gd in the marine environment.

Disorders induced by environmental stress in *Pyropia yezoensis* (Rhodophyta)

M. Kakinuma[1], S. Iwade[2], K. Suzuki[1], S. Iwata[1]
[1]Graduate School of Bioresources, Mie Univ., Tsu, Japan
[2]Suzuka Fisheries Research Division, Mie Pref. Fish. Res. Inst., Suzuka, Japan

In Japan, *Pyropia yezoensis* (*nori*) is one of the most extensively cultivated macroalgal species used as food, with about 340,000 tons (wet weight) harvested annually. However, *Pyropia* cultivation is greatly influenced by environmental conditions. Recently, *Pyropia* cultivation is affected by developmental and morphological disorders with rising seawater temperature. In order to understand the molecular mechanisms for response and adaptation to temperature changes in *Pyropia* thalli, we have investigated differentially expressed transcripts among the *P. yezoensis* standard "U51" and high-water temperature tolerance "MET11" strains, and the *P. suborbiculata* "PSI" and "PSO" strains isolated from northern and southern Japan, respectively. We report on the candidate genes related to adaptation and tolerance mechanisms to higher water temperature in *Pyropia* thalli. In addition, one of the most important environmental factors, which influences on growth and quality of *Pyropia* thalli, is nutrient condition. Discoloration of *Pyropia* thalli (*iroochi*) caused by decrease of inorganic nitrogen in seawater occurs in *Pyropia* cultivation farms every year. *Pyropia* thalli can utilize organic solutes like urea and certain amino acids as well as inorganic nitrogen. In these organic nitrogen sources, urea is the most effective to re-coloration of the discolored thalli. We report on the characteristics of three urea transporter genes, *PyDUR3.1/3.2/3.3*, isolated from *P. yezoensis*.

Marine microalgae as a sustainable source of bioactives for drug discovery

A. Ianora, G. Romano
Stazione Zoologica Anton Dohrn, Napoli, Italy

Natural products have been long been known to be major sources of medicinal medicines and many of these have gone on to become current marketed drugs. Drug discovery from marine natural products is much more recent with the first marine bioactive compounds isolated in the early 1950s. But some estimates suggest that the probability of discovering a drug from marine sources is approximately a thousand times more than that from terrestrial ones. This is due to the greater biodiversity of marine organisms and their chemical products, most of which have no terrestrial counterpart. Currently there are around 15 marine natural products in various phases of clinical development, mainly in the oncology area, with more on the way. But before marine drug discovery can reach its full potential two major bottlenecks must be overcome: sustainability and replicability. The possibility of obtaining a sustainable and continuous source of bioactives from marine microalgae that are more amenable to culturing in enclosed bioreactors compared to macro-organisms (sponges, corals, molluscs) may be able to meet the challenging demands of pharmaceutical industries. This would enable a more environmentally-friendly approach to drug discovery and overcome some of the bottlenecks such as the over-utilization of marine resources and the use of destructive collection practices.

Echinoderms as sustainable source of collagen for innovative applications in regenerative medicine

M. Sugni[1], C. Di Benedetto[1], T. Martinello[2], C. Ferrario[1], L. Leggio[1], A. Barbaglio[1], C. Gomiero[2] , F. Bonasoro[1], M. Patruno[2], M.D. Candia Carnevali[1]
[1]Dept of Biosciences, Univ. of Milan, Italy
[2]Dept of Comparative Biomedicine and Food Science, Univ. of Padova, Italy

Collagen-made materials and scaffolds are the most promising in tissue engineering applications, since they simultaneously offer biocompatibility and optimal mechanical performances. In this work we used common marine invertebrates (echinoderms) as an innovative and low-cost source of native intact collagen fibrils to develop ECM equivalents. Collagen fibrils were isolated from connective tissues of different echinoderms (sea urchins, starfish, sea cucumber), using putative by-products of the food industry or fishery. These fibrils were used to prepare films and/or 3D scaffolds which were characterized and compared in terms of ultrastructure and biomechanics. I*n vitro* biocompatibility tests were performed using mesenchymal stem cells isolated from horse peripheral blood; preliminary *in vivo* tests were assessed by subcutaneous implantation of the scaffold in rabbits and CAM tests for angiogenesis evaluation. The obtained fibrillar matrices strictly mimicked the native ECM and displayed mechanical performances in the range of natural tendon and skin. *In vitro* tests indicated that such substrates are not toxic for mammalian cells, which, after an initial "adaptation" stage, were also able to actively proliferate. *In vivo* tests suggest encouraging results related to biocompatibility and angiogenetic response, although further investigations are necessary. Overall echinoderm-derived collagenous matrices have the potential to be used for tendon or skin regenerative medicine.

Long-term preservation of echinoderm sperm under non-cryo condition for ecotoxicological bioassay

M. Kiyomoto
Graduate School of Humanities and Sciences, Ochanomizu Univ., Tokyo, Japan
Tateyama Marine Lab., Marine and Coastal Research Center, Ochanomizu Univ., Tateyama, Japan

We are examining the preservation of sea urchin gametes to make them more convenient material for education and research, especially on the embryology and ecotoxicology field. It is advantageous that a large amount of gametes they spawn and the simple and quick fertilization are available for the assay. If some organizations offer the supply of gametes possible to keep in appropriate period with sufficient quality, collection and care of adult urchins will not be necessary for each researcher. The possibility of egg preservation for enough period, two weeks to one month, were reported (Epel *et al*., 2004; Kiyomoto *et al*., 2013). The sperm storage is usually done without seawater (dry sperm) for several days. This storage period is lengthened by the addition of antibiotics to around 10 days (Hata, 1998). To maximize the preserved period, we examined the dilution or wash with antibiotics seawater. Because the activation of sperm is induced by dilution, some conditions to inhibit flagella motility were also investigated. The dilution of dry sperm with antibiotics normal seawater was enough to keep the motility and fertilization ability for longer period. Neither high potassium nor low pH improved the period of sperm preservation. In the condition, though sperm is diluted in seawater, the motility totally prevented provably by the effect of carbon dioxide

POSTERS

POSTERS

P.1S

MarLab: a marine research laboratory organized according to a quality management system

A. Bongiovanni[1], L. Caruana[1], L. Riccobono[1], M. Di Bernardo[1], L. La Gattuta[1], L. Anello[1], A. Pensato[1], A.M. Kisslinger[2], G. Liguori[3], G. Colotti[4], A. Lanati[5], A. Mascia[2], A. Cirafici[2], G. Lacerra[3], A. Digilio[6], M. Di Carlo[1]
[1]IBIM-CNR, Palermo
[2]IEOS-CNR, Napoli
[3]IGB-CNR, Napoli
[4]IBMN-CNR, Roma
[5]Valore Qualità, Pavia
[6]IBBR-CNR, Napoli

Quality principles and methodologies have been widely used in industrial and business applications, giving positive advantages in terms of good management, results improvement and cost reduction. This approach has long been ignored in scientific research which by their nature are often far from the industrial and business fields. Our aim was to generate and apply a Quality management system (QMS) for a research lab, working in the area of Life Sciences. Specifically, we have selected a research laboratory, called MarLab, working with marine animal models in the scientific area of drug discovery and embryonic development. From among various others (i.e., GLP, ISO17025, etc.), we chose to implement the ISO 9001:2008 Quality system. As specified by the ISO standard and in agreement with the heads of the laboratory, we first defined the Quality policy. Next, we identified operational and support processes to be managed, stakeholders, recipients, and suppliers. We also generated the procedures, operating instructions, guidelines and forms to cover all laboratory aspects. Recently, MarLab received the certification of all activities according to the ISO 9001:2008 standard. To facilitate the management of the QMS we have also developed a modular software, Help4Lab. We expected that this QMS model could be a new laboratory organization, motivating the staff towards a continuous improvement of shared operations and enhancing communication between all management levels and personnel. Such a system would also ensure the reliability of the results of research laboratories. Furthermore, the QMS model would increase the prestige of the laboratory and the Public Research Institution.

P.2S

Biomarkers of quality in commercially important Mediterranean species of crustaceans: a multiparametric approach

A. Pinsino, R. Bonaventura, C. Costa, R. Russo, F. Zito, V. Matranga
Istituto di Biomedicina e Immunologia Molecolare "A. Monroy", CNR, Palermo, Italy

In the framework of the DeCroMed Project, we used a multi-parametric approach to study the quality of the shrimp Parapeneus longirostris, and the lobster Nephrops norvegicus, during post-thawing periods. The quality of crustaceans as food products can be affected by environmental pollutants as well as by practices taking place during catching, handling, processing and storage. In this study we analysed by a multi-parametric approach the muscle exudates obtained by centrifugation from thawed specimens. We measured the water-holding capacity of P. longirostris and N. norvegicus and monitored changes in pH, protein content and composition of muscle exudates obtained by centrifugation. In addition, we evaluated the levels of a few candidate marker proteins, chosen from a list of biological markers known to predict the quality of food products and validated for their responsiveness to pollutants. Our results indicate that HSP70 protein levels and the analysed parameters are related to the quality and shelf life of crustaceans. Based on these results we defined a molecular assay that will be used to build a prototype device able to test the quality of crustaceans, and eventually their exposure to pollutants.

This work has been fully financed by the DeCroMed Project (http://www.decromed.it/) of the EU - Research & Innovation Strategy–Regione Sicilia–PO-FESR 2007-2013, CUP: G93F12000190007. The technical assistance of Mr. Mauro Biondo is acknowledged.

P.3S

An electronic nose based on MOS sensors for the quality control of deepwater rose shrimps

F. Lo Bosco[1], V. Guarrasi[1], F. D'Anca[1], A. Zaffuto[2,3], M. Zora[3], F. Librizzi[1], P.L. San Biagio[1]
[1]National Research Council, Inst. of Biophysics, Palermo, Italy
[2]Daimar S.R.L. Mazara del Vallo, Italy
[3]National Research Council, Inst. for Coastal and Marine Environment, Capo Granitola, Italy

The Mediterranean Sea represents one of the most important fishing area for *Parapeneus longirostris* (FAO Name: Deep-water rose shrimp). After being caught, the shrimps must be immediately frozen on board, otherwise they can go bad in few hours. Subsequently, the thawing process may change the organoleptic properties of the product; it is therefore important to establish good thawing procedures and to monitor the shelf life of the product after thawing. Moreover, the analysis of the aroma of shrimps can give important information on the presence of pollutants in the fishing areas and on their effects on marine organisms.
We used a laboratory Electronic Nose (EN) coupled with six different MOS sensors, to study the aroma of thawed shrimps and its changes after thawing. In parallel, a Gas Chromatographic analysis coupled to Mass Spectrometry (GC-MS) was carried out on the volatile fraction, to characterize the aromatic fingerprint.
The results show that a MOS based EN is able to clearly distinguish between shrimps of different quality. In addition, GC-MS data preliminarily indicate that the presence of pollutants may be detected in the aroma of the shrimps. Based on these evidences, we are currently developing a portable device for the rapid and accurate determination of the quality of crustaceans, that can be used *in situ* for the investigation of the effects on these organisms of both the thawing procedures and the presence of pollutants.

P.4S

Sea urchin embryo a model for aging research: use of oxidant and antioxidants molecules

P. Picone, D. Nuzzo, E. Messina, L. Cristaldi, V. Scafidi, M. Di Carlo
Ist. di Biomedicina e Immunologia (IBIM), CNR, Palermo, Italy

Free radicals are common outcome of normal aerobic cellular metabolism, imbalanced defense mechanism of antioxidants overproduction or incorporation of free radicals from environment to living system leads to oxidative stress (OS). OS plays a relevant role in triggering apoptosis and consequently is linked to pathologies such as neurodegenerative diseases (Alzheimer, Parkinson, Amyotrophic Lateral Sclerosis diseases) or others associated with aging. The use of antioxidants may play a pivotal role in preventing or slowing the progression of these diseases. In particular the use of natural molecules with antioxidant properties could be a suitable strategy for inhibiting the cell death cascade. Recently, sea urchin has been employed as a simple model system in aging and drug discovery research. This model system was, indeed, used to identify molecules and pathways involved in neurodegenerative processes [1-2]. Here we study the effect of oxidant (Hydrogen Peroxide (H2O2), β-amyloid, metformin) and antioxidant (ferulic acid, tocopherol, tyrosol) molecules related to neurodegenerative process on Paracentrotus lividus embryo. In particular, we investigated the effect of oxidants and antioxidants on morphology and biochemical mechanism.

1. Picone P., Nuzzo D., Di Carlo M. Ferulic acid: a natural antioxidant against oxidative stress induced by oligomeric A-beta on sea urchin embryo. Biol Bull. 2013 Feb;224(1):18-28.

2. Pellicano M, Picone P, Cavalieri V, Carrotta R, Spinelli G, Di Carlo M. The search urchin embryo a model to study Alzheimer's beta amyloid induced toxicity. Arch Biochem Biophys. 2009 Mar 1;483(1):120-6

P.5S

Studies on heavy metals effects on *Mytilus galloprovincialis* sperm chromatin proteins and on DNA

M. Piscopo[1], F. Caccavale[1], F. Pollice[1], G. Speranza[1], S. Avagnano[1], D. Drongitis[1], G. Guerriero[1], L. Fucci[1], J. Ausió[2]
[1]Dept of Biology, Univ. of Naples Federico II, Italy
[2]Dept of Biochemistry & Microbiology, Univ. of Victoria, British Columbia, Canada

Heavy metal pollution has become one of the most serious environmental problems in the world that brought threat to human health and ecological security. To monitor sea levels of heavy metals we used as specific bioindicator *Mytilus galloprovincialis* whose sperm chromatin is organized mainly by three protamine-like proteins (PLII, PLIII and PLIV). These proteins protect DNA from a variety of potentially dangerous reactive species but chromatin packaging doesn't protect DNA from metal ion-dependent damage in the presence of hydroxyl radicals. We report the effects induced by Cu^{2+}, Ni^{2+} and Cd^{2} ions on DNA and on *Mytilus galloprovincialis'* PLII and PLIII. Self-association ability of PLII and PLIII mediated by Cd^{2+}, Ni^{2+} and Cu^{2+} show that Cd^{2+} causes only PLII aggregation to a greater extend respect to Ni^{2+} that induces a mild aggregation of both PLII and PLIII; Cu^{2+}, instead, doesn't produce any type of aggregates. In vivo studies show that these heavy metals affect PLII, PLIII and PLIV state and properties. While nickel produces an increase of DNA binding affinity for both PLII and PLIII cadmium causes a decrease of PLII DNA binding affinity but an increase for PLIII. Copper and cadmium promote hydrogen peroxide damage of DNA also in the presence of protamine-like proteins while nickel doesn't. All these effects can lead an anomalous chromatin packaging and a reduction of the reproductive fitness in *Mytilus galloprovincialis* and other organisms exposed to the same toxicants.

P.6S

Analysis of the effects of co-exposure to antibiotics and cadmium on sea urchin embryos

M.A. Ragusa[1], A. Nicosia[2], F. Gianguzza[1]
[1]Dip. STEBICEF, Univ. di Palermo, Palermo, Italy
[2]IAMC-CNR di Capo Granitola, Torretta-Granitola, Trapani, Italy

In order to understand the mechanisms of responses to changes in the physical and chemical environment, as well as the mechanisms of developmental pathways, we investigated by RT-qPCR assays and light microscopy observations the impact of antibiotics and cadmium in *P. lividus* sea urchin embryos. In particular we inspected development and biomarkers for free radical damage and apoptosis.
During development embryos were exposed to an antibiotic mix (Ab mix, usually added to sea urchin cultures) or to sulfamethoxazole/trimethoprim mix (TMP/SMX, usually added to aquacultures) and/or levels of 10^{-5}, 10^{-4}, 10^{-3} M $CdCl_2$.
Even though treatment with TMP/SMX apparently did not affect development, it stimulated a remarkable molecular response to oxidative stress.
Moreover, when embryos were exposed to TMP/SMX and $CdCl_2$, even development was seriously impaired and the antioxidant defense was blocked.
On the other hand, Ab mix and Cd co-treatment made the cadmium effect on development worse, but the antioxidant molecular defense was unchanged, rising the possibility that another stress pathway is involved.
This study leads to the conclusion that co-exposure to antibiotics and cadmium induces synergistic effects on sea urchin embryos. Thus, in cadmium contaminated areas, antibiotic discharge can be an important environmental factor which might play an important role in survival of *P. lividus* populations.

PARTICIPANTS

PARTICIPANTS

Maria Agnello
maria.agnello@unipa.it
Palermo, Italy

Letizia Anello
anello@ibim.cnr.it
Palermo, Italy

Carlo Giacomo Avio
c.g.avio@univpm.it
Ancona, Italy

Teresa Balbi
teresa.balbi@unige.it
Genoa, Italy

Mauro Biondo
mbiondo@ibim.cnr.it
Palermo, Italy

Julian Blasco
julian.blasco@csic.es
Puerto Real (Cadiz), Spain

Rosa Bonaventura
rosa.bonaventura@ibim.cnr.it
Palermo, Italy

Chiara Bonaviri
chiara.bonaviri@unipa.it
Palermo, Italy

François Burgay
francois.burgay@gmail.com
Turin, Italy

Isabella Buttino
isabella.buttino@isprambiente.it
Livorno, Italy

Maria Byrne
mbyrne@anatomy.usyd.edu.au
Sydney, NSW, Australia

Marcello Cacace
mcacace@bo.ismn.cnr.it
Bologna, Italy

Laura Canesi
laura.canesi@unige.it
Genoa, Italy

Simone Cappello
simone.cappello@iamc.cnr.it
Messina, Italy

Tiziana Cappello
tcappello@unime.it
Messina, Italy

Luca Caruana
luca.caruana@ibim.cnr.it
Palermo, Italy

Eva Catanese
ecatanese@unime.it
Messina, Italy

Gary Cherr
gncherr@ucdavis.edu
Bodega Bay, CA, USA

Roberto Chiarelli
robertochiarelli82@libero.it
Palermo, Italy

Caterina Ciacci
caterina.ciacci@uniurb.it
Urbino, Italy

Ilaria Corsi
ilaria.corsi@unisi.it
Siena, Italy

Caterina Costa
caterina.costa@ibim.cnr.it
Palermo, Italy

Maria Costantini
maria.costantini@szn.it
Naples, Italy

Stefania D'Agostino
dagostino@ibim.cnr.it
Palermo, Italy

Marta Di Carlo
marta.dicarlo@ibim.cnr.it
Palermo, Italy

Narimane Dorey
narimane.dorey@gmail.com
La Rochelle, France

Marco Faimali
marco.faimali@ismar.cnr.it
Genoa, Italy

Edi Gabellieri
edi.gabellieri@pi.ibf.cnr.it
Pisa, Italy

Francesca Garaventa
francesca.garaventa@ismar.cnr.it
Venice, Italy

Agata Giallongo
agata.giallongo@ibim.cnr.it
Palermo, Italy

Paola Gianguzza
paola.gianguzza@unipa.it
Palermo, Italy

Adrianna Ianora
adrianna.ianora@szn.it
Naples, Italy

Siti Jaafar
tahirah@umt.edu.my
Cork, Ireland

Makoto Kakinuma
kakinuma@bio.mie-u.ac.jp
Tsu, Japan

Masato Kiyomoto
kiyomoto.masato@ocha.ac.jp
Tateyama, Japan

Keita Kodama
kodama.keita@nies.go.jp
Tsukuba, Ibaraki, Japan

Jerome Labille
labille@cerege.fr
Aix-en-Provence, France

Fabrizia Lo Bosco
fabrizia.lobosco@pa.ibf.cnr.it
Palermo, Italy

Maria Maisano
mmaisano@unime.it
Messina, Italy

Giuseppe Mancini
gmancini@dii.unict.it
Catania, Italy

Ilaria Marisa
ilaria.marisa@studenti.unipd.it
Padua, Italy

Chiara Martino
chiara.martino@unipa.it
Palermo, Italy

Valeria Matranga
matranga@ibim.cnr.it
Palermo, Italy

Oriana Migliaccio
oriana.migliaccio@szn.it
Naples, Italy

Elisabetta Morelli
elisabetta.morelli@pi.ibf.cnr.it
Pisa, Italy

Marco Munari
marco.munari@bio.unipd.it
Padua, Italy

Domenico Nuzzo
domenico.nuzzo@cnr.it
Palermo, Italy

Mari Ochiai
ochiai@agr.ehime-u.ac.jp
Matsuyama, Japan

Anna Palumbo
anna.palumbo@szn.it
Naples, Italy

David Pellegrini
david.pellegrini@isprambiente.it
Livorno, Italy

Alessandro Pensato
alessandro.pensato@ibim.cnr.it
Palermo, Italy

Pasquale Picone
picone@ibim.cnr.it
Palermo, Italy

Annalisa Pinsino
pinsino@ibim.cnr.it
Palermo, Italy

Marina Piscopo
piscopo@unina.it
Naples, Italy

Maria Ragusa
maria.ragusa@unipa.it
Palermo, Italy

Francesco Regoli
f.regoli@univpm.it
Ancona, Italy

Maria Roccheri
maria.roccheri@unipa.it
Palermo, Italy

Daniele Paolo Romancino
romancino@ibim.cnr.it
Palermo, Italy

Giovanna Romano
giovanna.romano@szn.it
Naples, Italy

Roberta Russo
rrusso@ibim.cnr.it
Palermo, Italy

David Sheehan
d.sheehan@ucc.ie
Cork, Ireland

Michela Sugni
michela.sugni@unimi.it
Milan, Italy

Maria Vitale
marvitus@yahoo.com
Villabate, Italy

Valeria Vitale
vvitale@unime.it
Messina, Italy

Hajime Watanabe
watanabe@bio.eng.osaka-u.ac.jp
Suita, Japan

Yukio Yokota
yokota@ist.aichi-pu.ac.jp
Nagakute, Japan

Francesca Zito
francesca.zito@ibim.cnr.it
Palermo, Italy

Design and layout

mail@azuleon.com