5th Bilateral Seminar Italy-Japan

Book of Abstracts

1st Marine NanoEcotox Workshop

Palermo 27th - 29th November 2012

Area della Ricerca CNR
Via Ugo La Malfa 153 - Palermo, Italy
BSIJ 2012 ORGANIZATION

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BSIJ-2012 Program

November 27th, 2012

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.30 - 9.00</td>
<td>Registration of Participants</td>
</tr>
<tr>
<td>9.00</td>
<td>Welcome of the Organizers</td>
</tr>
<tr>
<td></td>
<td>Valeria Matranga &amp; Masato Kiyomoto</td>
</tr>
<tr>
<td></td>
<td><strong>Opening Remarks &amp; Authorities Speaches</strong></td>
</tr>
<tr>
<td></td>
<td>Gianluigi Condorelli, Coordinator of the Department of Biomedicine, National Research Council (CNR)</td>
</tr>
<tr>
<td></td>
<td>Pier Luigi San Biagio, President of the Area della Ricerca, CNR, Palermo</td>
</tr>
<tr>
<td></td>
<td>Giovanni Viegi, Director of the Institute of Biomedicine and Molecular Immunology “A. Monroy”, CNR, Palermo</td>
</tr>
<tr>
<td>9.30</td>
<td>Foreword talk</td>
</tr>
<tr>
<td></td>
<td>Yukio Yokota (Aichi Prefectural University, Aichi, Japan)</td>
</tr>
<tr>
<td></td>
<td>Physical and Chemical Impacts on Marine Organisms.</td>
</tr>
<tr>
<td></td>
<td>- Retrospect and Foresight –</td>
</tr>
<tr>
<td></td>
<td><strong>Session 1</strong></td>
</tr>
<tr>
<td></td>
<td>Bio-materials from marine organisms, new bioassay models and materials</td>
</tr>
<tr>
<td></td>
<td>Chairs: Adrianna Ianora – Yukio Yokota</td>
</tr>
<tr>
<td>9.45</td>
<td>Salvatore Aricò (Coordinator of the UNESCO biodiversity initiative, Natural Sciences Sector, UNESCO, Paris, France)</td>
</tr>
<tr>
<td></td>
<td>The relevance of research on marine organisms for an enhanced effectiveness on policy measures related to the marine environment: The case of deep seabed genetic resources.</td>
</tr>
<tr>
<td>10.15</td>
<td>Adrianna Ianora (Stazione Zoologica, Naples, Italy)</td>
</tr>
<tr>
<td></td>
<td>The biotechnological potential of phytoplankton: new drugs and neutraceuticals from the sea.</td>
</tr>
<tr>
<td>10.45</td>
<td>Michela Sugni (University of Milan, Italy)</td>
</tr>
<tr>
<td></td>
<td>Innovative biomimetic materials from echinoderms: recent results and applied perspectives.</td>
</tr>
<tr>
<td>11.15-11.30</td>
<td>COFFEE BREAK</td>
</tr>
<tr>
<td>11.30</td>
<td>Masato Kiyomoto (Ochanomizu University, Tokyo, JAPAN)</td>
</tr>
<tr>
<td></td>
<td>Preserved echinoderm gametes as a useful and easy bioassay material.</td>
</tr>
<tr>
<td>12.00</td>
<td>Marco Faimali (Institute of Marine Sciences, CNR, Genoa, Italy)</td>
</tr>
<tr>
<td></td>
<td>Ephyra jellyfish as a new model for ecotoxicological bioassays.</td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>12.30</td>
<td>Poster Session</td>
</tr>
<tr>
<td>13.00-14.30</td>
<td>LIGHT LUNCH</td>
</tr>
<tr>
<td>14.30</td>
<td><strong>Session 2</strong></td>
</tr>
<tr>
<td>15.00</td>
<td><strong>Keita Kodama</strong> (National Institute for Environmental Studies, Tsukuba, Japan)</td>
</tr>
<tr>
<td>15.30</td>
<td><strong>Kei Nomiyama</strong> (Ehime University, Matsuyama, Japan)</td>
</tr>
<tr>
<td>16.00-16.15</td>
<td>COFFEE BREAK</td>
</tr>
<tr>
<td>16.15</td>
<td><strong>Bijoy Nandan</strong> (Cochin University of Science &amp; Technology, Cochin, India)</td>
</tr>
<tr>
<td>16.45</td>
<td><strong>Paola Gianguzza</strong> (University of Palermo, Italy)</td>
</tr>
<tr>
<td>17.15</td>
<td><strong>Valentina Asnaghi</strong> (University of Genoa, Italy)</td>
</tr>
<tr>
<td>17.45</td>
<td><strong>SPECIAL REPORT</strong> on pollution after the Fukushima accident</td>
</tr>
</tbody>
</table>

**November 28th, 2012**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
<th>Title</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.30</td>
<td><strong>Session 3</strong></td>
<td><strong>Toshihiro Horiguchi</strong> (National Institute for Environmental Studies, Tsukuba, Japan)</td>
<td><em>Comparison of vas deferens and penis development between the rock shell, Thais clavigera (Muricidae) and the ivory shell, Babylonia japonica (Buccinidae).</em></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Speaker</td>
<td>Title</td>
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<td>---------------------------------</td>
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</tr>
<tr>
<td>10.00</td>
<td><strong>Bijoy Nandan</strong> (Cochin University of Science &amp; Technology, Cochin, India)</td>
<td>Physiological and histopathological responses to sublethal concentrations of copper in the teleost fish, <em>Anabas testudineus</em> (Bloch -1972).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.30</td>
<td><strong>Cristian Mugnai</strong> (ISPRA, Roma, Italy)</td>
<td>Application of a biological multicriteria approach for the assessment of adverse effects of sediment pollutants: the Port of Trapani as an ongoing case study.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.00-11.30</td>
<td></td>
<td><strong>COFFEE BREAK</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.30</td>
<td><strong>Francesco Regoli</strong> (Universita’ Politecnica delle Marche, Ancona, Italy)</td>
<td>Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.00</td>
<td><strong>Roberto Chiarelli</strong> (University of Palermo, Italy)</td>
<td>Sea urchin embryos cadmium-exposed as an experimental model system for studying the relationship between autophagy and apoptosis.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.30</td>
<td><strong>Hiromi Seno</strong> (Tohoku University, Sendai, JAPAN)</td>
<td>Native specific reactions may cause the paradox of population control: A theoretical approach with mathematical model.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.00-14.30</td>
<td></td>
<td><strong>LIGHT LUNCH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.30</td>
<td><strong>Makoto Kakinuma</strong> (Mie University, Tsu, Japan)</td>
<td>Molecular analysis of physiological and developmental responses to water temperature changes in <em>Porphyra</em> spp. (Rhodophyta).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.00</td>
<td><strong>Hajime Watanabe</strong> (Osaka University, Suita, Japan)</td>
<td>Visualization of chemical impacts using <em>Daphnia magna</em>.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.30</td>
<td><strong>Camilla Della Torre</strong> (University of Siena, Italy)</td>
<td>ABC transporters research in aquatic toxicology: suitable model systems and novel assays.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.00</td>
<td><strong>Valeria Matranga</strong> (Institute of Biomedicine and Molecular Immunology &quot;A. Monroy&quot;, CNR, Palermo, Italy)</td>
<td>Cellular and molecular stress induced by chemical and physical agents in sea urchins.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.30</td>
<td></td>
<td><strong>General Discussion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.00</td>
<td></td>
<td><strong>Social Dinner</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Session 1

Bio-materials from marine organisms, new bioassay models and materials

Physical and chemical impacts on marine organisms - retrospect and foresight

Y. Yokota

Aichi Prefectural University

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The word “the mother sea” is quite symbolic of its nature. Rachel Carson named the first chapter of her book “The sea around us” “the mother sea”. We have been provided huge amounts and varieties of gifts, foods, craftworks, entertainments, stabilization of atmospheric environment and others from the mother sea since the prehistoric era. As the ocean occupies approximately 70% of earth surface and has three-dimensional expansion (approx. 1,350 mega cubic kms), changes in the marine environment will influence the terrestrial organism and environment. The existence of sea has been ever essential for us, is so today, and will be in the future, from multiple points of views.

Our mother receives and accepts children’s actions with her generosity and affections, giving gentle suggestions. Mother sea, however, receives results of our social activities, industries, agricultures, fisheries, etc. without any words. Consequently the marine environment is gradually worsened and its inhabitants, marine organisms, are more or less threatened. We are usually aware of the deterioration of marine environment by looking at the dramatic change of marine environment and the tragic animals and plants. Here, we have to learn from our history. In general, natural environment never unexpectedly falls in the grave conditions but has been gradually getting worse mainly by anthropogenic factors.

The bilateral seminar, Italy and Japan focused on “Physical and Chemical Impacts on Marine Organisms” was started from the concernment on the marine environment and organisms, 2004, and has been taken place biannually. Here, the past seminar was reviewed and prospective thoughts are discussed. We never forget that the sea contributes to the quality of human society from various points of view, material, spiritual, psychic, cultural, artistic, artistic, literary, etc.
The relevance of research on marine organisms for an enhanced effectiveness on policy measures related to the marine environment: the case of deep seabed genetic resources

S. Aricò
UNESCO Biodiversity Initiative, Natural Sciences Sector, UNESCO, Paris, France
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The oceans are rich in biological diversity, including organisms which are host to unique genetic resources. These resources are defined in an explicit manner under the Convention on Biological Diversity – an international legal instrument dedicated to the conservation and sustainable and equitable use of biodiversity concluded under the auspices of the United Nations. From an applied research perspective, including with a commercial intent, the deep sea and the seabed contain genetic resources of actual and potential interest. The combination of extreme conditions and potential for new discoveries in the deepest areas of the oceans make these environments potentially one of the largest reservoirs of genetic resources of major interest for commercial and industrial applications. Marine genetic resources hold a significant promise for helping meet some of the major current development challenges – combating diseases through the development of new drugs; helping in combating pollution of the marine environment through bioremediation; and stimulating the creation of public-private partnerships in the area of research and development. Yet, in spite of the increasing attention paid to issues related to marine genetic resources by industry and policy makers, information on research and development related to marine genetic resources, as well as on the modalities of the partnerships established between scientific institutions – largely public – and companies – largely private – is still poor. There is also uncertainty surrounding the legal regime applicable to marine genetic resources from areas beyond national jurisdiction. These factors may hamper the utilization of marine genetic resources in a manner that can be environmentally sound and socially equitable. Fundamental research in a clear enabling policy framework can help address some of the emerging and unresolved issues related to access to and the sharing of benefits related to the utilization of these resources, including from the standpoint of international scientific cooperation, technology transfer and capacity-building.
The biotechnological potential of phytoplankton: new drugs and neutraceuticals from the sea

A. Ianora, G. Romano, A. Palumbo

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In nature, chemistry-based interactions often regulate growth and survival of organisms, thus determining biological success and shaping community processes such as seasonal succession, niche structure and population dynamics. What makes these compounds of human and commercial interest is related to their bioactivity. Novel leads for the pharmaceutical industry are already based on the chemistry of ecological interactions among marine organisms. In this sense, chemical ecology principles have so far paved the way to marine bioprospecting, as proven by: (1) anticancer, antiviral and anti-aging products (ARA-A, ARA-C, Trabectidin, Resilience) from organisms which use these molecules, for example, to deter feeding by predators or to inhibit the growth of competitors, (2) analgesics that are much more potent than morphine (PRIALT), derived from the toxins that marine cone snails use to paralyze their prey; or (3) functional products, such as collagen and adhesives from the materials used by sessile organisms to colonize free surfaces.

Of the organisms that have remained largely unexplored are phytoplankton that comprise a vast group of unicellular organisms that are the base of the food chain in the marine environment. It has been estimated that between 22,000 and 26,000 species exist, of which only a few species have been identified to be useful for commercial application (e.g. Spirulina and Chlorella). Many species produce unique products like carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, and sterols with potential applications as neutraceuticals. As a group, phytoplankton are also of particular interest in the development of future renewable energy sources.

The aim of this presentation will be to focus on the ecological function and biotechnological applications of natural products originating from marine phytoplankton. Understanding the mechanism of action and natural function of these compounds can provide a basis for finding new applications of these products for commercial purposes.
Innovative biomimetic materials from echinoderms: recent results and applied perspectives.

M. Sugni\textsuperscript{1}, A. Barbaglio\textsuperscript{1}, M.Barbosa\textsuperscript{2}, F. Bonasoro\textsuperscript{1}, C. Di Benedetto\textsuperscript{1}, D. Fassini\textsuperscript{1}, A.P.Lima\textsuperscript{2}, A. Ribeiro\textsuperscript{2}, C.C.Ribeiro\textsuperscript{23}, S.Tricarico\textsuperscript{1}, I.C.Wilkie\textsuperscript{4}, M.D. Candia Carnevali

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Biomimicry is a promising sub-discipline of Biotechnology \[1\] that is addressed to novel material design inspired by animal tissues and has potential applications even in the field of tissue engineering (TE) and regenerative medicine. The MIMESIS (Marine Invertebrates Models & Engineered Substrates for Innovative bio-Scaffolds) project has been developed within this scientific context. In this project we take inspiration from natural tissues belonging to very common marine invertebrates, the echinoderms, with the final aim of developing innovative biomimetic biomaterials. Indeed, echinoderms possess very peculiar connective tissues, called Mutable Collagenous Tissues (MCTs). MCTs undergo rapid, drastic and reversible changes in their passive mechanical properties (stiffness, tensile strength and viscosity), these phenomena being under strict nervous control \[2\]. These striking mechanical properties exclusively depend on variations in the reciprocal interactions and cohesive forces between collagen fibrils, these changes being mediated by cellularly secreted protein effectors. Therefore MCTs (and their components) can be a source of inspiration and a supply of marine-derived resources for the ex novo design and development of biomimetic smart materials: these latter could be potentially used for in vitro and in vivo applications whenever a controlled and reversible plasticization and/or stiffening of the biomaterial (film, 3D scaffold, etc..) is required. The echinoderm model used under the MIMESIS project is the common sea urchin Paracentrotus lividus: this is a well known experimental model and an edible aquacultured species, that can potentially provide large-scale and economically advantageous amount of MCTs, derived from by-products of the food industry. Considering this background, the MIMESIS project is necessarily based on a strong multidisciplinary approach combining functional biology with biomaterial engineering. Here we present a brief review of recent results derived from the different MIMESIS project phases: 1) characterization of P.lividus MCTs by morphological, biomolecular, biomechanical and biochemical analyses and 2) first studies towards the development of MCT-derived substrata for cell culture studies.

Preserved echinoderm gametes as a useful and easy bioassay material.
M. Kiyomoto, G. Hamanaka, M. Hirose, M. Yamaguchi

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Bioassay of physical or chemical impact on the organism is important and indispensable to assess the effect on environment and our life. Model animals with several advantages have been proposed and used in various researches. In such model animals, sea urchins are distributed throughout the world ocean and have a long research history. But possibility of the sea urchin laying hundreds of thousands eggs has not fully utilized yet.

The spawned eggs of sea urchin usually lose the ability to fertilize in one night. The period maintaining the ability of the fertilization is lengthened by the addition of antibiotics. The cooler temperature is also effective to lengthen the period but the eggs of many species become broken in such condition. *Hemicentrotus pulcherrimus* is the most popular species on the research of cell biology and developmental biology in Japan. Perhaps because of the winter spawning season eggs of this species are fortunately possible to preserve in low temperature. The egg can be kept for one week or more in low temperature with antibiotics. In these years, we have examined these preserved gametes as an experimental material for the middle school education and demonstrated the practicality. This year we start to supply also for the education in university class and for the laboratory research, where sea urchins are kept in a marine laboratory for the use throughout the year, gametes are collected and shipped in accordance with the order from the researchers.
Ephyra Jellyfish as a new model for ecotoxicological bioassays

M. Faimali\textsuperscript{1}, F Garaventa\textsuperscript{2}, V. Piazza\textsuperscript{1}, E. Costa\textsuperscript{1}, G. Greco\textsuperscript{1}, V. Mazzola\textsuperscript{1}, M. Beltrandi\textsuperscript{3}, E. Bongiovanni\textsuperscript{3}, S. Lavorano\textsuperscript{3}, G. Gnone\textsuperscript{3}

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The aim of this study was a preliminary investigation on the possibility of using the ephyra of Scyphozoan jellyfish \textit{Aurelia aurita} (Linnaeus, 1758), the common moon jellyfish, as an innovative model organism in marine ecotoxicology.

To do that, in order to find a sensitive and reliable end-points in addition to mortality (M), a series of sequential experiments were carried out in the laboratory in order to study the jellyfish swimming performance and its variations in presence of stress; thus to verify the possibility to easily and correctly quantify two behavioural end-points: the frequency of pulsations (Fp) and the rate of sinking (Rs). Experiments have been performed both by direct observation of the ephyra motion and by an automatic recording system coupled with video graphics analyzer (Swimming Behavioral Recorded - SBR) already used with other marine organisms [1]. The SBR has been properly set to investigate the role of some methodological parameters on the ephyra swimming performance and then it has been used to analyse the acute and behavioural responses during static exposition to a reference toxic: cadmium nitrate.

The results of this work pointed out that between the behavioural end-points investigated only the frequency of pulsations (Fp) is an easily measurable one and can be used coupled with mortality.

The comparison of the EC\textsubscript{50} values obtained with cadmium nitrate [2] during this work with those obtained with other marine invertebrates, indicates that jellyfish are a promising model organisms for ecotoxicological investigation.

References


Impact assessment of hypoxia to the early life history of Japanese short-necked clam, *Ruditapes philippinarum*

T. Horiguchi¹, S. Kamohara², S. Yamada², M. Waku², R. Sone², Y. Iwata², M. Ishida², T. Ichikawa³, T. Suzuki⁴, H. Shiraishi¹

¹Center for Environmental Risk Research, National Institute for Environmental Studies, Tsukuba, Ibaraki 305-8506, Japan; ²Aichi Fisheries Research Institute, Gamagori, Aichi 443-0021, Japan; ³Science and Technology, Showa-ku, Nagoya, Aichi 466-0031, Japan; ⁴Graduate School of Environmental and Human Sciences, Meijo University, Tenpaku-ku, Nagoya, Aichi 468-8502, Japan

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A testing method with flow-through exposure systems was established to evaluate the effect of hypoxia (i.e., seawater with low dissolved oxygen (DO)) on the early life history of benthic organisms. Using the systems, observed values of the lethal concentration 50 (LC₅₀) were obtained for the early life history (i.e., D-shaped, umbo, and full-grown stages of larvae and settled juveniles) of Japanese short-necked clam, *Ruditapes philippinarum*. Other laboratory experiments were also conducted to evaluate avoidance by *R. philippinarum* larvae of hypoxic waters when they encounter them. Monthly field studies were carried out to assess the impacts of hypoxia on the early life stages (e.g., horizontal and vertical distribution of larvae and juveniles) of *R. philippinarum* in Mikawa Bay, Japan. Computersimulations using an ecological model for the early life history of *R. philippinarum* were performed to estimate impacts of hypoxia to larvae of *R. philippinarum* in Mikawa Bay, on the basis of results of laboratory experiments and field studies as well as observation data on hypoxia in the Bay. They implied that approximately 11-37% of larvae might have been killed by hypoxia in Mikawa Bay. Finally, it was suggested that water quality criteria values of bottom DO, which are to be set to start in the next fiscal year of 2013, for survival and reproduction of *R. philippinarum* should be 2 and 3 mg/L, respectively.

Acknowledgement: This work has been financially supported by Environment Research and Technology Development Fund of Ministry of the Environment, Japan (B-1003: “Development of Monitoring Technique to Assess Impact to Benthic Life by Hypoxia and of Achievement Evaluation Method for Bottom DO Criteria” from FY2010 to FY2012).
Changes in life history traits of megabenthic species in Tokyo Bay, Japan, concurrent with decrease in their stock sizes

K. Kodama1, J. H. Lee1†, J. C. Park1,2, H. Shiraishi1, T. Horiguchi1

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2. The United Graduate School of Veterinary Science, Yamaguchi University, Yoshida, Yamaguchi, Japan
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Stock size of the megabenthic species in Tokyo Bay, Japan, has been decreasing and the species composition has changed since late 1980s. We investigated changes in the life history traits between periods with different stock size for three dominant megabenthic species (mantis shrimp Oratosquilla oratoria, dragonet Callionymus valenciennei and marbled sole Pseudopleuronectes yokohamae).

Changes in reproductive pattern of mantis shrimp were evident between 1980s and 2000s. There were two spawning peaks; an early peak (May–June) by large females ≥1 year old and a late peak (July–September) by small females 0–1 year old. This general reproductive pattern does not differ between 1980s and 2000s. However, the monthly pattern in larval abundance changed with decline in the population size; larval abundance from the early spawning season was highest in 1980s, and it decreased substantially in 2000s, suggesting that decrease in the spawning-stock abundance of large females in 2000s.

There were differences in growth and reproductive pattern of dragonet between 1990s and 2000s. The growth of dragonet was higher in 1990s than that recorded in 2000s. The minimum body length at which they attain gonadal maturation was smaller in 2000s compared to that in 1990s. In addition, timing of the onset of first spawning became earlier in 2000s than that in 1990s.

We found changes in growth and feeding habit of marbled sole between 1980s and 2000s. The growth of marbled sole during 2000s was generally higher than that in 1980s. Weight of the stomach contents was significantly lower in 2000s compared to that in 1980s. In addition, the dietary composition changed substantially between 1980s and 2000s; main constituents of the stomach contents were annelids, mollusks and echinoderm during 1980s. In 2000s, however, marbled sole fed predominantly on annelids.

Causal mechanisms for the changes in the life history traits of these dominant species remain unclear, but it may be related to changes in biotic or abiotic factors, e.g., direct or indirect effects of fishing pressure, density-dependent competition for prey, shifts in the allocation of resource toward reproduction, and increase in water temperature as well as increase in duration and spatial extent of hypoxia in the bay since 1980s, or a combination of these factors.
Anthropogenic and naturally occurring halogenated phenolic compounds in the blood of cetaceans

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Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) are of particular interest because they elicit a variety of toxic effects such as disruption of thyroid hormone homeostasis and neurotoxicity in exposed wildlife and humans.

We determined the residue levels and patterns of OH-PBDEs, and related compounds, such as PBDEs, methoxylated PBDEs (MeO-PBDEs), and bromopheno-ls (BPhs) in the blood of eleven cetacean species stranded along the Japanese coasts. The dominant OH- and MeO-PBDE isomers found in all cetaceans were 6OH-BDE47 and 6MeO-BDE47. Additionally, 2,4,6-triBPh was dominant isomer in all cetaceans. In contrast, specific differences in the distribution of para- and meta- OH-PBDE isomers (PBDEs metabolites) were found among the cetacean species.

Residue levels of ΣMeO-PBDEs and 6OH-BDE47+2’OH-BDE68, and 2,4,6-triBPh and 6OH-BDE47+2’OH-BDE68 showed a significant positive correlation. These results may suggest that the large percentages of OH-PBDEs, MeO-PBDEs and 2,4,6-triBPh might share common source (i.e. biosynthesis by marine organisms), or metabolic pathway in cetacean species. Significant correlations were found between the concentrations of BDE99 and 2,4,5-triBPh. This result suggested that 2,4,5-triBPh in the blood of cetaceans could be a metabolite of BDE99.

Characteristic differences in the profiles of brominated phenolic compounds in cetaceans present the need for further studies on the exposure profiles, metabolic capacities and toxic effects.

References:
Toxicity due to copper and zinc on the fish *Puntius parrah* (Day, 1865)

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The toxicity of the heavy metals copper and zinc on the fish *Puntius parrah* was assessed based on standard renewal bioassay methods and the 96hr LC50 for copper was 0.5ppm and that for zinc was 9 ppm. The accumulation of Cu and Zn in water and sediment in five selected study sites of river Periyar in Kerala along with details on the physico-chemical parameters are also presented. Zinc and copper in water and sediment showed high concentration particularly in the highly industrialized area compared to the non industrialized zones of the river. The sediment samples in station 1 (Kuzhikundam creek) had an average value of 19.855±0.125 µg/g for zinc and that of 4.32±0.050 µg/g for copper. Similarly water sample from the same study area had an average of 16.26±0.250 µg/g for zinc and that for copper was 2.59±0.090 µg/g. The fish exposed to a sublethal concentration of 0.06ppm Cu and 0.9ppm Zn showed higher accumulation of both the metals in liver (Cu-78.824±0.052; Zn-1083.4±1.140 µg/g) as compared to kidney, gills and muscles. The histological changes induced by copper (0.04, 0.06, 0.12 ppm) and zinc (0.6, 0.9, 1.8 ppm) on the test organism under sublethal concentrations on 3rd, 7th, 14th and 28th days of exposure were also studied. Several histopathological changes were noted in gills, liver and kidney tissues attributable to Cu and Zn exposure. The histological sections are the clear indication of the gradual and severe histopathological alterations induced by copper and zinc during the exposure period.

At sub lethal concentration the total protein and fat content of the experimental fish decreased (protein- 50.2±2.236 to 32.5±0.291mg/100mg; fat- 22.38±0.228 to 12.02±0.630mg/100mg) but amino acids increased significantly in the control (66.49%) after 28 days of exposure period (94.84%). There was a significant decrease (p=0.001) in erythrocyte count of *Puntius parrah* after exposure to sublethal concentration of copper and zinc. The white blood cell count showed significant increase whereas hemoglobin, haematocrit, mean corpuscular volume and mean corpuscular haemoglobin concentration decreased significantly after 28 days of exposure to the heavy metals. The quintessence of the study is that, the specific problem associated with the heavy metals in the environment is their accumulation through food chain and persistence in nature. For this the collaborative and synchronized contributions of the policy makers, planners, scientists, government organizations and common man are needed. Thus effective management strategies are to be evolved and implemented to protect our water bodies and aquatic organisms from the arms of heavy metal pollution and its toxic effects.
Effects of temperature rising and ocean acidification on reproductive success of thermophilic sea urchin *Arbacia lixula*.

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Over the last few years, global climate change phenomenon have demanded more information on responses and effects of marine organisms regarding to temperature and acidification ocean variability to predict environmental evolution and exploitation of natural resources [1]. Reproductive processes are critical to understanding the success of a species influenced by environmental factors (e.g. temperature and pH) [2,3]. Within principal marine organisms, sea urchins play a crucial role both economic and ecologic in ecosystem control. Among the main Mediterranean sea urchins species, *A. lixula* is poorly studied because does not have economic importance and it is unpalatable. From ecological point of view, *A. lixula* seems to play an important role in rocky Mediterranean ecosystem due to its grazing activities [4,5]. Furthermore, according to several authors, this species is designed like a thermophilic species in spreading along Mediterranean coasts [6,7,8].

In order to address questions on the species future vulnerabilities, we examined the interactive effects of temperature rising and water acidification on fertilization and development success of *A. lixula*. Experimental treatments (20°, 24°, 26° and 27°C; pHNBS 8.1-7.8) were tested in all the T-pH combinations, with 20°C/pH 8.1 as a control. Percentage of fertilization was the same (<50%) across all treatments except for a total failure at 27°C. No effects were observed in pH treatments. Developmental success across all stages (gastrula (24h); pluteus (48h)) decreased with increasing temperature. At 26°C, the percentage of numbers of pluteus decreased with increasing acidification (pH 7.8). This could help to buffer the negative effects of acidification at the upper temperature limits of ocean warming. Results showed that, despite a thermophilic feature of *A. lixula*, to following a global climate change scenario, this species would be affected previous in reproductive success by temperature rising and after by acidification on larval survival strategy. This would probably lead to a population decline regarding to environmental changing [9].

References:


Ocean acidification effects on sea urchins and top-down control mechanisms along Mediterranean rocky shores

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Sea urchins are important grazers in the shallow subtidal and their grazing activity is among the principal controlling factors of the dynamic, structure and composition of macroalgal assemblages. The negative effect that ocean acidification is expected to exert on sea urchins could compromise their role in controlling macroalgae. Furthermore, macroalgae are differentially affected by a decline in pH depending on their calcium carbonate content. In this context, a laboratory experiment on Paracentrotus lividus juveniles, grazing on calcifying (Corallina elongata) and non-calcifying (Dictyota dichotoma, Cystoseira amentacea) macroalgae, was set up under different pH, corresponding to $pCO_2$ values of 390, 550, 750 and 1000 µatm. Results highlighted direct negative effects on sea urchins and on the calcifying macroalga under elevated $pCO_2$ (lower pH) conditions. Most interestingly, indirect negative effects mediated by the diet were observed in juvenile urchins: the carbonate content of calcifying macroalgae turned out to be important for strengthening the Aristotle’s lantern and the test of P. lividus. All the above suggests that, in a future scenario of ocean acidification, the decreased pH coupled to a reduced availability of calcifying macroalgae will have severe consequence for P. lividus, affecting both its grazing activity and its robustness to predation.

These results are particularly relevant as P. lividus and Arbacia lixula play complementary and synergic roles in the maintenance of barrens. A. lixula is generally more resistant to predation and feeds mainly on encrusting coralline algae. The decline of calcifying macroalgae will probably have consequences also for A. lixula, with severe implications on both macroalgae and sea urchin community structure. A future increase of $pCO_2$ will impact these top-down controls and, consequently, the ecosystem structure of rocky shallow areas in the Mediterranean Sea.
SPECIAL REPORT on pollution after the Fukushima accident

Surveys on environmental pollution and possible adverse effects by radionuclides in wildlife, after severe accidents of the Fukushima Dai-ichi Nuclear Power Plants, Japan

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Plenty of various radionuclides were released to the environment, through the melting down accidents of reactors in the Fukushima Dai-ichi Nuclear Power Plants (1F) of Tokyo Electric Power Company (TEPCO) in March 2011. Less is known about possible adverse effects by radionuclides in wildlife in Fukushima Prefecture and surrounding areas, although concentrations of radionuclides (mainly, ¹³⁴Cs and ¹³⁷Cs) in environmental samples, including aquatic and terrestrial organisms, have been reported in many sites/areas of Japan. We are now investigating environmental contamination levels and possible adverse effects by radionuclides in wildlife, such as amphibians, intertidal organisms, demersal fish and shellfish populations in, along the coastal line of and off Fukushima, as well as in Tokyo Bay. Here, we will briefly introduce our research designs/plans and a few results on the basis of ongoing surveys.
Comparison of vas deferens and penis development between the rock shell, *Thais clavigera* (Muricidae) and the ivory shell, *Babylonia japonica* (Buccinidae)

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The characteristics of the development of male genitalia (penis and vas deferens) in imposex-exhibiting female and male rock shells, *Thais clavigera* (Muricidae), were histologically examined using specimens from wild populations and tributyltin (TBT)-exposed females in the laboratory. Vas deferens and penis development was already observed in both imposex-exhibiting female and male rock shells even at approximately 6 months old. A variety of vas deferens morphogenesis patterns were observed in wild adult female *T. clavigera*, and the characteristics were summarised. The immature vas deferens at an initial stage, however, was only observed beneath or behind the penis, and no vas deferens was observed close to the vaginal opening (i.e., vulva) of the capsule gland in TBT-exposed females, which was different from the characteristics of vas deferens formation observed in wild females. Taking into consideration both the observed results from wild female specimens and from TBT-exposed females in the laboratory, the vas deferens sequence (VDS) index for *T. clavigera* was proposed as VDS 1-6. Meanwhile, we also histologically examined development of genitalia in the ivory shell, *Babylonia japonica* (Buccinidae), using 2-year-old shells from wild populations and laboratory-reared juveniles for 0-20 months of age. Differentiation of gonad (i.e., testis and/or ovary) was unclear before 14 months of age, and progressed after 16 months of age. Immatured vas deferens, however, was observed in males at 14 months of age, although no penis was observed in them. Formed penis was recognized in almost all males at 16 months of age, although vas deferens was not yet completed. Vagina, bursa copulatrix and capsule gland were developing in 14-month-old females. Albumen gland and receptaculum seminis were also developing in 16-month-old females. Differentiation and development of gonad did not precede the development of genitalia in the ivory shell, suggesting that regulatory mechanism of reproduction might be different between mollusks (prosobranch gastropods) and vertebrates. Moreover, there might be a considerable difference on vas deferens and penis development between the rock shell, *T. clavigera* (Muricidae) and the ivory shell, *B. japonica* (Buccinidae), even among prosobranch gastropod species.
Physiological and histopathological responses to sublethal concentrations of copper in the teleost fish, *Aanabas testudineus* (Bloch -1972)

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The present study was designed to evaluate the effects of sublethal concentrations of copper, an essential metal on the physiological and histopathological indices of the teleost fish, *Aanabas testudineus*. The 96 hr. LC$_{50}$ value of copper by static renewal bioassay method in *Aanabas testudineus* derived by probit method and was 1.7mg/L. The protective and therapeutic effects of vitamin C supplementation along with the toxicant were also determined. The free radical scavenging antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD) and hepatotoxic biomarker enzymes like alanine transaminase (ALT) and aspartate transaminase (AST) increased significantly in the serum of intoxicated fish. Significant reduction in erythrocyte count (RBC), haemoglobin (Hb), haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and oxygen carrying capacity (OC) in fish exposed to various toxicant concentrations in comparison with control group. Obvious symptoms of macrocytic hypochromic hypoxic anaemia and amitotic erythrocytes were detected in higher toxicant concentrations and long exposure periods. A significant elevation of serum glucose, cortisol, lactate dehydrogenase and alkaline phosphatase were detected in toxicant exposed samples as indices of heavy metal stress induced by hyperglycemia. However serum protein decreased significantly in fish exposed to toxicant over the control. The total protein content decreased significantly in liver, gills, kidney and muscle of heavy metal treated fish in comparison with the control. The enzymes CAT, SOD, AST, ALT and LDH increased significantly in the liver, gill, kidney and muscle of copper treated fish over the control. However the glycogen and protein decreased markedly in the tissues and organs under consideration. The lipid peroxidation (LPO) values decreased significantly in all the target tissues and organs under consideration. The fish treated with copper together with vitamin C showed significant improvement in enzyme levels, tissue metabolites and lipid peroxidation level. Histopathological studies clearly depicted degenerative changes in liver, gills, kidney and to some extent in muscle of fish under metal stress. The liver of copper treated fish showed degeneration in hepatocytes, cell necrosis and inflammation with sinusoid dilation and thrombus formation in central vein. The gill of metal intoxicated fish showed total degeneration of gill lamellae and oedematous changes characterized by epithelial detachment whereas the kidney under metal stress showed degenerative and necrotic changes in the nephrons, inflammation and haemorrhage, atrophy of tubular epithelium and glomeruli and oedema in Bowmans capsule. The muscle of copper treated fish showed degeneration of muscle bundles with inflammatory cell infiltration and necrosis. However the tissues and organs of fish exposed to copper along with vitamin C showed marked improvement towards control. The vitamin C restored the enzymatic activity in metal intoxicated fish to near control values by neutralizing the toxic reactive oxygen species (TROS). The present investigation clearly reveals the toxic effects imparted by the heavy metal, copper even at sublethal concentrations on the normal physiology and histology of aquatic animals in general and the fish, *Aanabas testudineus* in particular.
Application of a biological multicriteria approach for the assessment of adverse effects of sediment pollutants: the Port of Trapani as an ongoing case study

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The Port of Trapani is actually investigated as a model case study for the application of an integrated, multidisciplinary Weight Of Evidence (WOE) approach to improve criteria of quality characterization, dredging and management options for harbour sediments. The WOE is based on the evaluation of multiple Lines of Evidence (LOEs) including chemical, physical and biological investigations carried out on approximately 300 samples obtained from 100 cores up to 10 m long.

Among biological effects, particular importance is given to criteria for defining the ecotoxicological hazard. A battery of bioassays will be applied, including the marine bacteria Vibrio fischeri (on bulk sediment), the sea urchin Paracentrotus lividus and the alga Phaeodactylum tricornutum (on freshly extracted or frozen elutriate). The results will be elaborated according to a recently developed model which, based on logical flow-charts and mathematical algorithms, give different weights according to biological end-points and magnitude of responses, providing a synthetic index of hazard among one of five possible classes [1]. The integration of ecotoxicological results with those from chemical characterization (also elaborated within a specific module) will represent the first level of an Ecological Risk Assessment (ERA) procedure, according to the actual legislation and guidelines on harbour dredging and sediment management set by ISPRA [2].

The analysis of benthic communities (with particular focus on the study of marine seagrasses), microbiology and the development of some new biomarkers on P. lividus, will represent additional LOEs toward a second level of a detailed procedure to achieve a more comprehensive ecological risk analysis associated to polluted sediments.

In both levels of data elaboration, the previously developed conceptual and software-assisted model will be continuously validated and adapted for handling the critical issues of sediment characterization and management in harbors areas, also providing scientifically sound and tested hypotheses to revise actual normative guidelines. The model will aim to elaborate results from various lines of evidence, aggregate results into specific synthetic indices of hazard, and provide an integrated index of ecological risk, useful for environmental decisors and stakeholders.

References:
Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms.

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Several classes of chemical pollutants can act as pro-oxidant stressors increasing the intracellular generation of ROS and the onset of oxidative conditions through several, and often interacting pathways. Antioxidant defences are known for their responsiveness to environmental pollutants and frequently used as biomarkers at the transcriptional, translational and catalytic levels.

The aim of this talk is to briefly review the main oxidative mechanisms of chemical toxicity, and to use some experimental data for a general discussion on the modulation of transcriptional and catalytic responses of antioxidants, and the resulting implications for environmental monitoring.

Although molecular responses are often assumed to reflect similar changes in enzyme function, several factors can influence intracellular effects, including mRNA stability and protein turnover, signal sensing and transduction, post-translational modifications of proteins, and multiple mode of action of chemicals in complex mixtures. Relationships between transcriptional and catalytic effects are thus often inconsistent for antioxidants, confirming the complexity of interactions between exposure to chemical pollutants and regulation of oxidative stress responses that do not necessarily lead to transcriptional variations of genes, but rather to post-translational modifications of proteins.

These mechanisms are just beginning to be revealed in marine organisms, but different functional implications might be expected from variable gene expressions according to tissue, intensity of exposure and presence of close relationships between the substrates, activation of transcription factors and specificity of related genes.

In this respect, caution should be taken in monitoring studies where gene expression analyses might not reflect functional responses to oxidative challenge, and future studies should emphasize on the identification of post-translational mechanisms in marine organisms.
Sea urchin embryos cadmium-exposed as an experimental model system for studying the relationship between autophagy and apoptosis

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The sea urchin embryo is a suitable model system that offers an excellent opportunity to investigate different defence strategies activated in stress conditions. We previously showed that cadmium treatment provokes the accumulation of metal in dose- and time-dependent manner in embryonic cells and the activation of defence systems, such as the synthesis of HSPs and/or the initiation of apoptosis. Analysing autophagy, by neutral red, acridine orange and LC3-detection, we demonstrated that Cd-exposed embryos adopt this process as an additional stratagem to safeguard the developmental program. We observed that embryos treated with subletal Cd concentration activate a massive autophagic response after 18h of treatment. In addition, autophagy decreases between 21 and 24h, in the opposite of apoptotic process [1-2]. In order to investigate a possible temporal relationship between autophagy and apoptosis, we tested apoptosis by immunodetection in situ of cleaved caspase-3 and TUNEL assays. We showed that embryos activate a massive apoptosis after 24h of Cd exposure. In addition, a functional relationship between autophagy and apoptosis was estimated evaluating apoptosis in Cd-exposed embryos with inhibited autophagy, by treatment with 3-methyladenine (3-MA). We found that the inhibition of autophagy produced a reduction of apoptotic signals, suggesting that the two phenomena are functionally related. Considering the catabolic role of autophagy, an energetic hypothesis to explain this relationship was evaluated; in this case autophagy could contribute to apoptotic process providing ATP, necessary for the execution of the apoptotic programme. In effect, using methylpyruvate (MP), a substrate for ATP production, in embryos with inhibited autophagy, apoptosis was substantially restored. In this context, autophagy could play a crucial role in stress response of this suitable model system [3].

References

Native specific reactions may cause the paradox of population control: A theoretical approach with mathematical model

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One of serious problems in agriculture has been the pest outbreak. Usually some pesticides have been used against the pest. However, in some cases, such a pesticide is effective only in the early period of its application and results in an outbreak of the pest in the later period. Such phenomenon is often called the pest resurgence [1-4]. It could be caused by the emergence of a pesticide-resistant strain of the pest or by the decrease of its enemy population affected by the pesticide [1, 2]. Some researches showed that a small amount of pesticide could increase the pest fecundity whereas a large amount of pesticide decreases the pest population. Such phenomenon is called the hormesis or hormoligosis. Hormesis would play an important role to cause the resurgence [4]. In our work [3, 6], we analyze a time-discrete host-parasite system in order to consider the condition in which the harvesting of the host (pest) population results in the increase of the host population itself so as to cause the paradox of pest control, that is, the resurgence. Our model is a time-discrete population dynamics model extended from the Nicholson-Bailey model [5], introducing the host intra-specific density effect and the harvesting effect [3, 6, 7]. We could analytically prove that the resurgence occurs even when the harvesting does not directly affect the parasite (natural enemy) population at all. Our result suggests that such a paradox would not be necessarily caused by the reduction of the natural enemy population due to the harvesting itself (e.g., with a pesticide), or by the appearance of some pesticide-resistance or the pest hormesis. Instead, the purely ecological balance in the population dynamics may cause it[3, 6, 7].Our theoretical results can be easily extended to those for the other contexts including some biological conservation in not only land but also marine habitat. Our conclusion implies the importance of biological (ecological) researches for controlling ecological system.

References
Molecular analysis of physiological and developmental responses to water temperature changes in *Porphyra* spp. (Rhodophyta)

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The rhodophyte genus *Porphyra* (recently changed to *Pyropia*) includes more than 130 species, some of which are economically important as the edible “nori” in many places of the world, especially in Asia. In Japan, *P. yezoensis* is one of the most extensively cultivated seaweeds used as food, with about 350,000 tons (wet weight) harvested annually. However, the quality and yield of farmed *Porphyra* are greatly influenced by environmental conditions. In particular, growth and shape of *Porphyra* thalli are affected by changing water temperature. Under laboratory culture, thalli of the *P. yezoensis* “U51” standard strain show optimal growth at temperatures between 10 and 15°C, while thalli cultivated at temperatures over 20°C had decreased growth rates in proportion to increased temperatures, with obvious developmental disorders. As a first step toward understanding the molecular mechanisms for response and adaptation to temperature changes in *Porphyra* thalli, we have constructed subtracted cDNA libraries enriched for differentially expressed transcripts in the 10°C- and 20°C-cultivated thalli of *P. yezoensis* U51 and investigated the levels of transcript accumulation for each subtracted cDNA by dot blot analysis. In addition, the cDNAs corresponding to putative differentially expressed transcripts in the 10°C- and 20°C-cultivated thalli were sequenced to identify candidate genes important for response and adaptation mechanisms to temperature changes in *P. yezoensis*. Recently, physiological and developmental characteristics of *P. suborbiculata*, which is distributed along the entire coastline of Japan, have been investigated. Thalli of the “PSO” strain isolated from Okinawa Prefecture in southern Japan can grow in culture at temperatures between 15 and 25°C without morphological disorders, whereas thalli of the “PSI” strain isolated from Iwate Prefecture in northern Japan shows normal growth in culture at temperatures between 15 and 20°C. Growth rates of the PSI thalli are much less than those of the PSO thalli at the same temperature. In addition, a thermally selected “MET11” strain of *P. yezoensis* has been developed by traditional selective breeding at high temperatures in Japan. These strains are appropriate samples for investigations designed to elucidate adaptation and tolerance mechanisms to higher temperature in *Porphyra* at the molecular level. In order to fully understand such molecular mechanisms in *Porphyra*, further experiments on construction of subtracted cDNA libraries from the PSO, PSI, and MET11 thalli grown at different temperatures, and analysis of the cDNAs corresponding to putative differentially expressed transcripts, are currently being carried out in our laboratory.

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Visualization of chemical impacts using *Daphnia magna*.

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The water flea, *Daphnia*, has been the subject of study in environmental sciences for decades. Over the last few years, *Daphnia* emerged as a model organism that can link between environmental impacts and biological events in molecular level, because their expressed sequence tags and a genome sequence have been determined. In addition, functional approaches of overexpression and gene silencing based on microinjection of RNAs into eggs have been established. However, the transient nature of these approaches prevents us from analyzing gene functions in later stages of development. To overcome this limitation, transgenesis would become a key tool. Here we report establishment of a transgenic line using microinjection of plasmid into *Daphnia magna* eggs. The green fluorescent protein (GFP) gene fused with the *D. magna* histone H2B gene under the control of a promoter/enhancer region of the elongation factor 1α-1 (EF1α-1) gene, EF1α-1::H2B-GFP, was used as a reporter providing high resolution visualization of active chromatin. Transgenic lines were obtained from 0.67% of the total fertile adults that survived the injections. One of the transgenic animals, which exhibited fluorescence in the nuclei of cells during embryogenesis and oogenesis, had two copies of EF1α-1::H2B-GFP in a head-to-tail array. This is the first report of a transgenesis technique in *Daphnia* and, together with emerging genome sequences, will be useful for monitoring and evaluation of environment using *Daphnia*. 
ABC transporters research in aquatic toxicology: suitable model systems and novel assays

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The ATP-binding cassette (ABC) transporters is one of the largest known protein superfamily. Most of these proteins are transmembrane transporters which efflux various structurally unrelated compounds across cell membranes through binding and hydrolysis of ATP. These proteins play a crucial role in physiology, toxicology and disease of organisms from prokaryotic to humans. ABC transporters actively efflux toxic chemicals out of cells working as integral part of cell defence mechanism against cytotoxic compounds. The protective role of ABC transporters has been recognized in the last twenty years and the related phenomenon called the multixenobiotic resistance mechanism (MXR). Regardless their role in preventing accumulation and thus toxic effects of various contaminants the knowledge about the presence, regulation and function of these proteins in aquatic species is modest and more study are needed to address their ecotoxicological relevance.

Here we present three case studies using in vitro and in vivo systems as freshwater fish cell line and mussel and sea urchin embryos in order to select suitable model systems and to develop ecotoxicological assays that could be used to investigate the defence mechanism played by ABC transport proteins.

In the first study the Poeciliopsis lucida hepatoma cell line (PLHC-1) cells has been exposed to four cytotoxic metals (Cd, Hg, AsIII, and CrVI) and the gene expression levels of five ABC transporters and efflux activities has been analysed. Specific interaction of ABC transporters with Hg has been further investigated after both short and long-term exposure experiments.

In the second study a characterization of the response of ABCB and ABCC proteins in terms of gene expression and efflux activity was performed in gills, digestive gland and haemocytes of mussels exposed to Cd.

The last study aimed to develop novel assays in Strongylocentrotus purpuratus embryos, to investigate in hatched embryos the response of ABC proteins against the anticancer drug vinblastine and Hg. Toxicity of Hg - evaluated as morphological defects- and of vinblastine -evaluated as swimming performance- was measured in the presence or absence of MXR inhibitors as cyclosporin, PSC833 and MK571. MXR over-expression assay was also performed.

Our results indicate that some ABC transporters are actively involved in the response to metals in the tested models and highlight the importance of studying these proteins for a better understanding of disposition and toxicity of pollutants in aquatic organisms.
Cellular and molecular stress induced by chemical and physical agents in sea urchins


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Our research group has been classically involved in basic research studies on echinoderms, including the sea star Asteria rubens, the sea urchin Arbacia lixula and overall the Mediterranean sea urchin Paracentrotus lividus, the best known European established model system for studies on embryonic development.

The sea urchin embryo is a suitable model for the assessment of cellular responses to physical and chemical stresses at genomic and proteomic levels, since it has a complex network of genes involved in response, protection and repair of cellular damage. In particular, our interest is devoted to the identification of the molecular pathways involved in the stress response to environmental stimuli, such as ionizing radiations [1,2,3] or heavy metals [4,5]. Physical and chemical agents used in these studies are: UV-B, X-Rays, CdCl₂, MnCl₂, pH, Temperature, Mg deprivation. Results from this line of investigation are interesting from an ecotoxicological point of view, as they can have a predictive value in terms of protection of marine ecosystems. Lately, we focused our attention on the stress response of the sea urchin embryos to the combined effects of UV-B radiation and Cd exposure, at cellular, molecular and biochemical level. We obtained promising results indicating a synergistic effect of two different agents. Moreover, sea urchin embryos cultured in Mg-Free sea water show abnormal differentiation patterns and stress response.

In recent years we developed an interest for the understanding of basic mechanisms involved in biomineralization processes of sea urchin embryos and adults. Bio-mineralization, i.e. the formation of bio-nano-structured minerals by living cells, is an attractive area of the nano(bio)technologies. In particular, we have now identified a number of genes involved in the formation of the magnesium calcite skeleton of embryos, and we are monitoring their expression during physiological development or in pathological conditions, namely in skeleton-deficient embryos [6].

Taken all together, results described so far encourage the use of echinoderms for ecotoxicological studies and development of biomarkers. They can be used for fast, low-cost and reliable screening and testing of toxic agents. Results may provide important future directions for studies on environmental and human health.

References

Abstracts – Posters

Effect of the diatom aldehyde decadienal on metamorphosis in the sea squirt Ciona intestinalis

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Diatoms, a major group of microalgae, have been reported to produce a large variety of polyunsaturated aldehydes (PUAs) and other oxylipins which can compromise embryonic and larval development in benthic filter-feeding organisms such as the sea urchin Paracentrotus lividus. In particular, at high concentrations the PUA decadienal blocks cell cleavage and induces apoptosis, whereas at low concentrations this aldehyde induces teratogenesis and abnormal plutei formation [1,2]. These effects are mediated by the physiological messenger nitric oxide [3]. Here, we investigated the effect of decadienal on larval development and metamorphosis of the sea squirt Ciona intestinalis. After hatching, the mobile larva is transformed into a fixed juvenile through a remarkable regression of the tail occurring during metamorphosis. At the molecular level, many processes have been reported to occur during Ciona metamorphosis, such as caspase and MAPK activation [4], nitric oxide production [5] and oxidative stress [6]. Treatment of hatched larvae with decadienal resulted in a delay in metamorphosis with a concomitant reduction of ERK phosphorylation. Parallel experiments have revealed that the same effects are observed when hatched larvae are treated with the nitric oxide synthase inhibitor, TRIM, that reduces endogenous nitric oxide levels. The involvement of nitric oxide in the mechanism by which decadienal affects ciona metamorphosis will be addressed following the expression profiles of genes acting downstream of ERK activation.

References:
Microbiological approaches for the treatment of high salinity slops originated from marine transportation

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Waters (waste) originated by the operations in ship engine-rooms (bilge waters) and by the washing of oil tanks (slops) create a major disposal problem throughout the world because of the persistence and accumulation of xenobiotic compounds released from these waters in the environment. Many of these fluids are very stable emulsion, which make chemical treatment difficult and normal separation processes alone rarely effective. The conventional methods for treatment of emulsified oily wastewater is chemical de-emulsification followed by secondary clarifications, which requires the use of a variety of chemicals (e.g. sulphuric acid, iron and alumina sulphates...). The water phase from chemical pre-treatment has however to be further treated to meet today’s effluent standard for discharge into the environment. This can be achieved by granular activated carbon (GAC) filtration or by biological treatment including Membrane BioReactor (MBR). Once a GAC column is exhausted, the GAC must be replaced and disposed of or recycled in some way. Replacement and disposal of exhausted GAC is quite expensive. Encouragement of biodegradation - where one or more of the SOCs (Soluble Organic Compounds) are biodegradable - should lengthen the GAC service life for some SOC mixtures. Very little work has however been done on the biodegradation and adsorption of salty mixtures of biodegradable and nonbiodegradable SOCs. This lack of research is particularly problematic because such salty SOC mixtures are widespread in the environment, and many nonbiodegradable SOCs may cause adverse health effects.

In this work we have investigated the feasibility of different treatments including: i) A simple separation by gravity as a pre-treatment; ii) A chemical coagulation as a primary treatment; iii) A filtration on activated carbons as secondary treatment and the bio-regeneration (using inocula of oil degrading bacteria as Halobacterium sp and Alcanivorax sp.); iv) An MBR process as an alternative secondary treatment. The biological phase was operated inside a bench-scale reactor designed to allow at one time the development of suspended activated sludge and biofilm attachment on free floating carriers and using a submerged membrane unit as solid-liquid separation system. The process is an hybrid MBBR with a final ultrafiltration stage characterized by an hollow fibers module (BioFilm Membrane BioReactor – BFMBR). The membrane separation process and the high sludge retention time in the system enabled to remove a large part of Total Organic Carbon allowing to meet the limits imposed for discharge into the sea.

Sampled wastewaters are wash-waters originated by cleaning the ships’ fuel tanks (slops). Beside the presence of refractory compounds, these wastewaters show extremely high salinity levels (up to 25,000 p.p.m.), that prevent the possibility of their discharge into the sewer and address their disposal into the sea. This situation severely lowers the discharge limits for most of the “sensible” parameters, such as COD and hydrocarbons, thus requiring a high-level treatment.

The main objective of this study was to evaluate the feasibility of using biological processes with purposely acclimated microorganism for the treatment of high salinity oily wastewaters (slops). Results proved the feasibility of using of these methodologies for the reduction of the contaminants load.
Gene expression and stress response in sea urchin embryos with skeleton defects caused by magnesium deprivation.

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Echinoderms have an extensive endoskeleton composed of magnesian calcite, a form of calcium carbonate that contains small amounts of magnesium carbonate and occluded matrix proteins [1]. In the frame of the Biomintec European Project focused on the understanding of basic biomineralization mechanisms for the design of novel strategies in nano-biotechnology, we studied the effects of magnesium deprivation on Arbacia lixula sea urchin embryo development. Embryos were morphologically monitored, evaluating developmental abnormalities at different endpoints (3, 6, 24, 48 and 72 hours). In parallel, the spatial transcriptional levels of a skeleton matrix protein (msp130) and the protein synthesis levels of a signaling protein (p38MAPk) were detected by in situ hybridization (ISH) and Western Blotting (WB). The morphological analysis evidenced a general delay in development soon after the morula stage (6 hours), the absence of biomineral deposition after 24 hours and severe skeleton malformations after 48-72 hours. We observed the ectopic localization of primary mesenchyme cells (PMCs), the only cells in the embryo directing skeleton formation, by immunofluorescence with a FITC-conjugated lectin (WGA) able to bind specifically the PMCs. By ISH we detected the msp130 mRNA correctly in PMCs, although it was found persistently expressed even at 48h after fertilization, unlike control embryos where the messenger is down-regulated at this stage. At 48 hours we found high levels of p38MAPk by WB, if compared to control embryos; preliminary experiments showed hsp70 levels similar to controls. Further experiments will be performed to characterize the role of magnesium in the biomineralization process: we have now a toolkit of probes for various skeletogenic-specific genes (msp130, SM30, SM50, p16, p19) [2, 3] that will be used to study their embryonic temporal and spatial expression profiles. The internal calcium content and eventually the calcium carbonate polymorph forms [4] occurring in embryonic spicules in Mg-deprived embryos will be studied in comparison to control embryo.

References:
Involvement of nitric oxide on the effects of heavy metals on sea urchin development

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Nitric oxide, an important physiological messenger, has been shown to act as a cellular signal of environmental stress in different biological systems, such as marine microalgae, sponges and sea urchins. In particular, it has been reported that nitric oxide 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 [1]. In this context, our focus is to investigate if this messenger plays a role as a universal sensor of different environmental stress agents in the sea urchin embryo or if embryos display different responses following different treatments. To this aim, we used cadmium and manganese, well noted metals that induce developmental delay and abnormalities in sea urchin embryos, mainly in relation to skeleton elongation [2-5]. To understand the involvement of nitric oxide in these processes, embryos were treated with different metal concentrations under reduced endogenous nitric oxide 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. Further experiments have been performed to analyze the nitric oxide signalling in the sea urchin treated embryos by following the formation of nitrated proteins using specific antibodies.

References:
The sea urchin embryo: a model system to study effect of natural antioxidants on neurodegenerative diseases.

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Alzheimer's disease (AD) is a progressive, neurodegenerative disorder, characterized by loss of memory and impairment of multiple cognitive functions. Amyloid beta peptide (Aβ) is the main component of amyloid plaques observed in the brain of individuals affected by AD. Aβ is produced by γ- and β-secretase progressive cleavage of its Amyloid Precursor Protein (APP). Here, we use the Paracentrotus lividus embryo to identify molecules and pathways that can be involved in the degenerative process. We had, already, identified the presence of an antigen related to the human APP, called PlAPP [1]. This antigen is expressed in sea urchin embryo and after gastrula stage is processed producing a polypeptide of about 10 kDa [1]. By immunohistochemistry we localized the PlAPP antigen in some serotonin expressing cells, indicating that PlAPP is localized in primordial neurons. Moreover, oxidative stress and mitochondrial dysfunction, induced by Aβ, are among the earliest events in AD, triggering neuronal degeneration and cell death. Thus, natural molecules with antioxidant properties could be a suitable strategy for inhibiting cell death cascade. Here, by employing these urchin Paracentrotus lividus as a modelsystem, and Aβ oligomers, we tested the effectiveness of oleric acid (FA), a natural antioxidant, as putative AD neuroprotective compound. By microscopic inspection we observed that FA is able to revert morphological defects induced by Aβ oligomer on P. lividus embryo. In addition, FA is able to inhibit ROS generation, recover mitochondrial membrane potential and block apoptotic pathway. Moreover, the used modelsystem has allowed obtaining information about down- or up-regulation of some key molecules as Foxo3a, ERK, p53 involved in the antioxidant mechanism.

References
The Organizers of 1st Marine NanoEcotox Workshop
Valeria Matranga & Ilaria Corsi
EU NanoSafety Cluster - Marine Ecotox Focus Group Chairs

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**MANET – 2012 Program**

**November 29th, 2012**

8.30 – 9.00  *Registration of Participants*

9.00  **Welcome of the Organizers:**  
Valeria Matranga & Ilaria Corsi  
EU Nanosafety Cluster - Marine Ecotox Focus Group Chairs

9.10  **Opening Remarks**  
*Enrico Brugnoli*, Coordinator of the Department of Earth Sciences and Environmental Technologies, CNR  
*Pier Luigi San Biagio*, President of the Area della Ricerca, CNR, Palermo  
*Giovanni Viegi*, Director of the Institute of Biomedicine and Molecular Immunology “A. Monroy”, CNR, Palermo

9.20  **Georgios Katalagarianakis**  
(Directorate General for Research & Innovation, Bruxelles, Belgium)  
*EU research strategy for nanosafety in Horizon2020*

9.40  **Teresa Fernandes**  
(Heriot Watt University, Edinburgh, UK)  
Chair of the Hazard Focus Group of the EU Nanosafety Cluster  
*The EU Nanosafety Cluster mission and objectives: emphasis on aquatic toxicology*

10.00  **Gary Cherr**  
(University of California, Davis, USA)  
*Marine Nanotoxicology in the USA: the University of California Center for Environmental Implications of Nanotechnology (UC CEIN).*

10.20  **Jerome Labille**  
(CNRS - CEREGE, Aix-en-Provence, France)  
*The mesocosm approach to study the exposure, accumulation and toxicity of nanoparticles to aquatic/marine organisms.*

11.00 – 11.20  **COFFEE BREAK**

11.20  **Francesco Dondero**  
(Università del Piemonte Orientale “Amedeo Avogadro”, Alessandria, Italy)  
*Nanosilver effects in marine ectotherms: linking low and high informational levels.*

11.40  **Martin Hassellöv**  
(University of Gothenburg, Sweden)  
*Occurrence, identification, fate and behavior of engineered nanoparticles and nanoscale pollutants in marine systems.*
12.00  **Enrico Sabbioni**  
(European Center for the Sustainable Impact of Nanotechnology - Veneto Nanotech, Italy)  
*Presentation of the Marine NanoEcotox working group within the Italian Society of Nanotoxicology (SIN)*

12.15 – 13.00 **ROUND TABLE**  
*Common strategies and technologies for the assessment of impacts of emerging pollutants, including engineered nanoparticles, on the marine environment.*  
*Chair: Teresa Fernandes; Co-Chairs: Valeria Matranga*

13.00 - 14.20 **LUNCH**

14.20  **Ilaria Corsi**  
(University of Siena, Italy)  
*TiO2 nanoparticles and ABC transporters in marine organisms: the Trojan horse effect.*

14.40  **Giada Frenzilli**  
(University of Pisa, Italy)  
*Titanium dioxide genotoxic potential in marine organisms.*

15.00  **Laura Canesi**  
(University of Genoa, Italy)  
*Effects of TiO2 nanoparticle on immune and digestive gland function in the marine bivalve Mytilus.*

15.20  **Diana Boraschi**  
(Institute of Biomedical Technologies, CNR, Pisa, Italy)  
*Nano-immunosafety in marine organisms, a central issue in environmental nanotoxicology.*

15.40  **Annalisa Pinsino**  
(Institute of Biomedicine and Molecular Immunology "A. Monroy", CNR, Palermo, Italy)  
*Sea urchin immune cells activation in response to TiO2 nanoparticles.*

16.00 – 16.20 **COFFEE BREAK**

16.20  **Carla Falugi**  
(University of Genoa, Italy)  
*Developmental abnormalities in sea urchin larvae obtained from sperms exposed to engineered nanoparticles.*

16.40  **Franz Brümmer**  
(Universität Stuttgart, Germany)  
*Silver nanoparticle toxicity in sea urchin.*

17.00  **Francesca Garaventa**  
(Institute of Marine Sciences, CNR, Venezia, Italy)  
*Ecotoxicological screening on a ENPs pool: responses of different marine organisms and end-points.*
17.20  **Giovanni Libralato**  
(Veneto Nanotech, Rovigo and Ca’ Foscari University, Venice, Italy)  
*The challenge of ENMs in seawater: the case of nTiO2 and the main knowledge gaps.*

17.40  **Alessia D’Agata**  
(University of Messina, Italy)  
*Effects of sublethal concentrations of fresh, aged TiO2 and its bulk counterpart on marine mussels, Mytilus sp.*

18.00  **Concluding Remarks: current perspectives and future directions**

18.30  **Wine and cheese**
The EU NanoSafety Cluster mission and objectives: emphasis on aquatic toxicology

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The EU NanoSafety Cluster is a DG RTD NMP initiative to maximise the synergies between the existing FP6 and FP7 projects addressing all aspects of nanosafety including toxicology, ecotoxicology, exposure assessment, mechanisms of interaction, risk assessment and standardisation. Participation in the NanoSafety cluster was voluntary for projects that commenced prior to April 2009, but it has been compulsory for nano-EHS projects which have started since April 2009. The EU NanoSafety Cluster aims to facilitate exchange of information, data and knowledge across the different projects, sectors of activity and stakeholders. So far the work of members has taken the form of workshops focussing on particular issues, such as ecotoxicology and databases, has acted as a catalyst for the depository of materials, such as protocols, and has been the focus of key exchanges with workers in this field in the USA. This talk will provide an overview of the work of the EU Nanosafety Cluster, with a particular focus on aquatic toxicology, and future developments in the area of marine nano ecotoxicology.
Marine Nanotoxicology in the U.S.: The University of California Center for Environmental Implications of Nanotechnology (UC CEIN).

G.N. Cherr

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The University of California Center for Environmental Implications of Nanotechnology (UC CEIN; [1]) was established with funding from the US National Science Foundation and the US Environmental Protection Agency with the mission to study the impact of nanotechnology on the environment, including the identification of hazard and exposure scenarios that take into consideration the novel physicochemical properties of engineered nanomaterials (ENMs). The UC CEIN has made great progress in assembling a multidisciplinary team to develop the scientific underpinnings, research, knowledge, education and outreach that is required for assessing the safety of nanotechnology in the environment. The approach includes high-throughput/content screening approaches to develop structure–activity relationships that can be used to predict the impact of ENMs on organisms in freshwater, marine, and terrestrial environments. Marine studies include species of phytoplankton as primary producers and copepods and mussels as primary consumers linking photosynthesis as well as ENM transfer to higher trophic levels. The effect of ENMs on embryo development is also a focus of the Center [2, 3]. A powerful approach being used to model ENM impact in marine organisms is Dynamic Energy Budget (DEB) theory [4]. DEB theory focuses on the individual organism, with differential equations describing the rates at which an organism assimilates and utilizes energy and materials from food for maintenance, growth, reproduction, and development. The UC CEIN’s marine ecotoxicology efforts link ENM chemical properties, exposure, and biochemical responses of injury with the ecological and physical processes that ultimately regulate ecosystem-level impacts and ecosystem services.

References:
The mesocosm approach to study the exposure, accumulation and toxicity of nanoparticles to aquatic/marine organisms.

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Estimations give that 40 000 km³/year of freshwater flows into the worldwide oceans through the main rivers. Consequently, the Engineered Nanomaterials (ENMs) accidentally released or not in the aquatic environment will flow directly into the rivers, and be transported by waters, sediments, and organisms until reaching estuarine or coastal areas. Several questions rise regarding the effects of these ENMs towards media represented by a salinity gradient or salted aquatic media (seawater). The aims of this work was to elucidate using a mechanistic and holistic approach the principles governing ENMs behavior and ecotoxicity across a salinity gradient.

One novel aspect of this work is to use mesocosms to assess the impacts of ENMs across such a salinity gradient, taking into account both the exposure and hazards. The physico-chemical behavior of ENMs was followed across a salinity gradient, informing on the colloidal stability, heteroaggregation, complexation with natural organic matter, and consequently the exposure pathway at the marine-continental interface. This influences the bioaccumulation processes (trophic transfers) and the localization in the different tissues and cellular compartments of the exposed organisms.

Scrobicula plana bivalve was studied, covering a large salinity gradient under increasing realistic environmental exposure conditions (water, food-borne and mesocosms) [1]. The ecotoxicity and ecophysiology testings are performed across a salinity gradient in mesocosms using several biochemical and behavioral responses (multi-biomarker approach).

Such a degree of complexity in term of exposure to ENMs and of the related impacts is a real challenge for the scientific community, and will provide necessary data for Environmental Risk Assessment.

References:
Nanosilver effects in marine ectotherms: liking low and high informational levels

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The rising use of nanomaterials in several applications - including health care products and domestic uses- is posing new ecotoxicological concerns also for the marine environment.

Here we propose a framework of analysis for the assessment of biological effects of engineered nano-objects (ENO) in the marine ectothermic organism Mytilus galloprovincialis, a filter feeding mollusk with great ability to accumulate trace contaminants. The framework included high and low order level effect determination, spanning from bioaccumulation, bio-nano imaging, molecular and biochemical responses, life trait history till to fitness and population effects.

Two different nano-silver preparations (5 nm and 50 nm) were selected and further used in acute, chronic and microcosm exposures aimed to assess either ecotoxicological endpoints and mechanisms effects of nano-silver.

Results obtained within the VII UE Project nanoFATE will be presented and discussed.
Occurrence, identification, fate and behavior of engineered nanoparticles and nanoscale pollutants in marine systems.

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The oceans have been found to be the sink for many classes of persistent environmental contaminants, and it is likely that synthetic nanomaterials will also find its way to the sea, water borne or via sediment transport. In addition to synthetic nanomaterials, many other types of micro- and nanoscale pollutants have recently been identified as potential emerging pollutants, e.g. from road runoff, combustion, mining, waste and industrial processes. These incidentally produced nanomaterials can either be harmful themselves or carry toxic molecules adsorbed. The knowledge of potential exposure of pelagic and benthic marine organisms from nanoscale contaminants in the sea are limited by suitable measurement methods and fate and behaviour studies.

This presentations will review the potential methods for detecting and characterizing nanoparticles in seawater[1,2]. The limited knowledge on occurrence and size distributions of micro- and nanoscale contaminant particles will be discussed in relation to the natural particles. Further will be discussed fundamental behaviour and transport processes affecting synthetic nanoparticles in seawater, e.g. dissolution and complexation, interactions with natural organic matter[3,4], stabilization and agglomeration of natural and synthetic nanomaterials in estuarine mixing[5], sedimentation processes affecting the vertical distribution of particles in the sea.

References:
TiO2 nanoparticles and ABC transporters in marine organisms: the Trojan horse effect.

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The aim of the present study was to investigate the interaction of titanium dioxide nanoparticles (n-TiO$_2$) with the ATP binding cassette transport protein P-glycoprotein (P-gp) in the gills of the Mediterranean mussel *Mytilus galloprovincialis* as part of possible defense mechanism against NPs exposure in sea water. The Multidrug resistance mechanism (MDR) known also as Multixenobiotic Resistance (MXR) is a part of the detoxification mechanism based on trans-membrane transport proteins belonging to the ATP-binding cassette (ABC) superfamily, which actively pump a broad spectrum of chemicals including “xenobiotics” out of the cell preventing intracellular accumulation and potential toxic effects. The main representative of MDR/MXR-related ABC transporters is P-glycoprotein (P-gp/ABCB1) which act as a cellular first line of defence by preventing uptake of compounds by the cell (phase 0 of cellular detoxification). n-TiO$_2$ interaction has been investigated at gene level (ABCB1) and on efflux activity (pre-exposure and competitive binding assay) both in vitro (2h) and in vivo (96h) in gills of mussels exposed to n-TiO$_2$ (0.1-1 mg/L) and also combined with cadmium (0.1 mg/L). The aim to investigate exposure to n-TiO$_2$ coupled with toxic pollutant such as Cd may elucidate a possible Trojan horse effect played by n-TiO$_2$ in a marine model organism. A slight different behaviour of n-TiO$_2$ has been observed at gene and efflux activity levels in gill biopsies exposed in vitro. No modulation of ABCB1 gene expression was observed while a significant reduction in RhB accumulation compared to controls suggest a stimulation of P-gp efflux activity. Co-exposure to n-TiO$_2$ with Cd caused an increase in ABCB1 gene expression compared to single chemicals exposure suggesting a possible additive effect. In vivo n-TiO$_2$ (96h) caused a significant increase of ABCB1 gene expression (2-2.5 fold vs controls) also evident for Cd (3.5-4 fold vs controls) while no significant effects were observed at efflux level. Co-exposure to n-TiO$_2$ with Cd reduced significantly ABCB1 gene expression compared to single chemicals exposure suggesting a possible antagonistic behaviour. ABCB1 and P-gp may act as defence mechanism towards n-TiO$_2$ exposure in short term exposure condition while more complex and specific mechanisms might be activated in longer time exposure. The absence of a clear Trojan horse effect of n-TiO$_2$ towards Cd might be thus hypothesized.
Titanium dioxide genotoxic potential in marine organisms.

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Titanium dioxide (TiO₂) particles are used in many products, including industrial materials, cosmetics and pharmaceuticals. Published data raised concern about the toxic potential of TiO₂ for both human and environmental health. However, most information deals with mouse and human experimental models while scarce data are available for marine organisms.

This work is aimed at presenting preliminary results about the genotoxic potential of nanosized TiO₂ anatase in different organisms including mussels, fishes, fish cell lines, bottlenose dolphin leukocytes and fibroblasts.

Genotoxicity was detected by the alkaline Comet assay and RAPD-PC and the genotoxic effects of TiO₂-NPs was evaluated in Mytilus galloprovincialis and in Dicentrachus labrax both in vitro and in vivo. This effect has also been investigated by the co-exposure of TiO₂-NPs with Cadmium and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TiO₂-NPs resulted to induce a reduction of genome stability both in vitro (sea bass cell cultures) and in vivo (mussels). Comet assay only showed an induction of DNA single strand breaks at 10 mg/L and of double strand breaks at 0,1-5-10 mg/L in mussel gill cells treated in vitro. An effect of Cd and dioxin in inducing DNA damage both in vitro and in vivo was also observed, while no induction of DNA strand breaks was observed after TiO₂-NPs in vivo treatments in mussels. Even if referred to preliminary results, our data revealed that TiO₂ might possess a genotoxic potential, since a significant genomic stability reduction was found in sea bass cells and an increase of DNA strand breaks in mussel gill cells were observed after in vitro exposure. Moreover four days in vivo exposure to TiO₂-NPs gave rise to a 50% reduction of genomic stability in mussel digestive gland. Interesting data comes from exposures coupled TiO₂-NPs and Cd. Different times (4, 24 and 48 hrs) and doses (20, 50, 100, 150 µg/ml) were also tested in bottle-nose dolphin cell lines. TiO₂ particles induced DNA damage both in leukocytes and fibroblasts, even if depending on, the cell line and the exposure time. These are the first data regarding the genetic susceptibility of toothed cetaceans toward an “emerging” pollutant such as TiO₂, whose amount entering the marine environment is expected to increase in the next decade.
Effects of TiO$_2$ nanoparticles on immune and digestive gland function in the marine bivalve *Mytilus*

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Due to the increasing production of nanoparticles (NPs) and their potential release in the aquatic environment, evaluation of their biological impact on aquatic organisms represents a major concern. Suspension feeding invertebrates, in particular bivalve mollusks, may play a role in NP biotransformation and transfer through food webs and may represent a significant target for NP toxicity.

The results of a recent study are reported on the effects of titanium dioxide (n-TiO$_2$), one of the most widespread NPs in use, in the bivalve *Mytilus galloprovincialis*, largely utilized as a sentinel for marine contamination. Mussels were exposed for 96 h to different concentrations of n-TiO$_2$ suspensions (1, 10 and 100 $\mu$g L$^{-1}$) and multiple responses were evaluated in the digestive gland and immune cells, the hemocytes. In the digestive gland, n-TiO$_2$ affected lysosomal and oxidative stress biomarkers and decreased transcription of antioxidant and immune-related genes. In the hemocytes, n-TiO$_2$ decreased lysosomal membrane stability-LMS and phagocytosis, increased oxyradical production and transcription of antimicrobial peptides, indicating immunomodulation; moreover, pre-apoptotic processes were observed. The effects of n-TiO$_2$ on digestive gland and hemocytes were distinct, also depending on the endpoint and on nominal NP concentrations, with many significant responses elicited by the lowest concentrations tested. The results show n-TiO$_2$, at concentrations close to predicted environmental levels, significantly affected different functional and molecular parameters of mussel digestive gland and immune cells.
Nano-immunosafety in marine organisms, a central issue in environmental nanotoxicology

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Besides direct toxicity (relatively easy to detect and measure), nano-objects may affect the capacity of biological systems of maintaining homeostasis and performing their functions. This is particularly important in the case of the immune system, responsible for defending the organisms from external and internal dangers, where alterations in immune responses may lead to significant pathological consequences.

Nano-immunosafety is therefore a topic of central relevance in nanosafety regulations and needs to be seriously addressed. Indeed, the issue does not exclusively pertain human health, but it is of utmost importance also for the environment, since all living organisms (including worms, insects, and plants) have an immune system which ensures their health and survival.

Within the Working Group Hazard of the EU-sponsored NanoSafety Cluster, the Immunosafety Focus Group (led by D. Boraschi and A. Duschl) and the Marine Ecotox Focus Group (led by I. Corsi and V. Matranga) are working together for determining the impact of nano-objects on the immune defensive system of organisms in the environment (besides man), starting from marine invertebrates. The goal is two-fold:

1. validate the concept that the immune response of environmental organisms is a key element in assessing environmental nanosafety;
2. propose and develop the use of non-human immune cells/organisms for assessing nano-immunosafety for human beings.

The impressive similarity of several mechanisms of innate immunity across species warrants the possibility of developing predictive assays for testing the immunosafety of nano-objects in different settings (\textit{e.g.}, in the working place). The final goal is that of providing the regulatory bodies with valid and robust predictive assays to be adopted for both environmental and human nanosafety.
Sea urchin immune cells activation in response to TiO$_2$ nanoparticles

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Sea urchins are phylogenetically related to vertebrates and have been proven to possess a sophisticated, robust and sensitive immune system. Their immune cells carry out functions similar to those of their vertebrate’s immune system homologues, such as clot formation, phagocytosis, encapsulation, clearance of pathogens. Four different morphotypes have been described in the sea urchin Paracentrotus lividus, with the phagocytes as the most abundant type, accounting for approximately 80% of the total population (Matranga et al 2006, Pinsino et al 2008). The coelomic fluid, in which the immune cells reside and move, is governing the immunological capabilities of sea urchin, as it contains essential trophic and activating factors probably produced by immune cells themselves (Matranga et al 2005). In the last years, we demonstrated that sea urchin immune cells activate their immune response machinery in reply to different kinds of physical and chemical stresses, such as temperature shocks, pH drops, exposure to heavy metals, UV-B radiation and, recently tannum dioxide (SnO$_2$), cerium dioxide (CeO$_2$) and iron oxide (Fe$_3$O$_4$) nanoparticles (NPs) (Matranga et al 2000, 2005, 2006; Pinsino et al 2008; Falugi et al 2012). We established the utility of HSCP70/HSC70 as a general stress response marker to use for both acute and chronic environmental stresses: in fact it has been found an increase in HSP70/HSC70 levels in all cases, except for NPs exposure (Falugi et al 2012). Currently, in our laboratory, the interaction mechanisms between TiO$_2$NPs and sea urchin immune cells are under study at the cellular and biochemical levels. Preliminary results will give insights on the putative activation of sea urchin immune cells and provide a new tool for marine nano-ecosafety investigations.

References:
Developmental abnormalities in sea urchin larvae obtained from sperms exposed to engineered nanoparticles

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Engineered nanoparticles are increasingly produced to be employed in many commercial products of common use, but their possible toxic effects are not known enough. Preliminary studies report that they can play a role in damaging numerous important biological processes, including skeletogenesis on living organisms. That is very critical in vertebrates, especially during the developmental stages, when the skeleton develops in a genetically programmed way.

The aim of this study is to propose a model of nanotoxicity for aquatic organisms at the developmental stage. The research investigated whether the exposure to different concentrations of cobalt (Co), titanium dioxide (TiO₂) and silver (Ag) nanoparticles can induce skeletal damages in sea urchins (Paracentrotus lividus) at the pluteus stage.

P. lividus at pluteus stages were obtained from fertilized eggs exposing sperms at different nanoparticles’ concentrations (from 0.0001 up to 1 µg mL⁻¹). Non exposed sea urchins’ sperms served as controls. The pluteus were made permeable by treating them with glycerol solution and then they were treated in toto with the lectin wheat germ agglutinin (WGA), specific component for binding to the n-acetyl-glucosamine residues. WGA was fluorochrome conjugated, therefore, it was introduced into tissues and visualized through confocal (TCS SP2 Leica, Switzerland) and epifluorescence (Olympus BX60,Japan) microscopic investigations. Fluorescent WGA lectin-binding sites were observed in the mesenchyme cells and in the skeletal rods of nanoparticle-treated pluteus stages. On the contrary, no fluorescence was detected in the controls at pluteus stages. Morphological investigations of the pluteus by means of a Field Emission Gun Environmental Scanning Electron Microscope (FEG-ESEM Quanta 250–FEI, the Netherlands) coupled with an x-ray microprobe of an Energy Dispersion System verified morphological changes and the physical presence of the different nanoparticles in the bodies. No dose-dependent nanoparticle’s effects were identified. Morphological anomalies such as the asymmetrical rods of the skeleton and the irregular shape of the pluteus stage were found only in the exposed pluteus.

In conclusion, the present study suggests that nanoparticles interact with WGA lectin-binding sites in developing mesenchyme and skeleton, causing skeletal alterations and damages. That phenomenon induces malformations in the offspring.

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Silver nanoparticle toxicity in sea urchin

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Nanoparticles and especially Silver nanoparticles (AgNPs) are used already in many kind of applications e.g. in medicine and medical equipment and other everyday products (dishwashers, socks, computer keyboards). It is important to study health and safety issues associated with nanomaterials in the environment. We use the embryonic normogenese of the sea urchin Paracentrotus lividus to investigate the impacts of nanoparticles on the development of a marine organism.

AgNPs causes dose dependent developmental defects. The AgNPs are accumulated around the oral region and very likely on the ectoderm of the sea urchin. It has been observed that AgNPs are more toxic than their equivalent Ag⁺ ion dose.

To detect the subtle changes in the chemistry of AgNPs due to their agglomeration in sea water, within the sea urchin and on the ectoderm we apply a multi-technique sub-micro imaging (TWIN microscopy).

A decrease of calcite and excess of sulphur compounds within sea urchin as a result of AgNPs toxicity has been observed. The excessive sulphur compounds, concentrated around the oral region, are correlated with AgNPs concentration. The observed AgNPs agglomeration of AgNPs on the ectoderm is due to a biogenic process (i.e. agglomeration after cellular uptake in endosomes) by the excess production of the compounds in the sea urchin which involves sulphur/oxygen-containing substances.
Ecotoxicological screening on a ENPs pool: responses of different marine organisms and end-points.

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Nowadays, most of existing data about aquatic toxicity of Engineered Nanoparticles (ENPs) concern freshwater environment [1, 2]. The aim of this study was to contribute to fill this gap to more fully assess the effect of a panel of ENPs on different marine organisms. Organisms have been exposed to serial concentrations of ENPs: SiO₂, Ag, TiO₂, Fullerene (C₆₀), Carbon black and Graphene Oxide.

In particular, Echinoderms (Paracentrotus lividus) and crustaceans (Amphibalanus amphitrite and Artemia salina) were exposed to the whole panel of ENPs; in addiction, the effect of Silver ENPs have been investigated also on Algae (Dunaliella tertiolecta and Skeletonema costatum) and Cnidaria (Aurelia aurita).

In order to asses ENPs effect different end-points have been evaluated: algal growth inhibition, sea urchin sperm motility, jellyfish mortality, crustaceans mortality and swimming behaviour alteration.

The results show that the investigated ENPs have different effects on the tested organisms. In particular, SiO₂ and TiO₂ showed any toxic effect on the tested organisms. On the contrary, Fullerene, Carbon Black and Graphene Oxide caused a measurable toxic effect on sea urchin and crustaceans.

Finally, considering the results obtained exposing Silver ENPs to a wider battery of organisms, it was possible to point out that all the investigated end-points were able to underline a dose-dependent effect caused by Silver ENPs. For each end-point it was possible to quantify this effect by means of LC₅₀, IC₅₀ and EC₅₀. The results obtained, in particular those with Silver NPs, suggest that ENPs’ exposure can influence different trophic levels within the marine ecosystem, displaying dose-dependent effect on all the tested organisms.

References:
The challenge of ENMs in seawater: the case of nTiO$_2$ and the main knowledge gaps.

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Engineered nanomaterials are at the forefront of ecotoxicologist agendas due to their increasing use in a broad range of industrial and domestic sectors. Actually, they are manufactured in increasing amounts year-by-year. Particularly, nanoscopic titanium dioxide (nTiO$_2$) is adopted by a variety of industries mainly for catalysis and photocatalysis applications and as an additive in paints, papers, inks, plastics and various consumer products. So that it can be supposed that such widespread use of nTiO$_2$ may result to an increased environmental exposure that may reach unnegligible concentrations in surface waters posing a potential significant threat to aquatic ecosystems. Most part of literature on the ecotoxicity of nTiO$_2$ deals with aquatic organisms such as bacteria, algae, invertebrates, and fish, but there are also case studies considering cell lines and rodents. Mostly, testing species are from freshwaters. Little knowledge is available about saltwater species such as mollusc bivalves. For example, within our recent research activity on nTiO$_2$, embryo toxicity effects were highlighted for both dark and light/dark scenarios\textit{(in vivo) 48 h exposure of Mytilus galloprovincialis} zygotes.

Nevertheless, data about potential seawater effects are still limited and hard to understand and compare due to a lot of gaps in the knowledge. Practically, the scientific community has just started tackling with various seawater exposure scenarios and does not yet agree upon neither the way the exposure is carried on nor how ENMs are characterised during testing. For example, taking into consideration the ENMs dispersing methods authors might suggest behaving naturally, using synthetic or natural dispersing agents, sonicating, stirring or mixing. Moreover, many analytical challenges have to be solved. For example, the complete characterization of ENMs in exposure media and organisms has to be achieved, because it is extremely relevant for comparative purposes, and the quantification methods currently applied for ionic chemical species might have to be revised and validated. Indeed, it seems that ENMs tend behaving in specific ways that could not be approached in the traditional way.
Effects of sublethal concentrations of fresh, aged TiO$_2$ and its bulk counterpart on marine mussels, *Mytilus* sp.

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After their release into the environment, engineered nanoparticles (ENPs) age and tend to form aggregates, which might increase their size and could make them less mobile. There is, however, limited data on how these physico-chemical changes alter the potential of ENPs to induce biological damage. In this experiment, we studied the biological responses of a marine bivalve, *Mytilus* sp. at sublethal concentrations of an environmentally relevant ENP, titanium dioxide (TiO$_2$). Mussels were collected from a UK reference site, acclimatised, and exposed to 10 mgL$^{-1}$ of titanium dioxide (TiO$_2$), in either fresh ENP, aged ENP or bulk form, for 96h. Copper (40 µgL$^{-1}$) was used as a positive control. The ENPs and bulk TiO$_2$ were characterised using TEM. The biological responses included oxidative DNA damage, using the enzyme-modified comet assay, histopathological and histochemical analysis, metallothionein gene expression and fluorescent in situ hybridization, in gills and digestive glands. In addition, accumulation of metals in different tissues was determined using ICP-OES. Although the aged and fresh nTiO$_2$ showed significantly higher accumulation compared to bulk counterpart, the biological effects of several analyses suggest that the bulk form is more toxic than the two nano forms of TiO$_2$ (i.e. fresh or aged) used in the study. For oxidative DNA damage, however, all the treatment groups showed similar levels of damage compared to untreated mussels, suggesting a saturation level of induced DNA damage in these cell types for the concentration of TiO$_2$ used. Our integrated study suggests that for this ecologically relevant organism photo-catalytic aging of nTiO$_2$ does not significantly alter toxicity, and that bulk TiO$_2$ may be less ecotoxicologically inert than previously assumed.
ABSTRACTS - POSTERS

Toxicity of manufactured ZnO nanoparticles in the marine environment

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Due to the increased production of synthetic nanoparticles (NPs), the concern related to the occupational and public exposure to NPs is supposed to increase dramatically in the coming years as also by effect of their potential release in the environment. Nanoparticles of ZnO, having adsorbing properties in the UV wavelength range, are commonly added to sunscreens and cosmetics. Therefore, higher concentrations in coastal marine environment could be reasonably supposed especially during the summer season when a rapid washing of sunscreen in the seawater can be estimated. The fate and the behaviour of NPs in seawater strongly depend on different physic-chemical characteristics that could affect aggregations and colloid chemistry. It is well-known that NPs tend to aggregate in aquatic environments to form micrometer-sized particles and it is likely that this state of dispersion may reduce the influence of particle size, shape and surface properties on their ecotoxicity.

Our research work, about this peculiar environmental matrix, started with the evaluation of adverse effect of four different NPs (ZnO, SiO\textsubscript{2} Carbon black, SWCNT) towards marine organisms with different biological complexity (sea urchins, crustaceans and algae). Successively, we focused our concerns on nano ZnO effects for marine microalga \textit{D. tertiolecta}, and the sea urchin \textit{P. lividus} as they were the most sensitive among the tested species. The aim was to establish the main toxicity parameters and to evaluate whether nano-ZnO produced toxic effects which can be clearly ascribed to peculiarities of the nanostate. As far as the aggregation rate and size may be relevant to the bioavailability of the dispersed particles in the marine ecosystem, the size distribution of ZnO particles was monitored. Relying on \textit{D. tertiolecta}, which is a marine unicellular chlorophyta commonly used in standard chronic algal toxicity testing, we recorded dose response curves for exposure to nano-ZnO and calculated its main toxicological parameters. Dose response curves were measured also for exposure to bulk ZnO in order to check any specificity in the nanosized material with respect to its bulk counterpart. The findings were also compared to the toxic effects of free Zn\textsuperscript{2+}, to specifically investigate the metal ion contributions to ZnO toxicity. The early development, fertilization, and offspring quality of the Mediterranean sea urchin \textit{P. lividus} was also investigated. These findings are also compared to the toxic effects of Zn ions (ZnCl\textsubscript{2}), to specifically investigate the Zn\textsuperscript{2+} contribution to nano-ZnO toxicity and with bulk ZnO to evaluate the toxicity linked to the nanosize.
Toxicity of quantum dot nanoparticles to the marine microalga *Phaeodactylum tricornutum*

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The rapid increase of nanotechnology has raised the concern about the potential adverse effects of nanoparticles on the ecosystems and living organisms [1]. Quantum dots (QDs) are semiconductor nanocrystals, candidate to be widely used for biomedical applications and microelectronics, due to their unique optical properties. However, their environmental impact and the mechanism of toxicity still have not been fully elucidated. Phytoplankton represents the first link of the food chain in natural waters, thus the knowledge of the mechanisms of uptake, accumulation and toxicity of nanoparticles in these unicellular organisms represents a crucial point for understanding their ecotoxicity.

In this work we studied the chemical behaviour of water-soluble CdSe/ZnS QDs in seawater and their effects on the marine diatom *Phaeodactylum tricornutum*, which served as a model of biological receptor in the marine environment. We evaluated QD toxicity in terms of growth rate inhibition, oxidative stress, membrane damage. In addition, we used the synthesis of phytochelatin (PCs) as a biomarker of the presence of free Cd\(^{2+}\) ions released from QDs. The optical and chemical characterization demonstrated the propensity of QDs to aggregate, after dispersion in raw seawater [2]. Laboratory cultures of *P. tricornutum* were grown in culture media at increasing concentrations of QDs, from 0.04 to 2.5 nM, corresponding to a range of equivalent concentrations of Cd from 20 to 1200 nM. The results showed that algae accumulated Cd, but synthesized negligible amounts of PCs, thus suggesting that QDs did not release PC-inducing free metal ions. Our data also showed a gradual decrease in algal growth rate, till to reach an inhibition of 85% at 2.5 nM QDs. QDs were more effective in inhibiting the growth rate than an equivalent amount of CdCl\(_2\). Measurements of the activity of the antioxidant enzymes showed that superoxide dismutase (SOD) and catalase (CAT) activities were enhanced by exposure to increasing QD concentrations, whereas ascorbate peroxidase and glutathione reductase activities were not significantly affected. An increase in SOD and CAT activity can be considered a symptom of oxidative stress induced by an enhanced production of ROS. This hypothesis was confirmed by the concomitant increase in the intracellular ROS concentration. Our data also showed a dose-dependent increase of the cellular concentration of malondialdehyde, a product of membrane lipid peroxidation, indicative of membrane damage.

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Nanocrystalline surface layer of pure copper induced by friction

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Severe plastic deformation on the subsurface layer of metals by means of sliding under loading could lead to the formation of nanocrystalline surface tribolayer. In this work, a nanocrystalline surface layer with a thickness of 120 µm is formed on a coarse-grained pure copper sheet after oscillating sliding at room temperature in argon. In the as-formed top surface layer, the average transverse and longitudinal grain size is about 20 nm and 37 nm, respectively. This work provides experimental evidence that dry sliding friction can be developed as a surface nanocrystallization technology to produce nanocrystalline surface layer with a thickness exceeding 100 µm.
Study on growth process and porosity evolution of coating formed on titanium by micro-arc oxidation

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The micro-arc oxidation process of titanium foil can be divided into four stages according to the experimental observation and current density change in oxidizing titanium foil in phosphate electrolyte. The oxidation layer is composed of anatase titania and contains more P than Na. The coating thickness increases fast at the micro-arc stage with an average growth rate of 0.32 µm/min within the first 30 min, but the growth rate slows down at the following local-arc stage. The porosity (between 12%-16%) and pore diameter of the coating both increase with the oxidation time, but the increments are reduced after oxidation for 30 min. It is shown that it is a convenient method to determine the porosity of the micro-arc oxidation coating on titanium by analyzing the scanning electron micrographs with phase analysis software that is attached to the metallurgical microscope.

Key words: micro-arc oxidation; titanium; porosity
Review of available frameworks and tools for ecological risk assessment of engineered nanomaterials

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Despite the substantial potential of engineered nanomaterials (ENMs) to contribute to sustainable innovation, there is an extensive gap of knowledge regarding both their environmental health and safety profile and the suitable tools and frameworks for assessing their ecological risks. The scientific community has been long discussing the appropriateness of standard risk assessment approaches to apply to ENMs. Taking into account the novelties of ENMs, it has been widely accepted that the current European chemical safety legislation, mainly embodied by the REACH regulation, needs to be adjusted with respect to the data and information required for risk assessment [1]. In response to this need, the REACH Implementation Projects on Nano (RIP-oN 1,2 and 3) were launched in order to provide scientific advice on how to adapt the REACH guidelines to ENMs. Moreover, in the last few years several frameworks and tools aimed to support and speed up the risk assessment of ENMs have been developed.

In this context, the latest frameworks and tools for ecological risk assessment (ERA) of ENMs have been collated and evaluated with respect to their strengths and weaknesses according to predefined criteria. Most of the reviewed approaches are not intended to facilitate regulatory decision making, but they serve as preliminary hazard/risk screening and/or research prioritization tools. Since, with few exceptions, they all lead to qualitative results, the development of specific approaches for quantitative effects assessment appears necessary to complement the available toolset and allow low-tier quantitative ERA. In this context, the application of unconventional tools such as Weight of Evidence [2] and Multi Criteria Decision Analysis [3] seems to represent the best option for the development of a quantitative screening ERA tool able to integrate available ecotoxicological information and suitable for implementation in standard RA procedure.

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