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Primo simposio di Biologia Sperimentale applicata al mare e all'ambiente

First symposium on experimental biology: sea and environment

Trapani, Italy, 24-25 May 2019

ABSTRACT BOOK

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Massimo Cocchi *(University of Bologna, Italy)*

Primo simposio di biologia sperimentale applicata al mare e all'ambiente

First symposium on experimental biology: sea and environment

Trapani, Italy, 24-25 May 2019

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Primo simposio di biologia sperimentale applicata al mare e all'ambiente

First symposium on experimental biology: sea and environment

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FISH BLOOD PROFILE AS A MONITORING DEVICE IN ENVIRONMENTAL AND XENOBIOTIC STRESS-INDUCED RESPONSE

Caterina Faggio*

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Hematological parameters are very important in determining health and physiological status of the fish. Moreover, these parameters reflect the changes in the organism correctly and play an important role in the detection of disease and metabolism of fish living in different ecological environments. Fishes are poikilothermic creatures, in which changes are observed in hematological parameters due to environmental factors such as bacteria, parasites, water temperature, oxygen content, pH and so on. Hence, the variations of hematological parameters hematocrit, hemoglobin concentration, leukocyte and erythrocyte count have been used as pollution and physiological indicators of organic dysfunction in both environmental and aquaculture studies. These parameters are commonly applied as prognostic and diagnostic tool in fish health status. However, there are both extrinsic and intrinsic factors to consider when performing a blood test, because a major limitation for field researchers is that the "rules" for animal or human hematology do not always apply to wildlife. To understand how some environmental and xenobiotic factors are capable to modulating the hematic cells is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes.

EVOLUTION, ADAPTATION AND IMMUNE FUNCTIONS OF FISH LECTINS

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Fish are equipped with a complex lectin repertoire that, like mammals, are involved almost all the immune reactions. Carbohydrate recognition and interactions mediated by lectins have been recognized involved in vertebrate innate immunity, not only for recognition of potential pathogens, but also acting in the agglutination, immobilization and other functional steps. In fish, C, F types galectins, Rhamnose-binding lectin (RBL) and pentraxin have been identified in both cartilaginous and bony fish. In addition, selectins and other genes have been found in the currently available fish genomes. On the basis of our results about F-type and RBL lectins we showed that: lectin repertoires in fish are highly diversified and include not only representatives of the lectin families; described in mammals, but also members of lectin families described for the first time in fish species like the F type lectins and RBL; RBL and FTL have been identified in the eggs and embryos but they are also present in the serum. The characterization indicates that the tissue-specific expression and localization of the diverse lectin repertoires and their molecular partners is consistent with their distinct biological roles in innate and adaptive immunity; Therefore, in order to identify trends linked to cold adaptation in antarctic fish, we try, to compare Perciformes and the Antartic fish *Trematomus bernacchii* F lectins properties.

LIVING IN A HIGH CO_2 WORLD: EFFECTS OF OCEAN ACIDIFICATION ON PHYSIOLOGY, BEHAVIOUR AND ECOLOGY OF FISH

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Rising atmospheric carbon dioxide (CO_2) concentrations due to human activities are causing a pH drop and increased dissolved CO_2 in the oceans, a process known as Ocean Acidification (OA). OA is expected to have detrimental effects on several marine organisms including fish that may exhibit physiological and behavioural disruptions under elevated pCO_2 levels. The vast majority of the studies dealing with CO_2 effects on fish was conducted under controlled laboratory conditions and showed variable sensitivities among species. However, in-situ investigations could better reflect the complexity of organism's interactions, potentially further explaining the role of acclimation and adaptation processes in a context of a changing ocean. Field experiments off natural CO_2 seeps were carried out to evaluate direct and indirect effects of OA on fish populations chronically exposed to elevated CO_2 concentrations. Results showed that fish living under OA conditions exhibited increased growth and altered reproductive behavior, whilst embryos physiology and predator recognition ability were unaffected. Moreover, CO_2-mediated benthic habitat shifts led to changes of the structure and composition of fish communities, with potential repercussions on organism interactions and ecosystem functioning.

CAN MARINE PROTECTED AREAS RESTORE *PARACENTROTUS LIVIDUS* POPULATIONS?

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Can Marine Protected Areas restore sea urchin populations? Sea urchins have long attracted attention from scientists worldwide for their ecological role in coastal areas. Sea urchins, in addition, have been used as a food resource by humans since prehistory, and presently they are one of the important sea food consumed in many regions, but also exported-imported through the world. In the Mediterranean sea, the purple sea urchin *Paracentrotus lividus* (Lamarck) exerts a key ecological as a main regulator of the structure of coastal communities. At the same time *P. lividus* is recreationally and commercially exploited in many Mediterranean areas, as both male and female gonads are considered a delicacy in several countries. Presently the market of this sea food is expanding, and therefore major concern should be devoted to avoid overexploitation and possible communitywide effects of overfishing. The aim of the study is to figure out if well-

enforced MPAs can increase density, size and reproductive potential (gonad weight) of *P. lividus*, often important for the local economy. We examined the effects of *P. lividus* recreational harvesting on the species itself on rocky substrates of Ustica Island MPA. We compared the average density, size structure and gonad weight of *P. lividus* recorded at protected (no take zone) and fished sites (take zone C) in summer 2017 and 2018. *P. lividus* was always larger and more abundant at the protected sites than the fished ones. Results suggest that stronger management measures should be adopted by managers of MPAs to accomplish conservation of wild *P. lividus* populations also in the take zones.

HOW MANY NON INDIGENOUS SPECIES HAVE SPREAD ALONG ITALIAN COAST? THE ROLE OF SICILY AS CROSSROAD FOR MARINE BIOINVASIONS

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A recent paper by Servello *et al.* (2019; doi: http://dx.doi.org/10.12681/mms.18711), which involved the Taxonomy Lab of the University of Palermo, examined the state of art of the marine alien species in Italy. The research aimed to contribute to the effectiveness of descriptor of the marine strategy framework directive which contemplate the marine alien species presence in the Mediterranean Sea. More than two hundres non indigenous species have been counted, highlighting the threats towards the Mediterranean habitats. One hundred and eighty non indigenous species have established stable populations in Italian Seas; forty-four new alien species were recorded. Approximately half of the NIS recorded in Italy have most likely arrived through the transport-stowaway pathway related to shipping traffic. The actual non indigenous species distribution hotspots is located in the Adriatic Sea, Venice, which accounts for the highest number of alien taxa. In the Ionian Sea and Central Mediterranean Sea, Taranto and Catania can be considered hotspots as well. In the Western Mediterranean Sea, bioinvasion hotspots are the Gulfs of Naples, Genoa and Livorno. Regarding the Strait of Sicily it has been outlined its role aa crossroad between alien taxa from the Atlantic Ocean and the Indo-Pacific area. Species as the kyphosids, or the mussel *Brachidontes sp*, or some caprellids inhabit different habitats; however they have been recorded for the first times in the Sicilian coasts. The role of Sicily Island as sentinel for Mediterranean bioinvasions is discussed.

A NEW SUSTAINABLE FEED BASED ON AGRICULTURAL WASTES AND FISHING INDUSTRY DISCARDS FOR THE SEA URCHIN *PARACENTROTUS LIVIDUS*

Laura Ciriminna[1], Geraldina Signa[2], Giulia Visconti[1], Salvatrice Vizzini[1,2], Antonio Mazzola[1,2]

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Main goal of the echinoculture is the production of sea urchin gonads able to satisfy the market demands both qualitatively and quantitatively, and to reduce the impact of the harvesting pressure on natural populations. Moreover, the ingredients more frequently used for feed production, such as algae, vegetable meal, and oils and fishmeal, lead to further exploitation of natural resources, that are already stressed by human activities. To move towards a higher sustainability of echinoculture, this study proposes a new feed with a low environmental impact through the reuse of wastes and discards from the food industry. Two experimental formulations were obtained using endive (*Cichorium endivia*) market waste and anchovy (*Engraulis encrasicolus*) industry discards in different proportions, plus agar as a binder. After a preliminary evaluation of the feed stability, palatability and nutritional value, the two formulations were administered for 3 months to *Paracentrotus lividus* reared indoor. Gonads were analysed and compared between reared and wild sea urchins across time in terms of gonad somatic index, colour and fatty acids. At the end of experiment, all reared sea urchins showed a better gonad colour and a higher gonad somatic index and nutritional quality (PUFA, ω-3/ω-6) than wild specimens. Moreover, the formulation with a higher proportion of animal ingredient (60/40) showed the best performances in terms of gonad somatic index and colour, key factors in the assessment of the gonad commercial value. Therefore, the new feed, and especially the 60/40 formulation, represents a commercially valuable product.

A NEW COELOMIC CELL POPULATION IN THE REGULAR SEA URCHIN *ARBACIA LIXULA*: IMPLICATIONS FOR SEA URCHIN PHYSIOLOGY

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Phagocytes, vibratile cells and red and colorless spherulocytes are accepted as the only circulating coelomocytes in echinoids. Here, based on live and stained preparations, we characterize the cells of the echinoid *Arbacia lixula* and a new spherulocyte is described. Living cells were analyzed immediately after collection. Cytological preparations were made using a cytocentrifuge (10^5 cells per spot, 80 × g/5 min), fixed in formalin vapor and stained with Mallory's trichrome (MT) or toluidine blue (TB). Phagocytes are large cells with filiform or bladder-like expansions with no special affinity to stains, while vibratile cells are round coelomocytes with a remarkable flagellum and stain purple (weak metachromasia) with TB. Red spherulocytes are round cells filled with uniform-sized red vacuoles, which do not stain with MT, but show a brownish color. Colorless spherulocytes are elongated cells, filled with heterogeneous-sized colorless vacuoles, which stain light blue with MT. The new coelomocyte, named as granular spherulocytes, is a small rounded cell, with homogeneous-sized colorless spherules, which stain bright pink with MT. Phagocytes, red and colorless spherulocytes perform the most important immune functions in echinoids (*i.e.* phagocytosis, antibacterial activity and cytotoxicity, respectively), while vibratile cell's function remain is under debate. A cell similar to *A. lixula's* granular spherulocyte was described in *Eucidaris tribuloides*, however its function remains unknown. The new cell observed here shows the need for more accurate studies on sea urchin coelomocytes. Additionally, two important questions are raised: Are granular spherulocytes common in other sea urchins? What is the physiological function of this cell?

EFFECTS OF PERFLUOROOCTANE SULFONATE AND POLYETHYLENE MICROPLASTICS ON LIVER AND INTESTINE FROM EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX* L)

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Polyethylene microplastics (PE-MPs) are the most commonly found MPs on marine environment and, apart from been harmful by themselves, could increase the exposure of animals to chemicals associated with them. By its part, perfluorooctane sulfonic acid (PFOs) is highly toxic and bioaccumulates in the environment. The aim of the present study was to analyse the effects caused by the dietary administration of PE-MPs, PFOS and PFOS associated to PE-MPs on the status of liver and intestine status of European sea bass, selected as a model of marine fish. Fish were fed diets containing 0 (control), virgin PE-MPs (MP), PFOS (PFOs) or PFOS adsorbed to PE-MPs (MPs-PFOs) and liver and intestine samples were obtained at 7 and 21 days of the feeding trial. Samples were processed for histology, gene expression and antioxidant enzyme activities. The present results demonstrated that the exposure to different doses of MPs, PFOs and MPs-PFOs, was able to produce significant signs of inflammation in liver and intestine from sea bass. In liver, the expression of pro-inflammatory genes was up-regulated in fish fed the PFOs diet, while expression of antioxidant enzymes was down-regulated with all the diets. In gut, the expression of pro-inflammatory, antioxidant enzymes and stress genes was affected by all the diets at 7 or 21 days. PFOs adsorbed to MPs seem to exacerbate the negative effects produced on the inflammation and oxidative status, suggesting a synergic effect.

ACKNOWLEDGMENT: The authors thank Salvá A.I. for her technical support. The results from the present work were developed under the EPHEMARE Project (JPI Oceans) and funded by the Spanish Ministry of Economy and Competitiveness (MINECO) project code: PCIN-2015-187-C03-02) and *Fundación Séneca de la Región de Murcia* (*Grupo de Excelencia* grant number: 19883/GERM/15).

Session: Environment and health

Moderator: Marco Giammanco

HUMAN HEALTH RISKS FROM HEAVY METALS VIA CONSUMPTION OF MARINE SPECIES FROM AUGUSTA BAY (SOUTHERN ITALY)

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Human populations are exposed to different pollutants released in the environment from natural and anthropogenic sources. Food, particularly seafood, represents one of the main route of heavy metals transfer from the environment to consumers and a relevant exposure to chemicals. In the last decades, assessment of human health risk associated to seafood consumption emerged as a crucial aspect of public interest. Here we report on arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) concentrations measured in fish, crustaceans and cephalopods from Augusta Bay, a marine area characterized by a high anthropic impact. Cd and Pb are below the tolerable limits set by the European Union in foodstuffs. Mercury exceeded in most cases the threshold value indicated for all the species. The potential human health risk associated to consumption of the selected specie was calculated, for adults and children, using the estimated weekly intake (EWI) index, the Target Hazard Quotient (THQ) and the Lifetime cancer risks (CR) for the measured heavy metals. The EWI for As, Cd and Pb are below the PTWI, while the EWI for Hg showed values up to 10-fold higher than the tolerable limit. Similarly, a THQ >1 was registered only for Hg, indicating a potential health risks resulting from exposure to this pollutant. The CR was calculated in order to estimate the probability for an individual to develop cancer over a lifetime. The CR for As exceed the acceptable lifetime risk-ARL-of 10-5 for adults and children suggesting that a daily ingestion of these specie could increase the probability of carcinogenic risk.

THE NEHO COHORT: THE FIRST ITALIAN BIRTH COHORTS IN HIGHLY POLLUTED SITES

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Exposure to environmental contaminants during pregnancy is one of the determinants of a child's future health outcomes. The effects of environmental pollution on pregnant women living in heavily polluted areas is of special interest and, in this context, the Neonatal Environment and Health Outcomes (NEHO) cohort focuses on the investigation of: environmental toxicants transferred from the mother to the developing fetus; the role of placenta in fetal exposure; the influence of toxicants on pregnancy outcomes, fetal development, and health status during infancy. The NEHO cohort will enroll an estimated total of 800 pregnant women in three selected National Priority Contaminated Sites in Italy (Milazzo, Augusta, and Crotone). Epidemiological data collection concerning maternal health status, lifestyle, and preg-

nancy are obtained through survey questionnaires provided to mothers starting from the last two months of pregnancy. At the time of delivery, maternal blood, umbilical cord blood, and placenta tissue are collected to assess contaminant levels and to clarify how toxicants interact with the placental domain. Furthermore, placental transcriptome is studied in order to explore the interference of toxicants on the role of the placenta in maternal/fetal interplay. Regular follow-ups are planned at 6, 12, and 24 months. At the end of the first 67 weeks of enrollment the NEHO cohort reached 572 pregnant women: we therefore expect to reach the enrollment target in advance than expected.

IN VITRO EXPOSURE TO 2,2′,4,4′-TETRABROMODIPHENYL ETHER (PBDE-47) AFFECTS HUMAN IMMUNE RESPONSE

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Polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants that are added to numerous products to prevent accidental fires. Even though there is little information on the health effects of PBDE exposure, it is still of concern to humans because some types of PBDEs can build up in the fatty tissues of the several aquatic and terrestrial animals entering the food chain (EFSA Panel, 2011). Recently, their presence has been correlated to several pathologies but little is known about their effect on the human innate immune system activity. We investigated the effect of the congener 2,2′,4,4′-Tetrabromodiphenyl ether (PBDE-47) on the functional activity of the THP-1 human macrophages cell line and on ex vivo freshly isolated human basophils. Cytotoxicity and genotoxicity studies showed that PBDE-47 was able to induce toxic effects on the THP-1 viability at concentrations ≥25 μM. Immune function of THP-1 was studied after stimulation with bacterial lipopolysaccharide (LPS) and PBDE-47 exposure at concentrations granting macrophage viability. Two dimensional electrophoresis, Real Time PCR and ELISA demonstrated reduction in the expression of IL-1β, IL-6 and TNF-α cytokines. Furthermore, PBDE-47 perturbates genes involved in cell motility (CDH-1 and MMP-12) and microRNA expression. Finally, basophil activation assay showed reduced CD63 activation in PBDE-47 treated samples. In conclusion, our study demonstrated that PBDE-47 may perturb the activities of cells involved in innate immunity dampening the expression of macrophage pro-inflammatory cytokines and genes involved in cell motility and interfering with basophil activation suggesting that this compound can impair innate immune response.

USE OF *IN VITRO* SYSTEMS AND MOLECULAR MARKERS FOR THE EVALUATION OF THE EFFECTS INDUCED BY FLAME RETARDANTS (PBDE-47) ON ADIPOGENIC DIFFERENTIATION

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In recent decades there has been an increase in the incidence of obesity and all related diseases (diabetes II, cardiovascular disease, cancer) due to excessive fat deposition. Recent studies suggest that exposure to endocrine-disrupting compounds (EDCs) may play a role in the development of obesity. Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants that were extensively used in commercial products they are ubiquitous environmental contaminants that are both lipophilic and bioaccumulative. Considering their lipophilicity, PBDEs tend to bioaccumulate in the adipose tissue and interfere in particular, with the various stages of the adipogenic process by altering the cellular differentiation following the deregulation of physiological levels of some adipogenic markers. The aim of this study was to evaluate, *in vitro,* some of the main molecular markers involved in the various stages of adipogenic differentiation in order to identify any changes in the normal differentiation processes induced by PBDE-47. Effects of PBDEs on adipogenesis were studied in the 3T3-L1 preadipocyte cell model in the presence and absence of standard adipogenic inductors. The results showed that PBDE 47 is able to promote the differentiation of preadipocytes into mature adipocytes by showing that these pollutants are able to influence some key functions of adipose tissue in a pathological way and therefore represent a new risk factor for the development of obesity.

Session: Marine biotechnology and blue growth

Moderator: Concetta Messina

MACROALGAE AS BIOINDICATORS OF THE ENVIRONMENTAL STATUS IN MARINE COASTAL AREAS

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Bioindicators are living organisms that are useful to assess the health of the environment, and are also important tools for detecting environmental change. Among a vast array of bioindicators, macroalgae are very sensitive tools for prediction and evaluation of the environmental status of aquatic systems as they rapidly reach the equilibrium with their natural surroundings, and integrate ecological conditions over time. Community shift is a common responses to anthropic stress, hence macroalgae are frequently employed in costal monitoring programmes. Several macroalgae taxa have received also much attention in both field and manipulative studies, as well as in laboratory essays, for the great usefulness in the assessment of eutrophication and environmental contamination due to the high uptake and accumulation rates of nutrients and contaminants from the surrounding water. Moreover, the high potential of macroalgae biomass as a new feedstock for production of feeds, chemicals and biofuels opens new perspectives in the bio-based economy. Recently, macroalgae have been used to detect also the tourism impact on coastal areas in three Mediterranean islands, within the BLUEISLANDS project (2014-2020 INTERREG MED EU program). The occurrence and extent of plumes of nutrients and trace elements of anthropogenic origin, and their temporal and spatial variability, has been assessed through the nitrogen stable isotope composition ($\delta^{15}N$) and trace element concentration of brown macroalgae short-term deployed in tourist and reference sites. This approach represented an efficient early-warning system for seawater quality monitoring, helpful for environmental managers.

POSTERS

Session: Environment and health

DETERMINATION OF ABUSED SUBSTANCES IN BIOLOGICAL MATRICES FOR TOXICOLOGICAL AND FORENSIC PURPOSES

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During the year 2017, a research work was carried out at the Department of laboratory diagnostics - Toxicology and Biochemistry U.O.S. of ASP 6 in Palermo. The purpose of this investigation was to perform a second level analysis in order to look for abuse drugs. The sample used consisted of 73 serum tubes, which had previously tested positive for screening tests. The result of the analysis of these samples revealed a false positivity in most cases. From the information gathered through the sampling reports, it appears that the age group with several drivers, who are subject to checks by the traffic police, is the one from 17 to 30 years, with 35 samples analysed (48%). It also emerged that 92% are male and only 8% female. The percentages of substances detected are THC + Cocaine 8%, Cocaine 15% and THC 77%. Thanks to the processing of collected data, a statistic about the consumption of illicit drugs in the city of Palermo has been produced. Through the findings, it was possible to make a comparison with the incidence of abuse of these substances in Italy. Moreover, it was found that Cannabis is the illicit substance mainly used in the Italian territory. Due to the growing increase in drug use on our territory, it is necessary that the methods used for confirmatory analysis be accurate and precise, in order to provide reliable results on the presence of substances of abuse in biological matrices. In fact more and more sophisticated methods and analytical tools are being developed.

CAN PBDES AFFECT PATHOPHYSIOLOGY COMPLEX IN AIRWAY EPITHELIUM?

Giusy Daniela Albano[1*], Giulia Anzalone[1], Monica Moscato[1], Rosi Gagliardo[1], Marina Montalbano[1], Anna Bonanno[1], Daniela Giacomazza[2], Fabio Cibella[1], Mirella Profita[1]

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Exposure to polybrominated diphenyl ethers (PBDEs) can promote multiple adverse health outcomes such as cellular and molecular mechanisms involved in inflammation, mucus secretion, pH variations, and damaging the lung tissues. We investigated the effects of PBDEs (47, 99 and 209) on inflammatory and oxidative response (IL-8 and NOX-4), mucus production (MUC5AC and MUC5B), Trans-Epithelial Electrical Resistance (TEER), and pH variations, using an air-liquid-interface (ALI) airway tissue model derived from A549 cell line and from primary human bronchial epithelial cells (pHBEC). The effect of N-acetylcysteine (NAC) was tested. ALI cultures were exposed to PBDEs (47, 99 and 209) (0,01 to 1 µg/ml) for 24 hours with and without NAC (10mM) (30 minutes before the stimulation). We tested production of IL-8, Muc5AC and Muc5B (mRNAs and proteins) as well as NOX-4 expression (mRNA). TJ integrity was measured by TEER and pH measurements and rheological properties (elastic, G', and viscous, G'', moduli) in apical washes. Exposure of A549 cell line and pHBEC to PBDEs (47, 99 and 209) decreased TEER measurements, increased IL-8, Muc5AC, Muc5B (mRNAs and proteins), NOX-4 mRNA, and the measurements of pH and rheological parameters (G', G'') in comparison to untreated cells. The treatment with NAC inhibited the effects PBDEs-induced in epithelial cells. PBDEs likely induced loss of barriers integrity through activation of inflammatory responses and uncontrolling physicochemical and biological properties in airway epithelium affecting the lung health. N-acetylcysteine may be able to restore the effects of PBDEs.

Session: Marine biotechnology and blue growth

MULTIDISCIPLINARY ASSESSMENT OF PRODUCT QUALITY FOR THE IDENTIFICATION OF CRITICAL POINTS IN THE VALUE CHAIN OF SMOKED FISH PRODUCTS: A CASE STUDY ON SWORDFISH

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During the production process of smoked fillets of *Xiphias gladius*, lot of samples showed significant different sensorial properties even if treated in the same manner than others. In order to verify the origin of the problem and which parameters of quality resulted altered, a multidisciplinary approach to quality determination, including instrumental, biochemical and *shelf life* biomarkers, was adopted. The sensory analysis was conducted according to a specific scheme for smoked fish products, adapted to the species; TVBN was used to evaluate marker of degradation of the nitrogen component, while PV and MDA were used to estimate the markers of degradation related to the lipid component; the technological parameters measured were color and texture. The sensory, color and texture analyses revealed a significant difference in the quality of the analyzed samples. These results were confirmed by biochemical analyses, which further highlighted differences in the rate of sample deterioration, showing that the most spoiled lot presented a higher level of peroxidation respect the good quality lots. Since the success of processing fish, such as smoking, as a strategy to extend the shelf life of the product, is linked to the quality of the raw materials, and as all samples were treated in the same way in the industry (hygienic conditions and step of processing) we can confirm that the lots were different in quality from the origin and that the differences between the final products were not related to the processing in the factory.

ISOLATION AND CHARACTERIZATION OF HALOPHILES MICROORGANISMS FROM SOLAR SALTERNS OF TRAPANI, SICILY

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Microalgae are photosynthetic microorganisms including prokaryotes and eukaryotes that can be found in both aquatic (*i.e.* fresh and seawater) and terrestrial (*i.e.* rock, volcano, etc.) environment. They can be found also in extreme natural conditions (*e.g.* high salt concentration, high temperature, high pH, etc.) that allow the growth of only few organisms who are called extremophiles. Amongst this group, halophiles microorganisms are able to grow in high concentration of salt such as salt lakes, Dead Sea or salt evaporation ponds. These organisms are very interesting not only for ecological study but also for their several biotechnological applications. In the present work, we investigated the industrial potential of halophiles microorganisms, including microalgae and bacteria, that live in several solar salterns present at the Natural Reserve "Saline di Trapani e Paceco" in Sicily. In particular, after the isolation and the molecular characterization of these strains we tested the cytotoxic effect of their extracts on a small panel of human cell lines, and cell repair activity on the human epithelial cell line BEAS 2B. Cell extracts did not show significant cytotoxicity neither on normal nor cancer mammalian cells tested. However, almost all tested samples showed a moderate repair activity proving a potential use of these strains in cosmetics.

DEFINITION OF INNOVATIVE TECHNOLOGIES FOR MONITORING IMPACT AND BIOREMEDIATION INTERVENTIONS ON MARINE AQUACULTURE

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Aquaculture is the fastest growing food production sector in the world. Recently, the development policies of the sector have paid attention to the effects that aquaculture can have on the environment. The marine aquaculture inputs organic and inorganic compounds into the water column and sediments, that can have negative effects on the ecosystem. The aim of the current study is to define and transfer to fish farms, effective and low-cost tools which allow to monitor the marine aquaculture effect on environment and performance as well as to act with bioremediation interventions to mitigate these effects. Different techniques have been validated for the: electrochemical detection of total free sulphide (organic enrichment practical indicator); determination of the fatty acids profile of sediments, which has been shown to be useful for distinguishing organic matter coming from aquaculture, marine environment or from other anthropic sources of impact. In addition, the current study will provide for the validation of techniques for the characterization of sediments with NIR (to determine with a single reading different quality parameters); determination of chlorophyll-a with Remote Sensing techniques (to detect eutrophic trends); mitigation interventions with bioactivators (to limit eutrophication and increase O_2 availability). It is therefore important to provide, during fish farming, an adequate monitoring program for each marine cage farm, because based on its characteristics, it has a specific effect on the environment, with the aim of preventing, in a timely manner, the establishment of unfavorable conditions.

OPTIMIZATION OF CULTURE CONDITION OF DIFFERENT MICROALGAE STRAINS TO IMPROVE LIPIDS AND ANTIOXIDANT PRODUCTION: PRELIMINARY RESULTS

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Microalgae produce many interesting bioactive compounds such as polyunsaturated fatty acids (PUFA), sterols, pigments, vitamins, carotenoids, phenolic compounds. This research focused on the study of some bioactive compounds of the marine microalgae *Nannochloropsis sp., Isochrysis galbana* and *Phaeodactylum tricornutum*, in order to improve the knowledge for the optimization of culture condition and utilization of this algae in the pharmaceutical, nutraceutical, cosmetic, energy and aquaculture sectors. Total lipid content and fatty acid profile in all the species considered were evaluated. The content of total polyphenols (Folin-Ciocalteu reagent assay), total carotenoids (spectrophotometric method) and the antioxidant activity (by DPPH radical scavenging and iron-reducing power assay) of ethanolic algal extracts were also determined. The results showed that *Nannochloropsis sp.* has the highest total lipid content. *P. tricornutum* maintained under standard conditions has a higher polyphenols and carotenoids content with greater antioxidant activity than the other microalgae species considered. Nitrogen starvation condition stimulated lipid metabolism, as showed by significantly higher amount of total lipids, maintaining high levels of omega-3 PUFA and modified the quantity and quality of the antioxidant patterns in terms of contents of polyphenols and total carotenoids.

VALORIZATION OF ENDEMIC HALOPHYLES PLANTS BY THE CHARACTERIZATION OF THE ANTIOXIDANT POWER AND ITS POSSIBLE APPLICATIONS

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Antioxidants are known to have a great number of nutritional benefits, such as anti-inflammatory, anti-microbial, anti-cancer properties. The aim of this work was to determine how to obtain the highest quantity of effective antioxidants from endemic halophyles plant species: *Mesembryanthemum nodiflorum*, *Limoniastrum monopetalum* and four species of *Calendula* collected in Sicily *(C. maritima, C. suffruticosa subsp. fulgida, C. arvensis* and the hybrid of *C. maritima and C. fulgida)*. Five extracts for each species were obtained using solvents with increasing polarity (hexane, ethanol, acetone and water) and through extraction of supercritical fluids (SFE). Folin-Ciocalteu reagent assay was carried out to determine the total phenolic content and DPPH assay and an iron-reducing power assay, in order to test the free radical scavenging activity and reducing power of each extraction respectively. It was found that 80% (v/v) ethanol extracted the highest yield of phenolics, and also obtained the highest free radical scavenging activity and reducing power of all solvents tested. However, SFE extracts showed high antioxidant activity comparable to the ethanol extract. Extraction with SFE ensured a great selectivity by avoiding the use of toxic organic solvents, and thus consist of a promising technique for sustainable production of these extracts giving them added value.

AMPHIBIAN EMBRYOS AS ALTERNATIVE MODEL TO STUDY THE PHARMACEUTICAL'S TOXICITY

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Pharmaceuticals are becoming potentially ubiquitous pollutants because of their extensive use by man. One of the most frequent groups of pharmaceuticals that have been identified as being of particular concern is the non-steroidal anti-inflammatory drugs (NSAIDs) and chemotherapeutic drugs. In Albania, studies to determine the risk of pharmaceutical related with their occurrence in water bodies and their adverse effects on living organisms, including human, are scarce. The purpose of this study was to elucidate the possible toxic effects of ibuprofen and cyclophosphamide on cellular physiology of frog tadpoles. For this purpose, individuals of *Pelophylax shqipericus* belonging to stage 21 Gosner, were exposed to sub-lethal concentration (5 µg/L) of ibuprofen (IBU) and cyclophosphamide (CP) for 48 hours. Erythrocyte abnormalities and micronucleated cell frequency were evaluated as endpoints in blood smears prepared by Giemmsa-Romanowsky stain method. Parametric analysis of variance (ANOVA) or the nonparametric analysis (Kruskal Wallis test) based on the data distribution (normality and homogeneity of variance) were used. Differences were analyzed by the post-hoc Dunn's test. Simple linear correlation (Pearson test) conducted with the mean values, was used to establish significant relationships between biological responses. Blood smears from tadpoles exposed to CP for 48 hours showed a pronounced decrease in the number of RBCs and an increase in the percentage of the micro nucleated erythrocytes through chromatin fragmentation, while, cellular and nuclear vacuolisation, collapse and rupture of the cell membrane were abnormalities observed due to ibuprofen toxicity. Understanding the biological effects of these drugs on frog tadpoles, can help in using these animals as reliable bioindicator organisms in monitoring aquatic environments health.

INFLAMMATION AND TENTACLE REGENERATION RESPONSES IN *ANEMONIA VIRIDIS* (ANTHOZOA, CNIDARIA)

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The mechanisms for discriminating the "self" from "non-self" have evolved into a long history of cellular and molecular strategies, from damage repair to the co-evolution of host-pathogen interactions. Not all immune responses are due to the presence of genetically foreign entities, but to the emission

of danger/alarm signals from injured cells, such as those exposed to pathogens, toxins and mechanical damage. In this sense, the cnidarian capacity for regeneration could be considered an additional arm of innate immune defense. In this study, the immune responses and tissue regeneration in the temperate symbiotic sea anemone, *Anemonia viridis*, induced by cutting tentacles or foreign injection were investigated. Morphological observations of the inflammation phenomenon ando tentacles in state of regrowth, measurements of the expression of Proliferating Cell Nuclear Antigen (PCNA) as a regeneration marker, immune function and enzymatic activity detection were carried out. Starting from previous knowledge on the natural seasonal variability in biometric traits and enzymatic biomarkers of *A. viridis*, the activity of enzymes involved in inflammatory response such as protease, peroxidase and esterase were analyzed. We finally describe comparative evaluations and perspective of difference and the point of contact between regeneration and inflammation.

Session: Marine and aquatic environment

BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS AS TOOL TO ASSESS THE HEALTH STATUS OF TURTLES AFTER STRANDING

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Current evidence shows that the anthropological impact on aquatic organisms has many negative effects. This is evident with some species of sea turtles like *Caretta caretta*, reputed to be a vulnerable species according to the IUCN Red list of threatened Species 2017. The turtle *Caretta caretta* is the most abundant turtle that reproduces in the Mediterranean. The danger for these organisms is linked to the anthropization of the coasts, to nautical traffic, to the pollution of the sea, overall due to the presence of plastics and to the threats related to fishing. After the ingestion of materials suspended in the waters or wounds caused by these, the turtles are forced to run aground. With the aim of monitoring the species subject to these risks, the Experimental Zooprophylactic Institute of Sicily (Italy) has always studied the phenomenon of beaching for marine animals, an event that in some periods of the year manifests itself in a clear way, probably due to the privileged geographical position of the island. Value the state of health of these animals by biochemical and hematological parameters after stranding, is important to understand the survival of the individual or not and to highlight how the anthropic pressure continues to alter the typical ecosystems of the Mediterranean. The results obtained show that the hematological and biochemical parameters can be a good tool to evaluate the possibility of survival of turtles that are in serious health conditions.

EFFECTS OF ACUTE EXPOSURE OF *MYTILUS GALLOPROVINCIALIS* TO TWO NEONICOTINOID SUBSTANCES: THIACLOPRID AND CALYPSO

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Thiacloprid is a neonicotinoid widely used in agriculture, it has a high solubility in water and this poses a potential risk for aquatic environments. In fact, it can be found in surface waters following rainy events in particular. Thiacloprid is the basic component of Calypso, a widely insecticide that is very effective against a wide spectrum of pests. This compound can also be detected in surface waters. The effects of these two compounds were analysed in the laboratory on an aquatic filter-feeder species, namely

the mussel *Mytilus galloprovincialis*. The main objective of the study was to assess the acute effects of Thiacloprid and Calypso on *M. galloprovincialis*. Mussels were expose for 7 days to three different concentrations of these products (Thiacloprid: 1 mg/L; 5 mg/L; 10 mg/L; Calypso: 10 mg/l; 50 mg/L; 100 mg/L). The following biomarkers were then measured: cell vitality in haemocytes and digestive cells; antioxidant parameters, superoxide dismutase and catalase, in mussel digestive gland. Both the pure neonicotinoid and the Calypso showed the same mode of action. In fact, after acute exposure to the high concentrations, both cell lines maintained viability. As for antioxidant enzymes, activity of SOD and CAT showed significant (p <0.05) changes during the exposures to both substances, compared to controls. The results showed that these neonicotinoids, which alter physiological processes in evolutionarily more complex organisms, do not create obvious damage after acute exposure to *M. galloprovincialis*. In any case, results obtained do not exclude the possibility of more relevant damage following chronic exposure to pesticides.

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SYNERGIC EFFECT OF TOXICANT MIXTURE (CARBAMAZEPINE, CADMIUM CHLORIDE AND POLYBROMINATED DIPHENYL ETHER) IN MARINE FISH CELL LINE SAF-1

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It is a fact that human and their environments are exposed to a wide range of contaminants. Several compounds have been found in sea water overworld, like industry sub-products (as heavy metals, halogens…), plastics (microplastics, flame retardants…), or diverse pharmaceutic metabolites (paracetamol, atenolol, carbamazepine…). Although these elements are found at concentrations might be considered as sub-lethal, there is increasing concern about the potential adverse effects of the interactions between those substances when present simultaneously in a mixture. Under certain conditions, chemicals will act jointly in a way that the overall level of toxicity could be affected. To shed light into this issue, the cell line from fibroblast of *Sparus aurata* SAF-1 was exposed to increasing concentrations of carbamazepine (CBZ), polybrominated diphenyl ether 47 (BDE-47) and cadmium chloride ($CdCl_2$), until 72 h to evaluate the cytotoxicity and the expression of relevant genes (antioxidant defence, cell cycle and energetic balance) by real-time PCR. In general, both vitality and gene expression were affected by the exposure to the different toxicants, affecting the antioxidant defence and producing cell cycle disruption, showing the higher effects in the cells exposed to the toxicant mixture of three compounds. The continue exposure to the different mixture of contaminants severally increase the negative effects that produce the same compounds separately, increasing its toxicity and increase the impact of contaminants on health from marine organisms.

PLASTIC DEBRIS INGESTION BY DERMERSAL ELASMOBRANCH SPECIES FROM TYRRHENIAN SEA: *GALEUS MELASTOMUS, SCYLIORHINUS CANICULA* AND *RAJA MIRALETUS*

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In recent years, based on the plastic feature, many techniques have been optimised to the mass production for a large scale of different plastic materials. Despite the internationally laws regulate the plastic debris entering in the marine environment, up to 10% of plastic produced reaches the oceans where increase in concentrations and spread beyond all limits. In this study, three elasmobranch species have been considered: *Galeus melastomus, Scyliorhinus canicula* and *Raja miraletus*. The specimens were collected during May/June 2017 in the southernmost part of the Tyrrhenian Sea. A total of 88 specimens were sampled and the GastroIntestinal Tracts (GIT) were analysed using visual sorting under fume hood to prevent atmospheric contamination. The study confirmed the presence of plastic microfibers, macrofibers and macrofragment in all the three demersal elasmobranch species. Plastic particles extracted from GITs were black, white and red. In all, 21 plastic particles were found as follow: 6 in *G. melastomus*, 13 in *S. canicula* and 2 in *R. miraletus*. Thanks to Attenuated Total Reflectance Fourier Transform Infrared (ATR–FTIR) and micro-Raman spectroscopies, the composition analysis of plastic particles were performed. Results showed the presence of PA and PE (50% for both) in *G. melastomus*, PP (71.4 %), PTFE and CA (both 14.20%) for *S. canicula* and only kraton G in *R. miraletus*. Results reflected both primary and secondary uptake of litter in accord to the feeding habits of elasmobranchs studied.

PLASTICS INGESTION EVIDENCES IN TWO COMMERCIALLY IMPORTANT SPECIES FROM CENTRAL MEDITERRANEAN SEA

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Plastics and microplastics (MPs) pollution is an emerging

threat to marine environment. Microplastics were reported in several marine environments, from the surface to bottom. Moreover, is well known their occurrence in the gastrointestinal tract (GIT) of several fish species from oceans and closed and semi-closed basins. In order to explore the microplastics contamination of our seas and in particular of the central Mediterranean Sea, the present study focuses on two demersal fish species: *Zeus faber (John Dory) and Lepidopus caudatus* (silver scabbardfish) from a fishery exclusion area (Gulf of Patti, Messina, Sicily, Italy). Visual sorting, using a dissecting microscope of the gastrointestinal tract of 67 specimens (35 *Z. faber* and 32 *L. caudatus*), was performed under controlled conditions, to avoid atmospheric contamination. The characterization of extracted microplastics was performed using a Raman spectroscopy. The results showed that the 51.4% of *Z. faber* and 78% of *L. caudatus* specimens were positive to plastic particles, with 2.1 and 4.8 item/specimen respectively. Of these particles, mostly were represented by microplastic (98.4% in *Z. faber* and 94% in *L. caudatus*) and mesoplastics for the remaining percentages. Both fragments and fibers of various colors showed an overall composition of polypropylene (PP), polyamide (PA), nylon and, to a lesser extent, polyethylene (PE). From our results it is conceivable that also in Gulf of Patti plastics ingestion is a serious threat to marine species. Further studies are necessary to deepen the knowledge about microplastic intestinal uptake to comprehend the real risks for the final consumer.

MICROFIBERS OCCURRENCE IN THE MEDITERRANEAN SEA: EVIDENCE OF INGESTION BY DEMERSAL-SEMIPELAGIC (*BOOPS BOOPS*) FISH SPECIES

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Microfibers pollution has increased severely in almost every marine environment around the world, becoming a serious threat to marine habitats and biota. This study reported the presence of artificial cellulose fibers in the gastrointestinal tracts (GIT) of a commercially relevant demersal-semipelagic fish species (*Boops boops*) in the Mediterranean Sea. The samples were collected during an experimental trawl survey in the Fishery Exclusion Zone of the Gulf of Patti (38.19S–14.94W; 38.315S–15.06W; 38.17S–15.16W; 38.24S–15.21W), Messina Italy, on June 2017. Overall, 30 specimens of *B. boops* were examined. Results highlighted the ingestion of cellulose fibers in 63,3% of the total investigated bogues. Ingested fibers were detected at first using stereomicroscope and Scanning Electron Microscope (SEM), categorized according to size class, color and subsequently characterized using Raman spectroscopy technique. The study showed the presence of different colors and lengths of cellulose fibers with a maximum length of 30 mm and a minimum of 0,5 mm. All analyzed samples appeared black and red, 76 and 4 respectively. The study highlighted only the presence of cellulose fibers into the gastrointestinal tract of the bogues specimens and not plastic.

PRESENCE OF MICROPLASTICS IN THE GASTROINTESTINAL TRACT OF TWO SEABREAMS SPECIES (*PAGELLUS ERYTHRINUS* AND *PAGELLUS BOGARAVEO*)

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Microplastic pollution is probably one of the most discussed topics worldwide over the last few years. Due to their very small size (<5 mm), microplastics (MPs) can be ingested by a wide range of marine organisms, from zooplankton to large pelagic and benthic fishes. Once entered the trophic chain, MPs can be transferred along it through biomagnification processes not yet fully understood. Recent studies have shown how the ingestion of MPs can cause physiological injuries and general loss of welfare in aquatic organisms. However, not much is known about the actual damages caused by microplastics. The aim of this study was to investigate the presence of MPs in the gastrointestinal tract (GIT) of two high-values fish species in Mediterranean Sea, Pagellus erythrinus (Linnaeus, 1758) and Pagellus bogaraveo (Brünnich, 1768). A total of 39 specimens (15 P. erythrinus and 24 P. bogaraveo) were caught in the waters of Tyrrhenian Sea, between Rasocolmo Cape and Termini Imerese (Geographic coordinates: 38.350946S – 15.542023W; 38.306726S – 15.547955W; 38.229888S – 15.573224W; 38.0085189S – 14.589696W). During laboratory analysis, MPs were collected through visual sorting method, under controlled condition, to prevent airborne contamination. Microplastics composition were subsequently analysed using micro-Raman and FT-IR spectroscopies. The results showed that MPs were found in the GIT of 4 specimens; all microplastics found were black and fibrous. The most frequently observed polymer was Nylon 66 (Polyamide). The data collected in this study confirm that microplastics contamination is a serious and growing threat for marine ecosystems and their functioning.

EXTREMOTOLERANT BLACK YEASTS FROM THE DEPTHS OF THE MEDITERRANEAN SEA

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The black yeast-like fungus *Hortaea werneckii* was isolated for the first time in the Mediterranean Sea during two oceanographic cruises, from samples collected at different stations and depths (from surface to 3400 m) and resulted to

be the dominant fungal species (De Leo *et al.*, 2019). This ubiquitous fungus is known for its high halotolerance and for being responsible of the superficial mycoses "tinea nigra". The comparison of twenty-five strains of *Hortaea werneckii* isolated from seawater with a panel of strains isolated from different sources (environmental and clinical) and from different countries, using multilocus sequencing and AFLP analyses, showed that they were highly similar; in fact they consistently deviated from remaining strains not only molecularly but they also responded differently to combined temperature and salt stress (Marchetta *et al.*, 2018). The molecular differences might explain the separate cluster formed by these seawater-adapted strains as due to different ecological pressure than clinical and terrestrial ones.

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