present, simultaneously, in a mixture. In this study, specimens of sea bream were exposed, by food, to a mixture of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), cadmium chloride ($CdCl_2$) and carbamazepine (CBZ) for 15 days, and further maintained for 2 weeks without contaminants. In the liver, lipid composition, reactive oxygen species and oxidative-stress related genes, were determined. Both markers resulted affected after the exposure showing only a partial degree of recovery. In addition, the study of the observed relationships between the above patterns of biomarkers could be adopted to monitoring the health of aquatic organisms and aquatic ecosystem.

EFFECTS OF THE ENRICHED-ORGANIC DIETS COMPOSITION ON EUROPEAN SEA BASS (*Dicentrarchus labrax*) NEW TOOLS FOR FISH WELFARE EVALUATION IN AQUACULTURE

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In recent years, concern for the welfare of animals, including fish, has a strong impact on public opinion, putting pressure not only on producers and the scientific community, but also on institution to guarantee adequate "standards" for animal welfare. The market today challenges the production capacity of aquaculture by asking for products with consistently high quality. Unlike other farming methods, there is a lack of scientific information on the welfare of fish reared under intensive aquaculture conditions. Among the various aspects that characterize aquaculture, the increase in fish welfare has an impact on public opinion also for economic reasons whereby is very important to keep animals in good health, determine a higher feed conversion rate, lower mortality, optimal growth rate and high quality of the animal raised. Whereas stress events, acute and chronic, are reflected on the flesh, due to the increase in muscle activity (generally associated with this condition). We have firstly demonstrated that accelerometer tags are useful tools for welfare monitoring, indeed they do not affect welfare and health of implanted fish and that tagged fish can be sampled during experiments and be considered as representative of population, by displaying similar growth and physiological parameters compared to untagged fish. Consequently, the purpose of this study is to investigate the effect of two organic diets (contain organic vegetables and a natural antioxidant compound) on the welfare of the European sea bass compared to one conventional. A holistic approach was adopted, including the measurement of the primary stress response (cortisol), secondary (*i.e.* lactate, glucose, haematological parameters, lysozyme) and tertiary (*i.e.* swimming performance, muscle activity and growth parameters) indicators. In parallel, we evaluated the enzymatic activities of 7-ethoxyresoruphine-O-deethylase and glutathione-S-transferase, as index of function of the microsomal hepatic system of mixed function oxygenase (MFO), to evaluate the possible effects of pollutant contamination through the diet. In this study, multiparameter analysis approaches were performed to bring a better understanding of the effectiveness of a holistic approach to quantify welfare in organic aquaculture. The multi-parameter approach has outlined a complete picture of the physiological state of the sea bass. The Principal Component Analysis and the Multi-Criteria-Decision-Analysis have proved to be useful tools for an integrated assessment of the well-being of the fish, highlighting that the best condition was achieved in the experimental group fed with the organic protein-rich diet.

CHEMICAL COMPOSITION DETERMINATION OF MARINE SEDIMENT BY NEAR INFRARED SPECTROSCOPY TO MONITOR AQUACULTURE ENVIRONMENTAL EFFECT

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Aquaculture is playing an increasingly important role in compensating for fishery inability to meet fish product demand, accounting for 46% of global production and 52% for human consumption. However, aquaculture expansion activity determines increasing concerns about its detrimental effects on water, sediments and biodiversity. Marine sediments are composed by inorganic and organic matrix with complex physical, chemical and biological characteristics. Their alterations can record in space and time effects of ecological processes and human activities in the surrounding systems. Characterization of their composition variations is used to monitor the effects of fish farming on marine environment. Chemical analyses utilized to assess marine sediments quality can be very expensive and time-consuming. Near Infrared Spectroscopy (NIRS), a fast instrumental technique with a high accuracy could be a valid alternative to standard analytical techniques to evaluate physical and chemical composition of marine sediments, reducing the cost of routine analysis, to predict and follow eventual effects on in-faunal biodiversity. Based on spectroscopy in the near-infrared range, from 700nm to 2500nm, correlating by complex statistics absorbance by covalent bonds between H, C, O, N and S, to concentration of protein, lipid, carboxylic, amide aminoacids and other molecules or groups. After calibration equations between analytical data and spectral results, by a single scan numerous parameters can be predicted. Being sediment drying the only manipulation required. The aim of this study was to evaluate the potential of NIRS to monitor the effects of fish farming on marine environment, through the evaluation chemical composition of sediments collected beneath a bluefin tuna farm and a sea bream farm, located in the Castellammare Gulf (Trapani, Italy). 140 sediment samples were collected from sampling stations at different distances from the cages and from control stations, not influenced by farming. Total Organic Matter, Carbohydrates, Lipids, Protein, Total Sulphides contents were analysed by standard methods. Near-infrared absorbances (700nm-2500nm) were recorded by NIRFlex Solids (BUCHI, Milan Italy). NIRS spectra and analytical data were compared through scatter plots. The regression lines obtained showed a high dependence relationship between the two variables (predicted NIRS and analytical data), with coefficient $r^2 > 0.7$ ($p<0.05$) for each analysed parameter. Our results showed that NIRS is able to estimate several properties in marine sediments simultaneously in a short period of time and without chemical laboratory analyses.

A SCANNING ELECTRON MICROSCOPY PROTOCOL FOR THE STUDY OF MARINE MICROPLASTICS

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Microplastics (MPs) are widespread all over the world, representing the main class of debris present in the marine environment. The effects of microplastics on the marine ecosystem needs to be addressed in order to plan effective actions at global scale. The aim of this study was to obtain a specific Scanning electron microscopy (SEM) protocol, in order to deeply understand MPs structure and characteristics and acquire the best tools to deal with it. MPs may function as sites for the colonization of microorganisms and the subsequent formation of associated biofilms (the so-named Plastisphere). In fact, several microorganisms (bacteria, archaea, pico-eukaryotes) as natural biodegrades and recyclers, could also play a role in breaking down chemical compounds of plastics. SEM was used to examine the surface of floating microplastics collected in Tyrrhenian Sea and the procedure has been adapted to preserve the microplastic structure as well as the organic biofilm associated. For this reason, instead of the common procedures for SEM which involved washing in CO_2 or undertaken the plastic fragments at high values of pressure/temperature (80bar/40°), the samples were fixed in 70% alcohol for 48 hours. Samples were dehydrated in a graded series of alcohol, 1h in each solution from 70 to 100°. The drying critical point, in which the microbial biofilm integrity can be compromised, has been avoided and replaced with a procedure at temperature of 28°C for 12h, in order to preserve both microplastics and biofilm structure. The SEM photographs highlighted the presence of microorganisms on the Low-Density Polyethylene (LDPE) microplastics sample, previously characterized. Moreover, the collected data showed that the use of this protocol did not produce any kind of damage on both plastics and biofilm. The presence of *Diatomae* sp. was recorded and filamentous bacteria and some bacteria shapes, rods and cocci, and fungal hyphae were evident. The SEM represent an extremely effective tool when it comes to analyzing various pollutants, particularly microplastics. Making practical use of automated feature analysis can lead to the development of straightforward and accurate techniques to perform rapid analysis for the classification of microplastic particles in water samples.

NEW DATA ON THE DIET AND FEEDING HABITS OF THE BLACKMOUTH CATSHARK, *Galeus melastomus* RAFINESQUE, 1810 FROM THE SOUTHERN TYRRHENIAN SEA

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The knowledge of feeding habits is important to understand the trophic status of fish stocks. Furthermore, the availability of data like these is of particular relevance for the conservation and management of elasmobranchs' populations. Sharks are top predators in marine environments. However, despite they cover a key role for marine ecosystems, sharks and elasmobranchs in general are threatened by several human activities, such as fishing, pollution and habitat degradation. In the present study, we present data on the diet of the Blackmouth catshark (*Galeus melastomus*) from southern and central Tyrrhenian Sea. The stomach content of a total of 154 individuals collected in 2018 and 2019 was analysed. *Galeus melastomus* is a benthic feeder and scavenger whose diet considerably varies with seasons and areas. Hence, we can consider it as an opportunistic feeder. Specimens were collected in spring, summer and autumn by the MEDITS bottom-trawl survey and by commercial landings of the fishing fleets CAMPBIOL survey. Specimens were frozen on board to prevent digestion of the stomach content and immediately transferred in laboratory after landing. Each individual was subsequently measured (total length, TL) to the nearest cm, weighed (total weigh, TW) to the nearest g, and sexed. They showed a size range between 14.5 and 52 cm of total length (TL). Each prey item was identified to the lowest taxonomic level possible, counted and weighed (g). The 13% of the total examined stomachs was empty. The main prey items were: cephalopods, fishes and decapods. The percentage of occurrence of the different prey items varies in relation with the total length of specimens. Our results showed that small specimens of *G. melastomus* feed mainly on Euphausiacea and small fishes, mainly Myctophidae (*Electrona risso, Diaphus holti*). On the other hand, big specimens of *G. melastomus* feed on large-sized cephalopods (*Todadorades sagittatus, Eledone sp., Scaeurgus unicirrhus),* decapods (*Pasiphaea sivado, Pasiphaea multidentata, Robustosergia robusta*) and fishes (*Ceratoscolpelus maderensis, Macroramphosus scolopax).* Results of stomach analysis also showed the scavenger habits of this species, with the presence of fisheries discard and offal, like fish head, and sea bird remains. The feeding strategy of *G. melastomus* could be the result of an adaptation to the oligotrophic deep environment. In conclusion, we can consider this shark as a generalist opportunistic feeder, able to exploit the most common trophic resources of its environment.

NEW INSIGHTS INTO OTOLITHS ECOMORPHOLOGY OF TWO CONGENERIC SEABREAMS SPECIES, *Pagellus erythrinus* (LINNAEUS, 1758) AND *Pagellus bogaraveo* (BRÜNNICH, 1768)

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Over the last decades, scientific interest for fish otolith morphology and morphometry increased significatively. These

are useful tools to study many aspects of teleost life in marine environment. Several factors can affect shape and microstructure of otoliths, such as environmental parameters, feeding habits, ontogeny, physiology and phylogeny. Among different types of otoliths, sagittae is the largest and the most variable, with a high taxonomic value. In the present study, attention was addressed to two high-values fish species in Mediterranean Sea, *Pagellus erythrinus* (Linnaeus, 1758) and *Pagellus bogaraveo* (Brünnich, 1768). The common pandora (*P. erythrinus*) is a demersal species that lives mainly on rocky and muddy-sandy bottoms, with a high frequency of occurrence at a depth between 20 and 300m. It is a generalist predator and a benthic feeder, and it shows protogynous hermaphroditism. The blackspot seabream (*P. bogaraveo*) lives above all substrata, near offshore banks, on seamounts and in cold-water reefs. It is a benthopelagic predator and a protandrous hermaphrodite. The juvenile stage lives near the coast, whereas the adults live on the slope down the 800m. A total of 44 otoliths of *P. bogaraveo* and 71 otoliths of *P. erythrinus* were collected from trawled specimens at south Tyrrhenian sea (GSA 10) between March and October 2019 (belonging to CAMP.BIOL.19 project). Sagittae were removed from otic capsule, cleaned from tissue and stored dry inside Eppendorf microtubes. Morphometry and microstructure of all samples were analysed. Digital images of each otolith samples were collected by using a stereomicroscope with a built-in digital camera and subsequently binarized for contour detection by ImageJ 1.48p software. Statistical and shape analysis were performed using open-source software packages that run on the R platform. Moreover, a total of 16 otoliths (12 of *P. bogaraveo* and 4 of *P. erythrinus*) were used for SEM analysis, for the first time for these species. The results showed inter-specific differences between the two congeneric seabreams, certainly due to habitats and diet of these species. The *P. bogaraveo* specimens showed an elliptical otolith shape with crenate margin while *P. erythrinus* specimens showed a pentagonal shape of otoliths. An interesting bilateral asymmetry was detected between left and right sagittae of this latter species. All these features represent the prove of the eco-morphological of sagitta and sulcus acusticus in life and adaptation to the environment of fish.

CONTRIBUTION OF THE PROCESSING TECHNOLOGIES TO THE INCREASE OF ENVIRONMENTAL AND ECONOMIC SUSTAINABILITY OF THE FISHERY VALUE-CHAIN: EFFECTS OF AN OZONIZED SLURRY ICE SYSTEM ON SHELF LIFE OF *Sardina pilchardus AND Engraulis encrasicolus*

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Innovative technologies are necessary to support the fishery value-chain, in order to meet the increasing global demand for fish products, due to the greater awareness of the beneficial effects of seafood on the human health and the increasing global population. Innovation aimed to improve competitiveness, safety, environmental and economic sustainability of the sector must be driven by the market and sustainability needs, leading to adaptation of the products to consumer preferences, product diversification and reduction of losses between landing and consumption, still amounting to 27% of the fish landed. Indeed, fish products are extremely perishable and appropriate processing and preservation techniques must be undertaken. Among the new innovative techniques related to the cold chain, great attention could be devoted to slurry ice or liquid ice, in preventing fish spoilage and product loss. It shows several characteristics that make it a suitable technique for maintaining the product freshness over time, faster cooling rates, allowing temperatures between -0.5 and -1.5°C, unlike traditional flake ice by which the temperature can only drop to temperatures just above 0 °C. The use of ozone, as an antimicrobial agent, combined with slurry-ice has proven to be an equally successful technique in prolonging the shelf-life of several fishery species such as turbots (*Lepidorhombus whiffiagonis, Psetta maxima*) and the sciaenid *Collichthys niveatus* compared to flake ice or slurry-ice without ozonation. To investigate the effectiveness of a combined slurry-ice/ozone system (SIO) on prolonging the shelf life of two fishery species, *i.e.* sardines (*Sardina pilchardus)* and anchovies *(Engraulis encrasicolus*), a multidisciplinary approach was used. As regards both fish species, preliminary results showed that specimens stored under SIO were better compared to the specimens stored under the traditional flake ice (FI) in terms of sensory aspects (Quality Index Method; $p < 0.05$). Moreover, results of the main biochemical parameters related to the shelf life (total volatile basic nitrogen and malondialdehyde contents) suggested a longer shelf life for samples stored in SIO. In addition, pH values were statistically lower ($p < 0.05$) in the SIO specimens over the shelf life period considered, leading to the assumption of an inhibiting effect of the preservation system on the bacterial growth. Further analyses in progress will clarify the effectiveness of this technique in the shelf-life extension of these species, that are among the most important for the economy of our region.

SEA SALT: INNOVATIVE TECHNOLOGIES FOR TRACEABILITY AND SECURITY – INDUSTRIAL APPLICATIONS

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Sea salt is a renewable natural resource considered as a paradigm of sustainability. Sea salt production process, in fact, is based on the use of renewable resources, such as seawater, sun and wind, using simple technologies with a various degrees of mechanization for harvesting and subsequent industrial treatments. However, it is worth stressing that this process, defined by centuries of experience, does not require substantial technological innovations. The aim of the study is to contribute to the improvement of the image, and therefore of the added value of the product, through the possibility of certifying its geographical origin. From a methodological point of view, the innovation of product traceability consists in analyzing the microbial composition of salt crystal community, which has been found to be peculiar to each salt work. Halotolerant and halophilic microorganisms develop in saline waters. Archaea and Bacteria growing in brine, during crystallization process, can be entrapped in salt crystals, where they can survive for very long periods. By means of metagenomic analysis with 16S rRNA

gene extracted from Archaea and Bacteria recovered from salt crystals, it is possible to characterize the microbial communities of salt crystals and associate them to saltwork where the salt crystals come from. Keeping in mind this possibility, we analyzed the diversity of viable archea recovered from salt crystals originating from six Mediterranean Saltworks. Results of metagenomic analysis utilizing 16S rRNA gene showed that salt crystals from Mediterranean salt work considered are characterized by the presence of phylogenetically diverse populations of halophiles that are peculiar and methodologically identifiable. This results can be useful to thus trace the geographical origin of the salt.

FROM BY-PRODUCTS TO A FUNCTIONAL BIOMOLECULE: ASTAXANTHIN, A CAROTENOID WITH POTENTIAL IN HUMAN AND ANIMAL HEALTH AND NUTRITION

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One of the biggest challenges, nowadays, is the production of functional biomolecules through the valorisation of agro-industrial by-products or food waste. To date, these represent a growing concern not only from an economic point of view but also from an environmental and public health one. In this work the possibility to obtain added value natural compounds from industry by-products, through a simple aerobic cultivation of microorganisms, such as astaxanthin from *Xanthophyllomyces dendrorhous*, was evaluated. Astaxanthin is a natural pigment, with a strong antioxidant activity, that has been shown to be substantially greater than β-carotene and about a thousand times more effective than vitamin E. This carotenoid offers numerous health benefits to humans and animals and, consequently, has several uses. The most conspicuous sector of astaxanthin applications is its use in foods and feeds, mostly in marine aquaculture. The growth and the carotenoid biosynthesis of the yeast *X. dendrorhous* (ATCC 24202) were studied initially by a batch cultivation on a complex medium in 2L Erlenmeyer flasks with a working volume of 1L. The working conditions were: 22°C, agitation at 300 rpm, aeration at 4 L/min and pH 5, without further correction. The tests, carried out for a 10-day period, were conducted both in dark and illuminated conditions, performed by using a Phillips TLD 18 W/ 54 lamp. Then different food waste and agro-industrial by-products, such as: fruit and vegetable waste, collected from both large scale distribution and the agro-industrial sector, and pretreated whey were tested as cultivation media. These tests were conducted using the same conditions adopted in the first test. Extraction of astaxanthin was carried out using organic solvents together with ultrasound. Astaxanthin was determined by a UFLCXR liquid chromatograph combined with an LCMS-8040 (Shimadzu, Kyoto, Japan) equipped with a 100 x 2.1 mm, 1.7 µm C18 column. This study showed the possibility to produce astaxanthin from food waste and agro-industrial by-product as an alternative to expensive and non-eco-friendly synthetic products, thanks to microbial synthesis. Furthermore, the study confirms the important role of light in increasing the astaxanthin productivity from *X. dendrorhous*, even when agro-industrial by-products are used as growth media.

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METHYLMERCURY EFFECTS ON *Mytilus galloprovincialis* HAEMOCYTES ACTIVITY

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Bivalves, filter-feeding organisms, due to their wide distribution, are used by many countries in biomonitoring, particularly in the assessment of xenobiotics in the marine ecosystem. These sentinel organisms are able to accumulate trace metals and other substances in their tissues. Haemocytes are effectors of cellular immunity in bivalves and are capable of responding to stressors through activities such as phagocytosis or cytotoxicity. In order to identify cellular markers to investigate pollution, the effect of different concentrations of organic mercury (CH_3HgCl) on the morphology and responses of *Mytilus galloprovincialis* haemocytes was studied in this work. Sublethal concentrations of methylmercury, as evidenced by the Trypan blue exclusion test, were used to investigate its effect on morphology, the efficiency of phagocytosis towards yeast cells, the maintenance of the lysosomal membrane and the ability to release cytotoxic molecules. Alterations in haemocyte viability, morphological changes and alterations in the cytoskeleton were observed. The spreading ability, a cell morphometric parameter, was also used as an additional method. Exposure to CH_3HgCl influenced the percentage and index of phagocytosis. Finally, cytoskeletal and morphological modifications lead to a reduction in the ability to adhere to the substrate and incorporate the target. The cytotoxic activity of *M. galloprovincialis* haemocytes towards erythrocytes and the activity revealed from lysis plaque assay has not been modified by adequate concentrations of methylmercury in the medium. In addition, membrane permeability could be affected by methylmercury due to the reduced retention capacity of neutral red by the cells. This evidence confirms that the Mediterranean mussel *M. galloprovincialis* is a suitable model organism in the study of the state of health of the marine environment and in particular for investigation of pollution caused by xenobiotics.

EFFECTS OF MIXTURES OF EMERGING POLLUTANTS AND DRUGS ON MODULATION OF BIOMARKERS RELATED TO TOXICITY, OXIDATIVE STRESS, AND CANCER

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Over the years, there has been an increased interest in xenobiotic substances, including flame retardants, heavy