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Trapani, Italy, 27-28 October 2017

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Trapani, Italy, 27-28 October 2017

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FRIDAY 27 OCTOBER

ANTOLINI LECTURE (INVITED)

DISCOVERING BEE'S INTELLIGENCE: THE CONTRIBUTION OF TWO-PHOTON MICROSCOPY

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Fast two-photon microscopy not only allows to explore both the structure and functionality of the honeybee brain at different spatial resolutions, but enables to extend the functional analysis beyond static activity maps. Recording responses within the first neuronal network along the olfactory pathway, the antennal lobe, we investigate to what degree odour stimulus information is encoded in space, frequency and time features of the neuronal responses. In this way, it is possible to highlight the neural networks that are the basis of the amazing cognitive functions contained in a volume of no more than one cubic millimeter of brain and, perhaps, also contribute to counteract the very complex and partly unknown phenomenon of the Colony Collapse Disorder.

SESSION 1: ENVIRONMENTAL HEALTH AND ANIMAL WELFARE

MESSINA LECTURE (INVITED)

AQUACULTURE: FROM ANIMAL WELFARE TO QUALITY OF THE PRODUCTS

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The abolition of the trade barriers and the growing demand for fishery products, has inevitably led to a strong competition among the Mediterranean Countries. Sicilian productions, as well as the Italian one, are characterized by high quality standards of the value-chain. The standardization of the farming practices, together with the selection of adequate diets and the control of animal welfare, are able to guarantee a good health status in fish, that is related to the high quality of the products. It is therefore indispensable to distinguish our local production, characterized by high standard quality, from the farmed products, imported from others countries, that don't meet the same requirements, but compete in the common market. For this purpose, it is necessary to select some quality indicators of fish product, which give it distinctive, unique characteristics, useful for traceability actions. Polyunsaturated fatty acids of the omega-3 series meet these requirements. These components are more concentrated in fish products than other foods, because fish receive them from diet and accumulate it in tissues. The utilization of omega-3 as biomarkers and quality indicator has many advantages because it allows that the quality of fish product can be linked to the species and the production system. Over the past 15 years, our research team has monitored the quality of fish product farmed in Sicily, in comparison with products farmed in Italy and some Mediterranean Countries. Our studies have shown that fish farmed in extensive have peculiarities that make it similar to wild, rendering this production adequate to a niche market. On the other hand, fish farmed in intensive systems, have a constant and significant incidence of omega-3 throughout the year and a standard shelf life, which meets the consumer's expectations and are good requirements for certification actions, attesting the high standards of the Sicilian aquaculture value-chain.

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ORAL COMMUNICATIONS

CARCINUS AESTUARII AS AN INTERESTING BIOINDICATOR IN MONITORING ESTUARINE ECOSYSTEM POLLUTION. A MULTIBIOMARKER APPROACH

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An increasingly diverse array of toxic pollutants with uncertain ecological effects is being discharged into Albanian coastal waters, and detecting their impacts on the health condition of marine and estuarine biota is one of the most important and difficult challenges facing researchers and environmental managers. More than one third of the Adriatic coast in Albania is being eroded and accelerated by removing gravel and sand from beaches for construction industry, uncontrolled construction along the coast, deforestation of large coastal areas, and agricultural development. Increased migration to coastal areas has resulted in uncontrolled harvest of coastal and marine resources as well as in increasing pollution of marine and coastal waters, especially in lagoons. Most pollution comes from urban and industrial waste, sewage, and chemicals used in agriculture. Discharging the pharmaceutical waste into water is a relatively new phenomenon emerging nowadays, which of course affect biodiversity. Our approach was to establish a link among contaminants, molecular and cellular biomarkers, using crabs as resident sentinel species for assessing habitat condition and diagnosing stressors. In the present study, a suite of biomarkers like neutral red retention time (NRRT), glucose, total haemocyte count (THC), and oxidative enzyme activities (CAT, SOD), were evaluated in *Carcinus aestuarii* crabs collected from Lagoon of Narta, Vlora, Albania. Obtained results revealed a significant alteration of each specific biomarker due to presence of pollutants in water column and sediment. The integrated approach between biomarkers and chemical analysis of water and sediment, is a useful tool in biomonitoring estuarine environments and *C. aestuarii* can be considered a suitable bioindicator species.

PCB DEGRADATION POTENTIAL BY BACTERIA FROM LAKES OF THE EDMONDSON POINT AREA (ANTARCTICA)

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The use of polychlorinated biphenyls (PCBs) has been prohibited since the 1970s in western nations. However, their widespread use and chemical stability has led to extensive environmental contamination, even in remote areas. PCBs can be transformed into chemical substances by different microbial metabolic pathways. This work was aimed at analyzing Antarctic bacterial strains able to degrade PCBs at low temperature, with a focus on the metabolic pathways. Water and sediment samples were collected in four different lakes (EP-1, EP-2, EP-3 and EP-4) in the Edmonson Point area (Antarctica). Isolates were obtained from biphenyl enriched cultures, and preliminarily screened for their ability to use Aroclor 1242 as sole carbon source. Positive strains were identified by the 16S rRNA amplification and sequencing, and the presence of the *bphA* gene, involved in the first step of aerobic degradation of PCBs, was screened by PCR amplification. Results showed that 57 out of 192 isolates were able to grow in the presence of Aroclor 1242, with the 25.5% and 18.6% that were from sediment and water, respectively. Further, the 87.5% of the positive strains sowed the presence of the *bphA* gene and were mainly affiliated to *Actinobacteria* (mainly genera *Salinibacterium* and *Arthrobacter*) and *Betaproteobacteria* (mainly genus *Alcaligenes*). Obtained results showed a percentage of PCB-oxidizing bacteria higher than those previously reported in Antarctic environments. This suggests that the microbial communities of Antarctic lakes and ponds could be adapted to the presence of these pollutants.

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EXTRACELLULAR POLYMERIC SUBSTANCES WITH METAL ADSORPTION CAPACITY PRODUCED BY *PSEUDOALTEROMONAS* SP. MER144 FROM ANTARCTIC SEAWATER

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The EPS-producing *Pseudoalteromonas* sp. MER144 was selected among 606 isolates from Antarctic seawater for the highly mucous appearance of its colonies on agar plates. The production of EPSs was enhanced by a step-by step approach varying the carbon source, substrate and NaCl concentrations, temperature and pH. Optimal conditions for the EPS production resulted at 4°C and pH 7 in the presence of sucrose (2%, w/v) and NaCl (3%, w/v). EPSs produced under optimal conditions were chemically characterized, resulting in a moderate carbohydrate content (35%), uronic acids (14%) and proteins (12%). Monosaccharide composition was estimated to be Glu:Man:GluN:Ara:GluA:GalA:Gal (1:0.36:0.26:0.06:0.06:0.05:0.03), while the estimated molecular weight was about 250 kDa. The addition of sucrose in the culture medium, by stimulating the EPS production, allowed MER144 to tolerate higher concentrations of mercury and cadmium. This finding was probably dependent

on the presence of uronic acids and sulfate groups, which can act as ligands for cations, in the extracted EPSs. Monitoring EPS production under optimal conditions at different concentrations of mercury and cadmium revealed that EPS amounts increased at increasing heavy metal concentrations, indicating an adaptation to the stress conditions tested.

PERFLUOROOCTANE SULFONATE AND POLYETYLENE MICROPARTICLES ADMINISTERED IN DIET AFFECT THE IMMUNE SYSTEM AND STRESS MARKERS OF EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX* L.)

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Polyethylene microparticles (PE-MPs) are the most commonly found MPs on marine environment and could increase the exposure of animals to chemicals associated with them. Perfluorooctanesulfonic acid (PFOs) bioaccumulates in the environment. The effects of PE-MPs, PFOs and PFOs associated with PE-MPs on the expression of some immune-relevant genes from European sea bass were analysed. Fish were fed diets containing: 0 (control), virgin PE-MPs (MP), PFOS (PFOs) or PFOS adsorbed to PE-MPs (MPs-PFOs) for 7 or/and 21 days. Samples of skin mucus, head kidney leucocytes (HKLs), liver and gut were obtained. Cellular immunity (phagocytic activity, respiratory burst and peroxidase activity) was analysed on HKLs and found to be affected by MPs and MPs-PFOs diet at 7 and 21 days. Peroxidase activity and IgM was analysed on skin mucus and serum. Fish fed the PFOs diet for 21 days showed increased the peroxidase activity in skin mucus whilst serum IgM levels were not affected. The expression of nine relevant genes was analysed by qPCR in liver and gut. 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 were affected by all the diets at 7 or 21 days. PFOs attached to MPs seem to exacerbate the negative effects produced on the inflammation and oxidative status, suggesting a synergic effect.

Acknowledgements: Financial support by grants PCIN-2015-187-C03-02 MINECO, JPIOceans: Microplastics, EPHEMARE) and 19883/GERM/I5 (Fundación Séneca de la Región de Murcia, Spain) is gratefully acknowledged.

PCB-DEGRADING BACTERIA FROM THE ARCTIC KONGSFJORDEN (SVALBARD ISLANDS)

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Polychlorinated biphenyls (PCBs) were produced between 1929 and late 1970s and used in several industrial applications. They show bioaccumulation and biomagnification behaviours, so they pose a serious risk to the environment. Eight strains (*i.e.*. *Pseudomonas* sp. CIW-9, *Devosia* sp. DS-1, *Algoriphagus* sp. EW-10A, *Bizionia* sp. BS-8B, *Microbacterium* sp. C3W-24A, *Salinibacterium* spp. C3W-3 and ES-1, and *Leifsonia* sp. C1S-22B) isolated from the Kongsfjorden, and previously tested for the presence of the *bphA* gene, were selected to test their degradation efficiency in presence of Aroclor 1242 at 15 and 4°C. The composition and concentration of PCBs were determined by high resolution gas chromatography-mass spectrometry (GC-MS). Results showed that isolates were able to reduce between 3.5-60.1% of the PCB mixture. None of the congeners was totally removed. Tested strains had generally greater degradation efficiency at 15 than at 4°C, exception for strain C1W-9 that showed the maximum degradation efficiency at 4°C (37.1%). Conversely, strain DS-1 showed better ability to use (up to 60.1%) Aroclor 1242 at 15°C. Results clearly indicate that the biodegradative potential was not strongly related to the phylogenetic affiliation of the analyzed isolates. Furthermore, the outstanding degradation abilities exhibited by our isolates at 4°C, close to the *in situ* seawater temperature, reveal that the indigenous microbial community has a catabolic potential for PCB aerobic degradation that could occur at *in situ* environmental conditions.

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ANALYSIS OF THE TITIN-CAP GENE AS CANDIDATE FOR DILATED CARDIOMYOPATHY IN GREAT DANE

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Dilated cardiomyopathy (DCM) is an inherited disease of the myocardium, which is characterized by left ventricular enlargement and systolic dysfunction, resulting in impaired heart function that causes significant mortality and morbidity, including heart failure. It has been described in humans and several domestic animals, including the dog. Several genes have been identified in human and mouse as causal for DCM. These genes mainly encode proteins of the cytoskeleton of the cardiac myocyte, and titin cap (TCAP) is considered one of the main candidate gene. The aim of this study was to identify SNPs in two exons of TCAP gene, to evaluate their association with the DCM phenotype in Great Dane dog, using a direct mutation screening approach. Genomic DNA from 4 Great Dane dogs (2 male and 2 female with DCM phenotype and healthy) were used to PCR amplification and sequencing with the ABI PRISM® 3500 (Applied Biosystems). For all animals

physical examination, echocardiography and ambulatory electrocardiogram were performed. The DNA sequences of each fragment were aligned and screened for mutations using MEGA 6. In TCAP exon 2, a silent SNP (g. 29957 T>C of AAEX01022011) was detected in both DCM-affected and DCM-unaffected group. Our results showed no evidence that this gene is involved in DCM in the Great Dane dog. Detection of DCM-associated variants would contribute to development of genetic tools for rapid screening of families at risk for inherited disease and to improvement the methods of diagnosis and therapy.

NERO SICILIANO PIG'S INTESTINAL MYCOBIOTA: PHENOTYPIC AND MOLECULAR CHARACTERIZATION

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In this study we report a preliminary characterization of the intestinal mycobiota of Nero Siciliano pig, an autochthonous pig breed reared in the internal areas of Sicily island (Italy). A total of 21 pigs, from a swine farm in Messina, were collected using rectal swabs. For yeasts isolation, each swab was streaked on three Sabouraud's agar plates containing antibiotics and cultures were incubated at 30°C for 72 hours.

Forty-eight yeast strains were recovered from fecal specimens and initially, presumptively, identified using conventional morphological and physiological tests. Identification at the species level was then confirmed by PCR amplification and sequence analysis of the ITS1-5,8S-ITS2 region of the ribosomal DNA according to previous studies. Results showed that eight different fungal genera colonized the gut of our pigs and *Wickerhamomyces anomalus* and *Geotrichum candidum* (10/48; 21% each) were the most isolated yeasts followed by *Diutina catenulata* (8/48; ~17%), *Clavispora lusitaniae* (7/48; ~15%), *Trichosporon asahii* (5/48; ~10%), *Pichia kudriavzevii* (2/48; ~4%), *Rhodotorula mucillaginosa* (2/48; ~4%), and other species (4/48; ~8%). In particular, *W. anomalus, G. candidum, D. cutenulata, R. mucillaginosa* and *T. asahii* were already, previously, described as component of the mycobiota of other pig's breeds, instead *C. lusitaniae* and *P. kudriavzevii* appear to be exclusive of Nero Siciliano pig's mycobiota. However, other studies are needed to elucidate the complex structure of Nero Siciliano pig intestinal mycobiota and its effect on the well-being of this animal.

c-KIT MUTATION ANALYSIS AND ITS RELATIONSHIP WITH DEGREE OF AGGRESSION IN MAST CELL TUMORS IN TWO DOG BREEDS

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Mast cell tumors (MCTs) are one of the most frequent malignant cutaneous neoplasms in dogs. Therapeutic management is based to histological grade (Patnaik scale) and clinical stage. Some breeds showed a higher incidence of MCTs development than others, suggesting a possible genetic influence on both susceptibility to tumors and tumor aggressiveness. In this order, mutations in c-KIT, a gene that encodes Kit, a transmembrane tyrosine kinase receptor binding the ligand stem cell factor, have been identified and associated with the development of MCTs in both dogs and humans. The aim of this study was to identify mutations in the juxtamembrane region of c-KIT and to evaluate their association with grade of tumor and breed. Tissues samples from 2 dog (Whippet and Jack Russell Terrier) were used to extract DNA for PCR amplification and sequencing with the ABI PRISM® 3500 (Applied Biosystems) after cytological examination. The diagnosis was consistent with a II grade MCTs (according to Patnaik's histological grading). 2 healthy dogs were used to as control. The DNA sequences were aligned using MEGA6. Our analysis revealed that both tumors contained mutations: a duplication of 45 bp was detected in Whippet (in exon 11 but no change to the protein is made) while a deletion of 30 bp (encompasses intron 10 and part of the 5 end of exon 11) was identified in Jack Russel Terrier. Our results confirm the relationship between the histological degree of the tumor and the presence of mutations in c-kit.

WHOLE GENOME SNPS DISCOVERY AND ANALYSIS OF GENETIC DIVERSITY IN NERO SICILIANO PIG

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Nero Siciliano is a local pig breed reared under extensive or semi-extensive management mainly in the Nebrodi mountains of Sicily (Italy). This breed has been rediscovered by farmers who have established a consortium with the aim to protect, exploit and characterize the products obtained from it. In order to discover typical Nero Siciliano signatures, here we present a whole genome SNPs discovery investigation against the *Sus Scrofa* reference genome (SScrofa 11.1). Genomic DNA was extracted from leukocytes of fresh whole blood sample from a male of Nero Siciliano pig with Wizard® Genomic DNA Purification Kit (Promega Corporation), then quantified with Qubit 2.0 Fluorometer (ThermoFisher). DNA quality was evaluated by Nanophotometer P-330 (Implen GmbH) and 1 g di gDNA was used for TruSeq DNA PCR-Free Library preparation (insert size 350bp) following the protocol provided by Illumina. Illumina HiSeq X Ten sequencing produced 346.832.803 paired-end raw reads and after cleaning, performed with Trimmomatic (v.0.36), 346.573.870 (99,92%) good quality reads (phred score ≥ 30) were mapped using bwa aligner against the reference genome (Genbank: GCA_000003025.6). SNPs discovery was performed using SUPER-CAP and the potential effects of detected mutations were evaluated by SnpEff software (v4_3m_core). Analysis resulting in 9.302.670 SNPs, 1.336.511 insertions and 614.764 deletions. More than 5 million of those SNPs were alternative homozygous. Furthermore, over 40% of intronic variants, ~38% of non coding transcript variants and ~8% of intergenic variants were identified. The estimated variant rate was of 1 variant every 222 bases, showing a very high degree of variability among studied breeds.

SATURDAY 28 OCTOBER

SESSION 2: ENVIRONMENT AND HUMAN HEALTH

ESTEBAN LECTURE (INVITED)

BIOMARKERS OF WELFARE AND IMMUNOLOGICAL RESPONSE IN MARINE ORGANISMS

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All international food organizations agree on highlighting the enormous potential of the oceans to make a significant contribution to the food security and to the adequate nutrition for the world's population. In this context, the importance of fish farming is increasing all over the world. In addition, it is known that nutrition has a direct impact on the health of the individuals. Over the past decades the development of aquaculture was focused on improving feeding processes, prioritizing growth and biomass production over other factors. The rapid growth of this sector has forced to reduce production costs and adding value to their products, being fish health and fish welfare and the development of a sustainable business model and respected by consumers, two of its biggest challenges. This is especially important since the ban on the use of veterinary drugs by the EU, including antibiotics, so there is a special interest in having alternative natural methods for fish disease prevention and treatment in aquaculture. Thus, the use of functional feeds is a promising option. Concomitantly, we have been working in the establishment of different biomarkers to determine welfare and immune status, mainly in marine fish, including blood parameters, skin mucus parameters, immune activities or gut health which would be explained.

MARIOTTINI LECTURE (INVITED)

UTILIZATION OF BIOACTIVE COMPOUNDS FROM MARINE ORGANISMS: IS THIS AN UTOPIA OR A POSSIBLE RESOURCE?

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Starting from the ancient ages, and throughout all human history, humans have learned to utilize Nature to satisfy their basic needs, such as feeding, wrapping, inhabiting, etc.. Also for the treatment of diseases plants and also animals have been used to prepare extracts and medicines. Notably, plants have been the basis of traditional medicine for human populations since time immemorial, and the uses of plant-derived substances (a number of around 1000) is estimated to date from around 2600 BCE in Mesopotamia. As a matter of fact, extracts coming from a lot of micro-organisms, plants and animals have been a source of interesting and useful compounds having medicinal and therapeutic activity, several of them have been and are at present used to treat diseases or for other purposes connected with the maintaining of physiological functions of tissues, organs and apparatuses. A branch of science, named "chemistry of natural products" has been defined and organized starting from the study and the assessment *in vivo* and *in vitro* of natural compounds produced by organisms. During the past decades many organic substances having biological activity were recognized and developed and, at present, several of them are currently used as drugs or have other applications, for example as pigments, insecticides, substances of nutritional value, nutraceuticals, additives, cosmetics, molecular tools, fine chemicals, agrochemicals, etc. Furthermore, many natural compounds have been used as a basis to synthesize other bioactive molecules. Recent estimates emphasize that at present 60% and more than 75% of drugs having a natural origin are currently utilized in oncology and for the care of infectious diseases, respectively. Nevertheless, the main source of these compounds was historically the terrestrial environment, while the marine one was always less considered from this point of view. Oceans, and globally the marine environment, are the greatest environment existing on the Earth, occupying more than 70% of its total surface. Therefore, such enormous extension could contain more and more interesting compounds in comparison to those we know and utilize from the terrestrial environment. Many marine organisms are known to be able to produce a wide range of different bioactive compounds originating from metabolites, exudates, excretion, venoms, whole tissues, etc. which are utilized by organisms themselves for their life and for the relationships with other organisms (predation activity, getting food, protection purposes, etc.); so, the effectiveness for utilization of many of these compounds can be presumptively very high. For this reason, oceans are to be considered an enormous resource of bioactive molecules waiting for being discovered and potentially useful to be utilized as therapeutic agents. We can wonder because, heedless of their abundance and variety, historically marine organisms have been scarcely considered as a source

of pharmacologically-active substances. Indeed, the sampling of terrestrial organisms is easy, while strong difficulties to collect marine specimens may exist. Furthermore, scarce amount of extracts can be obtained from several marine organisms and their extreme variety presumes as much extreme variety of compounds. Anyhow, some recent reviews about marine pharmacology literature pointed out that in several countries findings on the preclinical pharmacology of 257 marine compounds having antibacterial, antifungal, antiprotozoal, antituberculosis, antiviral and anthelmitic activity have been reported. Other compounds were reported to have antidiabetic, anti-inflammatory or miscellaneous activities and to affect the immune and nervous system. Therefore, it is proper to wonder if this that can resemble an utopia could originate a new way, transforming also possible problems, such as those coming from venomous organisms, in a possible resource.

ORAL COMMUNICATIONS

APPLICATION OF MARINE COMPOUNDS TO COSMACEUTIC: ANTIOXIDANTS AND ANTIAGEING PROPERTIES OF SOME MEDITERRANEAN RESOURCES

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The research of the last twenty years has devoted considerable efforts to the study of the marine environment, as a valuable source of bioactive molecules, underlying the great potential in providing molecules at anti-inflammatory, antioxidant, immunostimulant and anticancer action. Among marine resources, marine plants, algae and shrimps, are valuable sources of structurally diverse bioactive compounds, such as antioxidants, that are of vital importance in counteract oxidative stress and correlated diseases, such as inflammation, and cancer. In addition, some marine organisms, that live in extreme or variables conditions, are able to synthesize some potential photo-protective compounds, which could be used in the prevention of cellular damage caused by ultraviolet radiation, as anti-photo-aging agents. The aim of the study was the discovery and the evaluation, *in vitro*, of new bioactive compounds from Mediterranean marine organisms and their potential utilization in the pharmaceutical, nutraceutical and cosmeceutical industries. The obtained results showed, in different cell lines, a significant improvement of the cellular antioxidant power, the ability to promote cells renewal and the induction of a skin–whitening effect. The obtained results provide some innovative applications of Mediterranean marine compounds in cosmeceuticals and medical research.

PRODUCTION AND BIOTECHNOLOGICAL POTENTIALITIES OF EXTRACELLULAR POLYMERIC SUBSTANCES FROM SPONGE-ASSOCIATED ANTARCTIC BACTERIA

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Antarctic biological matrices have been never employed for the investigation of extracellular polymeric substance (EPS) production by the associated cold-adapted bacteria, even if their biotechnological potential is very wide, and comprises a lot of advantages rather than synthetic molecules. Four sponge-

associated Antarctic bacteria (*i.e. Winogradskyella* spp. CAL384 and CAL396, *Colwellia* sp. GW185 and *Shewanella* sp. CAL606) were selected for their highly mucous aspect to investigate the enhancement of EPS production varying the carbon source, substrate, NaCl concentrations, temperature and pH values. Incubation at 4°C, 2% sucrose and 3% NaCl concentration in the medium, and pH 7 were determined as optimal conditions. EPSs were extracted in the phase of maximum production, allowing to a total amount of lyophilized exoproduct ranging from 34 to 130 mg/l. The chemical characterization of EPSs produced under optimal conditions resulted in a moderate carbohydrate content (range 15-28%), and the presence of proteins (range 3-24%) and uronic acids (range 3.2-11.9%). The chemical hydrolysis revealed galactose, glucose, galactosammine and mannose as principal constituents, with different sugar ratio for each strain. The investigation on their biotechnological potential showed an excellent emulsifying activity towards hydrocarbons by EPSs from *Winogradskyella* sp. CAL384, probably due to the high protein content. Moreover, EPSs showed the ability to protect cells from freeze-thaw and chelate heavy metals, probably related to the presence of uronic acids and sulfate groups. These findings suggest a possible EPS exploitation as cryoprotection agents, and the potential applications in cosmetic and food biotechnological fields as valid alternative to commercial polymers currently in use.

FROM BIOPROSPECTING TO APPLICATION: ANTIMICROBIAL PEPTIDES FROM SEA URCHIN *PARACENTROTUS LIVIDUS*

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Implant-associated infections are among the most serious complications in orthopaedic surgery. The use of orthopaedic devices, whose surfaces are an ideal substrate for the bacterial growth, has substantially increased the cases of infected implants. *Staphylococcus aureus*, *S. epidermidis*, and *Pseudomonas aeruginosa* are the most common bacterial strains that cause infection, adering on the surfaces of devices and creating a bacterial biofilm resistant up to 1000-fold to antimicrobial agents. A strategy to combat implant-associated infections would be prevention of infection at the implant site, thought local delivery of antibiotics. A solution to be preferred would be to employ non-conventional antimicrobial drugs that do not allow the development of resistant phenotypes. One example of such non-classical drugs are the cationic antimicrobial peptides (AMP). Recently in our lab was discovered an AMP from *Paracentrotus lividus*: Paracentrin-1 with antimicrobial and antibiofilm activity against *Staphylococcus* strains and *P. aeruginosa*. The aplication of this AMP represent the main aim of the ASTED project, funded by the PO-FESR. The project developed an orthopaedic devices capable of delivering Parcentrin-1 from their surface coated with a polymer of PDDLA containing the recombinant Paracentrin-1 (rP1) produced in Sf9 cells. Our results showed that rP1 were released from PDLLA and was able to kill planktonic bacteria wiht a MIC value of 12.5 mg/ml and inhibit the biofilm formation of *S. aureus* and *S. aeruginosa* at sub MIC concentration. Biotechnology skills of this study were transferred to the small enterprises, Ge.Me.S, to create a process of innovation and technological development.

NEUTRALIZING PROPERTIES OF PLANT EXTRACTS AGAINST JELLYFISH VENOM

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Cnidarian venoms are thought to have therapeutic potential so that the research about these compounds is a field of concern for applicative purposes. Nevertheless, at present the fine composition, the activity and the characterization of venom constituents are greatly unexplored. Cnidarian venoms are complex mixtures of thousands of components, mainly peptides, having specific bond targets. This study aims to evaluate the neutralizing properties of natural products from plants against cnidarian envenomation and induced pain, to develop protective products having topical utilization. The isolation of heterotrichous microbasic eurytele nematocysts from tentacles of *Pelagia noctiluca* collected in Eastern Tyrrhenian Sicily waters around Messina and venom extraction have been carried out according to published methods. Commercial extracts from *Ananas comosus* and *Carica papaya* were formerly evaluated on cultured mouse lung fibroblasts L979 to measure their cytotoxicity by MTT assay and here used to prove protection against cytotoxicity of eurytele nematocysts venom. Eurytele nematocysts induced cytotoxicity with an IC50 of about 40×10^4 N/mL. The extracts from *Ananas comosus* and *Carica papaya* resulted non-toxic, both with an IC50>2000 g/mL. *Ananas comosus* extract caused reduction of venom effects at 10 and 100 g/ml, doubling the amount of surviving cells at the endpoint, while *Carica papaya* extract was more effective at 100 g/mL than at 10 g/mL. These preliminary results show that the studied plant extracts can have an interest in fighting the effects of jellyfish stings and could be good candidates for the preparation of topical products. Further studies in progress in our laboratory will hopefully confirm these first heartening data.

SESSION 3: ENVIRONMENT AND HEALTH: EPIDEMIOLOGICAL AND LEGAL ASPECTS

DI LANDRO LECTURE (INVITED)

LA TUTELA DELL'AMBIENTE E DELLA SALUTE, DAL CASO PORTO MARGHERA AL CASO ILVA: PROFILI GIURIDICI

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La relazione partirà dal tema del principio di precauzione in sede penale, alla luce in particolare della norma attuativa in tema di obblighi di comunicazione (art. 301 Testo Unico Ambiente). Si tratteranno i profili problematici del caso del petrolchimico di Porto Marghera (ovvero anzitutto la questione dell'individuazione/descrizione dell'evento prevedibile ed evitabile); la differenza, centrale a livello normativo, tra «rischio che possa essere individuato a seguito di una preliminare valutazione scientifica obiettiva» e «rischio sufficientemente probabile che stia per verificarsi uno specifico danno ambientale»; i temi della prova scientifica nel processo penale e dell'accessibilità delle conoscenze scientifiche per l'operatore (con riferimento anche al caso-limite dell'agente con conoscenze scientifiche superiori). Dal problema della conoscibilità delle nuove acquisizioni scientifiche da parte dell'operatore, si passerà ad affrontare la questione di quando sorge per quest'ultimo l'obbligo di adottare tecniche più avanzate, in particolare laddove l'operatore agisca nel rispetto di norme giuridiche, divenute obsolete. Verranno analizzati, infine, i problemi delle difficoltà economiche e dei tempi per l'adeguamento dell'operatore alle tecniche più avanzate, recentemente posti dal caso ILVA.

ORAL COMMUNICATIONS

ENVIRONMENT AND HEALTH: FROM BASIC RESEARCH TO APPLIED RESEARCH

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The theme of research has always been an inseparable aspect of human existence, especially when we take into consideration the effect of the environment, we are surrounded by and we live in, on the human health. Nowadays, when it comes to research, obviously we look at University with reference to the totalitarian thought of the extended education system. Talking about University means ensuring the adequacy of a teaching finalized to the preparation and the access to work, but to do it we have to invest in the research. Research involves foreseeing, and foreseeing means to plan and therefore to esteem sums to place in the budget, which are intentionally reduced in favor of both an economic decrease and an intellectual impoverishment. Research means reading, studying and interpreting the scientific phenomena; interpreting man and environment within their reciprocal relationship; interpreting the literary thought by revisiting and re-examining the classics; comparing historical and current events; maintaining the freedom of the human being in the choice of his decisions. According to economic estimates due to basic structured flows, the big multinational companies influence the market and therefore the research. Actually, conducting research means discussing financial flows, productivity, business plans, profits on industrial base, not being interested in the phenomenon of study and scientific discovery at all; the value of research is reinforced by productivity and consequently poisoned by the economic profit that has more power on the country's political decisions.

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THE EXHIBITED BODY AND THE REAL BODY: THE SOCIAL ENVIRONMENT AND THE PSYCHOPHYSICAL HEALTH, @GENERATION

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In the childhood, regular physical and sport activity combined with proper nourishing habits is today an important prevention tool for the health of future generations. In relation to the 27th anniversary of the UN Convention on the Rights of the Child (UNCRC) the "General States of Italian Pediatrics" (SIP) have published a series of statements concerning the abandonment of sporting practice in pre-adolescent and adolescent ages which have had the effect of a high, in absolute, increase of sedentary. The phenomenon mainly affects girls, ranging from 24% (between 15 and 17 years) to 30% (between 18 and 19). Kids generally do a sport because they love playing, sports learning happening happily and becomes a way to experience the motor skills acquired so far. According to SIP data, 6 children out of 10 (57%) practice sports continuously, swimming and dancing, but after the primary school, about 11 years, starts moving away from practice. Between 2011 and 2012, the proportion of continuous practitioners decreased in the age group 11-14 years, from 56% to 53.4%. Percentage between 15 and 17 years becomes 48.5% and is 14 percentage points below, to 34.7%, between 18 and 19 years. The abandonment of sports practice could be attributed to the family crisis and the economic crisis, as families do not have the means to keep the children's membership fee. Another factor may be related to the fact that pre-adolescent and teenagers prefer to spend their time on computer tools to share virtual friendships and / or entertain themselves with video games. The *socials* such as Facebook, Instagram, Twitter exert a strong attraction especially on *@generation* Over 85% of teenagers have a profile matched with an image that usually does not match the actual one. Teenagers are looking for attention and approval, based on the clicks of post photos and comments received, but when "I do not like" and negative comments come up, problems arise. An increase in the number of teenagers living psychopathology related to the role of the physical appearance online. The use of *social* has significantly increased the symptoms of *binge eating* and *purging* by virtual image, real image. The use of sports practice is replaced by the use of *body building aesthetics*, self-compassion and body satisfaction. Here's the problem: "How many I like it has my picture?" It is concluded that Internet is a powerful socio-cultural medium of relevance for the body image of teenagers.

CULTURAL SELECTION AND HUMAN FOOD PREFERENCES

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Genes and culture may coevolve to determine variation in dietary habits. Our evolutionary heritage of food preferences and eating habits leaves us mismatched with the food environments we have created, which leads to problems such as obesity. Several hypotheses have attempted to explain the high rate of obesity present in today's world. The "thrifty genotype" hypothesis (TGH) suggested that obesity today is a throwback to our ancestors having undergone positive selection for genes that favoured energy storage. The "drifty genotype" hypothesis contends that the prevalence of thrifty genes is not a result of positive selection for energy-storage genes but, rather, is attributable to genetic drift resulting from the removal of predatory selection pressures. Both hypotheses focus on environmental changes over time, positive selection and genetic drift. While genetics plays a significant role, it is mostly major cultural shifts in post-communist countries that are responsible for obesogenic phenomena. Cultural transition in 1990s affecting the society after a long socio-cultural isolation offered different choices regarding food, so new consumption behaviours were formed. Consumption behaviours regarding food and health in Albania are the principal factors involved in the spread of obesogenic phenomena in the post-communist state. We believe that cultural selection is responsible of consumption behaviours or cultural norms regarding food not because they are adaptive but because they represent the historical and environmental conditions in which those norms were culturally selected.

REDOX STATUS OF ERYTHROCYTES IN METABOLIC SYNDROME: ROLE OF QUERCETIN

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Erythrocytes aggregation has been consistently associated with insulin resistance, central obesity and hypertension in the literature. Pro-oxidant generated in Metabolic Syndrome (MeS) decrease the erythrocyte deformability and increase the whole blood viscosity. The oxidative stress has a detrimental effect onto MeS due to the gross disturbance in microcirculation. Quercetin is power antioxidant contained in food. It has modulator effects on cellular physiology due to its membrane interaction ability and antioxidant potential. This pilot study was carried out the plasma antioxidant status and the Plasma Membrane Redox System (PMRS) activity of erythrocytes in patients with MeS compared to healthy individuals. Then the effect of quercetin at 50 M concentration on the PMRS activity has been evaluated. Subjects suffering from a MeS had poor antioxidant status as reflected by significantly low values of ferric reducing activity in plasma and erythrocyte respectively as compared with subjects not suffering from MeS. In addition, data show that the patients with MeS have a PMRS activity 13% higher than control group. Linear regression analysis revealed a negative and significant correlation between ferric reducing capacity and the PMRS activity of erythrocyte. Quercetin increases PMRS activity both in subjects with MeS (61%) and in control but there is no significant difference between control and subjects with MeS treated with quercetin. In summery, MeS patients exhibited evident systemic redox imbalance compared to controls. Quercetin could be used to stabilize the rheological properties of the blood in MeS.

IN VITRO AND COMPUTATIONAL STUDIES IN THE FIGHT AGAINST MALARIA

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Resistance to antimalarial drugs has spread rapidly over the past few decades. WHO recommends Artemisinin based combination therapies (ACTs) for the treatment of uncomplicated Malaria. In 2016, artemisinin resistance has been confirmed in 5 countries of the Greater Mekong subregion. We focused our study on different Syk inhibitors as new antimalarial drugs. Syk protein is present in human erythrocytes and membrane protein band 3 is its major target following activation by oxidant stress. Tyr phosphorylation of band 3 occurs during P. *falciparum* growth leading to the release of microparticles containing hemichromes and structural weakening of the host cell membrane. Syk inhibitors block these events interacting with Syk protein catalytic site. We have performed and compared the results of *in vitro*/proteomics and in *silico* studies. *In vitro* studies were based on the treatment of parasite's cellular cultures with different concentrations of Syk inhibitors and proteomics studies were focalized to study the modification of the Tyr phosphorylation residue of band 3 by Syk protein. *Silico* studies were based on different approaches of molecular modelling, to optimize the interaction ligand-protein and obtain the highest efficacy *in vitro*. In presence of Syk inhibitors we observed a marked decrease of band 3 phosphorylation according to the increase of drugs concentration. The proteomic data trend regarding the inhibition values IC_{50} correspond to the computational studies results. Our studies and the obtained results could be useful to the structural optimization of these compounds and to design new promising antimalarial drugs.

HOW ARE THE DIFFERENT COMMUNITIES LIVING IN THE GENOA AND TARANTO DISTRICTS (ITALY)? COMPREHENSIVE AND TIMELY ANALYSIS BY THE CITY EPIDEMIOLOGICAL REPORT (UPDATED JUNE 2017)

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Introduction and Objective. In many Italian cities, some districts have high environmental and health risks due to the presence of environmental pollution and other risks (socio-economic,..). To evaluate objectively and timely the overall mortality rate, adjusting by age, for gender and year in the different districts of Genoa and Taranto, we propose to use the "City Epidemiological Report" (Referto Epidemiologico Comunale - REC). *Materials and Methods.* From the statistical offices of the two municipality we received the overall data on mortality by districts, gender, age and period (2009 - June 2017). Then we estimated the number of *"expected"* deaths for both genders, periods and districts based to the entire municipal population (local reference). The Standardized Mortality Ratio (SMR) was calculated by dividing the number of observed deaths with the expected one. Finally, confidence intervals at 90% were calculated by Poisson method. *Results.* Many critical districts have been confirmed and quantified, but other issues deserve urgent insights. In Genoa, in the last two years, we found that the number of districts that had statistically worsened their SMR was higher than the improved ones. Also in Taranto, close to the industrial area (steel mills, refineries, cement factories, etc.), districts with higher mortality rates were observed. *Conclusions and Discussion.* Scientific deficiencies and conflicts of interest *(business bias)* drive some stakeholders to ignore and underestimate both avoidable health damages, environmental pollution and other risk factors. The REC helps to timely monitor the mortality inequalities between specific urban areas in a scientifically and economically way, by capturing the possible *"cocktail effect"* between socio-economic risks and different pollutants (not just carcinogens) which, even under legal limits, may cause additional deaths for pathologies not yet related.

NEW ANTIMALARIAL COMBINATIONS BETWEEN SYK INHIBITORS AND ARTEMISININS AGAINST *P. FALCIPARUM*

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The current therapy used for the cure of malaria is based on the administration of a series of artemisinin derivatives (ACTs) combined with longer half-life quinolines. The resistance to ACTs, is a major thread for the control and elimination of malaria due to *P. falciparum*. The need for new antimalarial drugs is more urgent than ever before, with emerging strains of the parasite now showing resistance against the best available drugs. Recently has been demonstrated that Syk inhibitors represent a new class of antimalarial drugs by suppressing merozoite egress by inhibiting the host target that cannot be mutated by the parasite to evolve drug resistance. In this study, our target is to evaluate the *in vitro* combination activity of Syk inhibitors with Artemisinins and understand their mechanistic interaction. We show that Syk inhibitors present an *in vitro* synergistic combination with all tested Artemisinins against *P. falciparum*. Furthermore, we observed that Syk inhibitors determine a massive accumulation of hemichromes in the parasitized erythrocytes triggering the activation of artemisinins and leading to a marked synergistic effect if administered in combination.

BIOINFORMATICS ANALYSIS OF NEXT-GENERATION SEQUENCING DATA IN MICROBIOLOGY

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Next-Generation Sequencing (NGS) technologies represent a high-throughput, automated, fast and economical tool in order to deep investigate whole genomes and transcriptomes. Here we present an overview of bioinformatics approaches that could be applied for NGS data analysis in

microbiology, spanning from *de novo* and reference-guided genome assembly and relative annotation, transcriptome assembly, differential gene expression analysis, SNPs discovery, to phylogenomics. After sequencing, short sequenced fragments (reads) are filtered to remove adapters and low-quality sequences; the remaining high-quality reads are then assembled (*i.e.* with SOAPdenovo2, SPAdes) or mapped against a reference genome (bwa, bowtie) or reconstructed based on this latter (IMR-DENOM, Reconstructor). BUSCO and QUAST programs can be used to evaluate the assembly results. Annotation can be performed using ab initio gene predictors, tRNA, rRNA and repeats searching tools, followed by homology search (Blast, InterProScan) to determine the function of predicted features. For transcriptomic data, if a reference genome is not available, high-quality reads can be de novo assembled (Trinity, Trans-AbySS), otherwise reads can be mapped on the reference genome (STAR). Then transcripts can be identified and count for differential gene expression purpose (Cufflink, EdgeR). An homology based search (*i.e.* Blast2GO) can be applied to make the functional annotation of detected features. Both in genomic and transcriptomic projects, SNPs discovery between samples and reference sequences can be performed using Samtools, GATK or SUPER-CAP softwares. Molecular phylogeny using a gene-by-gene comparison with a multi-locus sequencing typing (MLST) strategy can be performed on specific genes sequenced by target-sequencing or extracted from assembled genomes.

THE ROLE OF HUMAN SEMEN AS AN EARLY AND RELIABLE TOOL OF ENVIRONMENTAL IMPACT ASSESSMENT ON HUMAN HEALTH IN RISK AREAS. ECOFOODFERTILITY PROJECT

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A biomonitoring study (EcoFoodFertility Project) was conducted on blood and semen of healthy men living in the "Land of Fires"(High Environmental Impact-HEI) and in "Alto-Medio Sele"(Low Environmental Impact–LEI) areas of Campania (Italy) to assess environmental impact on fertility and human health. Two different groups no smoking, no habitual alcohol drinking, no occupational exposure, were recruited :i) first group of 110 males(28±5 yrs-old)from HEI (n=60) and LEI(n=50)areas ;ii) second group of 112 males (29.0±5.6 yrs-old)from HEI(n=57)and LEI(n=55) areas. In the first group,22 trace elements were analysed in blood and semen by optical emission spectrometry; sperm DNA fragmentation index(DFI)by Sperm Chromatin Dispersion test; total antioxidant capacity(TAC) and antioxidant enzyme activities in the semen(Glutathione reductase, Glutathione peroxidase) by spectrophotometry. In the second group, Telomere Length(TL)was assessed by quantitative Real-Time PCR on genomic DNA extracted from leukocytes(LTL)and sperm(STL). In the first group, HEI subjects showed significantly higher values($p<0.05$) for Al,Mn,Cr,Mg,Li,Co,Ca in blood, as well as for Cr,Cu,Zn in semen, while Fe was lower in the semen of HEI-group($p<0.05$).Immotile sperms and the DFI were both higher($p<0.026$ and $p<0.01$, respectively) in the HEI-group. TAC in blood showed no differences, while TAC, GSR and GpX in the seminal plasma were significantly lower in the HEI-group($p<0.05$). In the second group, STL was significantly longer in HEI subjects compared with LEI subjects(0.90±0.26 vs 1.15±0.51,$p=0.04$). No significant difference was observed between LTL and HEI/LEI areas (0.99±0.33 vs 1.00±0.38,$p=0.2$;). Human semen seemed a more early and sensitive source of biomarkers than blood to monitor high environmental pressure on human health.

RABINO MASSA LECTURE (INVITED)

MEDICAL ANTHROPOLOGY

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Medical anthropology has experienced remarkable growth in recent decades and thanks to its interdisciplinary approach has assumed its own identity. Overcoming the limits of medicine, which studies the individual as a representative of the human universe, medical anthropology seeks to fill the gap between individuality and universality by considering social and cultural groups as anthropological postulates; it provides the tools to conduct interventions aimed at knowledge of public health and the prevention of disease in different cultures and populations. For a holistic approach, this scientific subject must take into consideration the socioeconomic and historical dynamics of populations within a system of interactions between them and the natural and cultural environment. The relationship between health and environment is complex, and disease is no longer the case of a single ethnic group living within well-defined boundaries. Health is affected by a complex set of economic policies, international relations and population movements. The results of studies on immigrant groups in Turin will be presented in order to provide points of discussion for future developments of medical anthropology. The serological situation has been analyzed relating to the presence of some markers of hepatitis A and B in two groups of children living in two communities of gypsies, Roms and Sintis. The results have shown that almost all the children had the infection of the virus of hepatitis A, while the frequency of infections from virus of hepatitis B was higher in the Rom group because of the worse social and sanitary conditions.

In recent years, the importance of a correct diet, *i.e.* the correct input of nutrients and mineral salts, has been re-evaluated, especially in relation to the health status and longevity of subjects. It will be presented examples of the relationship between feeding behaviour and pathologies in immigrant populations in the Turin area. Our recent studies have demonstrated the correlation of vertebral paradysmorphisms with dietary deficiencies of vitamin and oligoelement in young nomads. In particular, it will be presented the results of clinical screening for hyperkyphosis and scoliosis in 101 young gypsies. The incidence of paradysmorphism was significantly higher in the nomads than in a control group of young Turinese. These paradysmorphisms are probably due to idiopathic factors and perhaps to the particular diet and the stress induced by exposure to the elements characteristic of a nomadic life-style. Finally, it will be presented our ongoing research project dealing with the analysis of feeding habits in Alpine populations. In these strongly endogamic populations, we studied the relationship between the feeding behaviour and the risk factors and onset of cardiovascular pathologies.

Since the study of feeding behaviour is relevant to programs for health improvement and for epidemiological monitoring, the results of our research can be applied to programs involving preventive interventions.

POSTERS

GRANZYMES ROLE IN THE CELL-MEDIATED CYTOTOXIC IMMUNE RESPONSE OF FISH AGAINST NODAVIRUS

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Granzymes (Gzm) are members of the serine protease family and major components of cytotoxic granules of professional killer cells. Multiple granzymes have been identified with different substrate specificities. Although the significance of granzymes A and B in cell-mediated cytotoxicity (CMC) has been extensively investigated,[1] recent reports suggest that other granzymes may have either equal or greater importance in mediating the immune response of fish. The aim of this work has been to evaluate the potential implication of several granzymes (GzmA, GzmK, GzmG, GzmB and GzmM) in Gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) upon infection with Nodavirus (NNV). In CMC assays we have found that the granzyme activity follows the following order: GzmA/K> GzmM > GzmB. At transcriptional level, the patter of expression followed the functional activity. This confirms previous studies in fish suggesting greater importance of granzymes A or K compared to lower GzmB in contrast to what happens in mammalian CMC response.[2]

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OXIDATIVE STRESS RESPONSE OF RAINBOW TROUT AFTER SUBCHRONIC TONALIDE EXPOSURE

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Polycyclic musk compounds are one group of synthetic lipophilic substances that are very important for commercial use, especially for cosmetics, detergents, fabric softeners and cleaning products.[1] After their use, these substances are only partially removed by wastewater treatment leading to a long-term exposure of the aquatic communities.[2] The aim of this study was to investigate the effects of subchronic tonalide exposure on oxidative stress indices in rainbow trout. During the toxicity tests fish were fed with feed supplemented with tonalide. Control (C) and two experimental groups (854 µg/kg for low concentration – LC and 8699 µg/kg for high concentration - HC) were used. After six weeks of exposure, the selected tissue samples (liver, gills, gonads and kidney) were homogenized and used to oxidative stress examination. Oxidative stress indices including glutathione S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR) and lipid peroxidation were measured spectrophotometrically using Varioskan Flash Reader. According to *Habig et al. (1974)*, GST was measured. According to *Flohé and Günzler (1984)*, the catalytic concentration of GPx was determined. The lipid peroxidation in the sample was evaluated according to the TBARS test described by *Lushchak et al. (2005)*.[3-5] In our experiments, we observed moderate reduction of GR activity in all tissues, a slight reduction in activity of GST for gills and gonads parameters. These changes were not significant. Moreover, after six-week exposure both in kidneys and in gills a moderate increase of the level lipid peroxidation was observed in the experimental groups compared to the control group. Many scientific studies confirmed that tonalide could be dangerous to aquatic organisms.[2]

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XANTHOTOXIN IMPROVES SCOPOLAMINE INDUCED MEMORY IMPAIRMENT IN PASSIVE AVOIDANCE TEST IN MICE – ROLE OF ACETYLCHOLINESTERASE

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Xanthotoxin (8-methoxypsoralen, 8-MOP) is a furanocoumarin found in many medicinal plants and is used in the treatment of psoriasis, vitiligo, and cutaneous T-cell lymphoma. This drug also possesses slight antioxidative activity

as evidenced from *in vitro* studies. Literature data have demonstrated that xanthotoxin possess inhibitory activities on monoaminooxydase (MAO), butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE). BuChE and AChE degrade acetylcholine that prevents the formation of senile plaques in Alzheimer's disease. The aim of the present study was to examine the effects of a subchronic administration of xanthotoxin on memory processes as well as on memory impairment induced by injection of scopolamine in the passive avoidance (PA) paradigm in male Swiss mice. We also assessed the relation of this drug in the level of ACHE in prefrontal cortex and hippocampus. Xanthotoxin by high-performance counter-current chromatography from methanol extract of fruits of *Angelica officinalis* was purified. Our results revealed that subchronic injections of xanthotoxin at the dose 1 mg/kg, i.p., improved processes of memory acquisition and consolidation in the PA task as well as increased the level of AChE. Moreover, xanthotoxin administered subchronically improved memory acquisition and consolidation impaired by scopolamine (1 mg/kg). Moreover, it was detected that acute injection of scopolamine decreased the level of AChE in the brain, whereas xanthotoxin reverse this action. The results obtained suggest xanthotoxin can be an interesting therapeutical option in disorders with memory deficits.

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APPRAISAL OF THE ABIOTIC AND BIOTIC FRAMEWORK OF FARO LAKE (MESSINA, SICILY)

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Faro Lake is a meromictic basin with singular characteristics in the Mediterranean. It is located in the northern part of Messina, and is part of the Natural Reserve of Capo Peloro (38°15'57"N; 15° 37'50"E). The brackish water of the lake is renewed thanks to the connection with the sea (both Tyrrhenian and Ionian) and with Ganzirri lake. In the study area traditional mollusc farming activity is present that has been recognized as ethno-anthropological heritage. The purpose of our study was to assess a start point for the monitoring of the parameters of the water and haemolymph of traditionally farmed *Mytilus galloprovincialis*. The determination of electrolytes (Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, P inorganic) and heavy metals in both Faro lake water and haemolymph have been carried out. The electrolytes have been determined using the method colorimetric and potenziometric and the heavy metals using ICP-MS method. Chemical and physical parameters of the water have been recorded during sampling, using a multiparametric probe (T, Sal, pH, Conductivity), while Winkler method was used to measure the O_2 present. The monitoring period was of one year (from April 2016 to March 2017) with monthly samplings in the same point of the Faro lake. *M. galloprovincialis* is a good bioindicator, ideal for assess levels of environmental pollution thanks to its biological, ecological and physiological characteristics (sessile, ubiquitous, filter-feeder and osmoconformer). The results of our study showed a typical fluctuation range in haemolymph parameters, related to the water ones. As osmoconformer organism, the haemolymph parameters were influenced by water parameter oscillation. Despite this, the variation of chemical-physical parameters affected the ions levels in some period of the year. The presence of heavy metals traces was constant during all the year. Our study reports for the first time the correlation between water parameters and haemolymph content of mussels farmed in the Faro lake, describing the trends and creating reference data for further studies.

MEPHEDRONE EXPOSURE IN MALE SWISS MICE: DAMAGES AND POSSIBLE SOLUTIONS TO ADDRESS DRUG ADDICTION

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Mephedrone (MEPH) [or 4-methylmethcathinone (4-MMC) or 4-methylephedrone], is a cathinone derivative and a synthetic stimulant that accelerates information sent from the brain to the body: induces the extracellular level of dopamine and the level of serotonin in the nucleus accumbens. It creates dependence as it induces a stimulating effect on the central nervous system (CNS). Addiction is an illness associated with functional disorders of the rewarding system with alterations in the structural remodeling of the synaptic connections, involving neuronal plasticity mechanisms with learning and memory phenomena. Key enzymes in remodeling are extracellular matrix proteases (ECMs) such as matrix metalloproteases (MMPs). MMPs play a key role in remodeling as well as being important for long-term learning, induction and maintenance as well as tasks of memory, all processes strongly associated with drug addiction. Therefore, the aim of our study was to study how subcronic exposure to three different doses (0.5, 2.5 and 5 mg/kg) of MEPH affects the activity of MMP-2 and 9 in the hippocampus and prefrontal cortex of mice, the structures involved in memory and learning, after subcronic administration of mefedrone. The results show that there is a significant increase in the activity of MMP-2 and MMP-9 in both structures at higher doses (2.5 and 5 mg/kg) of mefedrone, while the lowest dose of mefedrone (0.5 mg/kg) did not cause any significant changes. We also assessed the relationship with the memory consolidation processes evaluated in the passive avoidance (PA) paradigm in male Swiss mice, demonstrating that mephedron enhances the consolidation of memory and learning processes. Our results confirmed that treatment with three different doses of mephedrone resulted in an increased activity of MMP-2 and MMP-9. Therefore inhibiting MMPs activity, could possibly contribute to arrest the development of drug addiction.

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GAMBLING: SPREADING AMONG STUDENTS IN PALERMO

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Adolescents represent a particularly high-risk group and they are also vulnerable to the development of gambling related problems. Thanks to technological advances in gaming industry (slot machines, video lottery terminals, casino-types games) new form of gambling are continuously appearing.

With the purpose of having an idea about the spreading of gambling among the students of Palermo, a survey has been conducted through administration of questionnaires during the period from January to May 2017: this study involved 1622 students (686 boys and 936 girls) between the age of 14 and 19. In this survey it has been demonstrated that only 15% of students have never played; 36% declare they have played cards with money at least once in their life and 19% of them play lotteries and scratchers; some others have played sport betting (14%), bingo (7%), slot machines (5%), horse betting (4%). The money spent on a single day is between 1 and 10 euros (584 students); 530 teenagers spend 1 euro or less; 132 between 1 o and 100 euros; 16 students more than 100 euros/*die*. Moreover, most alarming is evidence indicating that there is a considerable number of students who have gambling players parents (father 18.65%; mother 16.4%; both of them 4.52%): this is considered an important vulnerability factor. The analysis of the picked data suggests that there is a widespread diffusion of gambling among the adolescent population. So, to conclude, the present research points out the need to complete immediate interventions on both levels of information and prevention.

STATISTICAL ANALYSIS OF A SURVEY ABOUT DIFFUSION OF BINGE DRINKING AND DRUNKOREXIA AMONG YOUNG PEOPLE

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Youth alcohol consumption is a major global public health concern. Previous reviews have concluded that exposure to alcohol marketing was associated with drinking initiation and higher alcohol consumption among youth. From January to May 2017, a survey has been conducted through administration of questionnaires about the diffusion of binge drinking and drunkorexia among the students of Palermo. Test was administered, during school time, to 2331 students between the ages of 13 and 20. Regarding the analysis of the questionnaires, 97.8% of student declare to have drunk at least once: their first taste of alcoholic drink happens at the age of thirteen/fourteen. They prefer to drink at the disco or pub (48% disco; 22% pub) in Friday and Saturday evening (92%). 52% of student also declare to associate alcohol to cigarette smoking. Another important statistic is that 736 girls and 61 boys declare to prefer to restrict food intake in order to consume greater quantities of alcohol and to avoid weight gain. In order to evaluate youth alcohol consumption in a different contest, it was created an online survey thanks to Google forms. This study involved 500 young people from Palermo between the ages of 19 and 30. The extrapolation of data confirmed the same results of the questionnaires on paper. In this test, however, it was also evaluated the spreading of drink-driving (68.5%). The present survey suggests that there is an expansion of such practices in young population. In conclusion, it appears necessary to adopt measures of information and prevention to reduce territorial diffusion.

STATISTICAL ANALYSIS OF THE CASES OF POISONING DETECTED IN THE EMERGENCY ROOM OF THE "SAN GIOVANNI DI DIO" HOSPITAL FROM 2012 TO 2016 AND IN THE TOXICOLOGY DEPARTMENT AND INTENSIVE CARE UNIT OF THE "BUCCHERI LA FERLA" HOSPITAL IN PALERMO

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Many clinical cases of intentional or unintentional poisoning have been analysed for every kind of xenobiotics in the period 2012-2016 observed at the "San Giovanni di Dio" Hospital in Agrigento, The same kind of study was carried out on some cases of the period 2000-2010 and 2010-2016 at the "Bucchieri La Ferla" Hospital in Palermo. At the "San Giovanni di Dio" Hospital in Agrigento individual and anonymous data from a sample of 264 individuals has been collected and they present a set of symptoms that are, in whole or in part, caused by the poisoning. Simultaneously, the survey carried out on the hospital in Palermo, which involved 96 individuals, gave particular attention to poisoning caused by food, pesticides, household products, cosmetic products, hygiene products, medicine, non-pharmaceutical chemical products, drugs and alcohol, along with animal and plant poisons, including mushrooms. The data has been compiled with respect to parameters such as: age, gender, aetiological agent responsible for the poisoning, route of exposure, major symptoms and medical implications. The rates have shown a decrease in the cases of alcohol toxicosis and of poisoning due to the use of antidepressant drugs and sedative hypnotics regarding the region of Agrigento, on the other hand, in the region of Palermo the rates have shown an increase of the cases of poisoning due to the use of antidepressant drugs and sedative hypnotics and an increase of the accidental poisoning derived from the use of cardio-vascular drugs. The overall cases of poisoning in Agrigento, from 2012 to today, are 264 with no mortality cases. However, from 1990 to today, in Palermo, the cases of poisoning are about 140 with a low mortality rate (about 7 cases out of the 140 taken into account).

SUBOXONE EFFICACY IN TREATING DRUG ADDICTION: COMPARISON WITH METHADONE AND MONITORING OF PATIENTS IN THERAPY AT SERT OF MARSALA

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Drug dependence and abuse is a public health problem as revealed by reports of state police that account overdose-related deaths for 305 during 2015. Most of the dead were caused by heroin. Despite evidence that methadone maintenance treatment is effective, it remains a controversial therapy because of its indefinite provision of a dependence-producing medication. The aim of this work is to evaluate clinically the effectiveness of Suboxone in maintaining abstinence and preventing relapse. Suboxone consists of buprenorphine and naloxone in the 4:1 ratio. The former is a partial agonist on the receptor and its absorption is rapid, while Naloxone is an antagonist on the receptor k and it is absorbed slowly. At SERT of Marsala 32 drug-addicted people were monitored. At the beginning of the monitoring, 23% of patients had already started Suboxone treatment, whereas the rest of the group was subjected to suboxone treatment for the first time. The monitoring activity included laboratory exams, questionnaires and weekly speeches. The evaluation were based on the patient's response to Suboxone, possible side effects and degree of patient satisfaction. Even if 15% of patients have interrupted the suboxone treatment, the success of the therapy over time has been satisfactory at the time of the last report. In particular, 82% of patients who have tried both treatments with methadone and suboxone find the latter more effective. Study findings suggest that Suboxone treatment improves the ability of patients to remain abstinent, having fewer side effects with respect to methadone and reducing the risk of relapse.

EFFECT OF OXIDATIVE STRESS ON $SO_4^=$ UPTAKE THROUGH BAND 3 PROTEIN IN HUMAN ERYTHROCYTES

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Human erythrocyte membrane consists of a lipid bilayer and integral proteins, amongst which Band 3 protein (B3p) playing a role in membrane deformability due to its cross link with cytoplasmic proteins, is responsible for Cl^-/HCO_3^- exchange, reflecting on gas exchange efficiency. The effect of oxidative stress in terms of lipid peroxidation, -SH groups oxidation, anion exchange capability and phosphorylation of Tyrosine residues of Bp3, modulating its function, has been here considered. Oxidative conditions have been induced by exposure to either H_2O_2 or $CdCl_2$ or NEM (N-ethylamelimide), with or without antioxidants such as Curcumin, Melatonin or $MgCl_2$. The efficiency of B3p has been verified by measuring the rate constant for $SO_4^=$ uptake, more easily estimated than Cl^- transport, while oxidative status was assessed by determining levels of MDA (malonyldialdehyde), membrane –SH groups and intracellular GSH (reduced glutathione). B3p expression levels have been also measured. Our results show that the exposure to all oxidative compounds used for the present protocol reduced $SO_4^=$ uptake *via* different mechanisms, as H_2O_2-induced damage was dependent neither on MDA production, nor on –SH groups and GSH levels reduction, contrarily to the other oxidants, while B3p expression levels were reduced after treatment with each oxidant. Moreover, phosphorylative pathways are involved in this inhibitory effect which is reversed by antioxidants. In conclusion, $SO_4^=$ uptake may be suggested as a tool to monitor erythrocytes function under oxidative conditions possibly deriving from environment (food, water, enspired air) and B3p may be a candidate for pharmacological targeting against oxidative stress-related pathologies.

THE EFFECTS OF ACUTE EXPOSITION OF CALYPSO 480 SC ON COMMON YABBY (*CHERAX DESTRUCTOR*) CRAYFISH

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Calypso 480 SC is an insecticide produced by Bayer containing the active substance thiacloprid (480 g/L), belonging to the family of neonicotinoid insecticides. Being crayfish an important component of aquatic ecosystems is considered sensitive indicators of water pollution. For this reason, the aim our study was to investigate the effects of acute exposition of Calypso on common yabby (*Cherax destructor*) at different concentrations (0.1; 0.5; 1; 5; 10; 25; 50 mg/L) for 96h. Both the percentage dead of animals and the LC50 at 24h, 48h, 72h and 96h were evaluated. Since the highest concentrations showed 100% mortality, three concentrations (0.1, 1, 10 mg/L) have been chosen for the measurements of oxidative stress and antioxidant parameters: thiobarbituric acid reactive substances – TBARS, superoxide dismutase – SOD, catalase – CAT, glutathione S – transferase – GST and reduced glutathione – GSH, in crayfish hepatopancreas, muscle and gills tissues. The 96h-LC50s at the concentration 7.65 mg/L of Calypso was considered. The effects of acute exposure to toxicant showed reduction of the level lipid peroxidation in hepatopancreas in all experimental groups compared with control. The results of antioxidant activity showed a significant change on GST in tissue of hepatopancreas while no differences on the others antioxidant parameters in crayfish's tissues were observed. The acute exposure (96h) to Calypso insecticide cause reducing the formation of reactive oxygen species in to cell lipids without determining an increase of antioxidant activity in tissues of common yabby (*Cherax destructor*).

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SEXUAL DIMORPHISM IN ENDLER'S GUPPIES (*POECILIA WINGEI*) – MORPHOMETRIC ANALYSIS

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Endler's Guppy is a small fish belonging to the family of Poeciliade, this group of fish is characterized by a marked

sexual dimorphism including differences in colour patterns, body size and genital morphology. The aim of the study was to determine the sexual dimorphism in adult Endler's guppies by morphometric analyses of the following parameters: weight (W), total length (TL), body length (BL), Fulton's Condition Factor (FCF), caudal fin length (CFL), dorsal fin length (DFL), pectoral fin length (PFL), head length (HL), and eye diameter (ED). A particular attention was given to the measurements of rays of anal fin: thickness ratio 3rd:4th ray and length ratio 4th:6th ray. In females, the rays of anal fin are of a similar size and structure, while, in males, the 3rd, 4th and 5th ray of anal fin developed into a copulatory organ, the gonopodium. Twenty fish (10 females and 10 males) stored in 10% neutral buffered formalin for a period of 100 days before starting the measurements were used. The results showed that males are smaller than females with significant differences in W, TL, BL and PFL. Females have bigger HL and ED compared to males, and these differences might be connected to the need of noticing the smaller and coloured males. In males, the measurements of anal fin demonstrated the development of anal fin in gonopodium. As the characteristic structures of gonopodium were as follows: fresh pulp on 3rd ray; spines on 3rd and 4th ray; terminal hooks on 4th and 5th ray. In conclusion, the morphometric analysis confirms sexual dimorphism in Endler's Guppy.The acute exposure (96h) to Calypso insecticide cause reducing the formation of reactive oxygen species in to cell lipids without determining an increase of antioxidant activity in tissues of common yabby (*Cherax destructor*).

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EVALUATION OF ANTIOXIDANT ACTIVITY OF ROSEMARY EXCTRACTS USING TWO DIFFERENT SOLVENTS: EDIBLE ALCOHOL AND ABSOLUTE ALCOHOL

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Rosemary (*Rosmarinus officinalis* L.) is an aromatic evergreen shrub. It is native to the Mediterranean and Asia and its fresh or dried leaves have a bitter, astringent taste and a characteristic aroma. Rosemary extract has been reported to have antioxidant, anti-inflammatory, antidiabetic and anticancer properties and contains many phenolic diterpene antioxidants (carnosol, carnosic acid, rosmadial, rosmanol, epirosmanol, and methyl carnosate). The present work was conducted aiming to evaluate the effect of different solvent extracts on the antioxidant activity of *Rosmarinus officinalis* leaves. Two solvents were chosen for the study namely: edible alcohol (40% ethanol – 60% water) and absolute ethanol (98%).The study started with the extraction procedures which is an important step in the processing of the bioactive constituents from plant materials. Three methods, the Folin-Ciocalteu (F-C), Ferric Ion Reducing Antioxidant Power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl Radical (DPPH•) assays were compared to determine antioxidant activity in Rosemary extract. Each method has variable sublimation and utilization of free radicals. Moreover, solubility, reagent pH and light also effect free radical utilization and formation of product. The FRAP assay is preferred, being more robust than the DPPH assay and giving a better indication of the concentration of potentially oxidizable polyphenols than the Folin-Ciocalteu method. Critical parameters seem to be temperature and time of extract, assisted arrangements, and time and temperature of evaporation procedure: the temperature was found to be the most prominent factor affecting the efficiency of extraction procedure. Future research should be addressed on the application of using *Rosmarinus officinalis* L. leaves as natural food preservative and to protect against peroxidative damage in living systems related to aging and carcinogenesis.

ANTIOXIDANT ACTIVITY AND CYTOTOXICITY IN *HORTAEA WERNECKII* GROWN UNDER SALT STRESS

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Hortaea werneckii is a worldwide black fungus present both in terrestrial and aquatic environments and responsible of a superficial cutaneous disease called Tinea Nigra. From a physiological point of view this fungus present peculiar characteristics of halotolerance with ability to grow in a wide range of salinity. For his rapid growth in culture conditions and yeast-like form, this fungus was proposed as model organism to study the halotolerance. In order to evaluate the effect of salinity stress on physiological mechanisms of adaptation of *Hortaea werneckii*, a strain isolated from seawater samples taken at 2500m depth in the Mediterranean sea was grown in liquid media at different salt concentrations from 0% up to 25%. After 3 days and 7 days of incubation at 25°C the number of viable cells were evaluated by Trypan Blue method, Neutral Red retention assay, and cultural analysis. Moreover, the presence of Radical Oxygen Species was also investigated by staining with fluorescent dye. Results demonstrated that the halophilic marine black yeast *Hortaea werneckii* was able to grow from 0% up to 25% NaCl. The salinity stress did not affect the viability of the cells up the salt concentration of 25%. Intracellular ROS increased with increasing of salt concentration, even though a peak of relative fluorescence was detected at 15% of NaCl concentration after 7 days. This is the first report on detection of intracellular ROS by fluorescence microscopy in the black yeast *H. werneckii* and thereby suggesting the applicability of this method in the study of *in vivo* generation of ROS in black yeast under stress condition.

MELATONIN INHIBITS INFLAMMATORY RESPONSE OF INTESTINAL EPITHELIAL CELLS

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Melatonin is the main secretory product of the pineal gland but it is produced also in gastrointestinal tract. In lumen

gut it reaches concentrations that can exceed the plasma levels by 10-100 times, but its local physiological role is poorly characterized. Melatonin, intraperitoneally-administered at high dose, has inhibitory effects in experimental models of intestinal inflammation, reducing mucosal inflammatory cells activation. However, the protective activity of melatonin in intestinal inflammation may depend also on a direct action of this indolamine on epithelial inflammatory response. The aim of this study was to evaluate in an *in vitro* model of inflamed intestinal epithelium the potential protective effects of melatonin. Differentiated monolayers of intestinal epithelial Caco-2 cells, in which the inflammatory response was induced by interleukin-1β and interferon-γ, were exposed at concentrations of melatonin in the range from 1 nM to 50 M. We also exposed differentiated monolayers to melatonin in presence of luzindole, an antagonist of melatonin membrane receptors, to determine whether or not potential effect of melatonin involve membrane receptors. Our results clearly show for the first time that melatonin decreased the release and the expression of some inflammatory mediators, including nitric oxide, interleukin-6, interleukin-8, cyclooxygenase-2 and that these effects are associated with a reduced activation of the nuclear factor-kB. Moreover, luzindole did not reverse the melatonin inhibition of stimulated- IL-6 release, indicating that the melatonin protective effect may be membrane receptor-independent. Our findings suggest that the assumption of pharmaceutical preparation of melatonin can also exert beneficial effects to gastrointestinal physiology.

ANTINFLAMMATORY EFFECTS OF GREEN *COFFEA ROBUSTA* L. ON RAT SKIN. MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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Coffea robusta L. (Rubiaceae) is cultivated in the eastern hemisphere. Coffee beans contain a large amount of compounds by beneficial activity such as phenols, diterpenes, xanthine and vitamin precursors [1, 2]. Anti-inflammatory and antioxidant activities of *C. robusta* L. have been widely demonstrated [3-5]. Aim of this study has been evaluate anti-inflammatory activity of green beans of *C. robusta* L. methanolic extract, added to vaseline to form an ointment, in a murine model (foot oedema test carrageenan induced). The specimens have been divided into groups: normal; oedema induced from 1h to 5h; oedema treatment by ointment containing green coffee beans extract (7%, 21%). Specimen from paw skin have been obtained and serial sections have been treated according to routinary protocols for light microscopy and immunohistochemistry. In carrageenin paw oedema dendritic, langerin and S100 positive cells, have been identified. It is widely known that DCs, important in immune response, supply epithelial cells to repair damaged tissue [6]. In specimens after application of ointment (green coffee beans methanolic extract) there was a clear oedema inhibition by 3h until the end of the experiment. These cells tend to disappear during treatment by green coffee beans extract. Moreover, fibroblasts -SMA expressing, to prove the tissue re-epithelialization process, have been shown. Fibroblasts, in the wound healing process, have been founded express -SMA [7]. The results suggest the beneficial activity of green coffee extract, in the treatment of inflammatory conditions of the skin.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF TOLL-LIKE RECEPTOR 2 AND S100 IN EPIDERMAL DENDRITIC CELLS OF MUDSKIPPER, *PERIOPHTHALMODON SCHLOSSERI*

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Mudskipper, *Periophthalmodon schlosseri* (Pallas, 1770), is euryhaline, amphibious, and air-breathing fish. These fishes live in close association to mangrove forests and often spend over 90% of time out of water, in adjacent mudflats. They are physiologically adapted to terrestrial life. Mudskipper's epidermis, consists by three layers: the outermost layer presents polygonal cells or rather flattened cells, in the middle layer there are voluminous cell with a large vacuole so-called swollen cells, that are a modification of epidermal cells for cutaneous air respiration; [1] and the stratum germinativum consists of a single layer of cuboidal or columnar cells [2]. The skin is the primary interface between the body and the environment, and has a central role in host defence. The initiation of immune responses to antigens in the vertebrate skin has often been attributed to epidermal Langerhans'cells (LC), that are dendritic cells (DC), antigen-presenting cells(APC) which reside in the epidermis [3]. A few previous studies showed the presence of DCs in fish [4]. In this study, we identified for the first time a specific DC-like subpopulation in *P.schlosseri*'s epidermis; the presence of these cells was demonstrated by immunohistochemical staining for the anti-TLR2 antibody and anti-S-100 antibody, which is considered a marker of Langerhans cells. These cells presented a polyhedral shape with long cytoplasmic processes extending among swollen cells, forming a delicate network in the middle layer epidermis, similar to Langerhans cells present in mammalian skin.

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ANTIMICROBIAL POTENTIAL OF BACTERIAL BIOACTIVE MOLECULES AGAINST PATHOGENS WITH RELEVANCE FOR AQUACULTURE

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A mucus sample obtained from an Antarctic sponge and a total of 98 bacterial isolates of different origin (32, 66 mesophilic and psychrophilic isolates, respectively) were randomly selected to perform a screening for bioactive molecule production, with particular regard to the antibacterial activity against bacterial pathogens with relevance for aquaculture. By the cross-streak method, about the 30% of screened isolates showed antibacterial activity against at least one target, and were successively subjected to the overlay test. Mesophilic strains generally resulted active exclusively against the target *Photobacterium damselae* subsp. *piscicida,* whereas thermophilic and psychrophilic strains showed a broader antibacterial activity. In a second step bacterial supernatants (pure and concentrated), obtained after incubation at different growth conditions, were screened for antibacterial activity, and in the case of a positive response the biomolecules were extracted. Among bacteria of polar origin, the concentrated supernatants of *Salinibacterium* sp. C3W3 and C2W9 resulted active against *Phobacterium damselae* subsp. *piscicida* after growth in presence of sucrose (10±0.0 mm). The same target was inhibited by concentrated supernatant of the mesophilic *Bacillus* sp. PS62 after growth in presence of sucrose and glucose (12±0.0 mm). *Bacillus* sp. PS62 produced a maximum extract amount of 20 mg/ml. The sponge mucus provided an extract that was active against *V. anguillarum*, *A. salmonicida*, and *P. damselae piscicida* (10, 15 and 20 mm, respectively). Our results suggest that bacteria could represent an optimal source of compounds active against bacterial pathogens relevant in the aquaculture field.

CULTURE CONDITIONS MODULATE LIPID METABOLISM AND ESSENTIAL FATTY ACIDS BIOSYNTHESIS IN THE MICROALGAE *PHAEODACTYLUM TRICORNUTUM*: POTENTIAL UTILIZATION IN NUTRACEUTICS

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Microalgae are receiving growing attention, due to its ability to produce many interesting bioactive compounds, such as fatty acids and antioxidants, useful for applications in many industrial sectors: pharmaceutic, nutraceutic, cosmetic and energetic. In relation to the culture conditions, nutrient availability and environmental parameters, the microalgae are able to modulate its metabolic machinery and to change the quantity and quality of some bioactive compounds, such as lipids and classes of fatty acids. The aim of this study was to evaluate the effects of changes in culture's environmental conditions, in the quali-quantitative modulation of the lipid accumulation, in the marine diatom *Phaeodactylum tricornutum*. The obtained results demonstrated that cells, maintained under stress condition, through nitrogen starvation, stimulate some biomolecular markers involved in lipid metabolism and produce a significantly higher amount of total lipids, maintaining also high levels of polyunsaturated fatty acids of the omega-3 series (omega-3 PUFA), that represent markers of lipid quality. The obtained results, therefore, seem to suggest that, as is well known, in studies from other microalgae, it is possible to modulate the lipid accumulation in *P. tricornutum* at different nutrient's concentrations to improve the quantity and/or the quality of the produced bioactive molecule.

BIOCHEMICAL MARKERS INVOLVED IN CELLULAR RESPONSE TO SUBLETHAL CONCENTRATIONS OF FLAME RETARDANTS: PRELIMINARY *IN VITRO* RESULTS

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The use of flame retardants (FR), to reduce polymers combustibility and smoke production, became a pivotal part of the development and application of new materials. Because of their chemical characteristics, such as high lipids solubility, resistance to degradation and persistence, FR are widely spread in the environment. Among FR, the class of Polybrominated diphenyl ethers (PBDEs) is often detected in the environment, wildlife and humans, representing a potential threat for the ecosystem and health. PBDEs can cause neurotoxicity, hepatotoxicity, endocrine disruption, cancer. However, data on the mechanisms of actions and induction of toxicity remain largely unknown. Most researches focused on the potential of these compounds in promoting oxidative stress. Our study focused on the identification of the mechanisms that, starting from oxidative stress, can promote variation on cell homeostasis, energy metabolism, cell cycle, cell damage, and proliferative events, *in vitro*. Preliminary results show that, at sub-lethal doses, different mix of PBDEs are able to induce oxidative stress and the modulation of some molecular markers involved in metabolic switch, stress, cell cycle and proliferation.

MUCUS AND BACTERIAL MICROBIOTA ASSOCIATED TO THE SKIN OF EXPERIMENTALLY ULCERED GILTHEAD SEABREAM (*SPARUS AURATA*)

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Interest in fish skin immunity and its associated microbiota has greatly increased among immunologists. The abundance of terminal carbohydrates, several enzymes (protease, antiprotease, peroxidase, lysozyme) and total immunologulin M levels were evaluated in skin mucus of experimentally ulcered gilthead seabream (*Sparus aurata* L.). Present results evidenced significantly decreases of terminal abundance of α-D-mannose, α-D-glucose and N-acetyl-galactosamine in skin mucus of ulcered fish, respect to control fish (non-ulcered). Furthermore, the levels of IgM and all the tested enzymes in mucus were decreased in ulcered fish (respect to control fish) although the observed decreases were only statistically significant for proteases and antiproteases. On the other hand, differences in the microbiota of non-ulcered and ulcered skin of *S. aurata* have been demonstrated. These changes were associated in the case of ulcered skin with increases of the level of genera which include species involved in skin lesions and reductions of the levels of microorganisms considered like beneficious such as those included in *Lactobacillus* genus. The results obtained in this study could be applied to design future prophylactic strategies such as use of probiotics to improve the skin mucosal system.

EFFECT OF *JASONIA GLUTINOSA* (ROCK TEA) ON *SPARUS AURATA* L. IMMUNE SYSTEM

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Jasonia glutinosa is a traditional medicinal plant belonging to the Asteraceae family commonly known as rock tea (RT), to which anti-inflammatory properties have been attributed. Therefore it is of growing interest to investigate it for new compounds with antioxidant, immune-stimulatory and anti-inflammatory properties for aquaculture. The aim of this study was to investigate the effects of RT on some immune parameters of the gilthead seabream. Thirty specimens were randomly assigned and divided in six tanks (n=5). Fish in each aquarium (two replicates) received one of the following experimental diets: 0 (control), 10% and 30% RT supplemented diet for a month. After 15 and 30 days of trial, head kidney leucocytes (HKLs) and serum were obtained. HKLs were used to determine the phagocytic, respiratory burst and peroxidase activities, and serum samples were used to evaluate the peroxidase activity and IgM levels. Our results demonstrated that fish fed 10 and 30% RT supplemented diets showed a significant increase in phagocytic ability and capacity at 15 but not at 30 days. Respiratory burst was increased in fish taken the 10% of RT supplemented diet for 30 days. Serum IgM levels were increased in fish taken the highest dose of RT for 30 days. No significant differences where observed in peroxidase activity (both in serum and leucocytes). These results suggested that dietary RT administration has an immunostimulant effect on some immune parameters of gilthead seabream at 15 days, but most of them disappear after 30 days of administration.

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EFFECTS OF DIETARY *WEISSELLA CIBARIA* ISOLATED FROM GUPPY *POECILIA RETICULATA* (CYPRINODONTIFORMES: POECILIDAE) AS PROBIOTIC ON SURVIVAL, GROWTH PERFORMANCE AND EXPRESSION OF GENE IGF II

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Guppy is a popular ornamental fish around the world, and in recent years, its culture has increased due to the high demand and the pressure on wild type of this fish. This study was conducted to investigate the effects of *Weissella cibaria*, a lactic acid producing bacteria with probiotic properties isolated from digestive tract of Guppy before, on growth performance and survival of this fish. To this end, 4 experimental groups were considered: group1 as control (only food), group2 (food and 10^6 CFU/g bacteria), group3 (food and 10^7 CFU/g bacteria), and group 4 (food and 10^8 CFU/g bacteria). After 8 weeks trial, growth parameters, survival and expression of IGF II were studied. Significant differences in growth parameters and survival rate were observed in all the groups fed with probiotic bacteria compared to control group ($P<0.05$), and the best results for final weight, BWI, FCR and SGR and the highest survival rate were related to group 2. In the study of gene expression, group 4 showed significant up-regulation ($P<0.05$), but no significant differences between other groups were observed ($P>0.05$). According to these results, *Weissella cibaria* could be recommended as an appropriate probiotic in the culture of Guppy. Furthermore, as finding an environment-friendly replacement for antibiotics has been the main topic of many studies in recent years, this bacteria could be applied as a food additive for other freshwater fish species, too.

PCB-OXYDIZING BACTERIA FROM THE FRESHWATER SPONGE *SPONGILLA LACUSTRIS* (LINNAEUS, 1759) (PASVIK RIVER, ARCTIC NORWAY)

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The Pasvik River is one of the most important rivers in Northern Fennoscandia. Emission Lake Inari (Finland) marks the border between Norway and Russia and flows into the Barents Sea. This has significant anthropogenic impact due to the factories and the strong presence of mining activities on the Russian coast, which involve waste and waste emissions. In the framework of the multidisciplinary SpongePOP project (EU INTERACT - July 2014), this study aims at characterizing the bacterial community associated with the sponge *Spongilla lacustris* (Linnaeus, 1759), as well as in sediment next to the specimens, also in relation to the occurrence of polychlorinated biphenyls (PCBs). During a first survey, the concentration of PCBs (by gaschromatographic analysis) and the structure of the bacterial community (by the Ion Torrent sequencing) were determined in both matrices. Obtained results showed a high concentration of PCB008 (2-4 'dichlorobiphenyl) in both matrices. Sediment and sponge samples were then enriched with biphenyl (BP; a PCB precursor) for subsequent bacterial isolation. A total of 72 strains (48 and 24 from sediment and sponges, respectively) were isolated from agar-enriched BP plates. Of these, 39 isolates (16 and 23 from sediment and sponges, respectively) were able to grow in the presence of Aroclor 1242, a mixture of PCBs. The amplification of the bphA gene, involved in the aerobic degradation of the PCB, confirmed the ability to degrade these pollutants in 39 isolates, mainly belonging to the Gammaproteobacteria and Actinobacteria.

A SIMPLE PROPOSAL TO ASSIGN A "*LIKELY SEX*" TO HUMAN SKELETAL UNCERTAIN REMAINS

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About 350 burials dating to the period between the 8th to 17th century were discovered in a wide sepulchral area, under the floor of the Cathedral of San Lorenzo and the neighboring Bishops'Chapel, in Alba (Piedmont) during an archaeological excavations undertaken in 2007-2011. A paleo-anthropological research program set up in order to define the biological profile of ancient human remains. Sex identification was performed according to anthropological literature and it was based on morphological and metric techniques. Individuals of both sexes were found: 104 males, 59 females. Due to fragmentary nature of skeletal remains, 37 adults are "not determinated" regarding sex. The aim of this study was to use a statistical procedure in order to reduce the number of indeterminates. We considered the anthropometric variables such as the lengths of the major long bones and the diameter of the femoral head. We performed a "likely sex" to the individual not determinated, considering the fact by taking account that men are on average taller than women. For every variable considered, largest values belong to male, and smallest values belong to female, most likely. We defined a "sex score" Y such that a "likely male" value scores +1 and a «likely female» value scores -1. Under normal distribution assumption for all the variables have a Gaussian distribution, if the total score is positive, we classify the remain as "likely male"; if it is negative, we classify it as "likely female". According with our method, it was possible to classify the sex-undeterminated remains into 12 "likely female" and 11 "likely male".

ANTIOXIDANTS FROM SEAGRASSES: *IN VITRO* EFFECTS AGAINST OXIDATIVE STRESS AND AGEING

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Marine plants represent a source of complex and unique molecules characterized by strong biological activity, therefore they are valuable allies for man. Peculiar feature of algae and plants is the production of secondary metabolites, such as polyphenols, synthesized as a form of adaptation to environmental stress. *Posidonia oceanica* is a Mediterranean endemic seagrass, fundamental for the marine ecosystem from biological, ecological and geological point of view. The aim of this study was to evaluate, in cell cultures, the antioxidant, photoprotective and antiageing properties of an extract, obtained from the leafs, naturally released from the plants, by the technique of Supercritical fluid extraction (SFE). The obtained results demonstrated that, starting from the straightening of the cellular antioxidant status, the SFE extract has a protective effect against induced oxidative stress and cell damage, as demonstrated by the regulation of some molecular markers related to both events. The sustainable exploitation of the marine biological resources can represent the basis for new applications in pharmacology, nutraceutics and cosmeceutics.

OPTIMAL HOUSING PROTOCOLS FOR THE SMALL-SPOTTED CATSHARK (*SCYLIORHINUS CANICULA*) IN CAPTIVITY

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Optimal housing protocols are the most investigated features in water animals rearing (for both aquaculture and aquaria). In water animals farming (especially for marine ones) the determination of optimal water parameters and feeding behaviours are of fundamental importance. For shark species the high sensitivity to different stressors is at the base of the difficulties encountered during housing of these ani-

mals. Stressed specimens release a great amount of ammonium (NH_4^+), that dissolved in a close system waters could also cause the dead of housed specimens. In our study the housing of small-spotted catshark *Sciliorhinus canicula* has been considered. *S. canicula* is a demersal species (maximum length 100 cm) inhabits muddy or rocky substrates from 10 to 400 m depth; its diet is composed of molluscs, crustaceans and teleost species. Wild specimens have been caught and transferred to C.I.S.S. (University of Messina, Department of Veterinary Sciences) and reared in 500L tanks. Weekly partial (20%) water exchanges were performed. Main water parameters (T, Sal, pH, NH_3) were daily monitored. This study investigates also on bite/bout ethology of the shark; the specimens were filmed using underwater camera (GoProHero4Silver) during feeding with different preys (shrimps, anchovies, squids). The feeding mechanisms resulted to be different depending on different preys proposed. When ammonium concentration was high, the swimming ability was reduced and a decreased interest towards food was noticed, confirming the high sensitivity of small-spotted catshark to this ions concentration. This study aims to create housing protocols of this shark species and to improve the knowledge about the almost unknown characteristics of the fascinating sharks.

AN EVALUATION OF THE NUTRITIONAL VALUE OF THE JELLYFISH *PELAGIA NOCTILUCA*

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Marine organisms represent a huge source of chemical compounds having biological properties. The phylum Cnidaria is one of the most important contributors in providing abundance of bio- and chemodiversity. Factors such as global warming, overfishing and water pollution, have caused a dramatic proliferation of Cnidaria, and in particular of Scyphozoa, indeed becoming an issue of social and economic concern (damages to tourism and aquaculture). For this reason, it seems important to find an alternative strategy for the exploitation of these marine species. In this study, a comprehensive chemical investigation on the nutritional properties of the most abundant jellyfish of the Mediterranean sea, namely *Pelagia noctiluca*, was carried out. The analytical protocol covered energy content, determination of polyphenols, fatty acids, proteins and nitrogen, heavy metals. The results obtained led us to conclude that there is a difference between male and female individuals:a different nutritional value was observed, depending upon tissue analyzed (*i.e.* bell and tentacles) and sex: female bell appears to have a higher nutritional value, probably due to the presence of lipids in oocytes. Proteins and nitrogen were more concentrated in tentacles than in bell, because of their characteristic protein-based structure in *P. noctiluca*. As regards polyphenols and heavy metals, no differences were highlighted that could be ascribed to the variable sex and/or organ. The results of fatty acids did not highlight differences in both sexes and in the two tissues the largest percentage is represented by SFA. Overall, data obtained from this research study suggest *P. noctiluca* as a potential and valuable food source, as it is already considered in the Asian continent.

ANTIOXIDANT AND ANTIAGEING PROPERTIES, *IN VITRO*, OF COFFEE EXTRACTS

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In order to protect their health, more and more people are interested in consuming foods that contain bioactive or functional components. The wastes obtained from food processing in the agri-food industry have a high potential, given by the presence of many valuable ingredients, that can be recycled to be used in various industrial sectors, such as nutraceuticals or pharmaceuticals. Coffee is a drink that is consumed by millions of people every day. Its beneficial effects are attributed to the precious elements contained in it and for this reason the by-products of its transformation can be considered potential functional ingredients for the food industry. Coffee by-products from the agro-industry produce valuable ingredients such as caffeine and polyphenols, important bioactive components at antioxidant action. Antioxidants are able to counteract oxidative stress and related diseases such as aging, inflammation and cancer In recent years, exhausted coffee has been increasingly studied as a precious source of bioactive molecules with antioxidant action, that can be also obtained with green techniques, such as Supercritical fluid extraction SFE. The aim of the study was to evaluate the antioxidant power of extracts, obtained by SFE from exhausted coffee capsules, for cosmeceutical applications, through the monitoring of biochemical markers *in vitro*. Human cell cultures were exposed to a standard protocol able to promote oxidative stress by UV, with or without preliminary exposure to a cream, including antioxidants extracted from coffee. The obtained results demonstrated the protective effects of the treatment with cream, including antioxidants from coffee, in the protection against the photo-aging and the subsequent cellular damage. The results suggest the potential use of exhausted coffee extracts in the cosmeceutics.

NEW ANTIMALARIALS DRUGS ON THE HORIZON?

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The increasing resistance of P. *falciparum* to all know antimalarial drugs, is rapidly emerging. For these reason considerable emphasis is now being focused on development of new therapies with novel mechanism of action. Band 3 is the

major attachment site of the spectrin-based cytoskeleton to the erythrocyte's lipid bilayer and thereby contributes critically to the stability of the red cell membrane. During the intraerythrocytic stage of Plasmodium falciparum's lifecycle, band 3 becomes tyrosine phosphorylated in response to oxidative stress, leading to a decrease in its affinity for the spectrin/actin cytoskeleton and causing global membrane destabilization. We hypothesized that this mechanism is involved in the parasite egress and the consequent dissemination of released merozoites throughout the bloodstream. We explored which tyrosine kinase inhibitors might block the kinase-induced membrane destabilization. We demonstrated that multiple Syk kinase inhibitors prevent parasite induced band 3 tyrosine phosphorylation and inhibited parasite-promoted membrane destabilization, suppressing merozoite egress near the end of the parasite's intraerythrocytic lifecycle causing their death. Syk inhibitors have displayed long-term safety in human clinical trials and constitute a promising class of antimalarial drugs that can suppress parasitemia by inhibiting a host target that cannot be mutated by the parasite to evolve drug resistance.

ADHESION TO A PROTOCOL FOR MICROBIOLOGICAL ASSESSMENT OF CARBAPENEMASE-RESISTANT *KLEBSIELLA PNEUMONIAE* EARLY IDENTIFICATION DURING CROSS-INFECTION DONOR-RECIPIENT IN KIDNEY TRANSPLANTATION

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Donor – derived bacterial infections (about 0.2%) cause severe complications of transplant and bad effects on prognosis. Multi-resistant antibiotics pathogens (MDR), like carbapenemase-resistant *Klebsiella pneumoniae* (KPC), current emergency it increases the risk of transmission. Donor selection (colonized or infected) and diagnostic recognition contribute to the safety of transplantation and to adoption of dedicated therapeutic protocols. In these phases protein chain reaction (PCR) and gel electrophoresis in pulsed field (PFGE) represent the best investigations for epidemiological and clinical studies allowing KPC genotypic identification. PCR, using validated primers for encoding carbapenemase genetic amplification (blaIMP, blaVIM, blaOXA-23, blaOXA-24 and blaOXA-58 blaKPC, blaOXA-48, blaOXA-51 and blaNDM1), allows both bacteriological diagnosis and possible carbapenemase production on the base of TaqMan real-time PCR described by Cole J.M. (2009) and subsequently validated by Centers for Disease Control and Prevention – USA. Genetic correlation between bacteriological isolates is analyzed with PFGE through genomic DNA extraction with Xbal (New England Biolabs Inc., MA, USA), Apal in according Tenover F.C. (1995) description and CHEF DRII (Bio-Rad, Milan, Italy) as already described by A. Marchese (2010). Cross – infection is confirmed when antibiotype and macrorestriction profiles possess 100% of match concordance, with pre - transplant infection absence in recipient. So as, in 2015 at our center we identified three kidney transplanted recipients with donor – derived infection (two sepsis, one urinary tract infection; two survived, one deceased). In agreement with Italian guidelines, the future target will be optimization of diagnostic, preventive and therapeutic timing.

EXPERIMENTAL APPROACH TO THE EFFECT OF AVAILABLE CARBON AS A STRUCTURING FACTOR FOR MICROBIAL POPULATIONS IN THE MEDITERRANEAN DEEP CHLOROPHYLL MAXIMUM

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The deep chlorophyll maximum (DCM) is a marine water-layer that is richer in phytoplankton than other layers, with relatively high rates of inorganic carbon assimilation. It is generally located towards the base of the thermocline in lakes and oceans during the stratification period. The increased carbon availability in this zone, as a consequence of photosynthetic activity, can affect the heterotrophic microbial assemblage (HMA). The aim of this work is to study how changes in depth and abundance of chlorophyll in DCM, and therefore in the available carbon, can affect the structure of the associated HMA. In the framework of the MEDIMAX project, Mediterranean seawater was collected from the vertical profile (up to 2,000 m) off the coast of Alicante, Spain, then used for experiments where the availability of DOC was manipulated. Water for the experiments was collected from a depth of 30 m where the CTD recorded the main DCM. The experimental procedure consisted in a factorial design with three replicates and two studied factors, the first factor was binary (light-darkness) while the second factor was carbon availability and presented 7 levels (1/4x,1/2x, Control without modifications, 2x, 4x, 16x, 32x) with respect to the original concentration. Microbial assemblages of the main bacteria that constitute DCM was determined by FISH, covering total bacteria as well as the most abundant groups of heterotrophic bacteria according to metagenomic determinations (Alpha-proteobacteria, SAR11, Beta-proteobacteria, Gamma-proteobacteria, Actinobacteria, - and specifically the ultra-small *Actinomarinaminuta* -, Bacteroidetes and Firmicutes). Maximum bacterial abundance within the vertical profiles was found at 30 meters, coinciding with the DCM. Experimental results show a significant increase in bacterial abundance associated with increased available carbon as well as changes in population structure.

IDEBENONE LOADED LIPID NANOPARTICLES: EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY AND *IN VIVO* TOPICAL EFFECTS

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Idebenone (IDE), a synthetic analogue of coenzyme CoQ_{10}, is an antioxidant agent that has been proposed for the treatment of skin disorders, including skin ageing. Due to its high lipophilicity, IDE is a poor skin permeants and it could benefits of being incorporated in suitable drug delivery systems to improve its topical bioavailability. Recently, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have emerged as promising carriers for topical drug delivery, owing to their many advantages compared to conventional formulations. The aim of this work was to assess IDE-loaded SLN and NLC *in vitro* antioxidant activity and *in vivo* effects after topical application in human volunteers. These nano-carriers were prepared by the phase inversion temperature method and showed spherical shape, small particle sizes (25-40 nm), polydispersity indices < 0.300 and good stability. *In vitro* antioxidant assays (DPPH and reducing power method) revealed a greater antioxidant activity of IDE-loaded SLN compared to IDE-loaded NLC, at both IDE concentrations investigated (1.0% and 1.5% w/w). *In vitro* experiments on fibroblast cells HS-68 showed that treating cells with IDE-loaded lipid nanoparticles (SLN and NLC), before the exposure to UV light, resulted in a significant protection of cells from oxidative stress.After a two-week topical application of gel vehicles containing IDE loaded SLN or NLC on healthy human volunteers, an increase of skin hydration was observed in comparison with control gel.These results suggest that loading IDE into SLN or NLC could be regarded a promising strategy to improve its topical effectiveness.

THE THERMAL RESPONSE OF A BACTERIAL EXOPOLYSACCHARIDE FROM SHALLOW HYDROTHERMAL VENT ORIGIN

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An exopolysaccharide (EPS1-T14), produced by the marine thermophilic *Bacillus licheniformis* T14, exhibiting interesting structural and bioactive properties with industrial potential, was investigated for its structural changing at increasing temperature. The whole biopolymer and its main monosaccharidic components (fructose, fucose and glucose) were analyzed by using the Attenuated Total Reflectance Infra-Red (ATR-FTIR) spectroscopy, in a spectral range spanning from 400 to 4000 cm^{-1}, at increasing temperature ranging from 20 to 80°C. To monitor the thermal behavior of the whole EPS1-T14 the spectra were analyzed by means of hypsocromic frequency shift and spectral distance whereas the wavelet cross-correlation was used to investigate the role, in the its molecular structure, of its three main component. The intramolecular OH-stretching frequency shift linearly increased by rising of temperature, suggesting that the whole EPS1-T14 system possesses a high stability until 80°C. Spectral wavelet analysis indicated that fucose, rather than fructose and glucose, mainly influenced the whole structure of EPS1-T14. Our results suggest that fucose is mainly involved in the thermal stability of the entire biopolymer, whereas glucose is responsible for its molecular flexibility. Our findings provide novel insights on the thermal stability of EPS1-T14, that could be useful in traditional biotechnological areas, including food, cosmetic and pharmaceutical applications, and novel biomedicine areas that require high-temperature processes.

MOLECULAR EPIDEMIOLOGY OF *CANDIDA* SPECIES IN VULVOVAGINAL INFECTION

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Vulvovaginal candidiasis (VVC) is common infection caused by *Candida* (*C.*) species that affects millions of women in the world every year and has been considered an important health problem. Knowledge of epidemiology and analysis of the spread of these pathogens is important in understanding and controlling their transmission. This study aimed to evaluate the incidence of different *Candida* species isolated from Tunisian women suffering from vulvovaginitis. In order to attain our objective, a total of eighty samples were collected by using vaginal swabs. Each isolate obtained was systematically cultured in Sabouraud dextrose agar and chromogenic agar medium. *Candida* species were initially identified by phenotypic methods like germ tube formation in serum and chlamydospore production on rice tween agar to separate *Candida albicans*/*Candida dubliniensis* from others non-albicans *Candida* species (NAC). Subsequently the species were confirmed by specific molecular PCR-based methods or DNA sequencing. The results indicated the dominance of NAC species (55% of the total) and *C. glabrata* was the most frequently isolated species (30/80; 37.5%) followed by *C. parapsilosis* (14/80; 17.5%) and *C. albicans* (36/80; 45%). In conclusion, the present study showed that NAC species are the main cause of VVC, especially *C. glabrata* and *C. parapsilosis*. This change of species increasing in VVC makes crucial to further investigate the epidemiology of these important human fungal pathogens.

EFFECT OF THE CHALLENGE WITH *VIBRIO ANGUILLARUM* ON MUSCLE CORTISOL CONCENTRATION IN EUROPEAN SEA BASS JUVENILES

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The establishment of an infection in fish involves not only the immune system but also the hypothalamus-hypophysis-interrenal tissue axis (HHI), which is usually triggered by several stressors. The aim of this work was to study the response of HHI consequent to a challenge with the pathogen *Vibrio anguillarum* in European sea bass (*D. labrax*), through the measurement of muscle cortisol concentration. The trial was carried out on 160 E. sea bass juveniles (13.67±0.7 g), divided in 2 groups (n=80): unvaccinated (C) and vaccinated with a commercial formulation against vibriosis (V). Eight weeks post-vaccination,

40 fish of C and 40 fish of V have been infected with a *V. anguillarum* strain. Dead fish and fish surviving the infection of both groups, C and V, were submitted to epiaxial muscle sampling for cortisol measurement by RIA. In spite of individual variability, differences in muscle cortisol content were significant between dead fish and survivors, 431.11±194.97 *vs* 15.55±14.98, (P<0,01), regardless the vaccination treatment. The values recorded in fish surviving to the infection are consistent with those reported by Bertotto *et al.* (2010), for healthy sea bass. On the contrary, infected dead fish showed a 28-fold concentration increase of cortisol. Therefore, it seems that in E. sea bass, an uneffective and non protective immune response is associated with a strong response of HHI in order to cope with the stressor. Such high values of muscle cortisol suggest an exhaustion of energy and protein stocks contributing to the negative outcome of the infection.

COEXPOSURE TO SULFAMETHOXAZOLE AND CADMIUM IMPAIRS DEVELOPMENT AND ATTENUATES TRANSCRIPTIONAL RESPONSE IN SEA URCHIN EMBRYO

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Sulfamethoxazole represents one of the most widely employed antibiotics. A considerable amount of sulfamethoxazole is introduced into the marine environment after utilization in aquaculture and its cytotoxicity is associated with the production of reactive oxygen species. Similarly, cadmium represents a metal largely employed in several anthropic activities which irreversibly accumulates into cells. In order to unveil the mechanisms of response to sulfamethoxazole and cadmium coexposure, *Paracentrotus. lividus* sea urchin embryos were used as model organisms. During development, embryos were exposed to sulfamethoxazole amount comparable to that usually used in aquaculture procedures and/or sublethal levels of cadmium chloride. Impairment of development and gene expression alteration of key factors in inflammation, detoxification, metal scavenging and cell death were inspected. Even though treatment with sulfamethoxazole apparently did not affect development, it stimulated a remarkable molecular response to oxidative stress. Simultaneous exposure deeply compromised developmental events and the defence mechanisms to cadmium exposure were blocked. Thus coexposure to sulfamethoxazole and cadmium induces neutralizing effects on sea urchin embryos.

LEUKAEMIAS, LYMPHOMAS, LYMPHO-MYELODISPLASIC AND PROLIFERATIVE SYNDROMES AND ELECTROMAGNETIC FIELDS

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In 1979 Wertheimer and Leeper revealed an increase of childhood leukaemia in populations living in Electromagnetic fields (EMF) exposed areas. Three years later they discovered an increase of adult leukaemias too. In 1990-2000 the reports became frequent and precise. Case-control studies performed in New Zealand also gave confirmation. The analysis of working expositions showed consistent and statistically significant (SS) increase of leukaemia in electric welders (OR=2.8) and electric lines employees (OR=5.8). The risk excess concerned mainly non-lymphocitic acute leukaemia (OR=2.3) and lymphoblastic acute leukaemia (not SS). On the whole, by analyzing the increase of occupational exposition as a function of work length and EMF intensity, dose-response relationship for acute leukaemia resulted statistically significant. SS increase of the incidence of leukaemias, lymphomas and acute/chronic lympho-myelodisplasic and proliferative syndromes have been repeatedly documented in adult males exposed both in living and working environments (train drivers and railway workers, electric welders and electric workers) to relatively modest EMF values (μT). Ionizing EMF radiations are well correlated to malignant brain tumours, for which the sensitivity to induction decreases with age (RR from 3.56 to 0.47; P=0.037). In contrast, benign meningiomas showing high and steady sensitivity in adults. In adults many reports of brain tumours associated to residential and occupational exposure are known. An increase of some tumours types, mainly leukaemias, and a more than triplicate risk of mortality due to SNC (SMR=3.7) and brain tumours (SMR=3,94) was observed among people living near high voltage electric lines to quite modest EMF values (0.34-0.44 μT). The increase of other malignancies such as melanomas, prostate, kidney, thyroid, digestive and pancreas tumours was also reported among people living in occupational and residential areas in which EMF levels were similar to those able to induce a SS doubling of childhood leukaemias (<1 μT).

COLLAGEN STRUCTURE IN DEPENDENCE ON HYDRATION CONDITIONS STUDIED BY MOLECULAR DYNAMICS AND INFRARED SPECTROSCOPY TECHNIQUES

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Collagen is a fibrous protein representing the main constituent of connective tissue in mammals. The basic structural unit of collagen is tropocollagen formed by 3 intertwined left-handed helices assembled in a triple right-handed helix. The amino-acid sequences of the helices are characterized by the repetition of triplets Gly-Xaa-Yaa, being one-thirds of the X and Y residues either prolines or hydroxyprolines (Hyp). Collagen triple helices associate in fibrils, and the union of the fibrils forms the characteristic fibres. Water molecules play an important role in maintaining the native conformation of collagen and dehydration is the main cause of tissue aging. In this study the role of collagen hydration was investigated by coupling molecular dynamics and infrared spectroscopy techniques. Two tropocollagen fragments with different hydropathy profiles were studied in three different hydration conditions with MD simulations. These fragments were built from Rattus norvegicus type I collagen sequence. In the same conditions, the aggregation of two fragments was studied. Type I collagen from rat tail tendon was contemporarily studied by IR measurements along a dehydration and rehydration treat-

ments. A collagen hydration model is proposed: at low hydration levels (aw=0.06-0.58), water is structured around charged regions, leaving exposed both hydrophobic and polar areas. This behaviour suggests the pseudo-fibrillar aggregation mechanism, showing that the aminoacidic composition of the two triple helix strongly influences their association propensity. Dehydration treatment does not induce collagen denaturation, but produces an irreversible elongation of the helix, which is revealed as an increase of IR b-sheet conformation percentage amount.

BIO-PSYCHO-SOCIAL ANALYSIS OF THE EFFECT OF SUPERJUMP TRAINING

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The purpose of this research was to evaluate how Superjump training can influence body composition, blood parameters and psychological aspects. Fourteen adult women healthy volunteers were recruited in this study (m±DS: age 29±9.50, weight 63.7±12.38, height 1.59±0.06, body mass index 25±3.67, fat mass% 20±5.80) and they performed a training protocol lasted 16 weeks, twice a week, 60 minutes per session. We analyzed body composition (body mass index, circumferences, fat mass), blood parameters (red blood cells, hemoglobin (HG), hematocrit, mean corpuscolor value (MCV), platelets, white blood cells, ALTs and ASTs, serum, total protidemy, creatinine, glycemia, triglycerides, total cholesterol, ferritin) and we administered two questionnaires about body self-esteem and behavioural regulation in exercise. Comparison of pre- and post-exercise results shows an improvement in anthropometric and hematochemical parameters. Statistically significant were: waist circumference (0.0001), tricep skinfold (0.0002), abdominal skinfold (0.005) and fat mass (0.007) as regards body composition; blood glucose (0.0004), total cholesterol (0.02), ALT (0.05) and AST (0.005) and basophil (0.06) concerning hematochemical evaluations. A positive outcome was also found in psychological assessment and in particular in self-esteem, since the increase of the latter has positively influenced the perception of self image. The results clearly demonstrated that Superjump is a workout that can improve the individual's bio-psycho-social aspects. It creates a positive psychological reinforcement, crucial to motivating to continue physical activity over time.

TRANSCRANIAL ALTERNATING CURRENT STIMULATION AND SPORT PERFORMANCE: AN EXPLORATIVE STUDY OF THE ASSOCIATION WITH THE GENETIC BACKGROUND

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In recent years transcranial alternating current stimulation (tACS) has emerged in clinical neuroscience. It consists in application of weak electrical currents on the head for several minutes (5-30'). The purpose of the study was to evaluate the effects of tACS stimulation on the sports performance investigating explosive strength of upper and lower limbs. We considered genetic background of participants, in particular ACE and BDNF polymorphisms. ACE gene encodes the enzyme of human angiotensin conversion and its association is with the resistance, while BDNF gene, coding for brain derived neutrophic factor, is associated with psychological attitude in Sport. In the research were involved 17 volunteers (7 males and 10 females) aged between 18 and 49 years (27.8±10.4) that were stimulated (treated group) or not (control group) with tACS for 10 minutes at 50 Hz. Before the stimulation it was collected a sample of saliva in order to extract genomic DNA for the analysis of the genotype by PCR technique. Explosive strenght has been evaluated with a set of tests before and after the tACS stimulation. ANOVA statistical analysis was performed and the results showed a significant negative effect of ID genotype of ACE gene ($p<0.05$) on the performance. Particularly, on squat jump, counter movement jump and counter movement jump arm swing, while BDNF GG genotype seems to be associated with a better performance only in counter movement jump arm swing. Further investigations need to better understand if the stimulation can be considered as a doping practice improving the performance.

THE ROLE OF POLYMORPHISM OF TIOPURINE METHYLTRANSFERASE IN THERAPY WITH AZATHIOPRINE. PRELIMINARY STUDY

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Azathioprine is an immunosuppressive medication used in the treatment of inflammatory diseases (MICI). Despite its extensive use in therapy, azathioprine can result in serious side effects such as myelosuppression. The likelihood of developing myelosuppression also depends on genetic factors: one of the enzymes involved in drug metabolism, methyltransferase tiopurine (TPMT), is subject to genomic polymorphism. To date, 40 polymorphisms have been identified, of which three are associated with a reduction in enzymatic activity. These are TPMT*2, TPMT*3A and TPMT*3C polymorphisms. TPMT*1 is the wild-type coding form for an enzyme capable of efficiently metabolizing the drug. The objective of the study was to determine the frequency of the three polymorphisms in a sample undergoing a TPMT genotype assay at the Molecular Laboratory of the "V. Cervello" hospital in Palermo. The test is carried out using DNA extraction procedures, PCRs, agarose gel electrophoresis and enzymatic digestion. From the analysis of 208 patients undergoing the test from 2011 to 2016, it was found that: 95.67% of patients did not have polymorphism; 2.40% had polymorphism *3A, 1.93% had polymorphism *3C. Therefore, 95.67% of patients may be exposed to azathioprine therapy without incurring side effects. Due to a reduced enzymatic activity, the remaining patients are able to be treated with azathioprine, provided that a lower dosage standard is used or is treated with another immunosuppressive drug.

ALIEN SPECIES: FRIEND OR ENEMY OF ENVIRONMENTAL HEALTH?

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Invasive alien species (IAS) can influence whole ecosystems altering habitats and local biodiversity, modifying the marine activities (including fishing and tourism) and introducing diseases that threaten human health. However, they may have positive impacts on the composition of local fauna and biotic interactions. The record and the spread monitoring in the Mediterranean Sea of the invasive aorid amphipod crustacean *Grandidierella bonnieroides* is an example of positive effect on local biodiversity. In 2014, the species was recorded with explosive abundance along Haifa Bay, in the north of Israeli coast, on soft bottom. Over the last two years, this species has spread throughout the Israeli coast, probably by ship fouling or ballast sediments, showing constant abundance so that it is possible considered established along the Israeli coast and part of the local fauna. The presence of *G. bonnieroides* did not induce a change on assessment of autochthonous species but only an increasing of local biodiversity. Soft bottom biodiversity is commonly lower than hard bottom; so, the new colonization of *G. bonnieroides* as possible read as an increasing of food available for the local fauna that feeding on macrozoobenthos. Finally, considering that this species has a short turn over and large range of tolerance (salinity, temperature and substrate) is a good candidate for food in aquaculture. This study showed that knowledge of invasive species is still underestimated and that, consequently, the direct or indirect introduction of alien species by human activities can bring benefits to local scale and be used as new resource for marine culture.

ASSESSMENT OF HEAVY METAL CONTAMINATION IN TERRACES FOR PUBLIC USE RECOVERED IN THE CITY OF PALERMO AND IN AGRICULTURAL TERRACES

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The contamination by heavy metals in the soil is nowadays considered an indicator of environmental pollution caused by to several factors, such as waste disposal, industrial discharges and the use of irrigation water. Developed in collaboration with *SO.GEST* Ambiente Laboratory, in Palermo, this study had as a purpose to monitor the levels of heavy metals - Cd, Cr, Fe, Mn, Pb, Zn -both in public lands and in agricultural terrains. All the soil samples collected have been subjected to an acid extraction process by using HCL and eventually to a mass spectrometer-based analysis for the quantitative/qualitative determination, specifically by the technique of flame atomic absorption spectrophotometer (FAAS) and graphite furnace (GFAAS). From the elaboration of the results, it emerged that all the soils in the city of Palermo feature heavy metals, with the predominance of Cd, Cr and Zn, respectively in the percentages of 5.69%, 64.06%, 1.40% on 60 soil samples analyzed, what is surely due to vehicular traffic, the use of irrigation water and also the use of fertilizers. Lead (Pb), instead, has not been detected in all public grounds, probably due to the switch-over to the lead-free gasoline, which has reduced the inflow of this metal. Lastly, the confrontation between the two types of soil showed a higher rate of contamination by heavy metals in the agricultural grounds, thus demonstrating how today the use of plant protection products has gained the upper hand, in order to increase agricultural production. In particular, the percentages found are the following: Cd 6. 37% Cr 210.06%, Fe 20.42, Mn 9.35%, Pb 125.1%, Zn 2.04 on 60 samples of soil. Although all metal concentrations have fallen within the limit values of the law, this will not exclude the fact that they are present into the soil and that even though in minimal concentration they are toxic to the man by contact, by ingestion or by respiratory action.

CONTAMINATION CONTROL OF AFLATOXINS IN MILK INTENDED FOR HUMAN CONSUMPTION: COMPARISON OF DATA OBTAINED BETWEEN 2014 AND 2017

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Aflatoxin M1 (AFM_1) is the hydroxylated metabolite on the hepatic level of aflatoxin B1 (AFB_1), found in milk when the animal is lactating after ingestion of zootechnical feeds contaminated with aflatoxin B1. Exposed with the present work is a study conducted at the laboratory So.gest s.r.l of Palermo, which lasted more than three years, on the presence of Mycotoxins in bovine milk which provenance is from farms in the Sicilian area of Catania, Enna and Caltanissetta in order to determine whether during this period some changes in concentration of aflatoxin M_1 (AFM) have occurred and access if the presence of the toxin is always within the legal limits. The analyzes were conducted with ELISA method on samples of milk just milked, and stored in the refrigerator of the laboratory some samples at 3°C±1°C. The study was carried out on the product to quantify Mycotoxins and ensure compliance with the limit imposed by EC Regulation 1881/2006, published in the EU Official Journal on 20.12.2006. The EC Regulation 1881/2006 establishes for aflatoxin M_1 (AFM_1) a maximum limit tolerated (LM) of 0.050 µg/kg (50 ppt) for milk and 0.025 µg/kg (25 ppt) for the milk intended for the first childhood. The analyses carried out in these four years have revealed that in all the samples the presence of AFM_1 has been found. In this last period, only one sample was found to have a mycotoxin concentration above the limit required by current regulations.

AQUACULTURE, IS EVERYTHING ALL RIGHT?

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Nearly half of the fish consumed all over the world comes from intensive sea fish fars, and the percentage is going to rise more and more. Criticalities are known: overexploitation of lesser value fish stocks, which become flour for most valuable species; genetic contamination of free range fish; con-

centration of bacteria and parasites and consequent dispersion of antibiotics and antifungal agents; high levels of fecal production remaining in water; all known but underestimated factors. The possible consequences for human health are poorly investigated, and there is almost no sample analysis of the water and the seabed underneath the cages. In addition, with the growth of fish farming, fishing opportunities will diminish, while fish farms need a few workers. The fish in the cages are crammed and cannot almost move, they are stressed and aggressions between animals are frequent; mortality is very high. Killing methods of normal use lead to a slow agony. In Italy, overall production is around 140,000 tonnes per year while imports are 80%; but recently the EU has called on the Italian government to increase the number of fish farms. With the exception of molluscs, 50,000 tonnes of fish per year are produced, which disperse 1.5 million tonnes of stools into the water. The European requests should be answered taking into account that, in addition to the above general criticalities, fish farms could, because of their characteristics, adversely affect see tourism, which is an important asset for our country.

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