

# Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale



**91<sup>st</sup> SIBS Congress on the role of the  
Italian Society for Experimental Biology  
in the Italian research**

**Ancona, Italy, 9-10 November 2018**

ABSTRACT BOOK

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Bollettino della Società Italiana di Biologia Sperimentale

eISSN 2284-0230

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## **TABLE OF CONTENTS**

Invited lectures .....	1
Oral Communications .....	3
Posters .....	9
Index of authors .....	15

## INVITED LECTURES

**HOW DID TRANSPOSABLE ELEMENTS IMPACT HUMAN DIFFERENTIATION AND EVOLUTION? THE CASE OF NON-LTR RETROTRANSPOSONS**

Alessio Boattini<sup>1\*</sup>, Etienne Guichard<sup>1</sup>, Valentina Peona<sup>1,2</sup>, Guidantonio Malagoli-Tagliacozzi<sup>3</sup>, Lucia Abitante<sup>1</sup>, Evelyn Jagoda<sup>4</sup>, Margherita Musella<sup>1</sup>, Marco Ricci<sup>1,2</sup>, Alejandro Rubio-Roldán<sup>5</sup>, Stefania Sarno<sup>1</sup>, Donata Luiselli<sup>1</sup>, Davide Pettener<sup>1</sup>, Cristian Taccioli<sup>6</sup>, Luca Pagani<sup>7,8</sup>, Jose Luis Garcia-Perez<sup>5,9</sup>

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Transposable Elements (TEs) are biologically important components of eukaryote genomes and their activity generated at least 46 % of the human genome. In Anatomically Modern Humans (AMH), only some non-LTR retrotransposons have recently been active. These elements often contain internal promoters, Transcription Factor Binding Sites and polyadenylation signals. They can drive adjacent gene expression, produce alternative transcripts for existing genes and contribute to the generation of new genes and pseudogenes. These characteristics make them one of the primary sources for genomic mutations and variability. This study is aimed at the identification of the role of such retrotransposons in the differentiation and evolution of the genus *Homo*, by comparing insertions in the genome of AMH (GRCh37-hg19) with those of our closest extinct relatives, Neanderthals and Denisovans, as well as with chimpanzees. We analyzed reference sequences of AMH and chimps and developed an in silico methodology for identifying species-specific insertions using ancient DNA sequencing data. These procedures led to the identification and confirmation of a large number of modern-, archaic- and chimp-specific insertions, the genomic loci of which have been characterized according to different approaches: variability in human populations, genetic and epigenetic features, selective pressures and regulatory/expressional alteration inference. Our results suggest that the activity of non-LTR retrotransposons throughout *Homo* evolution might have played an important role in AMH differentiation, generating new variability, influencing recent selective events and possibly affecting the human brain development. Further research on this topic can help Molecular Anthropologists and Evolutionary Biologists to better understand functional evolution of the AMH genome, while also opening new possibilities for studies in genomics and evolutionary dynamics. Guichard *et al.* (2018). *Impact of non-LTR retrotransposons in the differentiation and evolution of Anatomically Modern Humans*. *Mobile DNA*, 9:28.

**INTERACTIONS BETWEEN DIETARY POLYPHENOLS AND RUMEN MICROBIOTA**

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Plant polyphenols are largely present in the plant kingdom and, as a consequence, in the diet of ruminants. Polyphenols may interact with rumen microbiota, which is composed by the whole consortium of rumen microorganisms. The interaction between dietary polyphenols and rumen microbiota may result in changes of carbohydrates fermentation, protein degradation and lipid metabolism. Advancing in the analysis of rumen microbiota allowed to increase the knowledge about the microbiota composition as affected by dietary factors. In the present review, the effect of plant polyphenols on rumen microbiota responsible of unsaturated fatty acids biohydrogenation, fibre digestion and methane production was discussed. Overall, dietary polyphenols are able to perturb rumen microbiota composition, depressing or modulating the biohydrogenation of dietary unsaturated fatty acids. Large part of the literature is dedicated to the effect of a specific class of polyphenols: tannins. The effect of tannins on rumen microbiota largely depends to the nature of tannins and to the amount of tannins included in the diet. Condensed tannins have an inhibitory effect on the biohydrogenation of unsaturated fatty acids, whereas hydrolysable tannins have a modulatory effect on the biohydrogenation, by inducing the accumulation of different kinds of intermediates of the biohydrogenation process, maybe as metabolic response of bacteria to the stress induced by tannins. As regard fibre digestion, polyphenols are able to induce a depressive effect on Gram-positive fibrolytic bacteria and ciliate protozoa. This effect results in a reduction of volatile fatty acid production (mainly acetate). Reduction of fibre digestion is usually associated with a reduction of methane emission. This was demonstrated for condensed tannins, whereas the effect of hydrolysable tannins on methane reduction is probably due to a direct interaction with rumen microbiota without affecting dietary fibre digestion.

**THE UNIT OF EXPERIMENTAL RESEARCH IN BIOANTHROPOLOGY**

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In the framework of the study program on ancient and current human populations, the relationships between Biology and Culture are considered with the aim to define the population dynamics, by valorizing them as functional entities immersed in their environment. In terms of methodology, the study is carried out in order to demonstrate that the anthropological history of a population is the result of a complex process whose intelligibility lies both in the biological dynamics and in the social and cultural one. The peculiarity of the project consists in the investigation of the continuity between current and past populations through the analysis of the most strictly biological parameters of Anthropology (genetic characteristics, mitochondrial and nuclear DNA polymorphism) as well as the social and cultural factors (biodemography, eating behavior, living habits, migrations, state of health). To recon-



struct the dynamics of the populations of the past, human remains are used, which represent an important "Biological Archive". This material allows one to reconstruct the life conditions of the past by means of the paleodemographic, paleogenetic, paleodietary and paleopathological studies. In the biomedical field, the results of the bio-anthropological researches, by taking in consideration the factors of biological and social transformation, may have useful consequences in order to provide indications for possible preventive interventions.

### **TRADITIONAL AND EMERGING POLLUTANTS AS NEW CHALLENGES FOR MARINE ECOTOXICOLOGY: FROM THE MOLECULE TO ECOLOGICAL RISK ASSESSMENT**

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In the last 15 years the ecotoxicological approach based on the use of bioindicator organisms has allowed to understand mechanisms of bioaccumulation and modes of action of environmental pollutants, highlighting how these compounds interact with several cellular districts, induce the activation of detoxification pathways or the onset of various adverse effects. However, challenges for the marine environment are dramatically changing in the last decade both in terms of magnitude of disturbance, typologies of hazards, but also as new scientific and technological opportunities. Beside traditional chemicals, emerging pollutants like pharmaceuticals, microplastics (MPs), nanoparticles, algal toxins and new pathogens represent examples of worrying stressors for the health status of marine organisms and ecosystems. Characterized by different environmental sources and distribution pathways, these compounds have been largely ignored for a long time, representing now a growing concern for their widespread diffusion, the possibility to be absorbed by several species and possible deleterious effects. At the same time, the choice of bioindicators organisms is largely increased, and also methodological approaches greatly evolved, from the analysis of individual cellular responses or single-molecule screening, to quantitative high throughput analyses. We certainly changed our way to thinking ecotoxicology, moving from the initial paradigm of the exposure-response continuum to a more dynamic situation, in which biological inputs and normal biological functions can be modulated by compensatory or adaptive mechanisms. Individual components of the toxicity response pathway have been gradually characterized within highly complex and sophisticated network of interactions and cascade effects. Among emerging pollutants, recent data will be presented on the ecotoxicological potential of different pharmaceutical compounds in the marine environment, showing their accumulation in non target organisms, and the onset of biological alterations from changes in transcriptomic profile to potential functional alterations at cellular level. Also microplastics pose a remarkable hazard for their huge presence in the marine environment, uptake by several species along food webs, efficient absorption and release of different classes of pollutants after ingestion, tissue transfer and induction of transcriptional and cellular effects. Similarly, marine organ-

isms are experiencing the effects of global change, in particular ocean warming and acidification: beside direct adverse effects, such factors might influence the capability of marine organisms to cope with multiple stressors, including anthropogenic pollution. Climate changes are also influencing the occurrence of harmful algal blooms, biological invasions of alien species producing toxic metabolites, and new expansion of pathogens which had been considered as eradicated from coastal areas. In addition to significant ecological disturbance and human health concerns, specific ecotoxicological properties have been detected for some bioactive compounds, which are accumulated in invertebrates and fish tissues, causing molecular and cellular alterations, effects on fatty acids metabolism and reproductive potential of exposed organisms. The enhanced knowledge on these new threats is contributing to raise public awareness and promote new scientific or industrial initiatives. At the same time, the ecotoxicological approach has also become a key component of ecological risk assessment procedures and recently included in some national environmental law.

### **TOWARDS ELECTROMAGNETIC MEDICINE: ELECTRO-CONDUCTIVE PROPERTIES OF CYTOSKELETAL PROTEIN FILAMENTS EXPOSED TO TTFIELDS**

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We provide an overview of the modeling performed at both atomistic and coarse-grained levels in order to gain insight into electrostatic and electro-conductive properties of the cytoskeleton. Computer simulations and experimental measurements carried out for microtubules and actin filaments are presented. Charge and dipole values for monomers and dimers as well as polymerized forms of these proteins are summarized. Continuum approximations for cable equations describing actin filaments and microtubules compare favorably to measurements in buffer solutions showing soliton waves and transistor-like amplification of ionic signals. AC Conductivity and capacitance of tubulin and microtubules have been measured and modeled in the range of frequencies between 100kHz and 1 MHz. A dramatic change in conductivity occurs when tubulin forms microtubules. In living cells, this signals a conductive phase transition coinciding with mitosis in dividing cells. We conjecture that this process, coinciding with TTFIELD penetration into the cleavage furrow in a dividing cell provides the most significant mechanistic explanation of the observed effects. Finally, we provide estimates of the forces, energies and power involved in the action of TTFIELDS on microtubules and kinesin motors. These calculations are compared and contrasted with typical values experienced at a cell level and provide strong arguments for real physical effects of TTFIELDS in dividing cells. We also show results of DLS and TEM measurements on microtubules and tubulin oligomers in solution, which allow us to quantify these processes under controlled conditions.

## ORAL COMMUNICATIONS

### “DRINK AND SLEEP LIKE A FISH”- GOLDFISH AS A BEHAVIOR MODEL TO STUDY PHARMACEUTICAL EFFECTS IN FRESHWATER ECOSYSTEMS

Valbona Aliko<sup>1\*</sup>, Enis Mehmeti<sup>1</sup>, Mihallaq Qirjo<sup>1</sup>, Caterina Faggio<sup>2</sup>

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Behavior is a mechanism through which organisms react to internal and external stimuli to best cope with challenges in an ever-changing environment. The study of animal behavior patterns in response to environmental stress/threats, is relatively new and unexplored. The aim of this study is to offer a modest contribution in explaining the effects of pharmaceutical pollutants found in freshwater ecosystems, using the behavior patterns and physiology of *Carassius auratus*. Behavior changes were evaluated through swimming patterns, opercular and pectoral response, and rheological aggressivity. Animals were exposed for 5 weeks to water (as control), ethanol (0.25 and 1%, v/v), fluoxetine (100 µg/l) and caffeine (50 mg/l) and their short-term responses recorded. The video was analysed using the open-sourced software program Track3D and EthoVision XT, which objectively quantified swimming and social behaviors. In all treatments, fish shown significantly ( $p < 0.01$ ) high level of stress, aggressivity and hyperactivity, compared to control. It was interesting the fact that for each pollutant, fish exhibited different swimming patterns, beside the normal. These changes in the nervous system such as stressed behavior, irregular swimming patterns, hyperactivity and aggression, are consequences of pharmaceutical pollution in freshwater bodies and as such they can be used as suitable early physiological response biomarkers to environmental stress. Monitoring of altered behavior is a great early indicator of water pollution, which can easily be applied in the best aquaculture and fishery practices.

### TRANSITION FROM LATE LACTATION TO DRY PERIOD CAUSED SHIFTS IN METABOLICALLY ACTIVE RUMEN MICROBIOTA OF COWS REVEALED BY RNA BASED AMPLICON SEQUENCING

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This study aimed at monitoring Italian Simmental cows during 2 different physiological stages/dietary treatments, to observe possible modifications in their metabolically active rumen bacterial, archaeal and protozoal communities. Six lactating cows with close days in gestation were selected for this study. The experiment was divided into 2 collection periods: late lactation (248-332 DIM) and dry period (2-52 days before calving). During each period, single rumen fluid sample was collected with an esophageal probe from each cow, fed a specific total mixed ration (TMR) at least for 14 days prior to collection. RNA was extracted in duplicates and used as a template for cDNA synthesis, that was further amplified by PCR using gene specific primers. For bacteria and archaea, V3-V4 region of 16S rRNA genes, and for eukaryotes, V9 region of 18S rRNA genes were amplified, followed by sequencing using

300bp paired-end Illumina Miseq platform. In addition, the total protozoa No., and motility were also recorded using compound microscope. The transition from late lactation to dry period caused significant modifications in the commonly detected rumen bacterial phyla (*Bacteroidetes* & *Proteobacteria*), protozoal phyla (*Ciliophora* & *Amoebozoa*), and archaeal genera (*vadinCA11*, *Methanobrevibacter* & Unclassified *Methanomassiliicoccaceae*). In conclusion, dry period diet increased the protozoa No., and motility, and resulted in higher abundance of methanogenic microorganisms. This study has potential implication for microbial programming process in future, by controlled feeding managements in early life that persist in later life, thereby reducing methanogenic microorganisms and improving animal health and production.

### GRACILARIA GRACILIS: A POWERFUL PRODUCT FROM SUSTAINABLE AQUACULTURE

Gioele Capillo<sup>1\*</sup>, Caterina Faggio<sup>1</sup>, Serena Savoca<sup>1</sup>, Giuseppe Panarello<sup>1</sup>, Marilena Sanfilippo<sup>1</sup>, Nunziacarla Spanò<sup>2</sup>

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Algal species of the genus *Gracilaria* Greville (*Gracilariaceae*, *Rhodophyta*) being the main source of agar are of relevant economic importance. The increasing demand of agar have increased and encouraged studies on different agarophytes species. Among these, one of the most interesting in term of agar yield and quality is *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine & Farnham. Our study demonstrates the various characteristics of this alga thanks to different experiments. Growth potential of *G. gracilis* has been established in natural environment using two different culture methods: the square frames and the “reste” one. Results showed significant differences between the two culture methods with the “reste” with higher efficiency both for Daily Growth Rate ( $DGR = 4.56 \pm 1.19 \text{ kg m}^{-2}$ ) and Biomass ( $Y = 0.96 \pm 0.27 \text{ kg m}^{-2}$ ), defining an innovative method for seaweed culture. Then, the “reste” method has been used to assess “biofiltering” potential and growth of *G. gracilis* in aquaculture plant wastewater. While quite positive results regarding growth during the experimental period have been obtained the most interesting ones were related to reduction of dissolved “nutrients” as  $\text{NO}_2$ ,  $\text{NO}_3$ ,  $\text{NH}_4$ ,  $\text{PO}_4$  in water. Finally, the exploitability of *G. gracilis* as potential immunostimulants in *Danio rerio* (zebrafish) feed formulation has been established. As results upregulation of both antioxidant enzymes gene expression (CAT and SOD) and mucosal immune parameters (Total Ig, Total protein and ALP activity) in fishes fed on diet supplemented with *Gracilaria* powder were detected. Results obtained lead to the conclusion that *G. gracilis* could represents a very interesting organism to be applied in Integrated Multi Trophic Aquaculture procedures, being product exploitable as source of agar, feed supplement and others, derived from environmental friendly aquaculture.

### CHANGES EVOKED BY THE INDUCTION OF SYNTHETIC TORPOR ON THE GUT MICROBIOTA IN THE RAT

Pierfrancesco Chiavetta<sup>1\*</sup>, Claudia Sisa<sup>1</sup>, Timna Hitrec<sup>1</sup>, Emiliana Piscitiello<sup>1</sup>, Agnese Stanzani<sup>1</sup>, Fabio Squarcio<sup>1</sup>, Roberto Amici<sup>1</sup>, Silvia Turrone<sup>2</sup>, Marco Candela<sup>2</sup>, Patrizia Brigidi<sup>2</sup>, Matteo Cerri<sup>1</sup>



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The ability to induce a hypothermic state, mimicking torpor, would bring valuable benefits in medicine (1). During this state (called synthetic torpor) many organs undergo substantial adaptation. In natural hibernators, the microbiota was also changed both in quality and quantity. For example, the hibernating 13 lined ground squirrel gut microbiota showed evident alterations in Firmicutes and Bacteroidetes populations (2). Such changes may play a role in the metabolic adaptation to hibernation (3). The aim of this study was to assess whether such changes are also evoked by synthetic torpor. In this study, specific analysis on gut microbial modifications during synthetic torpor (4) was performed in several intestinal areas. The analysis performed included phylogenetic classification and inferred functional characterization. Although in both the cecum and the feces no changes were observed in treated animals compared to controls, interesting data resulted from the small intestine. At a class level, in this compartment, a strong tendency to decrease was recorded for Bacilli, from 48% to 19% in treated animals ( $P=0.07$ ), with a corresponding increase in Clostridia, from 47% to 79% ( $P=0.2$ ). In conclusion, the state of synthetic torpor affects the biodiversity of the microbiota in the small intestine even after only a few hours of metabolic suppression.

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#### THE CHOLERA EPIDEMICS IN TWO MUNICIPALITIES OF MOLISE IN THE NINETEENTH CENTURY

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Epidemics and famines have been the main causes of mortality crises in the past. In addition to changing the demographic dimensions of the communities, they have often disrupted the social life, developing new mentalities and innovating behaviors, especially those related to the mobility of individuals. In the nineteenth century the epidemics of plague ceased but cholera struck almost all the European populations since 1829. The governments tried to oppose the diffusion of the disease by adopting many precautions that intensified over time (sanitary cordons, quarantine, street cleaning, prohibition of throwing waste on the street, inspection of food, enlargement of hospitals), but the lack of knowledge of the transmission methods of the bacterium limited the effects of infection containment. The Molise study was done using death registers of civil status and comparing the characteristics of deaths in the years of crisis with those of previous years. All the epidemics that struck Italy also affected Molise. Despite the establishment of maritime sanitary cordons, deaths were more numerous in

the litoral municipalities. The most important epidemic was the first (1837). The number of deaths doubled in many municipalities and tripled in the coastal strip and in the immediate hinterland; moreover they died more females than males. Instead, in subsequent epidemics, the increase in deaths rarely exceeded 100% and died more males than females. The improvement in potable water network, sanitary services, food quality and precautions to limit the infection were the basis of the disappearance of cholera from Molise in the early twentieth century.

#### NUTRITIONAL FACTORS AND MULTIPLE SCLEROSIS: A CROSS SECTIONAL STUDY

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Multiple Sclerosis (MS) is a demyelinating, inflammatory and chronic Central Nervous System disease that has a significant impact on the National Health System. Data in the literature have shown that diet can affect the symptoms associated with MS but it is not easy to know which foods are useful and which are to be avoided to improve the patient's life, as well as finding the most suitable diet regimens in the various stages of the disease. This is a cross-sectional study which seeks to investigate the relationships between dietary habits, lifestyle and Expanded Disability Status Scales (EDSS). The study involved 100 outpatients who were followed at the MS clinic of the Villa Sofia-Cervello hospital in Palermo, Italy. Through a structured questionnaire and medical records consultation, we collected data on demographics and lifestyle including type and frequency of meals consumed and physical activity practiced. In addition, the nutritional status was evaluated on body mass index. The clinical data have included MS type, number of pulse therapies and type of pharmacological therapies, and the EDSS. Finally a correlation between food groups, (pro-inflammatory and anti-inflammatory), and disability index was made. The results show that there is an inverse correlation between the physical activity practiced ( $p=0.05$ ), the degree of satisfaction generated ( $p=0.009$ ) and the EDSS scale. Furthermore, there is a linear correlation between the frequency of meals consumed and disability index ( $p=0.042$ ). Finally, the consumption of milk and its derivatives was significantly associated with increased levels of EDSS ( $p=0.008$ ), while the fruits and vegetables intake is associated with lower levels of disability ( $p=0.05$ ).

#### RESEARCH, ENVIRONMENTS AND HEALTH IN THE MARCHE: EVIDENCE AND PROPOSALS

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The Marche Region (Italy) is a territory rich in natural resources but not without environmental criticalities that may impact on human health. One of the most critical area is the Site of National Interest (SIN) located adjacent to the city of

Falconara Marittima and not far from other small cities. Falconara Marittima has been the subject of at least five different epidemiological studies, for a total period of observation of about 20 years, which have highlighted several health problems considering both the deaths, "the tip of the iceberg", and diagnoses. Given the numerous and consistent evidence of a population that is sick and dies more than the regional average, nothing has yet emerged concerning the possible correlation between the environmental sources of pollutants with the excesses of diseases found in the territory. In fact, if on the one hand the epidemiological investigations have provided important observations, contributing to bring out the state of health of the Falconara M. citizens, the same cannot be said for the accumulated knowledge regarding possible environmental problems (e.g. mapping pollutants and their sources in the territory). The lack of attempts to correlate the pollutants (and their sources) with the greater incidence of certain diseases, determines a terrific lack of knowledge that cannot provide the crucial guidelines (for the legislator) for the necessary actions to be taken for a more effective primary prevention of the diseases. The citizens of Falconara M. have to be considered a real "biological model" to *in vivo* investigate the effects of the environment on normal biological processes and, beyond the limits of physiological adaptation, how pathogenetic process can be induced.

#### **CITY EPIDEMIOLOGICAL REPORT IN GENOA AND TARANTO. TO IDENTIFY THE INEQUALITIES IN MUNICIPAL GENERAL MORTALITY AND TO EARLY DIAGNOSE ENVIRONMENTAL CAUSES**

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In addition to regularly monitoring environmental exposures harmful to human health, we also suggest that human health itself should be timely and periodically monitored by space-time-age-gender epidemiological mortality studies. The "City Epidemiological Report (CER)" of Genoa and Taranto, using municipal administrative sources, could identify possible causes (i.e. environmental) monitoring the overall mortality by districts (25 and 6 districts, respectively). Descriptive analyses of the general mortality per year, gender and district, from 2010 to 2017, adjusted according to age, were performed by using Genoa and Taranto total mortality data. In both cities all population and deaths data of residents due to all pathologies in the district of the two cities, and in the regions of Liguria and Apulia respectively, were analyzed by gender, age and year of death. Standardized Mortality Ratio (SMR) for gender and district of residence with confidence intervals (90% CI) were calculated using internal (municipality) and external (region) reference population. In both cities statistically significant excesses were confirmed for whole mortality in the general population residing in all districts located in the areas closest to industrial plants. Space-temporal-age-gender analysis based on overall mortality

data has allowed a reliable, timely and parsimoniously monitoring the health status in each district of the two cities. Risk excesses were not reasonably attributable to demographic differences (age). The integration with different data related to previous studies and specific pathologies will help us understanding the specific etiological factors.

#### **GENETIC AND EPIGENETIC MODIFIERS OF NAFLD PROGRESSION**

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Non-alcoholic fatty liver disease (NAFLD), actually recognized as the most common liver disease worldwide, represents the hepatic manifestation of metabolic syndrome, being closely related to obesity, insulin resistance and oxidative stress. It includes a spectrum of conditions ranging from simple steatosis, characterized by hepatic fat accumulation with or without inflammation, to non-alcoholic steatohepatitis (NASH), defined by hepatic fat deposition with hepatocellular damage, inflammation and fibrosis. NAFLD is a complex disease where the interactions between the environment and the polygenic host background are able to determine disease phenotype and influence its progression. Recently, a lot of genome-wide association and large candidate gene studies have enriched our understanding of the genetic basis of NAFLD, which is useful to explain the substantial inter-patient variation in disease progression. The rs738409 C>G PNPLA3 variant, involved in lipid droplets remodeling, represents the major common genetic determinant of NAFLD, driving a normal liver to steatosis, NASH and cirrhosis. However, other gene variants involved in VLDL secretion (TM6SF2, APOB), *de novo* lipogenesis regulation (GCKR), mitochondrial lipid metabolism OxPhos (UCP2), phospholipid metabolism (MBOAT7) innate immunity (IL28B, MERTK) and hepatic stellate cells activation (MERTK, Irisin) have been implicated in the occurrence and/or the NAFLD progression. Nowadays the role of epigenetic modifications, DNA methylation and chromatin remodeling, on NAFLD progression is emerging. Finally, MicroRNAs, regulators of gene expression, commonly deregulated in pathological conditions, are currently the most intensely studied epigenetic factors in NAFLD (miR-122). Therefore, epigenetic changes interact with inherited risk factors to determine an individual's susceptibility to NAFLD.

#### **COMPUTATIONAL AND IN VITRO STUDIES OF SYK INHIBITORS AS NEW DRUGS IN P. FALCIPARUM MALARIA**

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Malaria remains one of the most devastating infectious diseases. Although the current therapies are working well, the WHO recommends Artemisinin Combination Therapies (ACTs) as the frontline treatments against *P. falciparum* malaria to limit the artemisinin resistance. In 2017 it has been confirmed in 5 countries of the Greater Mekong subregion. An alternative way to fight the parasite resistance could be use Syk inhibitors as new antimalarial drugs. Syk protein is present in human erythrocytes;

its activation by oxidant stress involves the band 3 (AE1) membrane protein. Tyr phosphorylation (AE1) occurs during *P. falciparum* growth leading to the release of microparticles containing hemichromes and RBCs structural weakening. Syk inhibitors block these events interacting with protein catalytic site. We have performed *in vitro* and *in silico* studies and compared the obtained results. *In vitro* we treated parasitized erythrocytes with different concentrations of Syk inhibitors and we evaluate the Tyr phosphorylation levels in Band 3 residues by proteomic analysis. In presence of Syk inhibitors we observed a marked decrease of band 3 phosphorylation according to drug concentration increase. The proteomic data trend relating to the inhibition values  $IC_{50}$  correspond to the computational studies. *In silico* studies were based on different approaches of molecular modeling aimed to deepen the knowledge about the ligand-protein interaction in order to obtain the highest efficacy *in vitro*. This study allow to optimize the structure of these compounds and to design and discover new promising antimalarial drugs.

### PRELIMINARY DATA ON THE MODULATORY EFFECTS OF DIFFERENT PLANT EXTRACTS ON JELLYFISH VENOM

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Jellyfish are toxic sea organisms representing a health issue for both recreational and occupational activities. Given that herbal products are attracting increasing interest, we examined plant extracts from *Carica papaya* L., *Ananas comosus* (L.) Merr., and *Bouvardia ternifolia* (Cav.) Schltdl., known for protective properties against different animal venoms. The interference of these extracts with the toxicity of tissue homogenates from the jellyfish *Pelagia noctiluca*, *Phyllorhiza punctata*, and *Cassiopea andromeda*, were evaluated on L929 mouse fibroblasts, used as an *in vitro* skin cell model. The quantification of jellyfish cytotoxicity was achieved by MTT cell viability assays, deriving dose response curves and  $IC_{50}$  values. Each of the extracts of *C. papaya* and *A. comosus* significantly lowered the cytotoxicity of *P. noctiluca* and *P. punctata*, but enhanced the toxic effect of *C. andromeda*. The extract of *B. ternifolia* reduced *P. punctata* cytotoxicity, showed no effect against *P. noctiluca*, and increased *C. andromeda* cytotoxicity. In summary, the modulatory effects of plant extracts on jellyfish homogenates cytotoxicity resulted to be species-specific, showing either protective or worsening effects. More specifically, extracts with protease activities, like *A. comosus* and *C. papaya*, seem to protect from jellyfish with toxic peptides like phospholipase A.

### EXTREMOPHILES ORGANISMS AS VALUABLE SOURCE OF RADICAL SCAVENGERS: APPLICATIONS IN THE BLUE-BIOTECHNOLOGIES

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A significant contribution to the discovery of new bioactive molecules was given by the study of species that have adapted to live in extreme environments, such as acidophilous organisms, halophilic, cryophilic and thermophilic. These are microorganisms that have developed unique physiological characteristics, which allow them to survive extreme physical and chemical conditions, such as high temperatures, high salt concentrations or extreme pH levels and therefore, can adapt itself and can proliferate in environments recognized as inhospitable to life. This is possible, because they are specialized to produce, in a modulated way, molecules that allow them to adapt to changes in the chemical-physical conditions of the environment. A large part of these microorganisms belongs to the domain of the Archea, ancestral bacteria that represent a considerable fraction of the prokaryotic world in terrestrial and marine ecosystems; their unusual properties make them a potentially valuable resource in the development of new biotechnological processes and industrial applications such as new pharmaceuticals, cosmetics, food supplements, molecular probes and enzymes.

### HYPERGLYCEMIA AFFECTS ANION EXCHANGE THROUGH BAND 3 PROTEIN: AN IN VITRO AND IN VIVO STUDY ON HUMAN ERYTHROCYTES

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Band 3 protein (B3p) accounts for erythrocytes homeostasis, namely ion balance, gas exchange and membrane deformability. In the present investigation, the effect of hyperglycemia, related to metabolic dysfunctions, has been evaluated on erythrocytes *in vitro* exposed to increasing concentrations of glucose (5-15-35-50 mM) for different time intervals (3-24 h) as well as on erythrocytes from patients with high glycated hemoglobin (HbA1c) levels. The rate constant for  $SO_4^{2-}$  uptake, accounting for the efficiency of anion exchange through B3p, along with levels of MDA, GSH, -membrane -SH groups and osmotic fragility have been measured. The incubation with high glucose concentrations (15-35 mM) for 24 h exacerbated osmotic fragility, compromising the measurement of anion exchange through B3p. When erythrocytes were exposed to high glucose for 3 h, being MDA, GSH and membrane -SH levels unchanged,  $SO_4^{2-}$  trapped by the cells was significantly reduced with respect to control (5 mM). Under high HbA1c levels, the rate constant was higher than control and  $SO_4^{2-}$  content higher than both control and high glucose-treated erythrocytes. The present findings show that: i) measurement of the rate constant for  $SO_4^{2-}$  uptake is a suitable tool to monitor the effect of hyperglycemia on erythrocytes; ii) hyperglycemia due to high glucose applied for 3 h affects anion exchange capability without producing lipid peroxidation; iii) high HbA1c levels seem to accelerate anion exchange capability through B3p. Future studies will evaluate whether this acceleration depends on an altered Bp3 conformation, affecting crosslink with Hb, or on altered phosphorylative signaling underlying B3p function.



## MEDIEVAL BURIALS IN THE CATHEDRAL OF ALBA (CN): PALEODEMOGRAPHIC REVIEW AND UPDATES

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During the archaeological excavations carried out from 2007 to 2011 in the Cathedral of Alba (Cuneo, North Italy), 377 human skeletal remains, dating back from the 8<sup>th</sup> to the 17<sup>th</sup> century, were exhumed. At the present, anthropological and paleopathological study was conducted on 302 individuals, according to morphological and metrical standard criteria provided in human osteological manual and paleopathology literature. In order to outline the biological and paleodemographic profile, sex, age at the death, stature, biomechanical stress and pathological conditions are described. The anthropological analyses reflected that 74 skeletons (24%) were non-adults, 228 (76%) adults; moreover, 109 individuals (48%) were identified as male, 62 (27%) female, and 57 (25%) unsexed, due to poor preservation. In our research program, we have tested the performance of statistics approach in sex estimation based on the metric observations on the long bones. We discuss the results obtained from applying statistical method in the group of unsexed adults.

## IMPORTANCE OF MUSSEL DIGESTIVE CELLS IN ECOTOXICOLOGICAL INVESTIGATIONS ON XENOBIOTICS

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Water environments, in particular coastal marine areas, brackish ecosystems and inland waters are subject to a strong impact linked to human activities. Heavy metals, radioactive pollutants and different xenobiotics can cause long-term adverse effects in the ecosystem. To control the well-being of these environments, the main control strategies involve the use of organisms that tolerate the presence of external pollutants and through molecular investigations on specific tissues. The most commonly used sentinel organisms are molluscs of the genus *Mytilus*. The target tissues in these organisms are gills, hemolymph and hepatopancreas. In recent years, hepatopancreas has been used as a good indicator for conventional pollutants, such as heavy metals, present in ecosystems. Digestive cells are excellent biomarkers, in fact cytotoxic, genotoxic and oxidative stresses alter the normal metabolic processes that can be easily identified. Changes in metabolic processes such as changes in antioxidant enzymes, are also indicative of the damage caused by emerging pollutants, by xenobiotics such as drugs, body care products, biocides, nanoparticles and microplastics, which released in small concentrations in the environment cause a chronic damage in mussels<sup>3</sup>. In addition, to the obvious environmental damage linked to these xenobiotics, the direct risk to humans through the food

chain should not be underestimated. Therefore, the importance of using this organ in the current environmental investigations on biomonitoring became important.

## ROLE OF O-GLCNACYLATION IN THE REGULATION OF CELLULAR VOLUME

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O-GlcNAcylation (O-GlcNAc) is a post-translational modification of proteins and occurs via conjugation of N-acetylglucosamine to serine/threonine residues. It is well established that O-GlcNAc is chronically elevated in diabetes mellitus, but the pathophysiological significance of this finding is not fully elucidated. Recently, the protein ICl<sub>n</sub>, crucial in the activation of a chloride conductance (I<sub>Cl<sub>swell</sub>) after anisotonic cell swelling, has been found to be O-GlcNAcylated. Nuclear magnetic resonance and bioinformatics show multiple O-GlcNAc modification sites, of which the functional roles are unknown. To explore the functional significance of O-GlcNAc modification of ICl<sub>n</sub>, the wild type and different mutant forms of ICl<sub>n</sub> have been characterized by patch clamp in the presence of normal or elevated O-GlcNAc levels. Our results show that: I) O-GlcNAc elevation suppresses I<sub>Cl<sub>swell</sub> and inhibits ICl<sub>n</sub> function; II) ICl<sub>n</sub>S67A is functional but insensitive to O-GlcNAc elevation; III) ICl<sub>n</sub>S67T function is lower compared to the wild type, and was not suppressed further by O-GlcNAc elevation; IV) ICl<sub>n</sub>T223A is functional and sensitive to O-GlcNAc elevation; V) ICl<sub>n</sub>S193X lost most of its activity, though the residual current was sensitive to O-GlcNAc elevation. Together, these results clearly show that O-GlcNAcylation of ICl<sub>n</sub> at S67 leads to I<sub>Cl<sub>swell</sub> suppression. Abnormally O-GlcNAcylated proteins involved in the regulation of cellular volume may contribute to the onset and progression of diabetic complications and may therefore represent novel targets in the prevention or treatment of these conditions.</sub></sub></sub>

## THE AGRICULTURAL ECOSYSTEM SERVICE: RESILIENCE AND ECOSYSTEM TIMELINE

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Resilience is one of the most fascinating concepts regarding sustainability, and mainly in the study of ecosystem services. Resilience in the study of ecosystem services requires a very crosscutting approach, because it concerns complex systems such as ecosystems and human social systems. Agricultural areas are fundamental components of sustainability; ecosystem services can provide links to integrate management and governance practices in the search for transitions towards greater sustainability in rural areas. The study concerns a micro-area in the province of Asti, using some typical tools of resilience and ecosystem services, trying to find a new methodological

approach. Without any better interconnection among research and related policy, technology might not have an impact on the real-world practical application. Indeed, every time the researchers will begin their search without focusing on how the data may be used, the final evaluation it fails to develop a new policy, which might be adapted to the new challenges. Whilst in the real politics, the under-estimation of the ecological studies leads to a significant environmental decline. A common approach should measure the ecosystem condition, according to a natural status comparable threshold values, in other words, on the basis of its similarity to a man-made environment least-impacted. However, Pristine agro-ecosystem concept is not credible nor does it provide an appropriate frame. Concerns that the pristine agro-ecosystem concept is not credible or that information provided is dubious may also dissuade the use. This article, is intentionally written in simple language, because the complexity can be explained only simply.

### **SOCIETY AND ECONOMY BETWEEN THE 17<sup>TH</sup> AND 18<sup>TH</sup> CENTURY THROUGH SOME PALEOPATHOLOGICAL INDICATORS: THE CASE OF THE MUMMIES OF ROCCAPELAGO (MO)**

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During the restoration of the church of the Conversion of St. Paul, located in Roccapelago, a village of the Emilian Apennines (Northern Italy), a chamber was found containing the remains of over 400 individuals who had lived between the 17<sup>th</sup> and 18<sup>th</sup> century AD. Some of them were naturally mummified. Mummification was possible due to the particular location of the crypt, which was built on the ruins of the medieval fortress of Roccapelago and was therefore equipped with ventilation slots. Thanks to the stratigraphy of the site and to the secure dating of the layers, it was possible to investigate the development of certain phenomena from the 17<sup>th</sup> to the 18<sup>th</sup> century. The aim of this study is to better understand the possible social changes underlying the development of some pathological characters in the ancient population of Roccapelago during the available temporal interval. The

social structure of the community of Roccapelago, during these two centuries, remains substantially the same. Anthropological analysis seems to highlight an initial phase of change in the food and occupational aspects, which perhaps going to consolidate during the 19<sup>th</sup> century. The prevalence of tibial periostitis remains stable, the study of clothes has in fact shown that there is no variation in the type of clothing used during the 17<sup>th</sup> and 18<sup>th</sup> centuries. The stability of the aspects considered, could be related to the persistence of the same activities in the sylvan environment and the same methods of protection of the legs by means of inadequate clothing.

### **MECHANISM OF SYNERGIC INTERACTION OF SYK INHIBITORS ON ANTIMALARIAL ARTEMISININ ACTIVITY**

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Artemisinin derivatives constitutes the major components of all antimalarial therapies. Although the mechanism of action of artemisinin-based combination therapies (ACT's) are still debated and unfortunately, in the Greater Mekong Subregion, the efficacy of ACT's has recently been questioned by resistance to both artemisinin derivatives and to the partner drugs. Therefore, the quest for new antimalarials that can be combined with an artemisinin to prevent the spread of drug resistance has heightened. We previously demonstrated that Syk kinase inhibitors block the phosphorylation of band 3, thereby interfering with the release of hemichromes and heme-enriched microvesicles. Those modifications are an essential prerequisite for parasite egression from the host cell constituting the theoretical background of the observed anti-plasmodial activity of Syk inhibitors. In the present study, we describe a marked, synergistic effect between Syk inhibitors and artemisinins. This synergistic effect appear to be causally related to the accumulation of hemichromes in parasitized red blood cells (pRBCs) and to their capacity to catalyze the activation of artemisinins.



## POSTERS

**LANGERHANS CELLS, MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION IN STRIPED DOLPHIN (*STENELLA COERULEOALBA*) EPIDERMIS**

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The skin is the primary interface between the body and the environment, and has a central role in host defence. Langerhans cells (LCs), play a central role within inflammatory and immune responses in the epidermis of terrestrial and aquatic mammals, through their specialized function in antigen capture. Langerin/CD207 is a cell surface receptor of Langerhans cells (LC) and represents a key molecule to mark LCs [1, 2]. The aim of this study was to characterize immunohistochemically with anti langerin/CD207 antibody, Langerhans cell in the dolphin *Stenella coeruleoalba* epidermis. An adult male striped dolphin, was found live stranded (length: 197 cm), on April 2011 in the Apulian coast (Adriatic Sea). The samples obtained were treated in accordance with protocol for optical microscopy. Some serial sections were stained with hematoxylin and eosin (H&E), [3]; for immunofluorescence investigation, serial sections were treated with langerin/CD207 antibody. In this study, the immunoreactivity of Langerin/CD207 antibody, revealed numerous Langerhans cells (LCs) with a polyhedral shape, located in the epidermal suprabasal layer (stratum spinosum). These cells present an irregular shape with long cytoplasmic processes extending among keratinocytes, forming a delicate network. Future studies will be aimed to characterize different dendritic cells populations present in the dolphin's skin.

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**MOLECULAR DYNAMICS AND UV SPECTROSCOPY TO INVESTIGATE COLLAGEN FIBRILLOGENESIS**

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Collagen is a fibrous protein representing the main constituent of connective tissue in mammals, with a basic structural unit called tropocollagen. Tropocollagen is a triple right-handed helix consisting of Gly-Xaa-Yaa repetitions, in which one-thirds of the X and Y residues are either prolines or hydroxyprolines (Hyp). Collagen triple helices associate in fibrils, and the alignment of them yields to the characteristic fibres, where tropocollagen molecules are staggered side-by-side with a shift of 234 residues

between two neighbours tropocollagens. MD simulations were performed to study tropocollagen aggregation in physiological conditions. Two tropocollagen fragments with different hydrophobic profiles were chosen and built from *Rattus norvegicus* type I collagen sequence. Other fragments were selected from the same sequence with a shift of 234 residues upstream and downstream of it. Association of two, three and four fragments in MD simulations shows that the amino-acidic composition of the triple helices strongly influences the assembly propensity. Fragments rich in charged residues needs a lateral addition of individual tropocollagens to self-assembly, while poorly charged segments easily associate in pairs. This behaviour suggests a cooperative binding mechanism at tropocollagen level. Rat tail tendon collagen was prepared at low temperature and at different pH. *In vitro* collagen self-assembly was monitored by measuring the turbidity changes of the solution as observed from the increase in absorbance at 310 nm. Curves of aggregate fractions vs time display a sigmoid profile, composed by three defined regions (lag, growth, plateau) indicating, according with literature, a cooperative process with a very short lag phase.

**EFFECTS OF MIXTURE OF ATRAZINE, DESISOPROPYLATRAZINE AND DESETHYLATRAZINE ON DEVELOPMENT OF EARLY LIFE STAGES OF ZEBRAFISH (*DANIO RERIO*)**

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Atrazine is one type of chloro-s-triazine herbicides and it is considered moderately toxic to aquatic animals. The aim of this study was to assess the acute embryotoxicity of a mixture of atrazine and two of its metabolites, desisopropylatrazine and desethylatrazine. As a model organism we used zebrafish (*Danio rerio*), which belong to one of the model fish organisms commonly used in toxicity tests to determine negative effects of various substances occurring in aquatic ecosystem. Toxic effects of were studied using evaluation of lethal endpoints, development disorder, and other sublethal endpoints such as hatching rate, formation of somites, and development of eyes, spontaneous movement, heartbeat, blood circulation, pigmentation, or edema at 24, 48, 72, and 96 hours post fertilization. The embryonal toxicity test was performed through the modified method of Fish Embryo Acute Toxicity (FET) Test (OECD guideline 236). Newly fertilized zebrafish eggs were exposed to various concentrations of a mixture, which include environmental levels in aquatic environment and multiples of environmental relevant concentration to find out if the negative effect is dose dependent. Our results showed that high concentrations of these compounds cause significant changes in development after 48 hours post fertilization.

**NEW SILVER NANOPARTICLES DEVELOPMENT STARTING FROM THE EXTRACT OF *ARTEMISIA ANNUA*: GREEN SYNTHESIS, CHARACTERIZATION AND ANTI-MALARIAL ACTIVITY**

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Among the metallic nanoparticles, interesting for their chemical stability, good conductivity and good biological activity, cover a primary role the silver nanoparticles. Their ability antibacterial, antifungal and antiparasitical it is already note in literature. In the last few years, the attention has been focused on the green synthesis of the nanoparticles to increase their biocompatibility in biological use. Following this tendency we have synthesized some silver nanoparticles using extracts of *Artemisia annua* and evaluated their possible use as antimalarial agents. Treating infected RBCs with this AgNPs no hemolytic effect was observed. Their TEM microscopy characterization displayed a homogenous size distribution in the range of 10-30 nm, useful for biomedical applications. Our *in vitro* test proves increased efficacy of *A. annua* AgNPs in comparison to silver nanoparticles synthesized with classical chemical method. Preliminary results show that *A. annua* AgNPs has a marked antimalarial effect on *P. falciparum* culture. All the experiments have been carried out under different dose response conditions. Considering the results obtained, it emerges that the potential of *A. annua*-AgNPs could be used as a novel nanotechnological strategy for malarial treatment.

#### BETA-ESTRADIOL AND ETHINYL-ESTRADIOL EFFECTS ON FEMALE HEMORHEOLOGY

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Estrogens are the primary female sex hormones. Among these, estradiol carries out the most powerful action. Estrogens are also the responsible for secondary sexual characteristics in women and affect the function of all major organ systems within the body [1]. It is also well-known that an estrogen-deficient state led to neurovascular and cardiovascular diseases [2]. In contrast, synthetic estrogens, like the ones used in oral contraceptives or hormonal replacement therapies, are associated with thrombosis and some other vascular diseases [3]. The aim of this study was to investigate the effects, in women, of  $\beta$ -estradiol and ethinyl-estradiol on some hemorheological parameters like blood viscosity, RBC aggregation and RBC deformability. The study was performed in accordance with the guidelines for hemorheological laboratory techniques and under the protocol established with the Portuguese Institute of Blood and Transplantation in Lisbon, Portugal. All donors were females and duly informed and signed their agreement. Blood aliquots were gently mixed and incubated with different beta-estradiol and ethinyl-estradiol concentrations, and RBC aggregation, RBC deformability and blood viscosity were assessed. The concentrations of  $\beta$ -estradiol tested were 50 g, 100 g, 150 g, 200 g and of ethinyl-estradiol were 25 g, 50 g, 75 g, 100 g. In our experiments, we observed a significant increase of the EEI for both compounds for some shear stress values. No significant differences in RBC aggregation and blood viscosity were found. These findings may be correlated with different patterns

of thrombotic and cardiovascular effects in different phases of the menstrual cycle or different dosages of oral contraceptive therapy used by some of the donors.

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#### ROLE OF 3,5-DIODO-L-THYRONINE ON THE PITUITARY-THYROID AXIS

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Studies have highlighted that the administration of 3,5-diodo-L-tyronine to rats fed diets rich in lipids induces a decrease of cholesterol and triglycerides plasma levels and body weight without inducing liver steatosis. On the basis of these observations we carried out some experimental *in vivo* studies to assess the effects of multiple high doses of T2 on the pituitary thyroid axis of rats fed diet rich in lipids. Fifteen male Wistar rats were divided into three groups of five animals each. The first group (N group) received standard diet, the second group was fed with a high fat diet (HFD group) while the third group (HFDT2 group), was additionally given with T2 intraperitoneally at a dose level of 70 g/100 g of body weight three time a week up to four weeks. At the end of the treatment period blood sample from each animal was collected, centrifuged and the resultant serum was stored at -20°C. The serum concentrations of TSH, T3, T4, ACTH, triglycerides, cholesterol, glucose, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase were then determined at various intervals. In addition, liver of rats was examined by histology order to assess the presence and degree of steatosis. The administration of T2 to rats fed with a high fat diet suppressed TSH secretion ( $p=0.013$ ) while no steatosis was observed in the liver of these animals. These preliminary data show that multiple administration of high doses of T2 to rats fed diets rich in lipid inhibits TSH secretion and prevents the onset of liver steatosis in these animals.

#### ANTHROPOMETRIC DATASET COMPARISONS: STATURE AND WEIGHT TREND IN ITALY

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Anthropometric features are inherited characters, that follow a polygenic model and are strongly influenced by environmental aspects as life style. For this reason Anthropometric characteristics are indeed very different by geographical area and change in time. Studying and monitoring the becoming of these variations, we search to verify the adequacy of the data sets to their use for ergonomic design. The focus on the human morphometric is current because of the interests of virtual design in digital simulations, playing attention to refer to an appropriate anthropometric database of "general population" rather than "working population". Italy has a tradition of anthropometric studies in the academic field of Ergonomics already applied since the '70<sup>es</sup> and integrated into the interests of production companies (Locati *et al.*, 1979) and until today (Micheletti *et al.*, 2018). Moreover starting from method by Grieco and Masali, 1972, we participated in the definition of the international anthropometric standards (today ISO 7250.1: 2017) and in the first national survey on general adults population ("L'Italia si Misura" project, 1990-91 by M.Masali \_University of Torino and G.Pierlorenzi \_Centro Sperimentale di Design now PoliArte of Ancona). More than 4.000 subjects were measured, males and females, representatives of different geographical regions (35 measurement). This survey is today a source of reference data. Italians anthropometric characteristics are reported, in particular relating to the estimates of increase in stature and weight conditions according to what emerges from the comparison of national databases of different periods to focus on changes in progress in Italy.

### OXIDATIVE STRESS INDUCTION BY THE INVASIVE SPONGE *PARALEUCILLA MAGNA* GROWING ON *PEYSSONNELIA SQUAMARIA* ALGAE

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The arrival of non-native species can produce significant adverse effect on the colonized environments. The new species can compete with autochthonous organisms for resources and/or space leading to stress that can compromise the survival of the affected organism. *Paraleucilla magna* is a calcareous sponge introduced to the Mediterranean Sea and Northeastern Atlantic. Eukaryotic cells contain a complex network of antioxidant defences that protects against the excessive production of reactive species induced by stressful situations avoiding damages to biomolecules. The aim of the study was to determine whether the new-come specie *P. magna* could induce the activation of the antioxidant defences in the native red algae *Peyssonnelia squamaria*. Individuals of *P. squamaria* growing isolated on rocky bottoms and individuals

epiphytized by *P. magna* were collected. The activity of antioxidant enzymes – Catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase – were significantly higher in the group affected by sponge compared to the control group, whereas the detoxifying enzyme GST did not evidence significant differences. Malondialdehyde levels, as marker of lipid peroxidation, were similar in samples epiphytized with the sponge when compared with the controls. GSH and polyphenol levels were higher in algae affected by the sponge compared to the control group. In conclusion, the arrival of the species *P. magna* is a threat for the native red alga *P. squamaria* inducing a situation of oxidative stress measured by means of biomarkers.

### CHARACTERIZATION OF THE METABOLIC PROFILE OF *PELAGIA NOCTILUCA* (SCYPHOZOA)

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The phylum Cnidaria is one of the most important contributors in providing abundance of bio- and chemodiversity. Jellyfish have to be considered a source of precious substances of diet and nutraceutical interest, such as collagen, oligosaccharides, fatty acids and bioactive compounds, including peptides and enzymes. This study elucidates the chemical composition of the tissues belonging to the jellyfish *Pelagia noctiluca* (Scyphozoa), which is the most abundant jellyfish in the Mediterranean Sea. For each sample (male and female) we have simultaneously extracted the water-soluble phase (namely A) and the CDCl<sub>3</sub> soluble lipophilic fraction (namely B), which were consequently analyzed by nuclear magnetic resonance (NMR) spectroscopy. The specific characterisation of two different tissues, bell and tentacles respectively, was carried out. As expected, samples showed the presence of aminoacids and other metabolites with similar concentration regardless of the kind of tissue. The B samples witnessed the presence of different fatty species among the tissues, as female bell is rich in simple triglycerides, which are not present in the other analyzed tissues. Male tentacles are characterized by the presence of polyunsaturated fatty acid esters (often embedded in macromolecular complexes) and animal sterols. We can conclude that there is a significant chemical and nutritional difference among the jellyfish tissues.

### MORPHOLOGICAL AND ULTRASTRUCTURAL STUDY OF *RHACOPHORUS REINWARDTII*, SKIN (SCHLEGEL 1840, RHACOPHORIDAE, AMPHIBIA)

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In this study we analyzed the morphological and ultrastructural characteristics of the *Rhacophorus reinwardtii* skin, a flying frog, which is one of the few species of amphibians that uses the "sliding flight" for movement. The 'sliding flight' of this



species is possible thanks to prominent membranes which create the surface, making it possible to overcome significant distances in the air. Using routine methods of LM, TEM and SEM we have studied the skin from gliding membrane, toe, and dewlap. We observed a conspicuous bundles of tonofilaments in the cytoplasm of epithelial cells, allowing this species the locomotion and the mechanism of adhesion to surfaces (gliding membrane, toe, dewlap). In the toe outer layer, are present prismatic cells; furthermore in the epidermis of the gliding membrane we have observed a layer of keratinized cells near the surface. In the connective tissue under the epithelium there are numerous mucous glands, an abundance of nerves and several types of cells including: melanophores, iridiophores and xanthophores. Our future studies are aimed to compare *Rhacophorus*'s skin structures with those of other species exhibiting similar behaviour (climbing/flying) (*Rana leucomystax*, *Litoria caerulea*) and to characterize Merkel Cells and Langerhans cells, both cells present in the skin of all vertebrates.

### THE COMBINED EXPOSITION TO POLYBROMINATED COMPOUNDS AND $\beta$ -BLOCKERS MODULATES VITALITY AND OXIDATIVE STRESS PARAMETERS IN THE MARINE FISH CELL LINE SAF-1: PRELIMINARY RESULTS

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Pharmaceutically active compounds have been detected in wastewater treatment plants and surface waters across the world. Among these compounds, some non-steroidal anti-inflammatory drugs as well as  $\beta$ -blockers used to treat human hypertension, such as atenolol (ATL), is one of the most abundant reported at a very variable range, in relation to the site. The uncertainty surrounding the behavior of these pharmaceutically active compounds could increase due to the presence of others pollutants in the marine environment. Polybrominated diphenyl ethers (PBDEs) are a class of brominated compounds which leads to accumulate in the marine environment, being ubiquitous and toxic. The interactions between PBDE and ATL and its effects on marine organisms are still unknown and *in vitro* system are ideal for screening of toxicity, dose-time exposure and studies on synergisms. In the present study are reported some preliminary results obtained in an experiment with the *Sparus aurata* fibroblast cell line (SAF-1), exposed to different concentrations of BDE-47, ATL and a mix of both, until 72 hours, to evaluate the cytotoxicity and reactive oxygen species (ROS) production. Evaluation of molecular markers related to toxicity, oxidative stress and cell cycle is in progress. At 72 hours the SAF-1 cells exposed to both compounds showed a decreased viability. Our preliminary results suggested the synergic effects of these class of compounds, which not only could remark the importance of the development of effective treatment strategies for remove these compounds from the marine environment, but also the prevention of the discharge.

### EFFECTS OF SUB-LETHAL DOSES OF THE POLYBROMINATED BDE-47 ON SOME INNATE IMMUNE RELATED PARAMETERS OF MYTILUS GALLOPROVINCIALIS

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Polybrominated diphenyl ethers (PBDEs) belong to the class of the flame retardants and are persistent and toxic pollutants, extremely stables. In fact, PBDEs have demonstrated to produce several negative effects on marine organisms. Marine bivalves are susceptible to be exposed to a large quantity of pollutants due to its feeding habits. However, the effects of PBDEs on the innate immune system from mussels remains unclear. With the aim to shed light on this issue, within the project CISAS, specimens of *Mytilus galloprovincialis* were allowed in aquaria, fed on microalgae contaminated with different concentrations of PBDE-47. The treatment was maintained for 15 days. Samples of haemolymph were obtained from T0 to 30 days of the experiment. Both immune and humoral related parameters were evaluated and demonstrated to be affected by the treatment. In addition, the results indicated that after 15 days of detoxification, immune parameters did not restore its basal levels, but some of them resulted overexpressed. BDE-47 exposure, at environmentally realistic levels, may affect various aspects of immune function in *M. galloprovincialis*, acting as a stressor that can compromise the ability to counteract external stimuli, such as pathogens, reducing animal welfare.

### ELECTROPHYSIOLOGICAL PROPERTIES OF CRUDE VENOM FROM PELAGIA NOCTILUCA (CNIDARIA: SCYPHOZOA) NEMATOCYSTS

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Cnidarians are known to negatively impact on ecosystems and human economic activities, and represent a health problem consequent to their stings. However, their venom is a rich source of bioactive compounds, and is only partially characterized. *Pelagia noctiluca* (Cnidaria: Scyphozoa) is the most abundant jellyfish in the Mediterranean Sea, and it is mostly found in the Strait of Messina (Italy). To investigate the properties of *P. noctiluca* venom, cultured human cells were exposed to the crude venom (0,025 ug/ul). Changes in the cellular volume were monitored by phase contrast microscopy and the membrane conductance was measured by patch clamp. The exposure of cells to the venom induced a dramatic cell swelling and profoundly altered the ion conductance of the plasma membrane, thus affecting the homeostatic functions of the maintenance and regulation of cellular volume. Venom-treated cells exhibited a large inwardly rectifying current, mainly due to permeation of Na<sup>+</sup> and Cl<sup>-</sup> and sensitive to amiloride. Cell swelling and the venom-induced current were completely abrogated following

removal of NaCl from the extracellular solution and thermal treatment of venom extract. The current-inducing activity was also observed following delivery of venom to the cytosolic side of the plasma membrane, consistent with a pore-forming mechanism. This is the first study exploring in detail the ability of *P. noctiluca* venom to modify the electrophysiological properties of mammalian cells. In this light, the study i) provides essential information on a possible use of bioactive substances; ii) provides new strategies in the treatment of envenomation.

### ANTIMICROBIAL ACTIVITIES OF EXTRACTS FROM *GRACILARIA GRACILIS* (RHODOPHYTA)

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Marine algae produce a wide variety of bioactive metabolites as antimicrobial, antifeedant, antihelminthic and cytotoxic agents. These compounds include alkaloids, polyketides, cyclic peptides, polysaccharides, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols. The antimicrobial potential differs among the different classes of seaweed, with the most promising being Rhodophyceae, followed by Chlorophyceae and Phaeophyceae. In this study, the antimicrobial potential of *Gracilaria gracilis*, from the Natural Reserve of Capo Peloro (Sicily, Italy), was evaluated. Soxhlet extractions was carried out using five different solvents (methanol, ethanol, acetone, chloroform and ethyl ether). Extracts were tested against Gram positive and Gram negative pathogens (i.e. *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Bacillus subtilis*, *Aeromonas hydrophila*, *Vibrio fischeri*). The antimicrobial susceptibility was assessed using the "agar disk diffusion method": 20 µl of each extract (at three different concentrations) were tested. Results showed that all the extracts were active against *B. subtilis*, with the best result that was obtained using the methanolic extract (inhibition halo diameter: 19 mm). A slight activity was also observed towards *Vibrio fischeri*. Our results enlarge the existing knowledge about the antimicrobial potential of red macroalgae. Further studies will be necessary to deepen the activity of *Gracilaria gracilis* against other Gram positive bacteria. Moreover, considering that *Bacillus subtilis* is often associated with food poisoning outbreaks, it could be suggested the use of *G. gracilis* extracts in the complexation of food preservatives, useful to reduce the risks deriving from the contamination by such microorganism.

### THE EFFECTS OF ACUTE EXPOSURE OF NEONICOTINOID INSECTICIDE ON DIFFERENT SPECIES OF CRAYFISH

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Neonicotinoids are one of the newest class of pesticides, used in agriculture like insecticides from the beginning of 90 years. They have become very popular and widely used all over the world for their low toxicity to vertebrates. Together, in last years some of the active substances in the neonicotinoid group (clothianidin, imidacloprid a thiamethoxam) have been banned for their secondary impact on non-target organisms and the environment. The aim of this study was to investigate the acute toxicity 96hLC50 of Calypso 480 SC (containing 48% neonicotinoid thiacloprid) on three species of crayfish. We used two different developmental stages: marbled crayfish (*Procambarus fallax* f. *virginalis*), red swamp crayfish (*Procambarus clarkii*) and common yabby (*Cherax destructor*), which were tested separately. The Calypso 480 SC had a more toxic effect on to younger stages of crayfish, the 96hLC50 in mg/L was: 1.60 and 27.30 marbled crayfish, 1.13 and 1.94 red swamp crayfish, 0.52 and 7.65 mg/L common yabby, values are sorted consecutively younger and then older. Also changes in behaviour in crayfish exposed to insecticide were observed, as is reduced aggressiveness, slowing movement of the limbs, reduced breathing, turning the crayfish on the back, apathy of organisms and subsequent deaths. This study provides and complements other important results for evaluating the toxicity effect of thiacloprid on non-target species, respectively crayfish. *Acknowledgements:* Supported by the Ministry of Education, Youth and Sports of the Czech Republic - projects CENAKVA (No. CZ.1.05/2.1.00/01.0024) and CENAKVA II (No. LO1205 under the NPU I program), and project Development of University of South Bohemia: International Mobility MSCA IF (no. CZ.02.2.69/0.0/0.0/17\_050/0008486).

### METHADONE VERSUS BUPRENORPHINE: DATA DETECTED FROM CALTANISSETTA SER.T

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In last years, heroin-addicted have exponentially increased: this has made it necessary to identify a pharmacological strategy as effective as possible. With this purpose, a statistical investigation was conducted in a sample of individuals, aged between 18 and 50. They were diagnosed and subjected to different treatments at Ser.T of Caltanissetta (Sicily-Italy) during the period 2013-2017. The analyzed patients were treated with three different pharmacological therapy: methadone 0.1%, methadone 0.5%, buprenorphine and suboxone. We obtained percentages of RESPONDERS, LOW RESPONDERS and NON RESPONDERS patients from data processing, based on used therapy. Considering pharmacological responses of the sample examined, it is possible to observe that the treatment with buprenorphine has led to 71.98% of RESPONDERS subjects, 23.52% of LOW RESPONDERS and 4.5% of NON RESPONDERS. Instead, the administration of methadone 0.1 % has produced 82.82% of RESPONDERS subjects, 11.08% of LOW RESPONDERS, 6.1% of NON RESPONDERS. The therapy with methadone



0.5% has resulted 88.98% of RESPONDERS subjects, 7.8% of LOW RESPONDERS, 3.22% of NON RESPONDERS. Finally, through the administration of suboxone, we obtained 86.34% of RESPONDERS subjects, 9.84% of LOW RESPONDERS and 3.82% of NON RESPONDERS. In conclusion, although it has emerged that treatment with methadone 0.5% is the most successful therapy, it is preferable to use suboxone (except in relapsing subjects) since it has also produced a high number of RESPONDERS subjects and a good safety profile for heroin addicted patients.

### STATISTICAL ANALYSIS ABOUT DIFFUSION OF EXERCISE ADDICTION IN SICILY

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Exercise Addiction (EA) is a behavioral dysfunction marked by uncontrolled compulsion towards all kinds of physical activity. With the purpose of understanding the epidemiology of this steadily increasing phenomenon, we have done a statistical analysis about some data obtained through the administration of an online-questionnaire (Google forms): the same one was also converted into paper-questionnaire and then it has been administered in many gyms in Palermo and Trapani (Sicily-Italy). The sample examined consists of 976 people aged between 14 and 65 (47.3% of them are women and 52.7% are men). For 53.8% of analyzed people, physical activity is one of the most important things of their life. In fact, 18% of them declared to have conflicts with family or friends, because of the excessive hours dedicated to sport. Moreover, 86.2% of them pointed out that physical activity is a mood-booster. Probably for this reason, 49.6% of them claimed that they have drastically increased the number of hours devoted to physical activities in last years. Now, the most impressive statistic is the following one: 23.5% of surveyed declared that they get restless and nervous if they are unable to perform the training. Thanks to the last data, a clear symptom of craving emerged. It is very complicated to make

a diagnosis of EA: however, through these questionnaires, the data indicate the presence of behaviors related to the Exercise Addiction. For this reason, it is necessary to act with preventive and information works in order to stem this not fully known phenomenon as much as possible.

### AMATEUR DOPING: A SURVEY AMONG SICILIAN PEOPLE

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In last years, Amateur Doping has caused many victims. In order to know the diffusion of this phenomenon, we have conducted an online-survey through Google forms. We also transformed the same questionnaire on paper and it was administered in many gyms in Palermo and Trapani (Sicily-Italy). The sample examined consists of 976 people aged between 14 and 65 (47.3% of them are women and 52.7% are men). We asked them if they ever took on substances to improve their athletic performances: 25.8% of them answered affirmatively and they declared to take on protein, amino acids but also Eca Stacks, which are hired on regular basis (34.6%). They bought this substances in sporting stores (32.2%), in pharmacy without prescription (26.8%), on internet sites (22.8%), in gyms (10.7%); 7.5% of them answered that they bought substances in other place. Furthermore, 17.9% of them declared that training companions, but also coaches and/or responsible of sports societies have proposed to them to take doping-substances (Winstrol, Tamoxifene, Testosterone, EPO, exc...). 16.8% of interviewed claimed that is right to take substance with the purpose to improve their performances. However, when we asked them if they would ever have taken substances to their children, 95.8% of them answered "absolutely not". They also declared to want more information about the real dangers related to uncorrected doping-substances intake. In conclusion, it appears necessary to adopt measures of information and prevention to reduce diffusion of this dangerous phenomenon.

## Index of authors

### A

Abitante, Lucia . . . . .	1
Aliko, Valbona . . . . .	3
Amici, Roberto . . . . .	3
Amin, Nida . . . . .	3
Aragona, Marialuisa . . . . .	9,11
Arena, Rosaria . . . . .	12
Avitabile, Elisabetta . . . . .	5,9

### B

Baiamonte, Concetta . . . . .	5
Bailoni, Lucia . . . . .	3
Bartolomeo, Giovanni . . . . .	13
Benazzi, Stefano . . . . .	8
Bernardinelli, Emanuele . . . . .	7
Blahova, Jana . . . . .	9
Boano, Rosa . . . . .	4,7
Boattini, Alessio . . . . .	1
Bortolini, Eugenio . . . . .	8
Bridelli, Maria Grazia . . . . .	9
Brigidi, Patrizia . . . . .	3
Brizzi, Maurizio . . . . .	7
Bruni, Antonella . . . . .	5
Buccioni, Arianna . . . . .	1
Burlando, Bruno . . . . .	6

### C

Caffaro, Federica . . . . .	10
Cancellieri, Giulia . . . . .	13,14
Candela, Marco . . . . .	3
Capillo, Gioele . . . . .	3,9,11,13
Cardazzo, Barbara . . . . .	3
Carraro, Lisa . . . . .	3
Casuccio, Alessandra . . . . .	4
Catania, Francesca . . . . .	14
Cavallo, Maria Chiara . . . . .	4
Cecconello, Edoardo . . . . .	7
Cerri, Matteo . . . . .	3
Cervellera, Stefano . . . . .	5
Chiavazza, Paola Maria . . . . .	7
Chiavetta, Pierfrancesco . . . . .	3
Cinti, Alessandra . . . . .	7
Cocchi, Massimo . . . . .	6
Cocilovo, Claudia . . . . .	9
Cornara, Laura . . . . .	6
Costa, Rosaria . . . . .	11,13
Cottone, Salvatore . . . . .	4
Cuesta, Alberto . . . . .	12

### D

D'Avino, Cristina . . . . .	5,8,9
-----------------------------	-------

Dalocchio, Roberto . . . . .	5
De lasio, Sergio . . . . .	4,7
Dessi, Alessandro . . . . .	5
Di Carlo, Annamaria . . . . .	13
Di Majo, Danila . . . . .	4,10
Díaz, Julio A. . . . .	11
Dossena, Silvia . . . . .	6,7,12

### E

Espinosa Ruiz, Cristobal . . . . .	12
Esteban, Maria . . . . .	12

### F

Faber, Paulo L. . . . .	10
Faggio, Caterina . . . . .	3,7,9,10,11,13
Fanelli, Mirco . . . . .	3
Fenoglio, Alessandra . . . . .	10
Ferriol, Pere . . . . .	11
Fiorino, Emma . . . . .	10
Fozza, Claudio . . . . .	8
Freitas, Teresa . . . . .	10
Fulcheri, Ezio . . . . .	7

### G

Garcia-Perez, Jose Luis . . . . .	1
Gennaro, Valerio . . . . .	5
Giammanco, Marco . . . . .	4,10
Gianicolo, Emilio . . . . .	5
Giordani, Paolo . . . . .	6
Girotti, Marilena . . . . .	4,7
Giustetto, Ambra . . . . .	10
Grimaudo, Stefania . . . . .	5
Guichard, Etienne . . . . .	1
Guida, Lucrezia . . . . .	6
Guzzetti, Eleonora . . . . .	11

### H

Hitrec, Timna . . . . .	3
-------------------------	---

### J

Jagoda, Evelyn . . . . .	1
--------------------------	---

### K

Kouba, Antonín . . . . .	13
--------------------------	----

### L

La Guardia, Maurizio . . . . .	4,10
Lauriano, Eugenia Rita . . . . .	9,11
Lavorano, Silvia . . . . .	6
Leo, Ludovica . . . . .	9
Li Volsi, Rosalia . . . . .	11
Liga, Anastasia Valentina . . . . .	13,14
Lo Giudice, Angelina . . . . .	13
Loddo, Saverio . . . . .	6

Luiselli, Donata . . . . .	1	Rizzo, Carmen . . . . .	13
<b>M</b>		Rotondo, Archimede . . . . .	11
Magazzù, Carol . . . . .	9,11	Rózański, Józef . . . . .	11
Malagoli-Tagliazucchi, Guidantonio . . . . .	1	Rubio-Roldán, Alejandro . . . . .	1
Manuguerra, Simona . . . . .	12	Russo, Antonello . . . . .	5
Marchetti, Giuseppe . . . . .	5,8,9	<b>S</b>	
Marino, Angela . . . . .	6,7,10,12	Salaberry, Eduardo . . . . .	11
Mariottini, Gian Luigi . . . . .	6	Saldanha, Carlota . . . . .	10
Masali, Melchiorre . . . . .	10	Sanfilippo, Marilena . . . . .	3
Medici, Serenella . . . . .	9	Santos Silva-Herdade, Ana . . . . .	10
Mehmeti, Enis . . . . .	3	Santulli, Andrea . . . . .	6,12
Mele, Marcello . . . . .	1	Sardo, Francesco . . . . .	12
Messina, Concetta M. . . . .	6,13	Sarno, Stefania . . . . .	1
Micheletti Cremasco, Margherita . . . . .	10	Savoca, Serena . . . . .	3,13
Morabito, Rossana . . . . .	6,7,12	Serra, Andrea . . . . .	1
Morghese, Maria . . . . .	12	Sisa, Claudia . . . . .	3
Mortillaro, Francesca . . . . .	13	Smeriglio, Antonella . . . . .	6
Murchio, Giovanni . . . . .	5	Sozzo, Sara . . . . .	7
Musella, Margherita . . . . .	1	Spanò, Nunziacarla . . . . .	3,11,13
<b>N</b>		Squarcio, Fabio . . . . .	3
Nagy, Tamas . . . . .	7	Stanzani, Agnese . . . . .	3
Nepveu, Françoise . . . . .	8	Stará, Alžběta . . . . .	7,13
<b>O</b>		Strouhová, Alžběta . . . . .	13
Orrù, Alessia . . . . .	4,7	Sureda, Antoni . . . . .	11
<b>P</b>		Svobodova, Zdenka . . . . .	9
Pagani, Luca . . . . .	1	<b>T</b>	
Pagano, Maria . . . . .	7	Taccioli, Cristian . . . . .	1
Panarello, Giuseppe . . . . .	3,13	Tejada, Silvia . . . . .	11
Pantaleo, Antonella . . . . .	5,8,9	Traversari, Mirko . . . . .	8
Peona, Valentina . . . . .	1	Trichilo, Vincenzo . . . . .	6
Pergolizzi, Simona . . . . .	9,11	Trombetta, Domenico . . . . .	6
Pettener, Davide . . . . .	1	Tsamesidis, Ioannis . . . . .	5,8,9
Pierlorenzi, Giordano . . . . .	10	Turrini, Francesco . . . . .	8
Pipitone, Rosaria Maria . . . . .	5	Turroni, Silvia . . . . .	3
Piscitiello, Emiliana . . . . .	3	Tuszyński, Jack A. . . . .	2
Plhalova, Lucie . . . . .	9	<b>U</b>	
Polverini, Eugenia . . . . .	9	Ugolini, Valeria . . . . .	4
<b>Q</b>		<b>V</b>	
Qirjo, Mihallaq . . . . .	3	Valente, Marcello . . . . .	7
<b>R</b>		Velíšek, Josef . . . . .	13
Rabino Massa, Emma . . . . .	1	Venturella, Fabio . . . . .	13,14
Rando, Rossana . . . . .	13	Viti, Carlo . . . . .	1
Realmuto, Sabrina . . . . .	4	<b>Z</b>	
Regoli, Francesco . . . . .	2	Zito, Rossella . . . . .	5
Remigante, Alessia . . . . .	6,7,12	Żuwala, Krystyna . . . . .	11
Renda, Giuseppe . . . . .	12		
Reybier, Karine . . . . .	8		
Ricci, Marco . . . . .	1		