Nervous system and arm regeneration in crinoids

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The crinoid *Antedon mediterranea* has remarkable regeneration abilities, making it a valuable model for the study of these post-embryonic developmental phenomena. Indeed, it can rapidly and completely regenerate arms, pinnules, cirri and the visceral mass, after autotomy or experimentally induced amputations. During regeneration a variety of neural regulatory molecules (neurotransmitters, neuropeptides and growth factors) are thought to play important signalling roles. Here we have analysed the ultrastructural features and some molecular aspects of the nervous system of *A. mediterranea* in normal and regenerating arms. Animals were collected from Baia delle Grazie in the Gulf of La Spezia (Ligurian Sea, Italy) and maintained in artificial sea-water. Experimental amputations were carried out mimicking natural conditions and regeneration was examined at early (72h post-amputation (pa)) and advanced stages (up to 4 weeks pa). Some tissue samples were processed for standard histological and ultrastructural studies whereas others were prepared for analysis of neuropeptide expression using mRNA *in situ* hybridization and immunocytochemical techniques.

In crinoid arms the main nervous component is the brachial nerve formed by a medulla mainly composed of neural processes and sparse chains of perikarya and an external cortex of perikarya. There is also some evidence of well-defined groups of perikarya at the level of muscle and ligament innervation, that could potentially be considered as a basic form of ganglia thus hypothetically suggesting metamerism of the nervous system. However, this “perikarya clustering” needs to be confirmed by further studies. In the early regenerative phase, the nerve has not yet fully regrown within the regenerate but a continuity can be observed between chains of nervous cells and the undifferentiated cells filling the blastema that is the characteristic structure of this phase. The advanced regenerative phase is characterized by the regeneration of the brachial nerve and the progressive differentiation of other tissues. Analysis of *A. mediterranea* transcriptome sequence data has enabled identification of transcripts encoding precursors of a variety of neuropeptides (SALMFamides, calcitonin-type, vasopressin-type) and on-going studies are investigating the expression of these and other neuropeptides, which will provide a basis for investigation of the physiological roles of neuropeptides in both non-regenerating and regenerating arms.

Hydrology, water chemistry and microbial community patterns in different urbanized areas of the Tevere river catchment (Rome, Italy).

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Urban rivers experience increasing water resource exploitation and urbanization impact, with important implications for ecosystem functioning and services. Most field studies reported that water quality and hydrological regimes are dramatically affected at low-land reaches by increasing anthropic impact and activities. There remains a lack of knowledge concerning the links between hydrology, water chemistry and microbial community structure, whose dynamics play a key role in organic matter decomposition and nutrient cycling along the river continuum.

The aim of this multidisciplinary study was to explore the hydrological, chemical and microbiological patterns along a severe urbanization gradient.

The catchment of the River Tevere (including the main stem, the stream Cremera, and the major tributary Aniene) was sampled at the closing section in differently urbanized areas at two contrasting seasons (winter/summer). The major hydrological, physical and chemical characteristics of river waters were measured directly or retrieved within datasets from monitoring agencies. The microbial community structure was analyzed to identify and quantify the aquatic prokaryotes and picoeukaryotes (i.e., heterotrophs and photoautotrophs).

Our results outlined recurrent patterns and quantitative changes of interacting microbial assemblages across the urbanization gradient at different hydrological settings. The total prokaryotic cell abundance increased toward the river mouth, with higher values registered downstream the city of Rome. The per-cell nucleic acid content, intended as a proxy of the cell metabolic activity, increased accordingly, while the ratio between photoautotrops and heterotrophs decreased downstream the confluence with main tributary.

Outcomes revealed that river microbes provide valuable indications on the ecological effects of urbanization and altered environmental conditions and could be also useful for studying different scenarios of water resources management in the basins involved.

First data on an urban population of Green Toad (*Bufo viridis*) in Milan.

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There are many problems in the management of populations of amphibians in the big cities. In 2016, data was collected in an Green Toad urban population located in the "Portello Park" in Milan, opened in 2011, following a large die-off of specimens due to lack of information on this population.
In the Park there are two fountains supplied with well water: the first, large circular shape connected to another half-moon and a small lying on top of a small hill in the Park. The depth of the two fountains is about 40 cm.
There is no plants and the two fountains are emptied and cleaned twice a year.
Daily data were collected in the period of spawning of the toads and weekly until the tadpole metamorphosis. They were detected biometric measurements of adults and tadpoles, recorded adult positions, eggs and tadpoles in the large fountain and minimum and maximum temperatures and humidity of the air.
The egg strings were laid from March 20 to April 1st, 2016 while the tadpoles were observed from 29 March 2016. The metamorphosis of tadpoles began in early June.
Some adults were observed throughout the sampling period and a couple was also observed on the 16 June 2016 without laying eggs. It was found a high mortality of adults in June, probably due to the limited output accesses by the two fountains. The population seems to be in good condition and made up of individuals of moderate size. The eggs have been laid in all portions of the large fountain, with preference in the half-moon and the north side (facing south). The growth of the tadpoles was regular.
Monitoring population will enable the knowledge and the management of this urban population of toad in a high traffic area visited by many people every day, and allowing the presence of amphibians in a large densely urbanized and cemented town like Milan.

Integrating LIM results in Ecopath: a working example on the Venice lagoon food web

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Quantification of energy and mass fluxes within food webs is of primary interest in system ecology since it allows one to calculate indexes of food web functioning, which can be included in the evaluation of ecosystem status. Different methodologies have been developed to reconstruct food webs based on incomplete sets of data (Steele, 2009). The most widely used tool nowadays is the Ecopath software, which provides a comprehensive and flexible modelling platform, including facilities to automatically perform ecological network analysis, and to calculate indexes of food web functioning. A critical step in the implementation of Ecopath models is represented by their balancing (i.e. finding the solution of the system of difference equations stating mass/energy conservation). Of potential help in this task can be an alternative tool, called linear inverse methodology (LIM) of constrained optimization, which allows to constrain a-priori the space of possible solutions based on a set of inequalities, which are imposed on the basis of general eco-physiological knowledge and site specific data on energy flows.

In this work we present the balancing of a steady state Ecopath model performed by applying a LIM. The application was carried out in the Lagoon of Venice. The trophic network was represented by thirty-two nodes, including single species compartments for the species of high economical or ecological relevance (e.g. Manila clam and seagrass). The food web model was constructed based on a set of data collected over the 2001–2005 time frame. Results shown that the solution obtained though LIM can be used to set up the Ecopath application. A comparison among balancing with and without LIM showed that most relevant differences are on the estimation of the fate of organic detritus, and its exchange at the bentho-pelagic level.

FIRST RECORD OF LEPTOCEPHALI OF THE FALSE MORAY, *CHLOPSIS BICOLOR* RAFINESQUE 1810 (ACTINOPTERYGII, ANGUILLIFORMES, CHLOPSIDAE) FROM THE ADRIATIC SEA: MORPHOLOGICAL AND MOLECULAR IDENTIFICATION

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Leptocephali are the characteristic larval forms of the superorder Elopomorpha, very difficult to identify at the species level and sharing few or no characters with the respective adult forms. In this study we used DNA barcoding coupled to classical taxonomy for the species identification of *Chlopsis bicolor* (Rafinesque, 1810) leptocephali. In the Mediterranean some individuals of *C. bicolor* in developmental stage, collected along the Sicilian shore of the Messina Strait, were already identified and their detailed morphological descriptions were provided. However, very few specimens of *C. bicolor* from the Adriatic Sea have been reported and this work represents the first record of its larval form. Three leptocephali of the false moray *C.bicolor* were morphologically identified among other eel larvae collected during scientific cruises performed in South Adriatic between 2010 and 2012. Moreover we sequenced a 655bp region of COI (cytochrome c oxidase I) in addition to classical taxonomy to provide a larval barcode voucher for species identification. COI barcoding has proven to be successful in larval fish identification, revealing also a good performance as a diagnostic method for the identification of leptocephali. Anyway, the limited number of anguilliform species annotated in the reference databases (Barcode of Life Data Systems and GenBank) allowed us to validate our barcode only at the family level (Chlopsidae). Nevertheless, the morphological description provided in this work together with the barcode sequence published in the project ‘Barcoding of the Adriatic Leptocephali’ (BAL) on the Barcode of Life Data System (BOLD, http://www.barcodinglife.org), represent a useful contribution to the knowledge of this species.

INSIDE THE BRAIN OF THE COLONIAL TUNICATE *BOTRYLLUS SCHLOSSERI*: WHEN BUDDING INVOLVES CYCLICAL NEURODEGENERATION

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Cerebral ageing is one of the biggest challenges of the twenty-first century. With improvements in modern medicine, the human lifespan has markedly increased, and so has prevalence of age-associated neurodegenerative diseases. Tunicate, the sister group of vertebrates, provide an excellent model for modeling human diseases. In the colonial tunicate *Botryllus schlosseri*, in the phase referred to take-over, all adults of the colony are synchronously resorbed by means of a massive and diffuse apoptosis, which involves also the nervous system. For this reason, the species represents a resource to investigate mechanisms of neurodegeneration. The central nervous system of *B. schlosseri* consists of a cerebral ganglion (the brain) located between the oral and the cloacal siphons. It is composed by an external cortex of two-three layers of neurons and an internal medulla of densely packed neuritis. We studied the process of neurodegeneration during the take-over at electron microscopy. We found an increase in neurons with polymorphic nuclei and condensed chromatin close to nuclear membrane. Moreover, we observed hemocytes within the ganglion. These were macrophage-like cells, containing in their phagocytic vacuole neural components, and morula cells. Some of the latter were degranulated and had already released the precursors of the cytotoxic enzyme phenoloxidase and its substrates in the ganglion. Since the cerebral ganglion undergoes size reduction during take-over, using confocal microscopy we quantified the neuron number during the mid-cycle, the phase preceding the take-over, and the take-over. We saw a progressive decrease in neuron numbers, which begins before the take-over and continues until the ganglion resorption. In the meantime, we detected phagocytes and morula cells increase. In conclusion, these morphodynamic data on neurodegeneration show that neurodegeneration begins before the take-over and involves inflammatory-related hemocytes.

GENETIC STUDIES OF THE WEDDELL SEAL IN THE ROSS SEA: A CLOSER LOOK ON THE COLONIES IN MARIO ZUCCHELLI STATION AREA

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Weddell seals (*Leptonychotes weddellii)* have the most southern distribution among all mammals, with breeding colonies that spread along the whole Antarctic coast. Several genetic, behavioural and population studies on this species can be found in literature, but almost all of them have been concentrated on the colony of McMurdo Sound. The present work is the first analysis of the genetic diversity of two colonies, Terranova Bay and Wood Bay, both located in the Ross Sea area. Their genetic structure was analysed and results compared with those already available from McMurdo.

Dloop and CytB (with different mutation rates) were used to estimate the effective number (Ne) of the whole Ross Sea population, test the possible recent expansion of the colonies and observe the variation and distribution of the haplotypes. 15 microsatellite markers were used to obtain the Ne for the colonies and tested for a possible genetic structure.

Both mtDNA fragments showed a Ne of around 50,000 females for the whole Ross Sea population. Expansion test using mismatch distribution was positive, and the beginning was around 58,000 years, a little later than McMurdo (81,000 years), but always during the last glacial cycle. Haplotype analysis showed a high diversity (Hd > 0.90), and the quantity of exclusive haplotypes varied from 43% to 81%, huge values, if we consider that all these colonies are very close to each other. So Antarctic seals tend to present a high intraspecific haplotype variation, with large populations that persist for long periods of time, perhaps due to the lack of human hunting and terrestrial predation. Microsatellites analysis showed very low differentiation between the colonies, confirming that they are indeed part of the same population. This was also confirmed by the number of most likely clusters (K=1). The Ne value for both colonies was estimated in around1,340 individuals.

Our results show that Weddell seals undergone through a demographic expansion since the last glacial cycle and that today they present a local remarkable genetic variation, with large populations that persist for long periods of time in the same area. These patterns are likely a consequence of their high site fidelity, lack of human hunting and terrestrial predation. Nevertheless, as a top predator mammal, the role of this species in the Ross Sea is crucial, and its demographic dynamics should be monitored to follow the future changes of such an important ecosystem.

The interplay between damages and adaptive strategies in *Q. ilex* plants subjected to heavy metal pollution

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Plants of the urban environment are exposed to a wide range of pollutants, including heavy metals (HMs). These latter may be very dangerous for the photosynthetic apparatus causing a decline of the primary production. The urban areas represent an open laboratory to successfully study the capability of biomonitors to endure the pollution and the specific strategies at basis of their adaptive success.

The aim of this research was to assess in *Q. ilex* L., a widespread species in the urban areas of Southern Italy, the contribute of photosynthetic regulation mechanisms and antioxidants in guaranteeing plant tolerance to HMs. At this purpose, leaves collected in two urban parks (UP) and two roadsides (RS) were analysed for HM content, leaf functional traits, photochemistry, photosynthetic protein expression and pigment content. In addition, the production of endogenous peroxide and antioxidant capacity was also evaluated. Our results indicated that RS leaves were characterized by higher HM concentrations than UP leaves. Whereas leaf traits were not affected by metal concentration in tissue, the photosynthetic pigment content as well as the levels of endogenous peroxide and antioxidants increased in RS, compared to UP leaves, indicating the occurrence of oxidative stress. As regards photosynthesis, in the two roadsides, the contaminated leaves exhibited a different photochemical behavior consisting in a diverse partitioning of absorbed light. In particular, leaves investing more light energy in photochemistry showed photochemical efficiencies comparable to those of uncontaminated leaves; on the contrary, leaves that diverted light mainly in dissipation mechanisms, were unable to maintain elevated photochemistry and photosynthetic protein levels, becoming more sensitive to HM injuries. The overall data indicated that the synergy between antioxidant defenses and the regulation of photosynthesis, achieved by the partitioning of the absorbed light in photochemical and non-photochemical processes, may contribute to the success of *Q. ilex* in the contaminated urban environment.

GENE FLOW AND REPRODUCTIVE SUCCESS ALONG AN ALTITUDINAL TRANSECT IN A MARGINAL NORWAY SPRUCE POPULATION

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Gene flow (GF) can be undoubtedly listed among the main forces acting on evolutionary processes, even if the magnitude and direction of its effects are still debated. Our study aimed at assessing historical and contemporary GF patterns in a peripheral Norway spruce population at the rear edge of the species' distribution, within the Campolino natural reserve (Northern Apennines, Italy). Within the study area, Norway spruce density decreases from the treeline to the mixed forest below; the complex structure along the altitudinal gradient calls for a deep investigation of GF dynamics shaping the population genetic structure. For this purpose we performed a parentage analysis in three plots (low, intermediate and high altitude), sampling a total of 321 adults and 311 juveniles. For each individual, we collected fresh needles and a wood core for dendrochronological analyses; we also recorded spatial position, diameter and height. We genotyped all individuals at 10 nuclear and 3 chloroplast SSRs. The age distribution of adult trees was similar between the high and low plots (average age of ~50 yrs) whereas the intermediate one had an average age of 107 yrs and 60% of individuals were >100 yrs. Genetic differentiation was low between the high and intermediate plots but increased between the intermediate and low ones. Such differentiation at a short distance is surprising considering previous results on alpine populations and might be interpreted as a signal of limited GF due to the local fragmented distribution. We estimated contemporary GF rates using both maximum likelihood assignment and fitting the neighbourhood model. Gamete immigration was similar among plots. However, analysing separately seed and pollen GF patterns, a lower seed immigration and a spatially restricted seed dispersal was found in the intermediate plot, highlighting the possible role of old individuals as dominant seed sources in shaping local dispersal dynamics. Using a fractional assignment of parentage, we estimated reproductive success for each individual, linking it with phenotype features (age, diameter and height) and plot. The most successful trees were the oldest among the largest. Our results emphasized the importance of carefully characterizing neutral processes to design effective conservation strategies and to correctly evaluate trees' adaptive response to ongoing environmental changes in future researches on local adaptation.

The haemocytes of the colonial ascidian *Diplosoma listerianum*: histochemical, histoenzymatic and ultrastructural studies

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In recent years, the attention of researchers towards members of the subphylum Tunicata, mainly ascidians, progressively grew, due to their recognised peculiar phylogenetic position of vertebrate sister group. In spite of the increased amount of studies on ascidians, mainly devoted to investigate the genetic control of development and/or allorecognition, haemolymph and its role in immune defense has been poorly investigated. Ascidian haemolymph contains a great variety of haemocytes with various roles. In compound ascidians, most of the studies on haemolymph and haemocytes have been carried out in members of the genus *Botryllus* and *Botrylloides* where a well-defined colonial circulatory system is present. The compound ascidian *Diplosoma listerianum*, easily found in the Lagoon of Venice, lacks a shared colonial circulation crossing the tunic and no description of the haemocytes circulating in the haemolymph is present in the literature. In this species, we started a characterization of the haemocytes based on their histochemical and histoenzymatic features and their ultrastructure. We were able to identify 7 morphotypes morphologically and histochemically referable to corresponding cell types described botryllids: undifferentiated cells (haemoblasts), storage cells (nephrocytes) and immunocytes. The latter are undoubtedly involved in immune responses, acting as phagocytes, involved in the clearance of foreign particles (hyaline amoebocytes and macrophage-like cells), or cytotoxic cells, able to induce cytotoxicity through the release of phenoloxidase (granular amoebocytes and morula cells). In addition, a spherical cell with small basophilic granules (round granulocyte) was recognized: they contain heparin and histamine, resembling the granular cell previously described in *Botrylloides* as a mast cell-like, able to infiltrate the gut epithelium.

A multi-temporal interdisciplinary approach between hydrogeology and microbial ecology for assessing the impact of a municipal waste dump on a volcanic groundwater system in Central Italy

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Groundwater (GW) contamination is an environmental and human health problem. GW is increasingly threatened by contaminant occurrence from human activities like storage of household and municipal solid wastes. The migration of a contaminant from surface to groundwater is controlled by various chemical and physical processes which take place in the sub-surface and are also linked to chemio-dynamic properties of a substance (e.g. sorption to soil organic matter) and to specific geological and hydrogeological setting. Volcanic areas are generally considered protective towards groundwater contamination due to their own low permeability even if the volcanic substrata, supposed to be un-deformed or blandly deformed, is intensely fractured and induced heterogeneous by brittle deformation processes, such as fractures and faults.

A municipal solid waste disposal located in Central Italy, has been studied for more than one year using a multi-temporal approach. Hydrological and hydrogeological dynamic of fluid circulation and microbiological parameters (cell viability, abundance, dissolved organic carbon) have been evaluated and related to the geological structures both in two and three dimensions. Moreover, contaminant occurrence was also evaluated. The overall results show that legislation limits were never exceeded (except for a contaminant in one well), while the multi temporal approach made it possible to depict not only the dynamicity and complexity of groundwater flux, but also to identify where the highest anthropogenic pressure is present using “non-conventional parameters” such as cell viability and DOC.

The microbial abundance, cell viability, Dissolved Organic Carbon (DOC) and all organic contaminant data were used for the geostatistical analysis in order to obtain 3D maps for investigating possible relationships between these biotic and abiotic parameters. Close relationships between geological heterogeneity, water circulation, pollutant diffusion, dissolved organic carbon and cell viability were found. The highest values of cell viability were found with DOC values ≤ 0.5 mg/L; above this value, DOC affected negatively the microbial community. The highest DOC values were found in correspondence of the most thick brittle levels (fractures and faults) indicating its surficial source from the waste disposal. This approach can be very useful to improve monitoring surveys and identify the most vulnerable sites in groundwater.

Evolution of vitellogenin gene family.

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The genes of vitellogenin, from which the main egg-yolk proteins of oviparous animal species arise, are an excellent example to understand how a gene family origins and has been changing during the evolutionary history. The coelacanth *Latimeria menadoensis* and the lungfish *Protopterus annectens*, considered living fossils, occupy a key phylogenetic position to investigate changes that have affected the genomes of the aquatic vertebrates that colonized dry land. In this study, transcripts of several vitellogenin genes in the two species of interest were isolated and their inferred amino acid sequences were compared to those of other vertebrates. The phylogenetic data suggest that the evolutionary history of this gene family was characterized by different duplication events that occurred in teleosts, aquatic sarcopterygians, amniotes and amphibians. The sequences obtained in the two sarcopterygians, compared to those of other oviparous vertebrates, present conserved domains but also some sites similar to those of teleosts and others to those of tetrapods emphasizing the intermediate characteristics of these living fossils.

Initial survey of the nocturnal macromoths of the Umbria (Central Italy)

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The species assemblages of nocturnal macromoths (Insecta, Lepidoptera) were examined in relation to the natural sensitiveness to photoreception using a 250W mercury vapor lamp, powered by a portable electric generator. The standardized sampling time was at least three hours of night investigation. Weekly samplings were carried out from October 2015 to May 2016 at twenty-six sites in Umbria (Italy) in the zone comprised between Lake Trasimeno (to the West) and Apennine Mountains (to the East). The aim of this research is to update the checklist of macromoths in Umbria, a region little known for Macroheterocera except the accurate study of Zerunian & Zilli (2014) conducted on Mt Subasio area (Perugia-Assisi). During our study 179 species comprising 9 families were collected, in particular Drepanidae (8 species), Erebidae (14), Geometridae (71), Lasiocampidae (3), Noctuidae (56), Nolidae (1), Notodontidae (19), Saturniidae (2) and Sphingidae (5). In the autumn-spring period, the attraction to artificial light has reported a new species to Umbria (*Eupithecia silenata* Assmann, 1848) and 18 species previously found only in Mt Subasio area. The survey through phototaxis highlighted the species phenological activity in the months between October and May, showing considerable monthly fluctuations: from 6 species in December to 98 in May. The first results of our study contribute to increasing the knowledge on Umbria Lepidoptera, an ecologically sensitive fauna, important bioindicator of environmental quality.

First attempt to measure adult neurogenesis in a lophotrocozoan (*Octopus vulgaris*) brain using flow-cytometry technique

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In the past decades, researchers provided an amount of significant information about the anatomical, molecular and functional mechanisms underlying neurogenesis in the adult brain.

Adult neurogenesis consists in proliferation, migration and differentiation of newborn cells that will be functionally integrated into the existing neural circuitry of adult brain. This process plays a crucial role in adaptation to the environmental challenges.

It occurs in animals with complex and centralized nervous system exhibiting cognitive capabilities and sophisticated behavioral repertoires, such as mammals, including humans, non-mammals vertebrates and, among invertebrates, it has been demonstrated in ecdisozoan taxa such as insects and crustaceans too. *Octopus vulgaris* is considered an "advanced invertebrate" for the size of its brain, the largest of any invertebrates. Evolved from the basal molluscan plan of tetraneury and characterized by a hierarchical organization. Octopus central nervous system is located around the esophagus, in a cartilaginous "cranium" between the eyes, and consists in a supra-esophageal and sub-esophageal masses connected to two optic lobes.

Octopus shows complex behaviours and unusual cognitive skills, as learning and memory, problem solving, individual personality and capabilities to play. For these reasons, it seems to be the most likely candidate for the neurogenic process among lophotrocozoans.

In our previous works, we found cell proliferation in specific areas of octopus brain involved in learning, memory and processing sensory information. Moreover, we demonstrated, using specific markers as PCNA, PARP1 and Oct-elav1 gene, that enriched environment increases proliferation and synaptogenesis.

Given that, we developed a protocol for flow-cytometry analysis to measure the proliferative activity in a faster and more reliable manner then the classical immunohistochemistry.

Dissociated cells from proliferating areas of octopus brain, vertical frontal system and optic-olfactory lobes, were exposed to BrdU and subjected to PI staining and FACS analysis. Bivariate distributions of BrdU content vs DNA content were analyzed and the G1/S subpopulation was determined. Univariate analysis of DNA content was used to determine the percentage of G2/S cells. Using this technique, we accurately quantified the effective number of proliferating cells in each areas, in order to determine the magnitude of the neurogenic process in octopus brain.

Biological clocks in cavefish: the evolution of circadian photoreception in darkness

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During evolution animals have adopted endogenous timing mechanisms that allow to anticipate cyclic environmental changes. This strategy permits to animals to coordinate physiological or behavioural processes to buffer environmental changes. The most common biological timing systems is the circadian clock which allows the anticipation of the day-night cycle. The behavioural and physiological mechanism whereby light regulates the clock is only partially understood. While in mammals the photic regulation of circadian rhythms depend exclusively by the lateral eyes, non mammalian vertebrates can rely on multiple extraocular photoreceptors, including deep brain photoreceptors. To address this question we have investigated at molecular and behavioural levels the circadian clock of cavefish, species evolved in environment typically characterized by continuous darkness, constant temperature as well as a scarcity of food and predators. Cavefish show common phenotypes, such as anophtalmia, depigmentation, low metabolic rate, and enhanced longevity. By comparing different species occupying very different habitats, the Somalian cavefish *Phreatichthys andruzzii* with the zebrafish *Danio rerio*, we investigated how molecular, cellular and behavioural mechanisms change during evolution in order to optimally adapt to their environments. In *P.andruzzii*, arrhythmic pattern of both locomotor activity and clock gene expression in central and peripheral tissues confirmed the prediction that the disappearance of photic entrainment in cavefish would be the logical consequence of exposure to constant darkness over the course of millions of years. However, cell culture and behavioural experiments showed that these cavefish still possess peripheral circadian clocks. Therefore, *P.andruzzii* has a clock, but this clock is completely blind. To understand the molecular basis of the blindness of *P.andruzzii* circadian clocks we cloned different non-visual opsin genes, and performed *in vitro* reporter and behavioural assays. Our investigations reveal the consequences of evolution in an extreme hypogean environment for the circadian clock and would be the basis to understand the regulation by light as well as for other mechanisms such as DNA repair, sleep and metabolism which directly or indirectly are affected by regular exposure to sunlight.

PATTERNS OF GENETIC AND MORPHOMETRIC DIVERSITY OF RECOVERED SPECIMENS OF *CARETTA CARETTA* IN THE ADRIATIC SEA

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The Adriatic Sea is an important feeding and overwintering area for the Mediterranean loggerhead turtle, *Caretta caretta*. Due to the increasing of human related impact in the area most animals are captured as by-catch from fishing activities, or found dead or alive stranded and floating in the sea. . Turtle mortality within the Adriatic Sea can act indirectly, can act indirectly on Mediterranean nesting colonies with low population sizes and exclusive haplotypes increasing their risk of genetic variability loss. Nevertheless, information about population structure and natal origin of loggerheads found in the basin are still limited. In this study, a comparative analysis of biometric data and DNA sequence polymorphism of the long fragment of the mitochondrial control region (815 bp) was carried out on 93 loggerheads recovered alive from three Adriatic feeding areas: North-western, North-eastern and South Adriatic. Differences in turtle's body sizes (i.e. Straight Carapace Length) among the three areas were investigated to understand the use of the Adriatic feeding habitats by loggerheads of different sizes. The origin of the Adriatic turtles stocks was assessed by means of a Bayesian mixed-stock analysis (MSA) to understand the migratory pathway of the species. The analysis of biometric data revealed a significant difference in turtle body size between the Northern and the Southern Adriatic. Five distinct haplotypes were detected. Among them, two are endemic to the Mediterranean nesting colonies and one was recently reported from the eastern Florida rookery. The remaining haplotypes are common both in Atlantic and in the Mediterranean nesting populations. MSA revealed a strong genetic relationship between the Adriatic specimens and the Mediterranean rookeries, while the contribution of Atlantic individuals was minimal. For the first time, within the Adriatic Sea stock Libyan contributors resulted more abundant than Greek ones. These results evidenced the presence of migratory pathways from Lybia and Greece towards the Adriatic Sea mainly related to the surface current system. In this scenario, reducing fishing effort and contrasting other threats faced by this species should be a prior intent to preserve these small and demographically vulnerable rookeries.

POSTER

LAKES AS AN ALTERNATIVE SOURCE OF ENERGY? PRELIMINARY IDEAS

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Eutrophication is a well-known environmental problem, which affects both the health of aquatic ecosystems and the anthropic use of water resource, with consequent environmental and health costs. Its negative environmental effects include the great increase of phytoplankton biomass in a water body, with further consequences such hypoxia, which can cause death to aquatic organisms. In mitigating the problem, a useful management of the vegetable biomass can be planned. Therefore phytoplankton blooms may represent an interesting source of carbon material e.g. for negative-electrode material for sodium-ion batteries.
Mono-species algal culture (*Pseudokirchneriella subcapitata*) were bred under controlled laboratory conditions and algae were characterized for their C, H and N content and by field-emission scanning-electron microscopy (ESEM). The algal biomass was then decomposed to carbonaceous species by heating in inert atmosphere up to 1000 °C. and the decomposition kinetics were monitored by thermogravimetric analysis (TGA) in order to find the best condition for carbonization to “hard carbon” species. “Hard carbon” species have a disordered structure of the graphite layers and voids between the random stacks of hexagonal carbon, making it much more suitable than ordered graphite for the storage of sodium. The structure of these carbonaceous species is therefore of primary importance to optimize their electrochemical behavior and will be studied by ESEM and XRD. The algae-derived carbon samples will be mixed with carbon black and PVDF binder at the mass ratio of 8:1:1 in an appropriate solvent. The slurry will be heated up, then coated on Cu foils as current collectors and then dried. The assembly of CR2032 coin cells in a glovebox will follow: metallic sodium foil will be used as negative electrode while fiberglass mat separator will be filled with electrolyte. This last will consist of 1M $NaClO_4$ in a 1:1 volume mixture of ethylene carbonate and diethyl carbonate. The coin cell will be tested galvanostatically at a current of 20 mA/g in a voltage window of 0.01-2.5V (vs Na/Na^+).
A further step could involve the use of phytoplankton bloom in eutrophic lakes: therefore a successful conversion of phytoplankton biomass into storage energy could represent a sustainable way to limit an environmental problem with some concrete advantages.

POSTER

TROPHIC ROLE OF CRUSTACEAN ZOOPLANKTON AND TRANSFER OF POPS THROUGH THE PELAGIC FOOD WEB

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Crustacean zooplankton plays a crucial role in the transfer of energy and matter and pollutants from primary producers to planktivorous fish in the pelagic community of lakes. They are composed by primary and secondary consumers and their population structure largely varies with the season. Contribution of primary consumers to total net zooplankton biomass being prevalent in spring and early summer and that of secondary consumers prevalent in autumn and winter. Despite comprising two clearly different trophic levels, zooplankton are often regarded as a one functional group, particularly in ecotoxicological studies and in models dealing with biomagnification and the role of zooplankton in transfer of pollutants to fish.
In the present contribution, we provide results of a pluriannual study on the deep subalpine Lake Maggiore (Italy), in which seasonal changes in carbon and nitrogen stable isotopes of zooplankton primary and secondary consumers were individually investigated. Results provide evidence that quantitative estimates of trophic roles within crustacean zooplankton are essential for understanding fluctuations in POPs (persistent organic pollutants) concentration in different compartments of the pelagic food web.

BioSpot, ladybirds and students for a citizen science project in urban environment

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The present paper aims at illustrating structure, objectives and results of the citizen science project BioSpot, developed at the University of Modena and Reggio Emilia in 2014.
BioSpot aimed to increase public awareness about human impacts on biodiversity studying ladybirds distribution in urban environments, with a focus on the invasive species *Harmonia axyridis* (harlequin ladybird).
The project follows the footsteps of two international projects, UK Ladybirds Survey and Lost Ladybug Project, for the analysis of the distribution of *H. axyridis* and other species of ladybirds in the United Kingdom and in the USA.
Over the project's first year (from February 2014 to June 2014) 30 classes from primary and secondary schools in Modena and Ferrara (Italy) have taken part in BioSpot. Indeed, together with the increase of public awareness, the aim of BioSpot was also to promote teachers' and student's interest in entomology.
This choice has been motivated by two reasons. Firstly, ladybirds are a useful model in educational projects, as they are commonly appreciated by children, thanks to their cute shape and bright colours. Secondly, school is one of the best targets for citizen science projects, as science and nature are generally very important topics in educational curricula and programs, and schools are usually very interested and open to projects in which scientists and researchers are involved.
Together with the collection of data on ladybirds, we conducted an evaluation (with questionnaires and interviews) to gain information about BioSpot's effectiveness and how to improve the project in the future. The present paper will present and discuss data collected through this evaluation.
BioSpot, focusing on ladybirds, has aimed at showing in the simplest and most practical way what the biological diversity really is, related specifically to local distribution. Together with this main aim, the evaluation has attempted to analyse promises and pitfalls of using an app in a citizen science project open to the schools.
Moreover BioSpot can be useful as an example for other projects linked to biodiversity with an important part of dissemination and communication to citizens, as for instance Climbio, a project about birds biodiversity in tropical environment.

The *Piwi* subfamily and genes involved in piRNA processing in *Protopterus annectens* and *Latimeria menadoensis*, two living fossil species: insights on the activity of the silencing pathway and evolutionary considerations.

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The subfamily of Piwi proteins is involved in several processes such as spermatogenesis, piRNA biogenesis, and transposon repression. Although this gene subfamily is under intensive scrutiny its evolutionary history is poorly understood, in particular with respect to their origin at the base of the vertebrate stem, leaving an important knowledge gap. Moreover the identification of evolutionary conserved structural features should generate useful information for better understanding their functions. We report here for the first time the transcript sets of *Piwi* genes in the West African lungfish *Protopterus annectens* and in the Indonesian coelacanth *Latimeria menadoensis*, two key species in the evolutionary lineage leading to tetrapods. The comprehensive phylogenetic analysis of 48 inferred amino acid sequences in 22 fully sequenced species provides new insights into the evolutionary history of the Piwi proteins of vertebrates. These results are also supported by the analysis of microsynteny performed in the major lineages. The expression levels of *Piwi* transcripts as well as of genes coding for proteins involved in piRNA biogenesis were investigated to detect the activity of the silencing pathways in which these proteins are involved. Our analysis suggests a low activity of the *Piwi* pathway in lungfish, in agreement with the hypothesis that large parts of its huge genome are made up of old and inactive transposons.

ORAL PRESENTATION

RESISTANCE TO PYRETHROIDS IN THE INVASIE SPECIES *AEDES ALBOPICTUS*

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The Asian tiger mosquito *Aedes albopictus* is an aggressive daytime biting mosquito, which arrived in Europe at the end of the ‘1970 from Asia. It is a competent vector for a number of viruses, including Dengue, Zika, Usutu and Chikungunya and the nematode *Dirofilaria immitis*.

Pyrethroids are widely used to control adult mosquitoes, imposing selection pressure for the emergence of resistance. Resistance to pyrethroids should be carefully monitored because mosquito control is the only method to prevent disease transmission in the current absence of vaccine and therapeutic treatment against viruses vectored by *Ae. albopictus*.

We surveyed mutations in the pyrethroid target site, the *para* sodium channel gene, of 597 *Ae. albopictus* specimens across Asia, Africa, America and Europe, including two samples from Italy. Additionally, we established an association between mutations at the *para* sodium channel gene and the resistance phenotype by genotyping two populations from China, which had been previously phenotyped using the World Health Organization standard tube bioassay for pyrehtroid resistance.

We detected two non-synonymous mutations in 5 populations across 4 countries. A novel mutation at position 1532 (I1532T) was found at a frequency of 19,7% in Rome. Most prevalent mutations were at position 1534, where we detected changes from phenylalanine to cysteine or serine or leucine. Positive association between pyrehtroid resistance and the presence of the F1532S mutation was established in two Chinese populations.

Resistance to pyretrhoids is emerging in *Ae. albopictus* and should be carefully monitored to favor the sustainable use of this type of insecticides. Mutations in the *para* sodium channel gene can be used as biomarkers for resistance surveillance, but not all mutations have the same predictive power.

Size classes distribution of benthic macrofauna along a disturbance gradient

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Aquatic ecosystem health and ecological status are ecosystem properties which can be measured from structural components of the ecological communities. Size distribution of biotic assemblages are important modifier of productivity, function and stability in marine benthic communities. With this study we investigate the size structure approach to ecological status assessment in a central Adriatic coastal ecosystem. The benthic community was studied along a depth gradient (from 7.5 to 130 m) in an area subjected to trawling fisheries. Six sites were sampled in winter 2015. Collected specimens were identified at the species level and sized. Length and weight size distributions for benthic fauna at each depth were calculated. Benthic fauna was also classified into ecological sensitivity groups. Ecological status was assessed through univariate and multivariate indices. Results of both approaches were then compared. At the shallower sites (depth < 20 m), the ecological status was High/Good, and size distributions of biomass and length tend toward smaller size classes. At medium-depth sites (40-50 m), ecological status was unsatisfactory probably due to the high disturbance provoked by fish trawling activity; size structure was characterized by small and medium classes. Deepest sites (>100 m) exhibited a satisfactory ecological status, and size classes were characterized by smaller classes for distribution of biomass and medium-large for distribution of length. The response of the size structure to gradient considered and the relationship with other indices were discussed.

First report of the fungus *Geotrichum candidum* Link as intimate colonizer of *Corbicula fluminea* (Müller, 1774)

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Corbicula fluminea is one of the most successful introduced species in the world, widely reported in Italy and in the Ticino River basin (Lombardy). Information about the fungal community associated to this bivalve are lacking and investigations focused on a deeper comprehension about the interactions among fungi and *Corbicula* are required.

Aim of the study was to confirm *in vitro* the deeper colonization detected in a previous investigation (submitted) between the Ascomycetes *Geotrichum candidum* (saprophyte fungus on a variety of plants) and specimens collected from Gaviola Stream (a semi-natural stream belonging to the irrigation system of the sub-lacustrine Ticino River).

Two experimental tests were performed:

1. fresh specimens were washed (running tap water, 1 min), crushed (sterile pestle), autoclaved, deposed onto TWA (Tap Water Agar) and inoculated with arthroconidia of *G. candidum* ($1x10^9$/ml); 2 controls were made (TWA + *inoculum*; TWA + sterilized specimen), 3 replicates each.
2. Both adult (10-15 mm length) and young (5-10 mm) fresh specimens were grown in dechlorinated tap water (500 ml) mixed to 10 ml of the aforementioned fungal *inoculum* (3 adults and, separately, 3 young/test, 3 replicates each); as control, similar specimens were maintained in fungus-free water. After 14 days at 20°C in natural day-night conditions, all the specimens were washed, crushed and analysed according to the standard damp chamber method (TWA).

For both tests, the growth of *G. candidum* was checked at 5× after 4-6-8-10 days of incubation at 25°C.

Results evidenced *G. candidum* ability to colonize both the crushed shells and the soft parts of *C. fluminea*, without added nutrients. In particular, starting from the 4th day of the experimental setting up, it appeared onto the 100% of the specimens of both tests, emerging from the soft part of the clams. During the 4 following days, the colonization spread to all the shells.

C. fluminea proves to be able to harbor the fungus. At the same time, *G. candidum* shows its adaptability in invading different kind of natural systems and, if appropriate conditions are present, permanently occupy them. Scientific evidences confirm the ability of *G. candidum* to penetrate a wide range of animals (i.e. Galapagos tortoises, snakes, gorillas, horses, canines,..), becoming an opportunistic agent of disease (i.e. renal geotrichosis, stomatitis, ophthalmitis,..). Consequently, the significance of this interaction deserves to be deeper evaluated.

INDICATORS OF SEDIMENT ORGANIC ENRICHMENT AND THEIR USE IN AQUACULTURE MANAGEMENT AREAS DEFINITION

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Making new space for aquaculture activities in the Mediterranean Sea is recognized as a priority for the future development of the industry and, therefore, for the implementation of the EU «Blue Growth Strategy». Zoning and site selection should comply with the ideas of the Ecosystem Approach to Aquaculture (Soto et al., 2008), in order to fulfill requirements from the Marine Strategy framework Directive. Of particular interest for an effective use of the Marine Space is the definition of Aquaculture Management Areas - AMA (Sanchez-Jerez et al., 2015). This task is carried out by considering the growth potential, the constraints imposed by conflicting uses of the sea, and, indeed, the long term environmental sustainability of the activity. The latter aspect is tightly coupled with the quantification of the organic enrichment induced by the farms, which provides the basis to assess degree and extension of modifications caused on sediment biogeochemistry and benthic community functioning. In this context, increasingly relevant is the use of virtual technologies in combination with operational oceanography, i.e. mechanistic models integrated in geographic information systems -GIS -, and using remote sensing products (Ferreira et al., 2012). This work focuses on the estimation of indicators of pressure of aquaculture farms on soft sediment benthic habitats, quantifying extension and characteristics of the area subjected to increased organic downward fluxes. These indicators allow one to synthesize the information produced by dynamic model simulations carried out at the farm scale, and to make it suitable as stationary GIS layers for AMA selection. Seabream-seabass net pens at sea were considered in the analysis. We compared the response of different indicators to variations in farm characteristics over the ranges typical for Mediterranean farms (environment, geometry, husbandry practices) by means of an integrated aquaculture mathematical model -FICIM - (Brigolin et al., 2014). We will focus our discussion on the following results: i) sensitivity of predicted pressures to threshold values adopted for categorizing organic matter downward fluxes; ii) use of mechanistic simulations to derive a meta-model for applications in data poor areas; iii) consistency of model derived trends with existing literature on benthic impacts.

Effects of artificial light at night (ALAN) on alpine stream periphyton: a flume simulation

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Artificial light at night (ALAN) is one of the most widespread human-induced alterations of the landscape, it is increasingly recognized as a contributor to environmental change and a threat to biodiversity at the global scale. Increasing research efforts have demonstrated numerous adverse effects on aquatic and terrestrial animals, microorganisms and plants. Aquatic primary producers, however, have rarely been studied, and our understanding of ecological effects on benthic autotrophs remains limited. Benthic autotrophs, such as diatoms, green algae and cyanobacteria, grow attached to underwater surfaces within complex periphyton communities and form the basis of the food web in many streams and clear, shallow waters. They use light both as a source of energy as well as a source of information for the regulation of physiological processes according to light/dark cycles. The main autotroph groups differ in their preferences for light conditions, therefore the alteration of light regimes may cause changes in periphyton growth and community composition. We conducted experiments in an outdoor flume system mimicking a sub-alpine stream (Trentino, NE Italy). We simulated the night-time light conditions of a waterbody in a light-polluted area (approx. 20 lux), and compared the biomass and community composition of benthic autotrophs in periphyton grown under ALAN with those grown under natural nights. The experiments were performed in two seasons in order to account for seasonal differences in community composition. The LED-based nighttime illumination resulted in a decrease of autotroph biomass and an increase in the proportion of diatoms. The effects depended on the season and the growth stage of the periphyton, indicating higher sensitivity to ALAN exposure in early growth stages (one to three weeks) compared to the later ones (four to seven weeks). Our results show that artificial light can have profound effects on the primary producers of aquatic ecosystems. By negatively affecting the biomass and altering community composition, artificial light at night may hinder primary production, which is a vital ecosystem function. Streams with frequent flooding events, droughts, sediment transport or physical perturbations that all reset the development of periphyton are expected to be more affected by ALAN than those with stable conditions. ALAN may therefore negatively impact the resilience of aquatic ecosystems.

FIRST RECORD OF TWO TOXIC DINOFLAGELLATES IN TIDE POOLS ALONG THE SARDINIAN COAST

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During the last years, Harmful Algal Blooms (hereafter HABs) have become more frequent in the Mediterranean Sea and nowadays they represent a serious problem for this biodiversity hot spot. Despite phytobenthic microalgae are the largest cause of HABs in the basin, only a few studies aimed to characterise such communities have been conducted till today and the knowledge on the presence of potentially toxic benthic microalgae in particular habitats, such as tide pools, is still scarce. In order to detect species of HAB producing benthic microalgae in tide pools of the rocky intertidal zone, a pilot study was conducted in Tavolara Punta Coda Cavallo Marine Protected Area (MPA) during the spring of 2016.

In a key site of the MPA (Punta Don Diego bay), where HAB producing benthic species have recently been observed, three different pools were sampled collecting water and ephilitic material with a brush-sampler (n=3), an efficient sampling device successfully used in other studies on benthic microalgae. Cell identification and count were conducted by means of an inverted microscope (three subsamples for each sample), according to Utermöhl's sedimentation method.

In all the collected samples, the two potentially toxic dinoflgellates *Prorocentrum lima* (Ehrenberg) F. Stein and *Coolia monotis* Meunier were recorded. The mean number of cells of the two species in the study area was of 12 cells ml^{-1} and 3 cells ml^{-1} for *P. lima* and *C. monotis* respectively.

These data represent the first record of the two species in tide pools and, even if the mean cell densities are very low, they prove the ability of these species to adapt to extreme environmental conditions, colonizing unique habitats such as tide pools, as already verified for other HAB producing benthic microalgae.

SEASONAL SEA-ICE DYNAMICS AND BIODIVERSITY ORGANISATION IN ANTARCTIC BENTHOS

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Global change is expected to affect resource-consumer interactions underlying stability in polar marine ecosystems, where modifications in sea-ice coverage and temperature will affect quality, quantity and timing of resource supply to consumers, with implications for species coexistence. Seasonal dynamics of Antarctic sea ice affects primary production patterns, impacting benthic communities fuelled by sympagic algae. We addressed the trophic organisation of benthic communities at Terra Nova Bay (Ross Sea), by means of stable isotope analysis on benthic macroinvertebrates sampled in five locations differing in sea-ice coverage during the Antarctic spring. We hypothesised an increased contribution of sea-ice algae to benthic food webs after sea-ice break-up and a predominance of detritus consumption and intraguild predation in ice-covered locations due to conditions of resource shortage. According to optimal foraging theories, trophic niche broadened at the community level and narrowed at the population one in ice-free conditions, as a consequence of increased quality range of resources. The only exception was found in the location where sea-ice break up occurred first, where we expected availability of sympagic algae to benthos to be at his maximum. Indeed, in this location trophic niche narrowed as all specimens were found to rely on sympagic algae as their principal food source. A gradient of contribution of sympagic algae to benthos was evident moving from the location with more persistent sea-ice coverage to that where sea-ice break up occurred earlier in the season. Inversely, intraguild predation increased in locations exposed to prolonged ice-coverage, resulting in longer food chains fuelled by detritus and benthic production. As a consequence of reduced quality range of resources, species packaging (i.e. niche overlap) within the isotopic niche space was higher in locations where sea-ice coverage was more prolonged, which could suggest a higher risk of species exclusion following long term changes in environmental conditions and/or resource inputs. These results suggest that studying the trophic-functional effects of changes in resource availability in benthic habitats could be a key for understanding food web reorganisation and stability in the face of future climate change-related variations in sea-ice coverage in Antarctica, and could provide important information for biodiversity management and conservation in the Ross Sea.

Avian brood parasitism in the new and old worlds

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Avian brood parasitism offers a convenient model to study coevolution mechanisms in terms of physiological and behavioural adaptations adopted by both parasites and their host species. We investigated two parasitic systems, one in Italy, involving the common cuckoo (*Cuculus canorus*) and its host, the reed warbler (*Acrocephalus scirpaceus*), and another in North America with the brown-headed cowbird (*Molothrus ater*) and its host, the yellow warbler (*Setophaga petechia*). We quantified the defensive efforts of parasite hosts as an antiparasite defense acquired either socially (i.e. by observing conspecifics), or individually (i.e. by trial-and-error attempts). We tested individual versus social learning and recorded defensive responses by these warblers to taxidermic mounts of the parasite presented at their nest. Individuals of both warbler species that adopted a strong defence showed a higher fitness than weak defenders. Contrary to yellow warblers, reed warblers acquired a specific antiparasite defence by observing conspecifics, i.e., by socially learning. Despite its fitness advantages, learning defensive responses was not commonly spread in populations. This last result indicates possible trade-offs in adopting contrasting responses effective to both parasites and nest predators. We finally discuss how the "arms race" between parasites and hosts may be explained by either an evolutionary lag or different coevolutionary paths.

Distribution of meiofaunal assemblages along different bathymetric transects at Terranova Bay (Ross sea, Antarctica).

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In the framework of the *Italian National Programme of Antarctic Research* (XXX Italian Expedition in Antarctica) sediment samples were collected along three bathymetric transects. The bathymetric transects were selected from coast to offshore in three different areas: one with high organic input due to the presence on the coast of the penguin assemblages (*Adelie Cove*), one in front of the Italian Research Base (*Rod bay*) and one in front of a bay without anthropogenic and organic inputs (considered as a control). Three stations at 25, 70 and 140 m-depths were identified in each transect where replicated (n=3) sediment samples were collected for the analysis of phytopigments, biochemical composition of organic matter (proteins, carbohydrates and lipids) and meiofaunal assemblages in terms of abundance, biomass and diversity.

We observed that the concentration of phytopigments, proteins, lipids and carbohydrates showed the highest values at the station located at 25-m depth along the transect in front of Adelie Cove, and increased with increasing water depth as well as in all other transects. Generally, in all stations and transects proteins were the most abundant component (53-70%), followed by carbohydrates (17-32%) and lipids (13-22%). Total meiofauna abundance ranged from 252.0 ± 102.7 to 5961.1 ± 658.8 individuals per 10 cm^2, and generally it increased with the increasing depth. Meiofaunal diversity ranged from 4 to 12 taxa and generally showed a similar pattern to that of the abundance.

Nematodes were the dominat taxon, ranging from 91.6% to 99.8%, followed by copepods (from 0.2% to 8.0%), and ostracods (from 0% to 0.5%). All other taxa (Priapulids, Acarians, Amphipods, Bivalves, Cladocerans, Gastropods, Isopods, Loriciferans, Oligochaetes, Polychaetes, Sipunculids, Tanaids and incertae sedis) were rare (<1% their contribution to the whole assemblages). These results suggest that food sources, even in terms of biochemical composition, play a key role in driving spatial distribution of meiofaunal assemblages in the Ross Sea.

Diazotrophy in chemosynthetic symbioses: sulphur-oxidizing bacteria of marine invertebrates are capable of nitrogen fixation

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Chemosynthetic symbioses are partnerships between invertebrate animals and sulphur-oxidizing bacteria, which are the primary producers providing most of the organic carbon needed for the animal host's nutrition. We sequenced genomes of the chemosynthetic symbionts from the lucinid bivalve *Loripes lucinalis* and the stilbonematine nematode *Laxus oneistus*. The symbionts of both host species encoded nitrogen fixation genes. This is remarkable, because no marine chemosynthetic symbiont was previously known to be capable of nitrogen fixation. Transcriptomics and proteomics in *L. lucinalis* revealed that in some individuals, the nitrogenase genes are as highly expressed as genes for carbon fixation and sulphur oxidation; nitrogen fixation is thus a key function of these symbionts. Mean stable nitrogen isotope values of *L. lucinalis* were within the range expected for fixed atmospheric nitrogen, further indicating active nitrogen fixation by the symbionts. The ability to fix nitrogen may be widespread among chemosynthetic symbioses in oligotrophic habitats, where nitrogen availability often limits primary productivity.

Biodegradation of xenobiotics as a regulation ecosystem service provided by natural microbial communities.

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Ecosystem functions (EFs) are the capacity of natural processes and components to provide goods and services that satisfy human needs, directly or indirectly (De Groot, 1992). Microbial communities provide several regulation functioning such as nutrient cycling, organic matter degradation and contaminant removal, ensuring soil fertility and water quality. Microbial communities are the main responsible of ecosystem homeostatic capabilities of biodegrading contaminants providing a key regulation service. In this context, the present work report two study cases of xenobiotic removal by the natural microbial communities, one in soil and the other in water ecosystem.

The biodegradation in soil of the anionic surfactant Sodium Lauryl Ether Sulphate (SLES) contained in a commercial foaming agent used in mechanized drills and in water of the sulphamide antibiotic Sulphamethoxazolo (SMX) are here reported.

Microcosm experiments were set-up with soil or water samples treated separately with the xenobiotics (85.0 mg/kg of SLES and 500 µg/L of SMX, respectively). Some soil/water samples were previously sterilized in order to evaluate abiotic degradation in absence of the microbial community. Moreover, control microcosms, consisting of un-treated soil/water, were also present in order to compare the microbial community before and after the contaminant addition.

At selected times (0, 7, 14, 21, 28 d) soil sub-samples were collected for assessing SLES concentration by MBAS spectrophotometric method and water samples for analysing SMX by a chromatographic method coupled to fluorescence detection (HPLC-FLD). Microbiological analysis were performed in order to assess microbial abundance (DAPI counts), cell viability (*Live/Dead* method) and dehydrogenase activity. Although an initial detrimental effect of SLES was observed on the microbial abundance, at the end of the experiment, SLES was no longer present in soil microcosms and it was completely biodegraded in 28.

Unexpectedly, the antibiotic SMX was also biodegraded during the experimental time. At day 28 the residual concentration was 23.6 % of the initial one, while 89.5% of the initial concentration remained in the sterile microcosms at 35 days. The overall results showed the role of the natural microbial communities in the biodegradation of compounds both considered “biodegradable” (SLES) and “non-biodegradable” (SMX).

THE INTERACTIVE EFFECTS OF MULTIPLE STRESSORS IN REGULATING CELL DENSITY MUCILAGE PRODUCTION OF THE INVASIVE MICROALGA *CHRYSOPHAEUM TAYLORII*

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The benthic mucilage producing microalga *Chrysophaeum taylorii* Lewis and Bryan (*Pelagophyceae*) is typical of tropical regions of the Atlantic and of the Pacific Ocean but it has recently received attention for its rapid spread in the Mediterranean Sea. As no information on *C. taylorii* response, especially in terms of mucilage hyperproduction, to different stressors actining toghether is available in literature yet, a manipulative field experiment was designed in Tavolara Punta Coda Cavallo Marine Protected Area to test the effects of nutrient enrichment, mechanical disturbance and hydrodynamics on *C. taylorii* benthic cell density and mucilage abundance.

Nutrient enrichment (addition of nutrients), mechanical disturbance (partial and total benthic organisms removal) and hydrodynamic stress (increased water flow) were simulated and treatments were randomly assigned to 20x20 cm plots following a full-factorial design (n = 3). Nutrient addition was obtained using a slow-release fertilizer in pellets (N:P:K); mechanical disturbance effect was instead obtained by scraping off the substratum using an iron brush and hydrodynamic styress was simulated by using a 10x10 cm plastic foil to create turbulence in the water (about half an hour every two days), removing mucilage aggregates.

The obtained results showed that both *C. taylorii* cell density and mucilage cover were remarkably affected by the interactive effects of the three considered stressors. However for each of them, different effcts were observed: while mechanical disturbance and high hydrodynamics produced consistent effects on both cell density and mucilage production (i.e. the former factor enhancing it and the latter decreasing it), nutrient enrichment produced contrasting effects, promoting cell abundance and inhibiting mucilage production. These results suggest that the higher amount of mucilage in nutrient poor water would serve to ensure a more favourable microhabitat to the cells embedded in the mucilaginous aggregates, proving that they act as a microhabitat, where cells can benefit from nutrient rich conditions. Therefore, important mucilage blooms are expected in oligotrophic sheltered coastal locations, especially where barren sheltered areas are present.

TIMES NEW GENOME ANALYIS OF BACTERIAL ENDOSYMBIONT *HOLOSPORA CARYOPHILA* FROM THE CILIATE PROTIST *PARAMECIUM*

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Holospora caryophila is an obligate bacterial endosymbiont of the ciliate *Paramecium octaurelia*, implying that it cannot reproduce in the absence of its host. It possesses a peculiar life cycle, involving two distinct shapes: the reproductive form (RF) and the infectious form (IF). The RF is a Gram-negative slightly curved rod (1-2 μm long), which is localised in the host macronucleus, and is able to multiply there. Under certain conditions, frequently triggered by host starvation, it differentiates into the elongated (up to 5 μm) IF, which does not perform division and is released by the host cells via vesicles. The IF is able to survive in the external medium, when another Paramecium cells takes it by phagocytosis, it is activated and becomes able to escape digestion and reach its target nucleus, where it produces again RFs. *Holospora* is also interesting for its evolutionary position, which is related to *Rickettsiales* (*Alphaproteobacteria*), an order of obligate intracellular bacteria with a broad host range including human pathogen. They also have been proposed to share a common ancestry with the mitochondrial predecessor.

In order to gain insight on the peculiar features of *H. caryophila* and its evolutionary relationships. we aimed to sequence its genome.

Currently, we have a draft assembly (111 scaffolds: 987,513 bp; N50: 105,107 bp; GC%: 33.6;), which was annotated through automatic pipelines and validated manually.

The metabolic capacity of this endosymbiont was inferred from its gene set, by comparison with that from selected *Rickettsiales* and free-living *Alphaproteobacteria*. As in other *Rickettsiales*, the metabolic repertoire of *H. caryophila* is highly specialised for an intracellular lifestyle, because several pathways typical for free-living bacteria are absent, in particular biosynthetic ones, which can be complemented by suitable membrane transporters. A distinguishing feature of *H. caryophila* is that functional ATP-producing catabolic pathways are missing. Therefore, this bacterium appears to rely directly on the host not only for the provision of most biosynthetic precursors, but also for energy supply.

Moreover, the analysis of the *H. caryophila* genome offered the chance to formulate some hypotheses on its evolutionary history, including the possibility of horizontally transferred genes, as well as on the interaction with its host, which would need confirmation by future analytical and experimental studies.

Endemism, allopatry and passive transport: the case of two *Proserpinicaris* (Crustacea, Copepoda, Harpacticoida) from Sardinia

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Proserpinicaris is a genus of Parastenocarididaewith wide geonemy,it includes specialized taxaliving exclusively in phreatic and interstitial continental groundwater. The only speciesknown for Italy are:*P. proserpina* (Chappuis 1938) from Southern and Central Italy,*P. amalasuntae* (Bruno and Cottarelli 1988) from Latium and Tuscany in Central Italy, *P.kalypso* (Pesce et al., 1988) from Sicily. In Sardinia,*P.admète* (Cottarelli et al., 1980) was collected from the hyporheic habitat of several streams in the island, and *P.ima* (Cottarelli 1989) from phreatic waters in the island of La Maddalena; a third species was collected in the Asinara island from the hyporheos at the estuary of the creek "BaddeLonga".This *Proserpinicaris* is new for Science and its complex of morphological characters highlight its remarkable affinity with *P. admète*.The Asinarawas the terminal portion of the Stintino Peninsula, separating from the mainland at the end of the Würm, when melting of the ice caps caused the rise of the sea level (Flandrian or Versiliantransgression).These geological phenomena are recent (the transgression peak occurred about 6500 years ago), but the time span seems to have been sufficient to allow the ancestor populations to undergo allopatricspeciation, resulting in the two strongly relatedspeciesResearch on species of Italian Parastenocarididaeat the molecular levelis currently in progress andone species of *Proserpinicaris* was already sequenced andmore molecular data will allow to clarify the framework outlined here.Although this speciation scenario seems very likely, other hypothesis such as passive anthropic dispersion can not be ruled out. In fact, Parastenocarididaedo not tolerate marine waters and are strongly linked to narrow environmental conditions. Laboratory experiments in progress at the University of Catania show that these harpacticoids can survive for several months, in small sealed containers with a small amount of water and without food or oxygen input.A possible transport through wet sands or containers containing water could explain the presence of a *Proserpinicaris*in Asinara, and of otherParastenocarididae in oceanic islands (Schabetsberger, 2009). Moreover, a newParastenocarididae was collected inSablayan, a volcanic island of the Philippines which hasnever been in contact with other mainland (Cottarelli, pers.com).

MONITORING THE TRANSPORT OF FISH EGGS AND LARVAE INTO A MEDITERRANEAN COASTAL LAGOON

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Fish community is a key element of the biodiversity of transitional water habitats. Many marine species migrate between the sea and transitional waters to take advantage of the trophic richness of these systems. In particular, the guild of marine migrant comprises species that enter lagoons and estuaries in the first stages of their ontogenetic development, with a relevant contribution in terms of abundance and biomass to the fish community of these systems. The connectivity between the sea and transitional water habitats is a crucial aspect of their nursery role, allowing the larvae and juvenile of marine fish to migrate into lagoons and estuaries and to subsequently colonise elective habitats. This process is closely linked to tide fluxes, even if it is not a solely passive transport. In this work we monitored the transport of eggs and larvae through the three sea inlets of the Venice lagoon, in the context of the environmental monitoring of the MOSE mobile dams (still under construction), that will prevent the flooding of the city by interrupting the high tide flux during extreme events. Seven sites along three sea-lagoon transects, one for each sea inlet, have been selected and the ichthyoplanktonic community have been sampled with bongo nets four times along the winter-spring migration of juvenile marine migrant species, from November 2015 till April 2016. During the samplings, water temperature, salinity, turbidity as well as dissolved oxygen and chlorophyll concentration were measured. Fish eggs and larvae were sorted and identified, when possible, at least at the family level. Seven species of marine migrant species have been identified in the samples. Data collected showed the transport of both eggs and larvae of these taxa through the sea inlets in all the three lagoon sub-basins, with no significant differences in density between sea and lagoon sampling sites. A simple accumulation index has been calculated to estimate the colonisation of lagoon waters by marine migrant early development stages. This index showed an accumulation of larvae, and to a lesser extent of eggs, in the lagoon waters, particularly in the case of the European Sprat *Sprattus sprattus* (L. 1758), with some differences among the three sea inlets. If carried on in the next years, this monitoring activity will provide a useful baseline to assess the possible impacts on fish fauna deriving from the closure of the sea inlet during extreme high tide phenomena.

Jellies-eating anthozoans

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Gelatinous plankton still plays a doubtful role in the trophic food chains of the oceans. In the pelagic realm, jellyfishes are among the major plankton consumers, but their role as food source for other jellies, fishes, turtles and humans is also documented.

The role of jellyfishes as possible food for benthic organisms is little explored, especially in the Mediterranean Sea. During their circadian and seasonal vertical migrations and at the end of their life cycle, jellyfishes can easily enter in contact with benthic assemblages. Through field and laboratory observations, we recorded several Mediterranean benthic invertebrates able to exploit jellies as food source. This documentation has been collected both in the wild and with jellyfishes kept in aquarium. Peculiar predation behaviours of anthozoans have been detected. When the jellyfish preys were larger than the oral disc of the predator, the jellies were shared and digested by several polyps. The anthozoans *Anemonia viridis*, *Parazoanthus axinellae* and *Leptopsammia pruvoti* were tested with three species of jellyfish (*Pelagia noctiluca*, *Rhizostoma pulmo*, *Aurelia aurita*). Moreover, the predation of ephyrae of *P. noctiluca* from *P. axinellae* and the scleractinian *Astroides calycularis* was also documented in natural habitat, suggesting that early developmental stages of the jellyfish life cycle could represent a consistent trophic resource for benthic organisms. On the other hand, these jellyfish eating anthozoans, affecting several phases of the life cycle of scyphozoans may represent a control factor at local scale towards coastal blooms.

These considerations highlight the necessity to understand to what extend jellyfish represent a trophic source for benthic organisms and re-define the role of jellies in the bentho-pelagic coupling.

A new taxon of freshwater gastrotricha macrodasyida: morphology and notes on its phylogenetic position

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Macrodasyida comprise about 350 worm-like species, hermaphroditic and all interstitial in marine an estuarine habitats except for three freshwater ones recorded only from their type locality: *Marinellina flagellata*, *Redudasys fornerise* and *Redudasys* sp. (Kånneby & Wicksten, 2014). They were respectively found from an Austrian stream, a Brazilian artificial reservoir and an USA aquifer. Here we describe a new freshwater taxon of Macrodasyida from different streams in Minas Gerais State, Southeastern Brazil, using Differential Interference Contrast (DIC), Confocal Laser Scanning Microscopy (CLSM) Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). The phylogenetic position of the new taxon within Macrodasyida was inferred by 18S rRNA gene, and the relationships with the other freshwater Macrodasyida species were investigated by COI mtDNA. The new taxon, 193 to 376 µm in total body length, is characterized by numerous cephalic cilia arranged into irregular bands, short around the mouth and longer on the anterolateral head margins, 6 pairs of lateral tactile bristles, one pair of anterior adhesive tubes and 2 pairs of posterior adhesive tubes of unequal length. Longitudinal muscles, inserted on the mouth rim, surround dorsally, laterally and ventrally the pharynx; the pair of main ventrolateral longitudinal muscles is inserted at the level of the anterior adhesive tubes. From TEM images the species seems to feed mainly on microalgae. Maximum Likelihood and Bayesian Inference analyses yielded topologies congruent with each other and support a position of the new taxon within the family Redudasyidae. The phylogeny based on COI mtDNA analyses clearly shows that the new Brazilian taxon is not *Redudasys fornerise* and that there is also a remarkable phylogenetic distance between the new taxon and *Redudasys* sp., according to the considerable geographical distance between the two type localities. Results strongly suggest new taxon as a new species, most likely belonging to a new genus among the family Redudasyidae,

Lateral Antimicrobial Resistance Transfer is active in the open environment

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Lately, there have been recurrent findings of antimicrobial resistance genes in natural habitats close to human activities. In most of these studies, the environment is viewed as a passive deposit of antimicrobial resistance mechanisms. It was also found that bacteria evidence biological cost and compensatory mutations in other parts of the genome for maintenance of these genes. Thus, in absence of antimicrobial pressure, it is expected that they disappear from environmental bacterial communities. In order to test this scenario, we obtained 11 class 1 integron-positive environmental strains from distant bacterial genera collected in Patagonian and Paranaense forests. We showed that 10 *intI1* genes were capable of inserting clinically relevant antimicrobial gene cassettes in the native *attI1* site without the need of antibiotic selection. The 46% (5/11) of the environmental strains, including an *Escherichia coli* sample, were found to be naturally competent for the acquisition of at least one of the *aadB* and/or $bla_{\text{VIM-2}}$ gene cassettes. The frequency of recombination in *Pseudomonas* sp. 1SL5 isolate was 10 to 1000 times higher than in the traditional laboratory model of *E. coli* TOP10::IntI1 co-transformed with *aadB* or $bla_{\text{VIM-2}}$ plasmid-borne. No significant fitness cost was found for the maintenance of both gene cassettes. Native *intI1* with inserted gene cassettes were stable in respective environmental strains for 30 days, proposing a scenario in which antimicrobial resistance genes can be stable and maintained in nature without antimicrobial pressure. Based on this data, a bi-directional flow of antimicrobial resistance genes between natural and nosocomial habitats is proposed, which implies the active role of the open environment as reservoir, at the same time as recipient and donor of resistance mechanisms along time.

DEEP-SEA CORALS AND SPONGES AS PODIUM FOR THE SUSPENSION FEEDER *OPHIOTHRIX QUINQUEMACULATA* (ECHINODERMATA, OPHIUROIDEA)

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Ophiothrix quinquemaculata (Delle Chiaje, 1828) is a large ophiuroid which can form dense aggregations on muddy environment. Even though it is usually reported as a soft-bottom species, this thorny brittle star may prefer small hard substrates (e.g. stones and big pebbles) scattered upon the sediment (Tortonese, 1965). During the research cruise MARINE STRATEGY 2015 the presence of *O. quinquemaculata* was recorded using ROV at about -290 m in the Bari Canyon (South Adriatic Sea, Italy). The sampling of four specimens in the area (D'Onghia et al., 2015) allowed the specific identification. Dense aggregations of *O. quinquemaculata* occurred on deep-sea sponges *Pachastrella monilifera* Schmidt, 1868 and *Poecillastra compressa* (Bowerbank, 1866) and on cold-water corals *Madrepora oculata* Linnaeus, 1758 and *Lophelia pertusa* (Linnaeus, 1758), this latter recently revised in *Desmophyllum pertusum* (Linnaeus, 1758) (Addamo et al., 2016). Two video transects were considered and all the frames where *O. quinquemaculata* was present were examined. The specimens observed were counted and their number was reported to the surface area thanks to a triangular metric laser beams mounted on the ROV. A total of 64 frames were analysed: 16 with *O. quinquemaculata* on rocky substrate, 25 on sponges and 23 on corals. No specimens were found on soft bottoms without hard substrata. From 1 to 12 specimens were observed on the rocky substrate or with their arms protruding from the base of hardground and pebbles, with a mean of 6.0±6.3 specimens/m^2. On the contrary, from 2 to 19 specimens were observed on sponges and from 1 to 45 on corals, with 11.3±7.0 and 31.4±20.6 specimens/m^2 respectively. The different densities of *O. quinquemaculata* observed on the three types of substrate resulted statistically significant (Kruskal-Wallis test, α=0.01), suggesting the use of corals and sponges as main feeding podium. Due to its suspension feeder trophic strategy (Domínguez-Carrió et al., 2014), *O. quinquemaculata* most probably uses corals and sponges to arise from the muddy seabed and to increase its food particles intercepting chances. Moreover, the three dimensional habitat provided by sponges and corals offered many grips to anchor against bottom currents. Corals' large size and branching might explain the higher density respect to sponges (α=0.001). This preference seemed to be not due to a specie-specific interaction, but rather to the height and the three-dimensionality of the feeding podium.

FOOD NICHE STUDY OF THE MUGILIDAE *Liza ramada* (Risso, 1826) IN GULF OF GAETA (It) BY STABLE ISOTOPE ANALYSIS.

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Costal ecosystems are among the most productive and exploited waters, providing a large variety of goods and services for the human populations including recreation and harvestable resource such as fish. The Mullet fish *Liza ramada* (Risso, 1826), is one of the most exploited fish in the word, and his trophic ecology is not yet completely understood. Generally it is considered a detritivore (very low position along food chain) but recent researches by stable isotope analysis assign to *Liza ramada* an higher trophic position. Specimens of fish community, benthic invertebrates and organic matter, (Sediment organic matter SOM, Plankton and Organic detritus) were collected in two sampling sites (Gaeta and Formia: North-West; Scauri and Garigliano: South-Est) in the gulf of Gaeta (Tyrrenian sea, Central Italy). The aim of present research was to test two hypothesis; i) had *Liza ramada* a significant different diet with respect to other fish species? ii) and what is the importance of organic detritus influenced its diet. The approach was to investigate the trophic plasticity of mullet fish by Stable isotopes analysis (S.I.A) of carbon (^{13}C) and nitrogen (^{15}N). Bayesian stable isotopes mixing models were used to quantify the proportional contribution of each resource to the population of *Liza ramada*. The niches space of the populations were calculated as the standard ellipse area (SEAc). The overall niche spaces of the mullet fish was characterized by higher ^{13}C range (-16.38‰) than the other fish species, consistent with the higher values detected in organic detritus. In particular the specimens come from the South-Est zone were strongly influenced by the ^{13}C range of the organic detritus; indeed the relative contribute of the organic detritus was very high (98.72%). Meanwhile, in the Nort-West zone the ^{13}C signatures of the mullet fish seems to be less influenced by the organic detritus, stressed from a lower relative contribute than in the other zone (54,48%). This study provide a novel information and highlting current lack on the trophic habits of this Mugilidae species. The Isotopic approach is a powerful tool to evaluate diet and trophic niche of an omnivorous fish as *L. ramada.*

The role of the antennae in sun and moon compass mechanism of *Talitrus saltator* (Crustacea, Amphipoda)

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To orientate along the sea-land axis of sandy beaches, the supralittoral amphipod *Talitrus saltator* (Montagu) employs several cues, such the sun and the moon whose use requires continuous adjustments to account azimuthal variations. The sun and the moon compasses rely on separate endogenous chronometric mechanisms (Ugolini et al., 1999) and the biological clock involved in the sun compass also regulates the circadian locomotor activity rhythm exhibited by this species (Ugolini et al., 2007). Our work wants to assess the existence of an antennal clock necessary for the proper functioning of the sun and/or the moon compass as demonstrated in the Monarch butterfly *Danaus plexippus* relatively to the sun compass (Merlin et al., 2009). The circadian expression of four canonical clock genes (*Per*, *Clk*, *Tim and Cry2*) in both the brain and the antennae was quantified and tests of solar and lunar orientation of intact and “antennaeless” individuals were conducted. The eventual impairment of the mechanism regulating the sun compass was also evaluated recording the locomotor activity rhythms of (intact and operated) animals subjected to different light:dark regimes. Molecular analysis revealed circadian oscillations of *Per* and *Cry2* expression in the antennae. As regard behavioural experiments, both intact and antennaeless individuals (exhibiting comparable activity rhythms) tested under the sun showed directional choices in agreement with the expected directions. Instead, the lunar orientation was highly compromised by the removal of these appendages and antennae-ablated individuals were uniformly distributed. Therefore, we concluded that antennae have a role in the functioning of the moon compass but are not necessary for a proper solar orientation.

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Unique marine benthic bacteria, archaea and viruses inhabiting the hadal biosphere

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Hadal trenches are the deepest (from 6000 m to more than 11000 m depth) and the most remote ecosystems on Earth representing the last frontier in marine ecological research. Despite there is evidence that these extreme ecosystems host life forms, the diversity and functioning of microbial assemblages are still largely unknown. In the present study, we carried out metagenomic analyses to investigate benthic bacterial, archaeal and viral diversity inhabiting the Izu-Ogasawara Trench (Pacific Ocean) at ca. 10,000 m depth. Our results, revealed the presence of novel, abundant and highly diversified hadal taxa, with unique genetic features, affiliating to JTB255 mgb (Gammaproteobacteria), MGI-Thaumarchaeota and Microviridae. Microbial metagenomes, including viromes, contained an exceptionally high portion of trench-specific genes, mostly of unknown function, and completely different from those found in the surrounding abyssal plains. Hadal microbial metagenomes were characterized by genes related to phages and lateral gene transfer (LGT) while viromes showed an over-representation of microbial-related functions. These findings suggest that virus-host interactions and gene flow can influence adaptation and evolution of microbial assemblages in hadal extreme ecosystems.

PRELIMINARY DATA ON THE PRESENCE OF *PINNA NOBILIS* (MOLLUSCA, BIVALVIA) IN AN APULIAN COASTAL BAY (IONIAN SEA, ITALY)

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Pinna nobilis Linnaeus, 1758 represent the largest Mediterranean bivalve mollusc, protected by European Council Directive 92/43/EEC (EEC, 1992) and Barcelona Convention. This species experienced the regression in its geographic distribution and density decrease, due to the deterioration of habitats and overexploitation. It is commonly found in the coastal soft-bottom areas, between 0.5 and 60 m of depth, mostly within seagrass meadows, but also on bare sandy bottoms. Recently, within the Porto Cesareo bay (LE), two sites colonised by a large number of the mollusc individuals were discovered. For each site, 4 areas (16 m^2) have been randomly selected and within each of them, all encountered individuals were counted and measured *in situ* by specialised SCUBA divers. The first site (about 4.000 m^2) was located at the depth of 1.5-2 m and characterised by a muddy sand substrate with the occurrence of a well-developed *Cymodocea nodosa* meadow. In this site, the high density of molluscs (100±15ind/100 m^2) that were smaller than 30 cm in average total length (ATL) was observed. The second site (about 7.000 m^2), located on the uncovered sandy bottom, at 2-3 m of depth, was characterised by lower densities (10±4.10ind/100 m^2) and larger mollusc specimens (ATL ~ 40 cm). The occurrence of juveniles, with a total shell length < 20 cm, proved that *P. nobilis* successfully reproduced in both of the investigated sites. Moreover, the remarkable frequency of large specimens in this area indicates the population's temporal persistence; according to the literature data, the estimated age of the largest sighted specimens could exceed ten years. Density values of *P. nobilis* observed in the study area are comparable to few littoral lagoons/sheltered bays of Corsica and Greece, but generally are higher than those usually recorded for the Mediterranean Sea, suggesting a largely positive effect of the bordering Marine Protected Area of Porto Cesareo. Peculiar characteristics of *P. nobilis* population in this area suggest that it should be considered for possible improvements in protection and conservation and in re-designing of the MPA borders.

Nestling sex and plumage color predict food allocation by barn swallow parents

Corti M., Romano A., Bazzi G., Caprioli M., Costanzo A., Rubolini D. and Saino N.

Despite parents are equally related to all of their progeny, they may differentially invest in offspring according to their reproductive value: this may result in a maximization of parental fitness. Sons and daughters can differ in reproductive value, especially in species where fitness is predicted by the expression of sexually selected traits. In many bird species, offspring plumage colouration functions as a honest signal of individual quality, thus allowing parents to differentially invest in male and female offspring according to their feather coloration.

In the present study, we tested whether parents allocate different amounts of food depending on plumage colour of their male and female offspring. We used as a model species the barn swallow (*Hirundo rustica*), a small passerine bird showing a large among- and within-brood variation in ventral plumage colour. In addition, male reproductive success varies according to ventral plumage colouration.

We recorded the proportion of feedings obtained from parents and body mass variation by dyads of same-sex and similar-sized nestlings subjected to either experimental darkening of their ventral plumage colour or to a sham treatment. Plumage darkening resulted in an increasing in food provisioning and in a body mass gain in males but not in females. These results suggest that parents modulate their parental effort in favour of the more valuable male offspring that are expected to provide the greatest fitness returns, because darker ventral colouration is associated with larger reproductive success in adult barn swallow males.

Our study thus provides evidence that parents are selected to differentially invest in offspring of either sex according to a trait expressed in early life which is relevant to intra-sexual competition for access to mates at sexual maturity.

LEAF-NETS (LN): A NEW QUANTITATIVE METHOD FOR SAMPLING BENTHIC INVERTEBRATES IN SPRING HABITAT

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Springs are peculiar ecosystems: despite the often limited spatial extension and the constancy of abiotic parameters, they host a well diversified and highly specialized fauna with many rare and endemic species. However, the full potential of springs remains relatively unexplored mostly because of the lack of standardized sampling procedures and difficulties to collect representative biological samples, especially in small-sized springs. Recent studies on sampling methodologies in spring habitats indicated that a modified Surber net, with a reduced frame area, reliably describes the structure and composition of spring communities. This method, however, is very invasive and may severely disturb the spring, especially when a large number of replicate samples is required. The main objective of the paper is to present a new quantitative method for sampling crenic invertebrates which combines a rather high efficiency with negligible impacts on spring habitat and biota. The new sampling apparatus is a modified version of the Leaf-nets originally proposed as an alternative to sample macroinvertebrates in non wadeable watercourses. The effectiveness of the new methodology was tested in a medium-size rheocrene spring in Central Apennine, where spring assemblages were sampled in parallel with a modified Surber net and with the new method (0.06 m^2 sampling area for both methods). Macroinvertebrate taxa richness and density were higher in Surber net, while no between-method differences were found for the number of insect taxa and Simpson diversity. Furthermore, the overall functional organization of Surber and Leaf-nets assemblages was very similar, although the relative abundance of shredders was higher in Surber samples. In conclusion, Leaf-nets sampled only 25% of the total individuals collected but 75% of the total taxa richness of spring assemblages. We think that in comparison with the Surber net, the negligible loss of information of the new method is highly compensated by its minor invasivity, lower impacts on spring habitats/microhabitas and invertebrate populations and by its higher versatility. The area of the sampling apparatus can also be modelled in relation to the spring size. The new sampling method also allows for a concomitant evaluation of the structural and functional (leaf litter breakdown) characteristics of spring ecosystems.

FIRST DATA FROM BIRD MONITORING AT KAREN MOGENSEN RESERVE, NORTHWESTERN COSTA RICA

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Due to its high sensitiveness, tropical biodiversity is a privileged target for the study of the effects of climate change; furthermore, birds (Aves) are considered excellent bioindicators, as climatic alterations strongly affect their life-cycle. The project CLIMBIO (*Climate & Biodiversity*), funded by the Fondazione Cassa di Risparmio di Modena, is aimed at the study of tropical ornithic fauna, together with the registration of weather parameters, in order to analyze the potential short and long term effects of climate change on birds. Within this project, we had the opportunity to undertake a periodic bird monitoring at Karen Mogensen Reserve, a protected area located in the Nicoya Peninsula, northwestern Costa Rica (Central America). This region is among the least known of the country in terms of ornithic fauna. The surveyed Reserve, an area of approximately 1,000 square hectares located between 100 and 500 m a.s.l., exhibits a complex of two main habitats: the widespread tropical dry forest and the wet forest surrounding some rivers and streams. These features make the area very suitable to host well diversified animal communities. For our study, fixed points and transects crossing the different habitat typologies and altitudes were identified and geolocalized with a GPS. Qualitative and quantitative data were collected based on direct observations and/or identification of bird songs. Here we provide results from a recent survey along with previous data collected over a 20 years period. The current checklist is made up of over 200 species, stressing the presence of a high species richness compared to similar areas in Costa Rica. We recorded 3 IUCN globally vulnerable or threatened species (*Crax rubra*, *Procnias tricarunculatus*, *Passerina ciris*) and reported for the first time 6 taxa from the Nicoya Peninsula, among which the icterid *Molothrus bonariensis*, a potential invasive species never observed before on the Pacific slope of Costa Rica. In addition, we revealed the presence of *Ortalis vetula* and *Catharus aurantiirostris*, two species that are characterized by isolated populations, restricted to the hills of the Nicoya Peninsula, and whose taxonomy needs further investigation. The information provided suggests that the Reserve represents a very important area for bird conservation within the country, and is confirmed as a suitable site for the continuation of the study on the influence of climate change on tropical biodiversity.

Toxicity evaluation of neonicotinoid pesticide, thiacloprid, in Zebrafish

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Neonicotinoid insecticides are widely used and quite effective for control of sucking-insects on crops and for flea control on cats and dogs. Neonicotinoids are classified as agonists of insect nAChRs (Group 4A) by the Insect Resistance Action Committee (IRAC). These insecticides act by interfering with the insect nervous system. Specifically, they exhibit a high degree of selectivity for insect nicotinic acetylcholine receptors (nAChRs). The nAChR target receptors are members of a family of ligand-gated ion channels, which are responsible for post-synaptic neurotransmission. The neonicotinoids are thought to have reduced toxicity compared to organopfosphate (OP) pesticides due to their presumed selectivity for insect over Vertebrate nicotinic cholinergic receptors. However, there is scant published literature concerning the neurobehavioral effects of developmental exposure of Vertebrates to neonicotinoids. Crosby et al. (2015) have observed in larvae, developmental neonicotinoid exposure significantly decreased swimming activity. The aim of this investigation was to assess the effects of neonicotinoid thiacloprid on *Danio rerio* or zebrafish embryo, using Zebrafish Embryo Toxicity Test (ZFET). *Danio rerio* was considered an excellent model for the environmental biomonitoring of aquatic environments and the ZFET is considered an alternative method of animal test. The use of this organism in conjunction with traditional *in vivo* developmental toxicity testing has the potential to reduce cost and to increase throughput of testing the chemical universe, prioritize chemicals for targeted toxicity testing, to generate predictive models of developmental toxicants and to elucidate mechanisms and adverse outcome pathways for abnormal development. For this reason zebrafish larvae were exposed to different concentrations of thiacloprid (0,001-1000 ppm). Endpoints evaluated are: viability, growth, morphology of brain and craniofacial structures, body shape, cardiovascular function, pigmentation, yolk sac, locomotor function and touch response. The results obtained by toxicity test have showed a high toxicity in function of time and concentration of thiacloprid, relating to the locomotor and cardiovascular functions. At higher doses mortality rate was 100%. Further studies will be carried out by our research team to understand thiacloprid mechanism of action. Furthermore, these results could be useful also to human health, considered our exposure to these insecticides.

Arm-tip regeneration in the spiny starfish *Marthasterias glacialis*: an integrated approach

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Starfish (Echinodermata, Asteroidea) possess striking regenerative capabilities being able to replace lost body structures after both autotomy and traumatic amputation. We selected the Mediterranean-Atlantic starfish *Marthasterias glacialis* (Linnaeus, 1758) as experimental model to study the arm-tip regeneration. The aims of this research are to describe in detail the complex cell and tissue patterning during *M. glacialis* regenerative process and to provide a complementary morphological perspective to previous and on-going proteomic studies [1].

Specimens of *M. glacialis* were collected in the west coast of Portugal (Estoril, Cascais) and subjected to traumatic arm-tip amputation. Regeneration tests were performed at different time-points: 48 hours, 13 days, 3, 6 and 10 weeks post amputation (p.a.). Regenerating samples were processed for both light and electron (TEM and SEM) microscopy analysis.

As for other Asteroids, in *M. glacialis* the process can be divided in three main phases: a) *repair phase* (until 48h p.a.) during which wound closure and healing phenomena take place; b) *early regenerative phase* (13d–3w p.a.) where dedifferentiation, rearrangement and first signs of differentiation occur and c) *advanced regenerative phase* (up 3w p.a.) characterized by complete differentiation,morphogenesis and re-growth of the lost body structures with the formation of a miniaturized arm.

Our results confirm that *M. glacialis* arm regeneration is mainly based on morphallactic mechanisms including 1) rearrangement and dedifferentiation of the stump tissues possibly used as source of cells (mainly from coelomic epithelium) for the subsequent regrowth of the new arm-tip, 2) lack of a well-defined undifferentiated blastema, although the presence of a widespread "blastemal connective tissue" is detectable, underlining the need to review classical epimorphosis/morphallaxis definitions. *M. glacialis* regenerative process can be well described by the "distalization-intercalation" regenerative model re-proposed for starfish by [2]: indeed, the first body structures reformed are the most distal ones and the new tissues subsequently differentiate by intercalation between these latter and the stump, following a proximal-distal gradient.

Current proteomic studies on coelomic epithelium/cells will help to clarify the role of this tissue during regeneration.

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CYSTOSEIRA HABITAT VALUE IN NW MEDITERRANEAN, AN EMERGY ASSESSMENT

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Increasing anthropic pressure on coastal zone is well reckoned as a major cause of habitat modification and loss worldwide. As a consequence, in the Mediterranean Sea, long-living sensitive species of macroalgae (Fucales) tend to disappear being replaced by stress tolerant algal turfs (e.g. Corallinales) or, in case of strong perturbation, opportunistic species (e.g. Ulvales).

In the Mediterranean Sea, Fucales are mainly represented by the canopy-forming macroalgae of the genus Cystoseira. Many studies show that Cystoseira dominated habitats are being lost in several areas of the Mediterranean Sea. Since the ecological consequences associated to Cystoseira belt loss are still little known, a system approach for a quantitative estimate is here proposed.

In particular an assessment of Cystoseira dominated habitats state along the Ligurian coasts is performed. Changes in complexity, ecological and economic value and the related ecosystem services are measured in habitats with different levels of regression and pressures by means of emergy analysis. Emergy, is an operative tool able to 1) measure the overall system functioning as well as system efficiency in exploiting available resources 2) provide a synthetic monetary assessment of ecosystem services and 3) make complex information easily accessible to different stakeholders, from general public to territorial managers. Results are obtained through a defined and repeatable procedure that allows monitoring the system through time. This approach will provide an overall picture of the value of Cystoseira dominated habitats, setting the basis for their management, through conservation and restoration plans.

Microbial biomass and activity of newly-shed leaf litter, forest floor and mineral soil of a Black locust monoculture and a coeval adjacent Black pine monoculture

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Alien plant invasions are known to alter community structure and biogeochemical cycles. Indeed, changes of plant cover may influence soil processes by controlling quality, quantity and timing of plant remains entering the soil. Aggressive native plant species may affect the diversity and structure of communities just as invasive alien species. The focus of the current research was to investigate by a paired-sites approach soil microbial abundance and activity in two sharply different types of forests under the same climate and soil conditions: a monoculture of the alien tree black locust and an adjacent coeval (40-years-old) black pine monoculture. The black locust stand had a dense understory of blackberry that contributed one half of annual leaf litter production. In order to check the relationships between plant litter and microbial communities, newly-shed leaf litter, forest floor litter and mineral soil were examined for abundance of fungal mycelium, total microbial biomass and mineralization rate as emission of CO_2 under standard conditions (25°C, in darkness). Microbial activity was also measured as the ability of microorganism to metabolize a set of 25 organic compounds. To assess the effect of black locust and blackberry on soil microbial activity, black pine mineral soil (i.e. soil not conditioned by the presence of any of the two species) was amended with water extracts from newly shed leaf litter of each one of the two species separately.
Newly-shed leaf litter of black locust had higher mineralization rate than newly-shed leaf litter of both blackberry and pine. Organic layer and mineral layer of the black locust forest had lower microbial biomass, fungal mycelium and mineralization rate than the black pine forest. The amendment of newly shed litter, forest floor litter and mineral soil with 25 C-sources induced an increase of respiration (catabolic response) that was higher for black pine than for black locust. The amendment of forest floor litter with water extracts from newly shed leaf litter of black locust or of blackberry resulted in a decrease of the mineralization rate. In conclusion, data suggest an allelopathic effect of black locust and/or blackberry on microbial community.

Microzooplankton community composition in the winter sea ice of the Northern Weddell Sea

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In winter microzooplankton represent an important fraction of the sympagic community in the Antarctic sea-ice, contributing as food supply for the upper levels of the trophic web. The composition of sea ice microzooplankton communities was analyzed during the winter period in the Weddell Sea. Samples were collected from 14 August to13 October 2013, with the oceanographic RV Polarstern.

Three transects were performed, 10cm diameter cores were drilled every 20m and cut in two parts dividing the bottom section (first 10 cm) from the remaining portion of the core.

A total of 52 ice saples were collected and analyzed at the inverted microscope.

Organisms identified in the ice layer include ciliates, heterotrophic dinoflagellates, foraminifers and first larval stage of micrometazoans. Total abundance ranged from 128 ind. L^{-1} to 1300 ind. L^{-1}.

Aloricate ciliates were the most abundant group with a maximum of 1017 ind. L^{-1} and 20 identified genera while tintinnids and heterotrophic dinoflagellates were almost absente or present with very low values.

Microzoplankton biomass ranged from 2.17 mg C L^{-1} to 28.2 mg C L^{-1}, higher values were mainly due to the number and the large size of aloricate ciliates as *Gymnozoum*, *Litonotus*, *Euplotes, Placus* and *Frontonia* genera. Also the naupliar stages of copepods and foraminifers *Neogloboquadrina pachyderma* contributed to the amount of carbon value.

Cluster analyses, performed on the abundances identified 4 main groups: A and C constituted by bottom samples, and B and D by upper samples. In most cases Top-core samples showed lower values of abundance and richness of taxa, highlighting a pattern of distribution probably due to the frequencies of brine channels, their dimension, and the degrees of ramification in the different ice portion.

ARTIFICIAL STREAMS FOR STUDYING THE RESILIENCE OF BENTHIC ASSEMBLAGES TO DROUGHT: A CASE STUDY FROM FERSINA RIVER BASIN (TRENTO, NE ITALY)

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The hydrological alteration of flow regime due to the combined effects of global warming and increased water abstraction represents nowadays one of the major threats to streams and rivers. Many lotic ecosystems, especially in alpine areas, are shifting from perennial to intermittent, due to the increased frequency and intensity of drought events. As a consequence, negative effects are expected on the benthic communities, which degree of resilience depends on the species-specific adaptations and the availability of in-stream refugia (for example, persistent pools). Despite the scientific relevance of this topic, accurate field studies involve several logistic and theoretical difficulties for freshwater ecologists. As pointed out by many authors, in order to provide generalizable results, future research on these topics should include a more quantitative approach. However, this is very difficult to achieve, because droughts are usually unpredictable, and also several confounding factors can interfere with the assessment of the sampling design. In this context, the use of artificial streams may represent a remarkable tool to study this type of disturbance. In fact, they allow to simulate and replicate drought events, varying their duration and extent in a manipulative and experimental way. In this study, the post-drought recovery pattern of benthic communities (both macroinvertebrates and diatoms) was investigated for three weeks after a 5-day drought, in relation to the presence of instream refugia (i.e. remaining pools). The innovative aspect of the research was the experimental setting, represented by four artificial streams (20 m long, 30 cm wide and 30 cm deep) directly fed by a second-order, pristine alpine stream (Fersina, Trento, NE Italy). This poster illustrates the preliminary results of this study.

Levels of endemism in the Italian tenebrionid beetles.

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Levels of faunal endemism are usually expressed as the percentage of endemic species among all species recorded in a given area. Geographical variations in endemism levels are largely studied in biogeography and conservation biology. Although it is well known that levels of endemism also vary among taxa, inter-taxon variation in endemism levels has received much less attention. Here we used the tenebrionid beetles of Italy (including Corsica and the Maltese Islands) as a case study to investigate how levels of endemism vary among different but related lineages. We excluded the genus *Lagria* and the subfamily Alleculinae because their ecology is completely different from that of all other tenebrionids. We evaluated variations in levels of endemism among taxa (i.e., genera, tribes, and subfamilies) both as a percentage of endemic species in a given taxon to the total number of species included in that taxon (which expresses the tendency of a taxon to produce endemics) and as a percentage of endemic species in a given taxon to the total number of species (which expresses the importance of that taxon to the overall endemic components). Because the number of endemic and non-endemic species may be not statistically independent, we also used as a measure of endemism the residuals of regression lines of endemics against non-endemics. Finally, we tested by ANOVAs the influence of phylogenetic position (expressed by subfamily and tribe nested factors), lifestyle (coded as xylophilous vs. geophilous) and body size (coded into four levels) on the percentage of endemic species within genera. We found that the use of percentages and residuals gave, in general, similar outcomes, with however important discrepancies in taxa that included only few species. Pimeliinae were the subfamily with the highest levels of endemism. At the tribe level, Erodiini, Pimeliini, Tentyriini, Asidini, Opatrini and Pedinini were among the lineages with the highest endemism levels. Among genera, *Asida*, *Pimelia*, *Tentyria* and *Opatrum* were those with the highest levels of endemism. Phylogenetic position and body size affected significantly endemism levels, with genera including larger species being also those with higher endemism, whereas lifestyle was not significant. This suggests that endemism in the Italian tenebrionid beetles is phylogenetically constrained and that lines including larger species (which are possibly less subject to passive dispersal) tend to be richer in endemics, independently from their lifestyle.

Pylon as a condominium: bird colonization of artificial structures occupied by White Stork in the Plain of Gela, Sicily.

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In the Plain of Gela poles of power lines are important structures available for nesting birds due to the absence of trees and other natural perches. The medium voltage pylons are frequently used by different species of birds for nesting and, since 2001, by the White Stork *Ciconia ciconia*. This research, conducted from 2014 to 2016, analyzes the colonization mechanisms of this artificial structure and interspecific interactions between nesting species. We have chosen 58 pylons, 13-18m tall. From January to June the following data were collected: no. of breeding species and pairs/pylons, type of nest (on platform, on abandoned nest of Magpie,etc.), reproductive biology of the species (arrivals/departures, nesting progress, fledglings), ecological and behavioral notes. 12 species have recorded: White Stork, Kestrel *Falco tinnunculus,* Lesser Kestrel *Falco naumanni*, Roller *Coracias garrulus*, Feral Pigeon *Columba livia* (domest.), Wood Pigeon *Columba palumbus*, Collared Dove *Streptopelia decaocto*, Magpie *Pica pica*, Jackdaw *Coloeus monedula*, Spotless Starling *Sturnus unicolor*, Spanish Sparrow *Passer hispaniolensis*, Tree Sparrow *Passer montanus*. Nesting confirmed was 532. Storks have earlier occupied nesting sites in January and in late December. Since February, storks have built or reorganize nests on top of pylons, using small olive branches, weeds, remnants of plants and plastic. In 2016, 70% of active nests of storks were occupied by Spanish Sparrow who nested inside. Breeding pairs of Spanish Sparrow in the nest of White Stork ranges from 2 to 13. Spotless Starling and Tree Sparrow have also nested inside the nests of storks. Except Spanish Sparrow, the other species do not seem to have changes in relation to the presence of storks on the same pylon. The Kestrel has occupied 9.76% of pylons and always colonized the same. The Magpie has reoccupied the pylons where the nesting of White Stork had not occurred for several years. The Jackdaw nested in nest-boxes; the artificial nests were occupied also by Roller and, previously, by Lesser Kestrel: these species tend to nest from April to May, when the nesting of Wood Pigeon and Spotless Starling have been completed. The tendency to occupy pylons at the same time can be attribute to the hypothesis of *Copy heterospecific habitats* (Cody, 1985). The study shows how pylons encourage the establishment of some species, but also constitute a risk of death by electrocution or collision with electric cables (Zafarana, 2016).

Effectiveness of high-flow releases in the recovery of a river environment compromised by fine sedimentation

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The study of dam impacts on river ecosystems has received increasing interest in recent years. In this context, along with significant morphological and hydrological transformations, a key issue is related to the management of fine sediments that accumulate in the dam basins. The spill, accidental or planned, of this material has often deep impacts on river ecosystems, especially in pre-alpine or alpine sections where streambed are coarse. Sometimes, after controlled openings, routine management operations, accidents or technical problems, important quantities of fine sediments can be released in the downstream sections. To resolve or mitigate this problem, specific high-flow releases, i.e. rapid and substantial increases in flow rates designed to move and flush away sediments, are often made. The effectiveness of these operations is usually monitored by the engineering point of view and ecological data are often lacking. The subject of this poster is a case study in the river Stura of Demonte (NW Italy). In January 2016, in the context of some maintenance operations of the Roccasparvera (CN) dam, large masses of fine sediments have been released, covering for kilometers the riverbed with a significant environmental impact. This poster shows the response of the benthic macroinvertebrate community to the siltation and the following high-flow release operations. Considering the significant unpredictability of these phenomena, this research represents a rare and interesting case of study, because we present a large dataset covering from the pre-siltation to the impacted conditions and to the series of recovery operations.

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Molecular taxonomy and distribution of barbels species in SCI protected areas of the Emilian tributaries of Po river inferred with mtDNA

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Barbels are freshwater fishes found in Emilia Romagna with two native species: the common barbel (*Barbus plebejus*) distribuited in the strip of plains and hills, and the canine barbel (*Barbus caninus*) in the Apennines. Both species are becoming rare so the updated IUCN Red List has recently increased the risk status of both species, respectively, to “vulnerable” and “endangered”. Therefore, these Barbels species have been involved in such European LIFE conservation and management project as LIFE13 NAT/IT/001129 “BARBIE” which has been carried out since 2014. The main goal of this project is the conservation of relict populations of barbels in 15 Italian Natura2000 SCI sites (Site of Community Importance) in the Provinces of Parma, Piacenza and Reggio Emilia (Central-Northern Italy). In many Italian rivers, the allochtonous species *Barbus barbus* was found and represents a threat for the native Barbels survival in particular, hybridization between *B. barbus* and the native species was hypothesized long ago on morphological basis and has been recently supported by molecular investigations. For this reason, a preliminary molecular characterization was carried out to assess the genetic diversity of the population with a view of conservation. Direct sequencing of a *cytochrome b* mtDNA fragment (728 bps) was performed on 255 specimens collected in 2014 and 2015. The *cytb* analysis detected 192 specimens belonging to *Barbus plebejus*, 43 belonging to *B. caninus* and 20 belonging to *B. barbus*. The network analysis showed 3 different haplogroups including 14 haplotypes of *B. plebejus* (5 newly described), 7 haplotypes for *B. caninus* (6 newly described) and finally 13 haplotypes for *B. barbus*. Molecular results confirmed a strong reduction of historical distribution of *B. caninus* in the study area and introgression by allochtonus species in the lowest part of tributaries of Po river. The results showed (a) the historical distribution decreased drastically for *Barbus caninus* (b) introgression of allochtonous species *B. barbus* focused on the lower part of tributaries of Po river (c) spatial overlap of habitat distribution between native species *B. plebejus* and *B. caninus* was founded probably due to climate changes and human activities. Conservation efforts within the Po drainage have to focus primarily on native populations. The future studies on nuclear markers could help to detect the hybridization level of the barbels population in the protected areas.

Expression pattern of extracellular matrix genes during arm regeneration in the brittle star *Amphiura filiformis* (O.F. Müller, 1776)

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Arm regeneration in Ophiuroids (Echinodermata) is a topic of interest from morphological, cellular, molecular and evolutionary perspective. During this process, different tissues are involved, among which connective tissue, whose main extracellular matrix (ECM) component is collagen. Despite its importance during both repair and regenerative phases, little is still known about ECM role in this process. In the present study, we employed the burrowing brittle star *Amphiura filiformis* in order to explore gene expression patterns of collagen and other ECM-related molecules during arm regeneration after traumatic amputation.

Different types of collagen, matrix metalloproteinases (MMPs), tissue inhibitor of MMPs (TIMPs), proteoglycans (PGs), proteins (e.g. ficolin) and glycoproteins (e.g. laminin) were selected starting from EchinoBase database with BLASTX search in the *A. filiformis* transcriptome available in Oliveri's lab. From the 39 selected genes, 11 were successfully cloned and tested, along with positive controls, by colorimetric whole mount *in situ* hybridisation at early (stage 2), intermediate (stage 4) and advanced regenerative stages.

Our results indicate that selected collagen genes are expressed in different structures at intermediate and advanced stages, whereas they are not expressed at early stage and in the distal undifferentiated arm-tip at advanced stages; some are not expressed in any considered regenerative stage, thus indicating their diverse spatial and temporal contribution in ECM deposition and maturation throughout the regenerative process. Ficolin is expressed only at advanced stages at the level of the vertebrae, whereas laminin is expressed at the tip of the spines, in both epidermis and dermis and in the radial nerve cord at different regenerative stages, therefore suggesting their importance in skeleton and nervous system regeneration. No expression pattern is detectable at the examined stages for the selected TIMP.

Further molecular analyses on ECM-related molecule and collagen genes, together with ultrastructural and immunohistochemical investigations, are necessary in order to better understand the role of the connective tissue during *Amphiura filiformis* arm regeneration.

POTENTIAL DEGRADATION OF CHLORPYRIFOS IN REMOTE HIGH-ALTITUDE COLD SITES: MICROCOSM EXPERIMENT ON FORNI GLACIER, ITALIAN ALPS

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Mountains act as “cold traps” for semi volatile compounds, and this favors contaminant accumulation in glaciers. Glaciers are ecosystems hosting microbial communities adapted to scarcity of available nutrients and cold conditions. Bacteria living on glaciers are well-known to be versatile with regard to carbon and energy sources. In addition, they can degrade contaminants at low temperatures. Hence, organic pollutants, which can reach glaciers through atmospheric transport processes, may be biodegraded *in situ* because they may represent a source of nutrient for microorganisms in a naturally carbon-poor ecosystem. We studied the degradation of chlorpyrifos (CPF), an organophosphate pesticide found in Italian alpine glaciers melt-water, by *in situ* microcosm experiment carried out on the Forni Glacier (Italian Alps). Degradation of CPF was studied in light and dark conditions, and in abiotic and biotic environments. The results indicated that the largest decay rate of CPF occurred under light biotic conditions (decay rate= -0.028 ± 0.004 d^{-1}). Moreover, the differences between the decay rates at the four groups showed that bacterial activities contributed more to CPF degradation under light than under dark conditions. At the same time, the photolysis process involved in CPF degradation was more efficient in unsterilized than in sterilized conditions. Importantly, no toxic effect of this pesticide was observed on microbial communities at the experimental concentration, which is higher than environmental concentration in melt water from glaciers on Italian Alps.

In conclusion, this study suggests that microbial degradation can occur *in situ* also on glaciers and remove contaminants able to reach remote cold sites. This process can therefore contrast the accumulation of pollutants transported on glaciers. The presence of xenobiotic-degrading microorganisms also implies that glaciers may represent reservoirs of bacterial genes and strains with potential applications in bioremediation of cold environments.

“ANTHURID IVASION” ALONG THE MEDITERRANEAN COASTS: THE SPREADING OF *MESANTHURA* CF. *ROMULEA* AND *PARANTHURA JAPONICA*

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The Anthuroidea is a superfamily of elongated isopods belonging to the mesofauna, mainly distributed in the austral hemisphere and able to colonize a variety of benthic habitats. In recent years, two species from the families Anthuridae and Paranthuridae have been reported as non-indigenous in the Mediterranean Sea: one species of *Mesanthura*, most likely conspecific with *Mesanthura romulea* native to Australia, and *Paranthura japonica,* native to the Asian Western Pacific. Both these species were initially recorded along the Italian coasts, in the harbour of Salerno (2000) and in the Lagoon of Venice (2005), respectively. Since these recordings, their distribution has been considerably expanding. In this study, we present an update on their current known distribution in the Mediterranean Sea from literature records and unpublished data acquired from a range of surveys conducted by the authors across the Mediterranean Sea. Thus far, *M.* cf. *romulea* has been found in 13 Italian localities, and dubiously in Tunisia. *M. romulea* was found in harbours and marinas, as well as in natural habitats (such as seagrass meadows and algal canopies), suggesting shipping as the most likely vector of introduction and highlighting its capability of further expansion both by natural spread or secondary translocation. Furthermore, *P. japonica* has been reported in 20 localities from four countries (Italy, France, Greece and Tunisia), in several important shellfish farming localities (Venice, Taranto, Olbia, La Spezia, near Thau lagoon), suggesting aquaculture to be the main vector of introduction for this non-indigenous species. In addition, the subsequent findings for these two species in ports and on boat hulls highlights the role of vessel traffic in their further spread across the Mediterranean.

Despite their different introduction histories, the simultaneous occurrences of these non-indigenous anthurids in numerous Mediterranean localities may stem either from their recent spread in the basin, or from an improvement in taxonomic expertise. Since anthurid isopods are from a taxonomically challenging taxon, these two non-indigenous species may have gone either unnoticed or misidentified for a long time.

Earthquake-related niche overlap changes in groundwater copepods inhabiting springs fed by the Gran Sasso Aquifer (Central Italy).

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Subterranean waters host unique, stable animal communities comprising species able to survive in these habitats characterized by challenging conditions that can potentially determine severe inter- and intraspecific competition. However, virtually nothing is known about how groundwater species use food and space. We analysed here niche overlap in species spatial distribution (Pianka O index based on species abundance) in the subsurface copepod community in the springs of the River Tirino at the boundary of the Gran Sasso Aquifer (central Italy) in low-discharge (1997), high-discharge (2005), and post-seismic, very high-discharge (2012) years. The aquifer was hit by a 6.3-Mw earthquake in April 2009 that led to a crash in subsurface copepod abundance. To assess if mean niche overlaps differed from those expected by chance, we compared the observed values with the means expected from simulated null-assemblages. We used repeated-measures ANOVAs to analyse differences between years in both pairwise overlap values (O) and species mean overlap values (O_{sp}, calculated as the average of the pairwise O values of all pairs containing a certain species). We distinguished between stygobites (i.e., obligate groundwater species) and non-stygobites. We found that niche overlap values were not significantly different from null expectations in 1997. A significantly larger mean niche overlap in 2012 suggests an anomalous concentration of species, also indicated by a species segregation analysis. O values varied significantly among years and species pairs. O_{sp} values were higher in 2012, but the difference between 1997 and 2005 was not significant and there was no effect of species specialization. Therefore, differences in the mean aquifer discharge may generate changes in niche overlap between species pairs (O), even when the mean interaction of a species with the others taken together (O_{sp}) remains similar, as happened between 1997 and 2005. The 2012 post-seismic scenario was different, because mean species niche overlaps increased significantly, as a result of the exceptional high discharge triggered by the 2009 earthquake. To conclude, despite a general crash in abundance of stygobites, the 2009 earthquake had profound impacts on species segregation and spatial niche overlap.

SPERM-LESS MALES MODULATE FEMALE BEHAVIOUR IN *CERATITIS CAPITATA*

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In the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann)(Diptera: Tephritidae), mating has a strong impact on female biology, leading to a decrease in sexual receptivity to remating and increased oviposition. Previous studies suggest that sperm transfer may play a role in inducing these behavioural changes. Here we report the identification of a medfly innexin gene, *Cc-inx5*, whose expression is limited to the germ-line of both sexes. Through RNA interference of this gene, we generated males without testes and, consequently, sperm, but retaining all the other reproductive organs intact. These sperm-less males were able to mate and, like their wild-type counterparts, to induce in their partners increased oviposition rates and refractoriness to remating. Interestingly, mating to sperm-less males results in oviposition rates higher than those induced by copulation with wild-type males. In addition, we found that the female post-mating behavioural changes we observed corresponded to variations in transcript abundance of genes known to be regulated by mating in this species. Our results suggest that 1) sperm transfer is not necessary to induce remating inhibition and increased oviposition rates in medfly females, and 2) sperm presence affects the regulation of oviposition. Our results pave the way to a better understanding of the role/s of seminal components in modulating female post-mating responses.

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Floodplain protection initiatives increase carbon storage and sequestration to mitigate climate changes. The case study of Natural Reserve of Paul do Boquilobo (Central Portugal).

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Floodplain wetlands are among the most human-impacted ecosystems, supplying a wide range of ecosystem services (ES), including carbon storage and sequestration, which are an important process in climate regulation.

The current study aims to assess the spatial-temporal variation of carbon stored and sequestered by the Natural Reserve Paul do Boquilobo (NRPB). NRPB is a protected wetland floodplain of 817 ha in Central Portugal, which was declared protected area in 1980. The analysis was performed based on land cover maps of three dates (1967, 1990 and 2015), using InVEST model. Moreover, two alternative scenarios (Business and Environmental scenarios) allowing a projection to 2040, were used in order to evaluate to the future evolution of the area, contemplating different scenarios.

Two different scenarios were proposed, one assuming the continuous trend of natural habitat evolution (Natural scenario), and another assuming that private property within the reserve area would be used for agriculture (Economic scenario)

The results highlighted that the activation of habitat protection in 1980 favoured an increase of natural habitats extension at the expense of croplands, resulting as the direct consequence, the carbon storage increase over time. During 1967 the carbon stored in NRPB was 52,685.11 Mg, increasing up to 57,072.53 Mg and further to 63,394.79 Mg during 1990’s and 2015, respectively.

Regarding the future projections, carbon storage was increased in both scenarios. According to Business scenario, the NRPB would store 68,916.04 Mg (+8.71% if compared to 2015), with a carbon sequestration value of 2,219,939.65 US$, while the Environmental scenarios would account for 96,688.02 Mg (+52.52% to 2015), with a carbon sequestration value of 13,386,282.56 US$.

The establishment of RNPB led to an increase of natural habitats and carbon storage service. The increase of carbon stored in RNPB was mainly due to the ecological successions of natural habitats, particularly those related to riparian zones, highlighting their ecological and economic importance. The analysis of future scenarios shows the potential contribute of NRPB to climate regulation in the context of European targets, thus providing an excellent management tool.

Evaluating groundwater dependent ecosystem quality by biodiversity analyses: a proposal from the EC-AQUALIFE Project.

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The groundwater dependent ecosystems (GDEs) are under increasing anthropogenic impacts, yet they are still not object of specific regulations. Also, whereas surface water bodies are subject to biological monitoring programmes worldwide, the ecological aspects of GDEs are never considered in monitoring issues. EU regulations look at the groundwater only for its role in sustaining surface terrestrial and aquatic ecosystems, without consideration for its intrinsic ecological value. The AQUALIFE project aims at including routine monitoring programs within groundwater studies by developing a user-friendly software package for the assessment of biodiversity status and trends in the GDEs. The pilot area involved in the AQUALIFE project is the Abruzzo region (central Italy), where 550 sites were sampled from three main GDE types: (1) entirely, (2) highly, and (3) opportunistically groundwater dependent ecosystems. These sampling sites were selected to reflect impact gradients on the basis of the location of the main pressures known at the regional scale, according to the classification given in the Water Information System for Europe (WISE) and the water physico-chemistry per site. We found that both surface-water insect larvae and the meiofauna were sensitive to high concentration of ammonium detected at several sites in the hyporheic zone of the Sagittario River, the Tirino River and the Gamberale stream. Our preliminary analyses also indicated a significant reduction of species abundance in sites polluted by ammonium ($N\text{-}NH_4^+ > 0.0653$ mg L^{-1}, the minimum threshold value fixed by most EU countries for groundwater). Coastal aquifers, as in the Vomano River catchment, were affected by groundwater pollution by nutrients (ionized ammonium and nitrates) and other organic compounds as PAHs. In the Vibrata River catchment affected by groundwater withdrawal, we observed a decrease in species abundance with increased dominance of euryhaline species in groundwater sites where marine intrusion was detected. In conclusion, these preliminary results indicate the importance of considering groundwater species as bioindicators in monitoring programmes.

BORIS AN EASY-TO-USE, OPEN SOURCE EVENT LOGGING SOFTWARE

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Even if 'more' is not necessarily 'merrier', large amounts of behavioural data have the potential allow developing new frameworks and dramatically improve hypothesis-testing designs. Behavioural scientists, increasingly often, are facing situations in which there is a large amount of video and audio data to code, with the need to go over relatively inflexible software, often constrained by species-specific options. BORIS is a free, multiplatform, open-source program that allows a user-specific coding environment. It can be set for a computer-based review of previously recorded videos or live observations. BORIS is open to user-specific settings and allows a project-based ethogram to be defined, imported, and then shared with collaborators. The observations may include one or two videos (e.g. simultaneous screening of recordings from different sides of an aquarium). The coding can be performed using previously assigned keys on the computer keyboard. BORIS allows the definition of an unlimited number of events (states/point events) and subjects. Once the coding is completed, BORIS can extract a time-budget and present a summary of the main behavioural features.

POSTER

MERCES: MARINE ECOSYSTEM RESTORATION IN CHANGING EUROPEAN SEAS (H2020 FUNDED PROJECT)

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The H2020 project MERCES "Marine Ecosystem Restoration in Changing European Seas" (Grant Agreement 689518) is focused on the restoration of different degraded marine habitats, with the aim of: 1) assessing the potential of different technologies and approaches; 2) quantifying the returns in terms of ecosystems services and their socio-economic impacts; 3) defining the legal-policy and governance frameworks needed to optimize the effectiveness of the different restoration approaches. Specific aims include: a) improving existing, and developing new, restoration actions of degraded marine habitats; b) increasing the adaptation of EU degraded marine habitats to global change; c) enhancing marine ecosystem resilience and services; d) conducting cost-benefit analyses for marine restoration measures; e) creating new industrial targets and opportunities. To achieve these objectives MERCES created a multi-disciplinary consortium (28 Partners from 15 Countries) with skills in marine ecology, restoration, law, policy and governance, socio-economics, knowledge transfer, dissemination and communication. MERCES work plane consists of 10 Work packages on: the inventory of EU degraded marine habitats (WP1), pilot restoration experiments on soft and hard bottoms, including deep-sea ecosystems (WP2, WP3, WP4), and the effects of restoration on ecosystem services (WP5). The legal, policy and governance outputs will make effective the potential of marine restoration (WP6) and one dedicated WP will assess the socioeconomic returns of marine ecosystems' restoration (WP7). The transfer of knowledge and the links with the industrial stakeholders will be the focus of WP8 and the results of MERCES will be disseminated to the widest audience (WP9). The project will be managed through a dedicated management office (WP10). MERCES project will contribute to the Blue Growth by: i) improving the EU scientific knowledge on marine restoration, ii) contributing to EU Marine Directives; iii) implementing the Restoration Agenda, iv) enhancing the industrial capacity in this field, v) increasing the competitiveness of EU in the world market of restoration, and vi) offering new employment opportunities.

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RANKING DRIVERS OF NATIVE FISH COMMUNITY IN LOWLAND RIVERS OF NORTHERN ITALY (EMILIA-ROMAGNA REGION)

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The ecosystem decline, particularly in freshwater ecosystem, is a common phenomenon that follows a global trend. Habitat degradation caused by urban and agricultural pollution, damming, removal of riparian vegetation and excessive water abstraction, affect severely most of the rivers in industrialized countries and under industrialization.

This study aims to provide information on the effects of abiotic parameters (latitude, longitude, altitude, ammonia and nitrate nitrogen, BOD_5, COD, conductivity, total phosphorus, suspended solids and water temperature) and biotic factors (exotic fish community) on native fish community of Emilia-Romagna lowland rivers.

The study considers the sampling sites in the most important rivers of region, the sampling sites are representative of geographical zone named B Zone, with respect to fishing regulations (L.R. 11 07.11.12). The relative environmental characterisation is taken by monitoring sites of Regional Environmental Protection Agency of Emilia-Romagna (ARPA). Statistical analysis of data is performed using CANOCO software 4.5 for Windows in order to study both native community distribution along variables considered and how parameters contribute to determine the distribution of native community.

The results show that the native community rarefaction is mainly due to alteration of habitat, reduction of aquatic vegetation, introduction of exotic species and increase of exotic bentivorous cyprinids already introduced time ago and to the interactions of these factors.

Effects of extremes climate events on intertidal rocky shores

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The role of biodiversity in modulating ecosystem functioning is widely accepted but to date no model has been tested in intertidal habitats and on a large geographic scale. Moreover, this relationship is not well documented in ecosystems affected by extreme climate events. Here, we investigate effects of heat wave on metabolic functioning of rocky intertidal communities and species as expressed by measurements of oxygen consumption (μmol O_2 $l^{-1}h^{-1}g^{-1}$) and compare responses on a large geographic scale.
We selected 13 sites on a latitudinal gradient (spanning 9 degrees). In each site, we sampled randomly the whole assemblage by scraping 10 chips (10 x 10 cm), 10 individuals of *Patella spp*. and 10 individuals chosen from the most abundant macrobenthic organisms belonging to species of *Mytilus galloprovincialis*, *Stramonita haemastoma* or *Mytilaster minimus*. Chips and animals were first placed in tanks filled with seawater and left undisturbed to acclimatize. Then in order to simulate low tide, water was removed from the experimental tanks. Chips and animals were divided in two groups, the first exposed to increasing temperatures (HOT SPOT), and the second was maintained at environmental temperature and used as a control treatment (CTRL). The experimental temperatures for each treatment were site-specific and corresponded to the highest air temperature (T °C) recorded in the last 10 years. After 4 hour of emersion, chips and animals were placed singularly in respirometric chamber filled with filtered seawater. Once submerged, we recorded by mean of an optode the heterotrophic oxygen consumption (t_0) and the measurements were repeated hourly for 3 times (t_1, t_2, t_3).
Our results show that heat wave affects individuals and communities by accelerating the metabolic rate, and that both latitude and the magnitude of the hot spot (i.e. the difference between air and water temperature) experienced by animals, influenced such responses. However while the metabolic debt payed differed when the individuals rate was taken into account, the response was buffered by the presence of entire community. Lastly, our results show a significant relationship between functioning and biodiversity: the larger number of specie in the community, the higher is the resistance. The only exception from this pattern is represented by mussels bed, suggesting the need for considering functional aspects of biodiversity in addition to taxonomic identity.

A cross-community approach to energy allocation among lagoon macro-invertebrate guilds

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Decomposition processes are main sources of energy in lagoon ecosystems but little quantitative information is available on their relative importance with compared to autochthonous primary production and on the energy allocation among different detritus feeder guilds.

Here, we analyse the energy pathways at the first level of benthic food chains in lagoon ecosystem using size patterns at the community and guild levels, i.e., the cross community scaling relationships (CCSRs). The study has been done on macroinvertebrate guilds in fifteen Mediterranean and Black Sea lagoons. Four guilds have been considered: scrapers/shredders, gatherers collectors, filtering collectors and predators. The former two guilds have also been merged as detritus-feeder guilds and, including filtering collectors, as prey guilds.

Different guilds had clearly different taxonomic composition but also different average individual body size, with predators larger than scrapers/shredders and gatherers/collectors but not larger than filtering/collectors. CCSRs clearly differentiated predators and prey guilds, with prey specific density being an order of magnitude higher than predator one, but also detritus feeder and filtering collector guilds, reaching the former higher specific density than the latter. CCSRs did not differentiate scrapers/shredders and gathering/collector guilds.

These results extends previous findings on the use of CCSRs to quantify vigour of different ecosystem types to within ecosystem comparisons, demonstrating the potential relevance of CCSRs as synthetic tools for quantitative assessments of energy flows among different pathways in lagoon ecosystems.

Comparative analysis of fatty acid profiles in three eutardigrade species

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Tardigrades colonize a wide range of habitats representing an important component of the meiofaunal communities. They can be predators, preys or primary consumers in food webs. Most species are herbivorous, feeding on cell fluids of algae and mosses, while others feed on bacteria, or prey on micrometazoans. Despite the wide range of food sources, details on food preferences and on consequent lipid composition of tardigrade species are in practice unknown. Aiming to fill this gap of knowledge, we investigated the fatty acid composition of three eutardigrade species, since fatty acids are the main component of lipids, and they play an important role in the cell membrane functions and in the physiological responses of organisms. The analyzed species, differing in colonized habitat and probably in diet, were: *Acutuncus antarcticus* (Hypsibiidae), a freshwater Antarctic species cultured using *Chlorococcum* sp. as food source, and the moss-dwelling *Macrobiotus macrocalix* and *Richtersius coronifer* (Macrobiotidae). For each species, lipids were extracted from ten replicates of 150-250 animals with chloroform/methanol and the total extracts were used to obtain the fatty acid metylesters that were injected into a gas chromatograph. In all species, the same 21 fatty acids belonging to saturated, monounsaturated (MUFA) and polyunsaturated (PUFA) groups were identified. In *A. antarcticus* the most represented fatty acids were: palmitic (C16:0), stearic (C18:0), oleic (C18:1n-9), and myristic (C14:0) acids; saturated fatty acids (56.6%) were more abundant than MUFA (22.3%) and PUFA (21.1%). In *M. macrocalix* the most represented fatty acids were: oleic (C18:1n-9), palmitic (C16:0), stearic (C18:0), and linoleic (C18:2n-6) acids; the saturated fatty acids (38.4%), MUFA (28.8%) and PUFA (32.8%) were uniformly distributed. In *R. coronifer*, *alpha*-linolenic (C18:3n-3), palmitic (C16:0), stearic (C18:0), and arachidonic (C20:4n-6) acids were the most represented; the percentage of PUFA (52.8%) was higher than that of MUFA (8.2%) and saturated fatty acids (38.9%). These data showed clear differences in the fatty acid composition and amount among different tardigrade species. The fatty acid profiles mirrored the food source and could be used as indicator to assess the feeding diet of tardigrades. Interestingly, species inhabiting the same substrate and eating the same food (moss cell content) use/transform the fatty acids in different ways, thus indicating different biochemical needs.
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How to inform Maritime Spatial Planning from an ecosystem services approach? Challenges from the case of the Adriatic Ionian Region.

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The European Integrated Maritime Policy suggests the use of an Ecosystem Based Approach to reach objectives of sustainable uses in the marine domain. The legislative framework derived from the IMP is structured with respect to two Directives: the Marine Strategy Framework Directive (MSFD) 2008/56/EU, aiming at ensuring that the good environmental status is achieved and marine ecosystems are kept healthy and productive; and the Directive on Maritime Spatial Planning (MSP) 2014/89/EU establishing that blue growth should be pursuit without harming the capacity of marine ecosystems to provide marine goods and services. However, the MSP directive is basically focused on the allocation of human activities for blue growth, considering that MSP, and marine management in general, can only plan and manage human activities in marine areas, not marine ecosystems or components of ecosystems.

The present work reflects on the application of a framework based on ecosystem services concept to inform MSP, considering that human activities are, on one hand, drivers of change and impacts on marine ecosystems, but on the other hand benefit from the delivery of marine ecosystem services.

The proposed framework is applied to inform a Maritime Spatial Planning process in the Adriatic and Ionian Region, capitalizing on the ADRIPLAN project (adriplan.eu) commissioned by the DG MARE, in order to reflect on the relationships between hotspots areas for the delivery of multiple services in relation to the analysis of cumulative impacts, as main threats on biodiversity and ecosystem functioning in the same areas.

Results are discussed considering knowledge gaps, barriers and bottlenecks along with the MSP process in a transboundary area, in the background of the parallel implementation of MSFD. The needs of establishing a monitoring program and a permanent dialogue of science and policy emerge as main challenges in the case study area.

Effects of artificial light at night (ALAN) on alpine stream periphyton: a flume simulation

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Artificial light at night (ALAN) is one of the most widespread human-induced alterations of the landscape, it is increasingly recognized as a contributor to environmental change and a threat to biodiversity at the global scale. Increasing research efforts have demonstrated numerous adverse effects on aquatic and terrestrial animals, microorganisms and plants. Aquatic primary producers, however, have rarely been studied, and our understanding of ecological effects on benthic autotrophs remains limited. Benthic autotrophs, such as diatoms, green algae and cyanobacteria, grow attached to underwater surfaces within complex periphyton communities and form the basis of the food web in many streams and clear, shallow waters. They use light both as a source of energy as well as a source of information for the regulation of physiological processes according to light/dark cycles. The main autotroph groups differ in their preferences for light conditions, therefore the alteration of light regimes may cause changes in periphyton growth and community composition. We conducted experiments in an outdoor flume system mimicking a sub-alpine stream (Trentino, NE Italy). We simulated the night-time light conditions of a waterbody in a light-polluted area (approx. 20 lux), and compared the biomass and community composition of benthic autotrophs in periphyton grown under ALAN with those grown under natural nights. The experiments were performed in two seasons in order to account for seasonal differences in community composition. The LED-based nighttime illumination resulted in a decrease of autotroph biomass and an increase in the proportion of diatoms. The effects depended on the season and the growth stage of the periphyton, indicating higher sensitivity to ALAN exposure in early growth stages (one to three weeks) compared to the later ones (four to seven weeks). Our results show that artificial light can have profound effects on the primary producers of aquatic ecosystems. By negatively affecting the biomass and altering community composition, artificial light at night may hinder primary production, which is a vital ecosystem function. Streams with frequent flooding events, droughts, sediment transport or physical perturbations that all reset the development of periphyton are expected to be more affected by ALAN than those with stable conditions. ALAN may therefore negatively impact the resilience of aquatic ecosystems.

Exposure and post-exposure effects of chlorpyrifos on the gills of *Carassius auratus.*

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Chlorpyrifos (CPF) is a widely used non-systemic organophosphorus insecticide frequently detected in surface waters around the world. The goal of this study is to evaluate both direct and post-exposure effects of CPF on the gills of *Carassius auratus*. The fish were exposed to a single short-term contamination event (96 h) at three nominal concentrations (1,4 and 8 µg/L) in semi-static renewal tests. This was followed by a recovery period of 7 days during which the animals were kept in pesticide-free water. By using both an ultrastructural and morpho-functional approach, we demonstrated that a short-term exposure to CPF negatively affected the gill apparatus of *Carassius auratus*. We observed several histopathological alterations such as epithelium detachment, formation of oedemas and extensive cell degeneration primarily in the Interlamellar Cell Mass (ILCM). The severity of these modifications was dose dependent. Through confocal analysis we also detected the decreased signal intensity of Na^+/K^+-ATPase in the treated specimens, along with the induction of the inducible isoform of nitric oxide synthase (iNOS). Following the post-exposure period, the effects remain, but there are some areas with a normal histological appearance. In these areas it is possible to detect the resumption of Na^+/K^+-ATPase expression along with an increase in iNOS immunoreactivity.

In addition, the amount of lipoperoxide in the gills, in both the exposure and post-exposure groups, was evaluated. A statistically significant increase in the values of lipid peroxidation were observed in exposed groups compared to the control group, at all tested concentrations. After the recovery period the levels of lipid peroxidation were similar to those found in basal conditions, except for the group exposed to the highest concentration. It was observed that after 7 days in a free-pesticide medium the gill apparatus is partially able to renew damaged cells compromised by exposure to pesticide. These results highlight the importance of performing post-exposure tests when attempting to assess pollutant effects.

ANALYSIS OF THE CURRENT STATUS OF ANTICOAGULANT RESISTANCE IN NORWAY RAT (*RATTUS NORVEGICUS*) IN ITALIAN POPULATIONS

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The Norway rat, *Rattus norvegicus*, as well as other synanthropic rodent species, is an important pest and it is essential to find efficient strategies to control wild populations. The introduction of the anticoagulant compounds in the early 1950s produced a significant change for rodent control practices. However, after some years, first resistance events were observed in wild rodent populations. In the Norway rat, anticoagulant rodenticide resistance is mainly associated with mutations in the third exon of the Vitamin K epoxide reductase complex subunit 1 (VKORC1) gene. Rats carrying these mutations, may lose their susceptibility to rodenticides and become resistant. The identification of the resistant wild populations is very important to improve the control practices and to limit the damages due to a not adequate use of the anticoagulant rodenticide. In fact, they can be dangerous for predators that consume rats that have eaten the anticoagulant. Furthermore, many rodenticides tend to bioaccumulate. The environmental damage caused by resistance are very important, not only for a secondary poisoning but also because some resistant strains are very difficult to eradicate and can enhance the spread of zoonotic diseases. Studies on anticoagulant resistance in wild populations have been made in many countries, but are completely lacking for Italy. In this preliminary work, the anticoagulant resistance in the Italian wild populations of *R. norvegicus* is tested searching mutation (SNPs) of the third exon of the VKORC1 gene known to be associated with the anticoagulant resistance (resistance SNPs, r-SNPs). We obtained sequences of the third exon for 64 Norway rats from eight italian regions (Abruzzo, Calabria, Campania, Lazio, Piemonte, Sicilia, Toscana and Veneto) and San Marino Republic. We found a single missense mutation (Y123S) in 6 individuals come from the same Venetian locality. This homozygote mutation, isn't know to be associated with resistance in literature. However, its high local frequency makes it a candidate for future test. We are planning to sample other Norway rats from areas of use/not use of anticoagulant rodenticides to understand if there is a relationships with the use/not use of those compounds and the presence/absence of the mutations known to confer the resistance. This work was partially supported by A.N.I.D., Associazione Nazionale delle Imprese di Disinfestazione.

ENERGY PRODUCTION, DNA DAMAGE AND CELL VIABILITY

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Gametes are highly differentiated cells that, after fertilization, give rise to an embryo in which new undifferentiated cell lines are produced and generate the tissues that build the new individual. To do this, the genetic information carried by the gametes has to be preserved. In this process, besides the nucleus, the mitochondrion contributes with its genome and functions to the new individual development, sustaining its viability throughout the whole life span.

The presence of high membrane potential in gamete mitochondria poses a question on how the mitochondrial genome can cope with energy production byproducts. Indeed, mitochondrial membrane potential is often used during oxidative phosphorylation (OXPHOS) to produce ATP, but this pathway could also raise the rate of oxygen reactive species production. Furthermore, the contribution of glycolysis, at least in some species, was also considered as energetic pathway in gametes.

In the present work, we analyzed data coming from a species, *Ruditapes philippinarum*, in which mitochondria are transmitted both through sperm and eggs. This system can be particularly useful since the mitochondria that populate the germ line in males derive only from sperm mitochondria. So, when analyzing them, we are sure that we are focusing on mitochondria that will be actually transmitted across generations. With this in mind, we compared transcriptomes of male and female gonads searching for transcripts involved in OXPHOS, glycolysis, alternative pathways of ATP production, and DNA protection. For comparison, we also used data from a relative species with strictly maternal inheritance.

Considering the obtained results, for both gamete types mitochondrial respiration appears to be one of the metabolic pathway for energy production, possibly flanked by adenylate kinase shuttle, gluconeogenesis and glycolysis. We found male-biased transcripts involved in defense against oxidative stress and mitochondrial quality control. Defective gametes are eliminated by apoptosis, or fail to perform fertilization. Mechanisms of mtDNA protection from reactive molecules as well as mechanisms of repair can also act to maintain mitochondria and, consequently, cell functionality. Understanding how energy is produced in different cell types, and which molecules protect nuclear and mitochondrial genomes, can help finding how to avoid accumulation of oxidative damage, a common facet of living beings, as predicted by the free radical theory of ageing.

GENETIC PECULIARITIES OF THE WILD BOAR IN ITALY

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The wild boar is an important game species and the ancestor of domestic pigs. In Italy, recent introductions of exotic individuals and hybridizations have resulted in an uncontrolled population growth, which in turn has threatened the survival of native stocks.

Proper management programs are highly improved by genetic characterization. We present here the first molecular study of the wild boar in Umbria. A total of 251 samples, collected in different hunting areas, were screened by analyzing both nuclear microsatellites and mitochondrial DNA (mtDNA). Eighteen nuclear loci were analyzed on 163 samples. Data analyses show three distinct groups clearly separated from the domestic pig. These three groups are geographically separated and divide the Umbria region in three different areas. Boar mtDNA haplotypes (obtained by sequencing 708 bps of the control region) cluster into haplogroups D1, D2 and D4 with the latter being typical of Italy. A fine phylogenetic characterization of entire mitogenomes reveals an ancient divergence of D4 from the other branches and, provides new clues about the ancestral origin of the Italian wild boar.

The Taxonomy Lab: a lookout in the Mediterranean Sea

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The unusual title, we propose, refers to the current trend of marine research that poses two simple questions: "who?" and "where?". Albeit an Italian well developed taxonomic tradition, the Mediterranean Sea may be still considered a box full of "zoological surprises". It appears incredibly little explored to completely unexplored. Gaps in the achievement of a satisfactory understanding of its biodiversity are evident by the high number of newly described; or by the need of taxonomic revision for many taxa, as several case studies disclose. Among the ones carried out in the Taxonomy Lab of the University of Palermo, the record of the crustacean amphipod *Grandidierella bonnieroides* Stephensen 1948, an alien invasive species recently observed along the coast of Israel. Or, the record of the Atlantic invasive amphipod *Ptilohyale explorator* Arresti 1989 along Turkish coast, actually a clear mis-identification, which caused a domino-effect on check-lists of alien species. The Turkish specimens do belong to *Parhyale plumicornis* (Heller, 1866), a Mediterranean endemic species. The lack of information is dramatic regarding rare species. *Caprella santosrosai* Sanchez-Moyano et al 1995 is a rare amphipod up to now included among the Mediterranean endemic species: its record along the Atlantic coast of Portugal changed its status of endemicity. Uncertainties also affect the identity and status of dominant species, such as the emblematic case of the polychaete *Eulalia ornata* Saint-Joseph, in 1888, being considered an exclusively Atlantic species, but only recently reported for the Mediterranean Sea being dominant in the sabellarid reefs. In the past, *E. ornata* had been probably misidentified and confused with the congeneric *E. viridis* (Linnaeus, 1767). Again, the record of *Kyphosus vaigiensis* (Quoy & Gaimard, 1825), one of the kyphosid fish species reported in the past, more than a hundred years ago; a group of species having vagrant habits, which used to occasionally enter the Mediterranean from the Atlantic or from Indo-Pacific area.

These and other studies carried out at the Laboratory of Taxonomy of the University of Palermo will be presented at the joint Congress SITE-UZI-SIB.

δ^{13}C and δ^{15}N seasonality of zooplankton predators and prey to investigate the pelagic food web of lakes with different trophic level.

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Seasonal changes in trophic position and food sources of net zooplankton taxa of two deep, subalpine lakes, characterized by a different trophy (Lake Maggiore and Lake Iseo), were investigated during the year 2011.

Stable isotopes ratios of primary and secondary zooplankton consumers in the pelagic zone and their population structures were estimated seasonally, collecting samples with a large (opening mouth Ø= 58 cm), 450-µm mesh nylon net. Crustacean zooplankton taxa composition were quite similar in the two lakes, although differing for population density and standing stock biomass. Two main phases were identified: a spring-summer phase, during which primary consumers prevailed and peak SSB and population density were attained; and an autumn-winter phase, during which contribution of secondary consumers to zooplankton SSB was far from negligible.

We observed a strict dependence of changes in ^{13}C from the temperature of the sampled layer (0-20 and 0-50 in Iseo and Maggiore respectively), confirming the previous results obtained in lakes of different typology and trophy.

In both lakes, seasonal changes in carbon baselines isotopic signatures were tightly linked to changes in water temperature, with increasingly less depleted values along with thermal stratification. Range of taxa specific nitrogen isotopic signatures was wider in Lake Maggiore, ranging between 0.8‰ to 17.1‰ in the former and between 2‰ to 10‰ (for *Eubosmina* and *Bythotrephes*, respecitvely). In both lakes, in spring, *Eubosmina* was less δ^{15}N-enriched than *Daphnia*, most likely because of exploiting nitrogen fixing, cyanobacteria colonies, commonly detected in lakes with the onset of thermal stratification. The observed differences of δ^{15}N enrichment can be related to a better efficiency of oligotrophic lake in organic matter recycling into the water column. In Lake Iseo, a smaller stepwise N enrichment than in Lake Maggiore confirms the hypothesized link between nitrogen isotopic signature and lake trophy: the higher the trophy the lower is the enrichment. Two major links, between *Daphnia* and *Bythotrephes* in Lake Maggiore and between *Eubosmina-Leptodora* in Lake Iseo are likely related to different fish predation pressures and size structure of crustacean populations in the two lakes.

Morpho-functional alterations induced by lead in the gill apparatus of *Thalassoma pavo*

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Lead is a naturally occurring nonessential metal present in the earth's crust, rock, soil and water, but most waterborne lead comes mainly from human activities such as mining and smelting, coal burning, cement manufacturing, use in gasoline, batteries and paints. Like all heavy metals, lead is extremely stable and persistent in the environment and it is also very toxic and bioaccumulative. Among marine animals, fish are most susceptible and more vulnerable to metal contamination than any other aquatic organism.

With this in mind, the aim of this work is to evaluate the effects of lead on the respiratory system of *Thalassoma pavo*, a common fish of the southern Mediterranean. In this work the gills of *Thalassoma pavo*, were examined, under basal conditions and after 192 h of exposure to three sub lethal concentrations of lead (800, 2000, 12000 µg/l).

Histological and ultrastructural alterations induced by lead were analyzed by both light and electron microscopes. Moreover some functional parameters through their differential expression in basal and experimental conditions were evaluated. In detail Na^+/ K^+ ATPase and Aquaporin 3 (AQP-3) were considered to assess the putative alteration in ionic and osmotic equilibrium and the metallothioneins (MTs) as markers of detoxification after exposure to lead.

The analysis clearly showed that short-term exposure to sublethal concentrations of lead induced numerous and significant alterations in gill morphology and ultrastructure. The main effects recorded were: formation of aneurysms with infiltration of erythrocytes in the distal portion of secondary lamellae, hyperplasia, cellular degeneration, hypertrophy of chloride cells and alterations of the vascular component at level of secondary lamellae. Lead is also able to significantly affect the ionic balance as evidenced by the modulation in the expression pattern of Na^+/K^+ ATPase and AQP-3. The induction of MTs expression confirms their role as a marker of exposure to heavy metals. The results highlight the risk posed by heavy metals to aquatic biota and demonstrate the importance of environmental monitoring to establish limits for heavy metal use.

Cave-dwelling planarians and biodiversity from North Italy: preliminary species richness assessment of the genus *Dendrocoelum* (Platyhelminthes, Tricladida, Dendrocoelidae)

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Groundwater is a major source of potable water supply at global level, but it is also a vulnerable biotope with a largely underestimated biodiversity. Among the stygobiont fauna of northern Italy one of the less studied group is represented by cave-dwelling planarians. The focus of this study is to identify the role played by cave features in the occurrence of stygobiont planarians, assessing abundance and conservation status of the known species of the genus *Dendrocoelum* (Dendrocoelidae) and evaluating the geographic distribution of other cave-dwelling dendrocoelids in different subterranean systems. In North Italy the genus *Dendrocoelum* is represented by only 4 species in caves from Liguria, Lombardy, and Friuli. More than 50 natural caves where surveyed (March-December 2013; October 2015-June 2016) in several areas of these regions including the type locality of *D. italicum* (Lombardy) and *D. beauchampii* (Liguria), and the single Italian cave harbouring *D. collinii* (Friuli). The rich collection of triclads allowed us to estimate population abundance and to define environmental features of caves and microhabitat inhabited by freshwater planarians. The new records notably increased biodiversity values for underground ecosystems and enlarge the geographic range of dendrocoelids species. Future studies should focus on understanding the level of endemism in northern Italy Karst, on the potentiality of freshwater flatworms as bioindicators and on their functional role in the cave ecosystems.

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DISTRIBUTION AND POPULATION SIZE OF *Androsace brevis* (Hegetschw.) Ces.

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Androsace brevis is a protected species according to Regional Law 10/2008, included in the National Red List because of its status as an endemic and as a rare species in its distribution range. It is a high altitude, light-demanding cushion plant, present above 2000 m.a.s.l. on rocky ridges exposed to strong winds, on prealpine terrigenous substrates. The distribution range of *A. brevis* is not yet well defined, and is peculiar compared to the distribution range of other Lombardy endemic species; it consists of three large discontinuous populations separated by Lake Como, on the Western Orobian Alps (1), the Lugano Prealps and a portion of the Lepontine Alps, in addition to a disjointed populations in the Rhaetian Alps (2). The University of Milan started a research project to define the ecology of the species, its conservation status and the causes of its present distribution:as a preliminary work, we verified the distribution range of this species and the numerical consistency of its populations. To define the distribution range, we started in 2013 a thorough exploration of the sites known from the literature and neighbouring areas. To verify the populations size, we counted all individuals and, for every cushion, we counted the number of rosettes and number of fruits. To compare all the variables recorded among the different pupulations we performed an analysis of variance (ANOVA), followed by a post-hoc Tukey HSD test

Distribution Range

The easternmost population is located on Monte Fioraro (Orobian Alps), the westernmost is located on Monte Camoghè, (Lugano Prealps), the northernmost is located near Rifugio Gianetti (Raethian Alps), and the southernmost is located on Pizzo dei Tre Signori (Orobian Alps).

Populations Consistency

The biggest population (193 cushions) has been found on Passo di San Jorio (Lepontine Alps) We observed significant difference in the medium number of rosettes of cushions into the three sectors of areals (ANOVA $_{\text{test}}$ $F_{36,724}$ P < 0,0001), with the biggest ones in the Rhaetian Alps (Tukey HSD test P < 0,0001). Also the production of fruits showed significant difference among the three sectors of areals (ANOVA $_{\text{test}}$ $F_{13,674}$ P < 0,0001) with the Western Orobian Alps indicated as the more productive sector (Tukey HSD test P < 0,0001).

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GLOBAL INVASION OF *Aedes albopictus* AS A VECTOR OF ARBOVIRUS: WHAT CONSEQUENCES FOR DISEASE OUTBREAKS?

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Invasive species represent a global concern for their rapid spread and the possibility of infectious disease transmission. This is the case of the global invader *Aedes albopictus*, the Asian tiger mosquito, vector of more than 20 arbovirus, notably chikungunya (CHIKV), dengue (DENV), Zika (ZIKV). The identification of important invasion corridors has great potential for mitigating invasion and disease risks. Combining classical population genetics and Approximate Bayesian Computation (ABC) approaches with historical records we provide a comprehensive picture of the demographic history of populations from the supposed native range, South East Asia, and from the invaded regions in Indian Ocean, Mediterranean Basin and USA. In South East Asia, the low differentiation and the high co-ancestry values identified among China, Thailand and Japan confirm that, in the native range, these populations maintain high genetic connectivity, revealing their ancestral common origin. China appears as the oldest one. Outside Asia, the invasion process in Reunion, America and the Mediterranean Basin is supported by a chaotic propagule distribution, which co-operate in maintaining a relatively high genetic diversity within the adventive populations. We propose that the demographic history of the populations may contribute to create the conditions for efficient arbovirus transmission and, consequently, for outbreak establishment.

Sunscreen products affect reproduction and larval development of *Paracentrotus lividus*

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Coastal areas are among the marine ecosystems more threatened by anthropogenic impacts, including those due to micro-pollutants contained within personal care products such as sunscreens, which can be released in marine environment even in large quantities. In the present study, we assessed the impact of three different sunscreen products, at different concentrations, on reproductive capability and larval development of *Paracentrotus lividus*, one of the key species of the Mediterranean costal ecosystems. In particular, we tested the effects of solar products largely used in Europe and U.S.A. characterized by a different composition, and a new eco-compatible sunscreen. We found that European and American sunscreens determined a higher fraction of anomalies in the embryos and larval development of *Paracentrotus lividus* than the ecofriendly product. However, a negative, immediate effect was observed after the addition of all the three sunscreens, particularly on the early developmental stages of *Paracentrotus lividus*. Findings reported here expand our understanding of the impact of sunscreen products on marine life and underline the importance to identify and use personal care products respecting marine ecosystems.

Sessione tematica: Ambienti di transizione e marino costieri

How to communicate research achievements: experiences from a European project

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Science plays a central role in our lives. Therefore, the communication of scientific findings to a wider community and the transfer of knowledge should be present in all research programmes. Here we present the experience accumulated during the EU funded project DEVOTES (DEVelopment Of innovative Tools for understanding marine biodiversity and assessing good Environmental Status) with the aim of assisting other researchers in developing a successful dissemination strategy to communicate project achievements. Both traditional (e.g., peer reviewed publications, stakeholders workshop and participation to scientific conferences) and innovative (e.g., social networks, smartphone applications) dissemination tools have been used to engage the different categories of stakeholders identified during the development of the dissemination plan. All the planned dissemination activities were addressed keeping in mind the main aim of DEVOTES, to build knowledge on the functioning of marine ecosystems (i.e., improving ocean literacy), and to raise awareness on the implications of human activities on marine ecosystems. Without a solid understanding of this topic, policy makers and the wide public cannot make informed decisions to solve environmental issues. Using social network and web-based analytic tools, the DEVOTES Dissemination Team performed a constant evaluation of the effectiveness of the different means of communication, of their impact and usefulness. The development of a detailed dissemination plan and the constant evaluation of the dissemination results, during and after the project lifetime, is fundamental for the achievement of an effective and timely communication of scientific findings.

INSIGHTS INTO POPULATION GENETICS, CONNECTIVITY AND PHYLOGENETICS OF THE LONG-NOSED SKATE *DIPTURUS OXYRINCHUS* (LINNAEUS, 1758).

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The present work aims at providing information on the genetic variability and the genetic structure of the near-threatened long-nosed skate *Dipturus oxyrinchus* (L. 1758) in order to contribute to the formulation of an effective management plan for the species. A total of 210 specimens were collected in six sampling sites along the coasts of the island of Sardinia (Western Mediterranean), where the species is still abundant, contrary to what it is reported for other areas of its distributional range.

A portion of mitochondrial control region (CR, 666 bp) and the Cytochrome Oxidase I (COI, 561 bp) genes were sequenced, and analysed singularly and concatenated (1227 bp).

Using the concatenated dataset, a high haplotype diversity (Hd= 0.819) and low nucleotide diversity (π= 0.0016) were identified, with a total of 27 haplotypes. The Spatial Analysis of Molecular Variance indicated the occurrence of three distinct genetic clusters (SAMOVA: Φct= 0.0801, P-value= 0.0196), corresponding to northeast, northwest and south Sardinian sites. Several significant pairwise Φst values confirmed potential limitations to gene flow.

Considering the markers singularly, the mitochondrial control region (CR) showed a higher degree of nucleotide polymorphism than COI. Nevertheless, the occurrence of three distinct genetic clusters was confirmed based on sequence variation of the both CR and COI.

The comparison of Sardinian sequences to those obtained from public repositories (GenBank and BOLD) for the Atlantic and the Western Mediterranean pointed out the sharing of haplotypes between these areas, challenging the validity of the hypothesis of complete genetic isolation of the Mediterranean stocks proposed in previous studies.

Besides, the taxonomic and evolutionary status of *D. oxyrinchus* was also examined based on the COI gene. Specimens of *D. nidarosiensis*, *D. laevis*, *D. cf intermedia*, *D. cf flossada* were sequenced and compared to the available sequences of over 20 species of the genus *Dipturus*. The occurrence of only two well-distinct species (*D. oxyrinchus* and *D. nidarosiensis*) was confirmed for the Mediterranean, while shared sequences among putative real distinct species (e.g., *D. chilensis/D. trachyderma*, *D.laevis /D.cf flossada*) were found in the adjacent Atlantic Ocean. This result suggests potential misidentifications and/or the lack of resolutive power of the COI marker along with potential introgression/hybridization between closely related species.

Soil quality along two roads of the Vesuvius National Park exposed to different vehicular traffic.

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Vehicular traffic releases into the atmosphere pollutants that affect air quality. Air pollutants can move at brief and long distance from the emission sources and, through dry and wet depositions, can reach the soil surface, modifying its quality in terms of chemical, physical and biological characteristics. Chemical and biological indicators are used to highlight soil quality. The aim of this study was to assess the impact of two traffic flow typologies on the soil quality of the Vesuvius National Park. In order to reach the aim, surface soils were collected inside the Riserva Alto Tirone (an undisturbed site, as far from the roads) and along two roads, leading to the cone: Ercolano, characterized by continuous and various traffic (cars, motorcycles, buses for the whole year), and Matrone, crossed by a limited period and only by a traffic typology (tracked vehicles). The soils were collected at the end of the touristic season (autumn 2015) and at the beginning of the next one (spring 2016) at two altitudes (approximately, at 600 and 900 m). The soil physico- chemical parameters (*i.e.* pH, water, organic carbon and nitrogen contents) slightly varied between seasons and among the roads. Besides, soil metal (Cr, Cu, Ni and Pb) content was lower at the undisturbed site as compared to the roadsides. Among the metals, only Pb showed differences between the soils collected at different altitudes with lower values at the lowest altitude, where similar values were detected for the two roadsides. These findings highlighted that the microclimatic conditions and topography affected Pb deposition on soils; in addition, the tracked vehicles would seem to affect heavily the soil Pb content. At the end of the touristic season, it was evident a stress condition for soil microorganisms as higher values of the metabolic quotient (qCO_2) were measured in autumn than spring; in addition, the soils collected along the road Matrone showed the highest stress conditions. The lowest microbial and fungal biomass in autumn confirmed the stress conditions of the soils at the end of the touristic season. Besides, the microbial and fungal biomass appeared less active at the undistributed site as compared at the roadsides. In conclusion, the various traffic typologies would seem to affect soil Pb concentrations with heavy effects due to tracked vehicles, and to induce stress conditions for the soil biota, more evident at the end of the touristic season.

Do bioactive molecules exsist in *Armadillo officinalis* Dumèril, 1816? (Crustacea, Isopoda, Oniscidea)

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Bioactive molecules are chemical compounds which exist in all organisms and can be used for the development of new molecules with biological activity (e.g. drugs). Several studies showed that these molecules had antiproliferative effects in tumor cells.

The Aim of this study is to identify bioactive molecules from hepatopancreas of the terrestrial Isopods. Kobert (1903) et al. identified the presence of enzymes able to digest fibrin, starch and amygdalin in *Armadillo officinalis*. Specimens collected in a natural reserve were divided into males, females and gravid females from *Armadillo officinalis*. We evaluated the antiproliferative effects on Human multiple myeloma cell lines (U266) by using the samples extratc from specimens *in toto*, specimen without hepatopancreas and only hepatopancreas. After 24 h ATPlite assay (Luminescence ATP Detection Assay Sistem, Perchin Elmer) was performed in order to evaluate cell viability of MM cells.

Significative results were observed in the condition with hepatopancreas of gravid female with a U266 viability reduction of about 80%. Moreover, the females without digestive gland and females *in toto* showed a decrease of cell survival of 30-40%. No significant results were observed with the extract of the males.

These preliminary results suggest that bioactive molecules extracts by female specimens of *Armadillo officinalis* are able to reduce proliferation of multiple myeloma cells.

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Effects of bisphenol A on early development of the ascidian *Phallusia mammillata* (Chordata, Tunicata)

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Bisphenol A (BPA) is an organic compound used in the manufacture of polycarbonate plastic and epoxy resins that is released into the environment from sewage treatment effluent, landfill leachate and degradation of plastic products. BPA can act both as a teratogenic substance and as an endocrine disruptor. The phylogenetic position of tunicates as sister group of vertebrates and their cosmopolitan distribution in marine ecosystems coupled with their ecology and easy manipulability make them reliable model organisms for ecotoxicology bioassays. Here we evaluated the effects of different concentration of BPA (0.1, 0.5, 1, 5, 10, and 20 µM) on ontogenetic processes sensitive to environmental pollutants in the ascidian *Phallusia mammillata*. To test the effects of this substance on sperm viability we fertilized eggs with sperm pre-exposed for 30 minutes to BPA. One hour post fertilization (hpf) we calculated the percentage of eggs that reached the 2-cell stage compared to control. Then we analyzed the consequences of a fertilization performed directly in BPA solutions, counting the percentage of 2 cell-stage embryos 1 hpf. To test the effects on embryogenesis, we analyzed hatched larvae developed from 2-cell embryos exposed to BPA. We evaluated the number and type of malformations under a dissection microscope and we performed immunostaining of central nervous system (CNS). Exposure of sperm to BPA did not influence fertilization rate. Co-exposure of eggs and sperm at concentrations higher than 5 µM caused incomplete division of eggs, producing heart shaped embryos that did not develop further. Regarding embryogenesis, 10 µM BPA caused specific malformations at central nervous system, with an increased distance between otolith and ocellus, or an extrusion of ocellus from the sensory vesicle. Embryonic development was significantly altered at 20 µM concentration. 97% of the larvae presented a severely affected phenotype with short and kinked tail. Immunostainig with anti β-tubulin antibody showed an altered pattern of fibers in CNS. These results showed that the most sensitive process to BPA is the first cell division. When the 2-cell stage embryos are treated, higher concentration are required to alter the correct development of CNS. BPA confirmed its teratogenic effects on ascidians and its interference with CNS development even if the mechanism of action in this group is still to be clarified.

Project “Biodiversity Mare Tricase” - A biodiversity inventory of the coastal area of Tricase (Apulia, Lecce, Italy) in the Otranto Channel

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“Biodiversity is maintained” is the first descriptor of EU’s Marine Strategy Framework Directive. Nowadays several taxonomic inventories for the Mediterranean Sea are available, but there are still important gaps on the basic information about taxonomy, distribution, abundance, and temporal trends of several groups. Biodiversity is rapidly changing due to habitat loss, overfishing, pollution, climate change, eutrophication etc. Long-term biodiversity monitoring at high taxonomical resolution are crucial to understand these patterns of biodiversity change.

Thanks to the joint support and patronage by the Municipality of Tricase, the “Magna Grecia Mare” Association, and the Mediterranean Agronomic Institute - CIHEAM Bari, a preliminary checklist of the coastal and marine fauna and flora of the Tricase area (southern Apulia) is being carried out during April - October 2016 within the project “Biodiversity Mare Tricase”. Sampling is carried out by visual collection, substrate scraping, and collaboration with fishermen and local citizens. Sample processing and taxonomic identification are undertaken in the new marine station “MARE outpost”, a laboratory dedicated to biodiversity research and conservation. The MARE outpost is located in the Otranto Channel, a strategic biogeographical crossroad between Adriatic, Ionian, and Aegean Seas, representing a monitoring hotspot for both native and alien species diversity.

Until now 285 marine species, 238 genera, 192 families and 20 phyla were found; in addition, 52 species of coastal plants were identified. Particular attention is paid to identification of key taxa such as hydrozoans, molluscs and other invertebrates of commercial interest, and fish.

The project also include:

- The study of the phenology and life cycle of marine organisms.
- The creation and updating of a biodiversity website (www.biodiversitymaretricase.org) to make data and high-resolution pictures freely available.
- The organization of educational workshops and training courses for kids and the general public to enhance awareness on marine biodiversity.

Inspired by the seminal work by Salvatore Lo Bianco in the Gulf of Naples, this biodiversity project will represent a basis for further studies in the area and, at the same time, will promote nature and biodiversity conservation and the development of sustainable tourism in the Salento peninsula.

WHAT MAKES *HALOPHILA STIPULACEA* A SUCCESSFUL COLONIZER IN A WIDE RANGE OF ENVIRONMENTAL CONDITIONS? A LOOK INTO ITS ECOLOGICAL PLASTICITY IN ITS NATIVE SITE USING A MULTIDISCIPLINARY APPROACH

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The ability of seagrasses to undergo specific morphological and biochemical adjustments in response to environmental gradients is an important adaptive trait. Parallel to these adjustments, shifts in the associated microbial communities can also influence host's health and its capacity to withstand environmental changes, helping colonization and expansion in new territories. Integrated assessments of seagrass ecophysiology and associated microbial community structure can help understand the capacity of a seagrass species to persist and adapt. *Halophila stipulacea* is a tropical seagrass with a native range east to India, west to eastern continental Africa, south to Madagascar, and north to the Red Sea and Persian Gulf. It is a Lessepsian migrant in the Mediterranean Sea, and an invasive species in the Caribbean Sea where is a threat to native species. This widespread distribution and pervasiveness is due to its ability to thrive in a wide ecological range. In this study, we use a multidisciplinary approach to investigate the changes in morphology, biochemistry (pigment and phenol content) and epiphytic microbial community structure of the tropical seagrass *H. stipulacea*, in response to local environmental gradients due to depth, location and season, in the northern Gulf of Aqaba, Red Sea.

The entire suite of descriptors was considerably influenced by depth, location and seasonality, confirming the high ecophysiological plasticity of *H. stipulacea*. Leaf morphology and photosynthetic pigment content were modulated in response to light availability and hydrodynamics. The highest leaf surface area and photosynthetic pigment contents were observed at the lowest irradiance and hydrodynamics. While, at these conditions, biomass, density, percent cover and total phenol content showed lower values. Although the bacterial community composition differed among depths, sites and plant compartments, the epiphytic bacterial communities showed a well conserved core bacteriome, with a high incidence of the classes *Alpha-*, *Gamma-* and *Delta-Proteobacteria* both above and belowground across depths and sites. A shift in the structure of the *H. stipulacea* bacteriome was observed under low light regimes, with a high incidence of *Gamma-Proteobacteria* and *Bacteroidetes*, while *Cyanobacteria* and *Rhodobacteraceae* thrived in conditions of high light availability and hydrodynamics. These shifts are consistent with the different ecophysiological conditions of the plants and again throw spotlight on the functional relationship between seagrass and microbes.

To the best of our knowledge, this is the first time that such descriptors have been used in an integrated approach. Results help to gain a better understanding of the optimum growth patterns of *H. stipulacea* and its ecological resilience, in order to predict potential future expansion of its geographical range. In general, this approach can improve the effectiveness of seagrass monitoring programs, in particular those aimed to estimate seagrass plasticity across different habitats.

Example of use of a simulation tool in the assessment of exposure to chemicals

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The assessment of exposure to chemicals is one of the fundamental stages of the environmental risk assessment; however the environmental samples are limited in time and space and they do not measure the bioavailable dose for biota. Recently has been developed the tool MERLIN-Expo that contains several models to simulate the fate of pollutants in the environment and within the living organisms and provides an estimate of exposure which can be used in the process of Risk Assessment.

This study is an example of use of MERLIN-Expo starting from the environmental monitoring data performed on the Po river waters by ARPA Emilia from 2007 to 2014 as part of the controls required by Decree n° 152/06 to simulate the concentrations of pollutants in river sediments and biota; among the pollutants that can be used in MERLIN-Expo, it was chosen the arsenic because it shows concentration values almost always greater than the detection limit and quite variable between samples.

It was considered a simplified food chain consisting of diatoms, copepods, carp and catfish, assuming that each group only eats the previous.

Starting from the concentrations of As in the water, MERLIN-Expo simulated the concentration of metal in the river sediments and in the elements of the food chain; the results were compared with those obtained in previous studies carried out on the Po river and available in the literature. The values calculated by MERLIN-Expo in sediments are slightly smaller but of the same order of magnitude as those of literature. Instead the values calculated in fish were found much larger than the experimental ones, leading to an overestimation of the concentration in biota.

The results show that the use of simulations in the study of the distribution of pollutants in the environment can be a useful instrument to support environmental monitoring and risk assessment, but they emphasize the need to use the input data as possible concerning the reality of the sampling site and to compare the results with data from the same site and the same period.

Long-term ecological research and benthic diversity changes in the Valli di Comacchio

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An essential prerequisite for the assessment of the ecological quality of lagoon ecosystems is the understanding of the natural variability and its effect on the performance of quality indices. This study is focused on the long-term variability of richness and diversity by using macrobenthos data gathered from a Long-Term Ecological Research (LTER) Network site (IT07-002-M: Valli di Comacchio). The Valli are one of most representative choked (i.e. not influenced by the tidal regime) lagoons in the Mediterranean and Europe. The Valli have always been home to anthropogenic activities, like fisheries, tourism, etc., which, sinergistically with eutrophic water inflows, led to quite high levels of disturbance for the whole ecosystem and particularly for the benthos. However, natural events (e.g. extremely hot or low temperature) cannot be disregarded because of their possible influence on the benthic communities of shallow lagoons. The structure and composition of the macrobenthic community was studied in the Valli at permanent sampling sites for almost twenty years (1996-2015). Analyses of water parameters disclosed significant differences in their temporal (but not in the spatial) variations, and we were not able to classify the sites according to any expected degree of stress. The analysis of species number and univariate indices revealed similar general patterns across all sites, albheit strong interannual variability was evident. Benthic invertebrates were categorized into five ecological groups, from EG-I, sensitive, to EG-V, first order opportunists. EG-III to EG-V, with very few exceptions, dominated the benthic community over the long period. Despite no clear relationship was found between community descriptors and general climate trend (as summarized by NAOI), the variability of the community was high after severe events, as severely cold winter or severely hot summer. Multivariate analysis revealed how the presence of some taxa (e.g. *Streblospio shrubsolii*, *Cerastoderma glaucum*) was particularly related to salinity fluctuations. The M-AMBI index was used to assess the ecological quality status, which resulted often unsatisfactory. Differently than in other lagoons (e.g. Tortolì, Orbetello, Lesina), no remarkable differences in status assessment were found when using biomass instead of abundance in index calculation. The overlapping effects of natural and anthropogenic disturbance on benthic communities may challenge the environmental assessment of the Valli, since stress is an intrinsic characteristic of this shallow ecosystem. This study showed that caution should be taken when implementing environmental policies (e.g. WFD 2000/60/EC), so as not to disregard the importance of the different events (natural and human-caused) on the ecosystem health: the long-term variability exhibited by the benthic community in the Valli di Comacchio should be considered within ecological quality assessment schemes.

Biotic invasions in an Adriatic lagoon

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Introduction and spread of non-indigenous species are considered one of the main threats to biodiversity at different scales and extent. Aquaculture and related activities are recognized as one of the most important drivers of non-indigenous species in the Mediterranean Sea. The north-western part of the Adriatic Sea is characterized by a large number of brackish coastal lagoons, that are recognized as highly vulnerable to species introduction as a consequence of their environmental instability, low number of species, exploitation for aquaculture and shellfish farming, and the presence of not saturated benthic communities. The Sacca di Goro is a shallow-water embayment of the Po River Delta approximately triangular in shape with a surface area of 26 Km^2, an average depth of 1.5 m, and is connected to the sea by a 2.5 km large, very shallow (avg 0.5 m depth, with the exception of two narrow waterways) mouth. Its western and central areas account for one half of the total surface, and host the most flourishing clam farming ground of Europe. The eastern zone is very shallow and is a sort of *cul-de-sac* of the lagoon, characterized by dramatically poor water renewal. Within the wider framework of a LIFE project (LIFE13 NAT/IT/000115 AGREE), we monitored the benthic biodiversity in the eastern part of the Sacca. Ten sites were sampled in triplicate in 2015. The bottoms were muddy: RPDL ranged between 0 and 1 mm depth, while the content of sedimentary organic matter ranged between 1 and 6%. Overall, about 60 taxa of benthic macroinvertebrate were gathered, with diversity (as Shannon's index) ranging between 0.9 and 2.4. Multivariate analysis showed that sample points segregated apart in three clouds reflecting a spatial gradient. Non-indigenous species were defined according to EU databases (CIESM, DAISIE, IMPASSE, WoRMS) and recent literature. Their number resulted to be high, since almost 25% of the total taxa collected were allochtonous. Figures were different among taxonomic assemblages, with Polychaeta, Crustacea and Bivalvia showing the highest number of non-indigenous species. Among these, the presence of the indopacific aorid *Grandidierella japonica* Stephensen, 1938 was detected for the first time in the Mediterranean Sea, while the finding of a reproducing population of this species suggested that *G. japonica* has become well established in the Sacca di Goro. Our results suggest that the Sacca is an inherently unstable and variable environment subjected to multiple introductions. The great majority of non-indigenous species collected was of indopacific origin. Aquaculture and release through ballast waters seemed the commonest ways of introduction, and the possible role of LNG-carriers at the nearby gas terminal is discussed. The factors favouring non-indigenous species introduction and mass development in this particular area thus seem: (i) the occurrence of intentional introductions and transfers, with the consequent accompanying species, (ii) large volume of maritime traffic, (iii) environmental stress and high nutrient load of both natural and anthropogenic origin.

Protist ciliates as natural reservoir for bacteria potentially pathogenic for metazoan: preliminary results of the experimental infection of the planarian *Dugesia*.

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Most of the microorganisms belonging to genera responsible for vector-borne diseases (VBD) has hematophagous arthropods as vector/reservoir. Recently, many new species of such microorganisms were found in a variety of terrestrial and aquatic eukaryotic hosts: in particular, numerous new bacterial species belonging/related to the genus *Rickettsia*, *Alphaproteobacteria* of the order *Rickettsiales* with an obligate intracellular lifestyle (e.g. the etiological agents of epidemic typhus and spotted fever), were discovered in protist ciliates. Although their pathogenicity for either humans or animals is still under study, these bacteria could actually act as etiological agents of possible VBD of aquatic environment. Indeed, an increasing number of massive fish death was recorded in intensive aquaculture facilities during the lasts years due to epidemics caused by *Rickettsia*-like bacteria with unknown natural vector. As ciliates could vector pathogenic organisms possibly responsible for zoonosis, we intended to verify the transmission of the *Rickettsiales* endosymbionts hosted by two species of ciliates to a metazoan model, the planarian *Dugesia japonica*. The ciliates were *Euplotes woodruffi* and *Paramecium multimicronucleatum*; the first hosts in the cytoplasm two different *Rickettsiales* endosymbionts, "*Candidatus (Ca)* Megaira polyxenophila" and "*Ca.* Bandiella woodruffii", in addition to the betaproteobacterium *Polynucleobacter necessarius*; the second hosts in the macronucleus the *Rickettsiales* endosymbiont "*Ca.* Trichorickettsia mobilis". Ciliate monoclonal mass cultures were set up to perform the following infection experiments: 1. Ciliate washing, concentrating, homogenizing, and addition to regular food for planarians. 2. Antibiotic-treated planarians feeding on ciliate-enriched food or regular food (as control), then washing and letting digest for 24, 48, and 72 h; 3. Comparative multidisciplinary investigation: molecular analyses, whole mount *in situ* hybridization, and TEM observation. Preliminary results are encouraging: we recovered endosymbionts up to 72 h after feeding, and identified them in planarians' intestine and digestive vacuoles in TEM material. Endosymbionts were frequently observed near vacuole edge, with vacuole membrane sometimes somehow interrupted. TEM results were compared with literature data where endosymbionts were demonstrated to escape vacuoles invading the cytoplasm. Financial support: PRA 2016, University of Pisa.

Effects of ecological restoration works on the benthic diversity of the Valli di Comacchio

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The Valli di Comacchio are the main chocked lagoon of the North Adriatic coast. The Valli are characterized by a hydraulic regime regulated by man, and by strong seasonal temperature and salinity excursions. These factors, including human disturbance, have led to a drastic selection of the biotic communities of both plants and animals. The Valli have always been home to anthropogenic activities, particularly fish farming, which led to hypereutrophication and frequent and intense blooms of phycocyanobacteria. These events lead to reduced functionality and were particularly negative for the benthic component. As part of the project LIFE09NATIT000110 (Natura 2000 in the Po Delta) aimed at improving the water circulation in the major basins (Magnavacca and Fossa Porto) of the Valli through recalibration of channels and construction of sandbanks, the macrobenthic community was studied for a two-year period (2013-2014). Sampling was carried out at four stations. The analysis of the benthic community showed low values of diversity. In 2013 were found 22 taxa, with two species numerically dominant, *Streblospio shrubsolii* and *Chironomus salinarius*: these species are typical of reduced, organically enriched sediments. In 2014, the taxa found were only 14. The ecological quality status, calculated using the biotic indicators required by law (DL260/10) was unsatisfactory. Further stations were sampled in the main basin (Valle Magnavacca) in summer 2014, to assess the effects of the drainage of an impounded channel (Gobbino) which connects the central area of the Valli with the sea. The benthic community was investigated immediately before and three months after the opening of the channel, and the effects were evident. Before the excavation, we found only 13 taxa, with *S. shrubsolii* numerically dominant. The opening of Gobbino resulted in an increase in the number of taxa (43) and different dominance relationships (*Neanthes succinea*, *Heteromastus filiformis*, Crustacea, etc.) within the macrobenthic community. The ecological quality status resulted satisfactory. Vivification given by Gobbino is vital to improving the ecological quality of the Valli di Comacchio, and any efforts should be made to keep the channel working. This statement is important to land manager and conservationists, since results from this study suggest that, at least from the benthic point of view, the protected Valli di Comacchio (IT40600002) are probably a sink habitat, while the adjacent marine habitat (the source) is not.

Effects of a penguin rookery on macrobenthic diversity in the Ross Sea (Antarctica)

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Adelie Cove (Ross Sea, Antarctica) is a small 70 m-deep V-shaped bay, covered in the innermost part by permanent fast ice. The sides of the cove are characterized by stony shores, while the rocky cliff at the northern cape of the cove is colonized by an Adelie penguin (*Pygoscelis adeliae*) rookery. Penguin rookeries are major sources of nitrogen and phosphorus input and may create marine hotspots of eutrophication generated from excreta that enrich surrounding areas through deposition processes and surface run-off. The effect of nutrient and organic loading from rookeries and its impacts on the coastal benthic fauna have not been quantified previously. Here we report the preliminary results of a study in which the macrobenthic community structure is investigated in the vicinity of the penguin rookery. Quantitative samples of benthos were collected during the austral summer 2014-2015. Three transects along a depth gradient were sampled.

Sediment texture differed among stations: the site closest to the rookery, showed the highest content of silt and clay and of organic matter (2.3%). The analysis of macrobenthos revealed great differences in composition and abundance among the stations. About 2,501 individuals belonging to 67 species were identified. The macrobenthic community was dominated by Polychaeta (44.1%), followed by Anthozoa (21%) and Malacostraca (20.3%).Multivariate analysis segregated station points into two main clusters according mainly to depth. Dissimilarity among stations was high: benthic communities differed in structure and composition along the spatial gradient but also along the depth gradient. Despite the relatively high biodiversity recorded, only a few species were numerically important. Analyses evidentiated that the differences among stations were mainly due to the local dominance of one or a few species, such as *Edwardsia meridionalis*, *Eudorella gracilior*, *Aphelochaeta marioni*, and *Leitoscoloplos mawsoni*, which was particularly abundant almost in all samples. In the Ross Sea coastal zones, it is reported that benthic community organization shows two contrasting structural patterns: biologically-controlled assemblages, with high diversity and structural and functional complexity, and oligospecific, poorly structured, physically-controlled assemblages, with species adapted to high levels of disturbances. From our preliminary results, benthic assemblages at Adelie Cove seem to be part of this latter type.

Is the size of wetland important to macrozoobenthic biodiversity?

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Wetlands are one of the most important ecosystems on earth and since 1976, they are protected by the RAMSAR Convention. They are complex, highly diverse, and constantly changing in relation to the variations of abiotic parameters (e.g hydrology, climate, nutrient fluxes etc). A hypothetical inversion in the concept of the theory of islands isolation and biogeography by MacArthur and Wilson (1967), could be comparable for the analysis of geographic isolation in wetlands. The aim of this work is to determine how important the size of a pond or lake is for the macrozoobenthic biodiversity. Freshwater invertebrates were sampled from 14 lakes that belong to Danube Delta and from 10 ponds from Po River Delta. The surface area of the sampling sites ranged between 34 sqm and 1445 ha. Correlation analysis between the surface area and the value of the diversity (Shannon-Winner Index) was performed. The results showed an opposite trend of the correlation in small ponds and large lakes when they analyzed seperately. The correlation was positive in small ponds, which suggests that small areas contain less biodiversity than large area ponds. On the other hand, large lakes showed a negative correlation with the macrozoobenthic diversity decreases with the increase of the surface area. This results may emphasize a threshold above which the diversity decreases.

Identification of migratory routes of European blackbird (*Turdus merula*) and song thrush (*Turdus philomelos*) in Lombardy from ring recoveries.

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Migration is a fundamental phase of the life cycle of many birds. Detailed knowledge of seasonal movements is important for understanding the ecology of migratory species, the dynamic of their populations, and for planning effective conservation actions for threatened species. In spite of the great interest of this topic, migration timing and routes are poorly known particularly for small-sized birds. Birds ringing data have proven helpful in studying bird migrations, but their potential is underused so far.

We used an automatized and repeatable procedure to identify and represent autumn migration directions of the European blackbird (*Turdus merula*) and the song thrush (*Turdus philomelos*), across Lombardy (northern Italy) based on ring recoveries provided by Istituto Superiore per la Ricerca Ambientale (ISPRA), Swiss Ornithological Institute and Max Planck Institut für Ornithologie.

For each bird captured at least twice during autumn, we identified a hypothetical move of the individual by linking together the places of its capture. We then divided Lombardy in cells and calculated the mean migration direction within each cell by averaging the angular directions of the moves crossing that cell. Finally, we simulated flyways by letting hypothetical birds move across Lombardy according to migration directions at each cell. Results show that both species move through Lombardy mainly in the prealpine zone close Maggiore, Lecco and Garda Lakes. Alpine passages are important for populations breeding in Switzerland and Austria. Valleys are used as preferential flyways for crossing Alps. Routes cross in the prealpine area, where short northward movements also occur.

This procedure can be easily replicated for several small sized migratory species for which large datasets of ringing data are available. The maps of migration directions produced by this procedure offer a clear representation of the expected movements of birds during migratory season using exclusively information about their spatial distribution. The results thus obtained may have important application for the conservation of species of hunting interest.

A comparative morphological-molecular study of two free-living plagiopylids and their archaeal endosymbionts from anthropized areas of Kolleru Lake, India

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Members of the family Plagiopylidae are ubiquitous and most commonly live in various anaerobic environments, either as endocommensals in the intestine of higher invertebrates such as Echinodermata, or as free-living anaerobic ciliates. They live in the saprobic part of water column of freshwater systems or even in brackish and seawater environments. Sampling was carried out in different areas of Kolleru Lake, the biggest water body in Andhra Pradesh state. An effective culture method was developed for growing plagiopylids ciliates within original samples, which were subsequentially employed for complete morphological and molecular characterisation. We focused our attention on two populations of *Plagiopyla*, namely population TBS19 (species1) and population MMP17 (species2), representing two different morphotypes. The main objective of this study was to describe these two populations using traditionally employed and newly introduced morphological features, molecular data, and symbiont characterization. For the morphological descriptions, living cells were observed using light microscope and differential interference contrast (DIC) microscopy, the total infraciliature and nuclear apparatus were revealed by staining with silver impregnation and feulgen reaction, respectively. SEM study was performed to reveal the structure of somatic and buccal infraciliature. From morphometric data we obtained measurements for both populations: species1 measured about 66.8x29.3 µm, whereas species2 about 92.8x62.5 µm. Species2 exhibited a peculiar sac-like deep buccal furrow, more elongated in comparison to species1. The nuclear apparatus and extrusomes of species2 closely resembled *Plagiopyla nasuta* structures, having large ovoidal macronucleus single compact-type micronuclues (17.8x16.0 µm) and straight extrusomes (4.0 µm). Instead, species1 exhibited 2-4 vesicular-type micronuclei and curved extrusomes. The cytoproct showed 3 dense rows of cilia on the right side and one row on its left side in species1. In species2 the cytoproct exhibited 3 strict rows of dense ciliature on the right side but none on the left. The contractile vacuole exhibited 3-5 pores in species1 and only 2 pores in species2. Morphological data sets were complemented by molecular data obtained by 18S rRNA gene sequencing and phylogenetical analysis. Archaea endosymbionts were observed in both species and are currently under investigation. Morphological features of investigated populations are presently compared with descriptions available in past literature. Molecular data already suggest that we are dealing either with new species or with species that has been characterized only morphologically.

***Ago* genes in two living fossil species, *Protopterus annectens* and *Latimeria menadoensis*: evolutionary considerations.**

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The argonaute subfamily proteins play many roles in epigenetic programming, genome rearrangement, mRNA breakdown, and inhibition of translation. We report the transcript sets of *Ago* genes in the West African lungfish *Protopterus annectens* and in the Indonesian coelacanth *Latimeria menadoensis*, two key species in the evolutionary lineage leading to tetrapods. The phylogenetic analysis provides new insights into the evolutionary history of this subfamily in vertebrates. These data are supported by the analysis of microsynteny performed in the major lineages. Expression assessment of *Ago* genes and genes coding for proteins involved in small RNA biogenesis evidences a low activity while the analysis of dN/dS ratio suggests that genes involved in small RNA processing evolve more rapidly than *Ago* genes.

What is the matter with these specimens? Unusual shell erosion of the *Corbicula fluminea* (Veneroida: Corbiculidae) (Müller, 1774) in two small streams of the sub-lacustrine Ticino River basin

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Corbicula fluminea is an exotic bivalve, native to southern and eastern Asia, considered to be among the 100 worst invasive species in Europe due to its economic and ecological impacts in freshwater ecosystems. In old bivalves, when the periostracum has been slightly eroded, the white calcareous internal layers of the shell become visible, especially near the umbos; in these cases, erosion can also lead to changes in shell thickness.

In 2015, two distinct populations of *C. fluminea* with apparently abnormal external shell erosion were found in two semi-natural small streams of the complex irrigation system of the sub-lacustrine Ticino River.

In order to describe shell damage, morphological variability and physical condition, we measured the shell length, the thickness of the right valve, the shell ash free dry weight and the dry weight of the flesh of each bivalve in the laboratory. Subsequently, so as to highlight any morphological differences, we compared the morphological characteristics of these two populations with previous data of a population collected in a third stream in the same area, which did not show any shell damage. Our results confirmed that there was shell erosion and reduction of its thickness in both populations.

We hypothesized various explanations for the cause of this erosion: our first idea was related to the pH of the water, but data did not show any differences between streams (DF=2; F=1.44; p: 0.293). Secondly, we hypothesized that there may have been differences in the level of calcium or the total hardness of the water, but this was not the case either (Ca: DF=2; F=1.65; p: 0.250. CaCo3: DF=2; F=1.67; p: 0.248). Our third suggestion was that the erosion may have been caused by predators, but only a few animals actively prey on these bivalves (e.g. herons or rats) and the marks they leave on the shells are completely different from the ones on our specimens. Lastly, we hypothesized that the bivalves may have been affected by a disease or parasitic infestation that had weakened their shells or prevented them from self-repairing. However, it would be very unlikely for all the specimens to share the same disease or infestation in two different areas at the same time.

In conclusion, a valid explanation for the morphological alteration that our study revealed is yet to be found, leaving the following question unanswered: what is the matter with these specimens?

Hindwings and elytra: shape and size evolution in dung beetles.

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Most of the dung beetles are excellent fliers. This skill enables long dispersal movements, and makes effective the search for mates and ephemeral and/or unpredictable food sources. Besides, the ability to fly may be lost as a consequence of adaptation processes to peculiar habitat and niche conditions. We studied Scarabaeidae species that recover hindwings below the elytra at rest (27 species of two subtribes: 11 Drepanocerina and 16 Onthophagina). In these dung beetles the hindwing folding mechanism is basically the same and is articulated in at least five major steps. The geometric morphometrics approach was applied to examine the overall shape variation of hindwings (landmarks method) and right elytra (semilandmarks method). The relative warps analysis highlighted that Drepanocerina and Onthophagina displayed two distinct morphological hindwing patterns (54.35 % the overall shape variation explained by RWs 1 and 2), but with low degree of variation, within a framework of relative hindwing shape consistency probably due to functional constrains. The first two RWs of the right elytra represent instead more than the 88% of the overall shape variation, evidencing thus different pattern in the two subtribes. The analysis of shape covariation by partial least-squares analysis gave significant results (r= 0.84, cross set analysis = 95% and permutation tests = 0.10%), being the two structures strictly correlated. Furthermore, the two subtribes were clearly separated in the plot of the first vectors correlation. Analyses of regression scores on log centroid size (as a measure of size) evidenced that hindwings allometric trajectories of the two subtribes are similar (they are roughly parallel), whereas elytra trajectories are very different (they diverge at increasing log centroid size values). This suggests that the evolution of elytra shape-size relationships of the two groups diverge, whereas the evolution of hindwings shape-size relationships do not, maybe due to functional constraints. To visualize in detail the evolutionary history of phylomorphospace changes and evaluate the diversification of related Onthophagina taxa, the hindwing and elytron shapes were mapped onto a molecular phylogenetic tree (COI sequences). This study suggests that the puzzle of Scarabaeidae evolutionary history can be powerfully elucidated with researches of hindwings shape and size, reflecting or not the evolution of the elytra morphology and the environmental scenarios in which the dung beetles have differentiated.

Ecological characterization of the Clitunno River (Central Italy): a lowland stream fed by limnocrenic spring

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The Clitunno River (Umbria, Tiber River basin) is a small river of great historical, landscaping and environmental value. The stream originates from a lowland spring system, characterized by a regular flow which is not able of removing the considerable deposits of fine sediments accumulated over the decades due to input of civil and productive discharges. The alteration condition has worsened in 2006 following the spill of oily substances and solvents due to a serious accident of an oil mill. Since 2011, the river is subject to hydraulic and environmental recovery by "Consorzio di Bonificazione Umbra" based mainly on the partial removal of sediment up to "Casco dell'Acqua" locality (floodgate site, 10 km from the spring). The aim of the study is an environmental quality assessment of the medium-high reach of Clitunno River. Nine monitoring stations were identified between the spring zone (St.1) and "Casco dell'Acqua" locality (St.9). In June 2011 and 2014, water and sediment samples for ionic and heavy metals analyses were collected, respectively. In March, June and September 2014 macroinvertebrate samples were collected, a total of about 49,000 specimens, belonging to 64 taxa. The limnocrenic spring affects the waterway until the St.3 (high levels of calcium, sulphates, magnesium and conductivity) with respect to the remaining downstream stations characterized by high values of sodium, nitrates, pH, water temperature and dissolved oxygen. The organic pollution is manifested in the downstream sites (St.7-St.9), while the heavy metal contamination is evident in the reach river near a small industrial area (St.5). The peculiar ecological condition of the upstream reach, i.e. minor amount of oxygen and high storage level of organic material of natural origin, showed an habitat similar to that of the downstream reach. However, the approach based on biological and ecological traits of benthic freshwater macroinvertebrates has allowed to differentiate well the natural condition status of the upstream reach from the pollution condition of the downstream reach of the Clitunno River. The study highlighted the peculiarity of Clitunno River biotope, practically unique in Central Italy, contributing to the knowledge of this particular freshwater ecosystem and therefore to its protection and conservation.

The distribution of the non-native *Gammarus roeselii* Gervais 1835 and native *Echinogammarus stammeri* (Karaman 1931) in the irrigation system of the sub-lacustrine Ticino River (Lombardy, Northern Italy)

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One of the most successful invaders in freshwater ecosystems is the Balkanian amphipod *Gammarus roeselii*, which has a long invasion history in Eastern and Central Europe, where it is now considered as a well-established exotic species.

Up to 2005, in Italy, its presence had only been reported for the Sile River basin (North-Eastern part of Italy); subsequently, in 2014, it was discovered by our research team in the Ticino River basin (Po River floodplain, Northern Italy), despite the lack of a direct connection between these two basins.

In this paper, we update the distribution of *G. roeselii* in the southern part of the sub-lacustrine Ticino River basin, paying particular attention to the small semi-natural streams of its complex irrigation system; furthermore, we compare the density of the non-native amphipod with the native species *Echinogammarus stammeri*.

With this aim, we selected 13 semi-natural main tributaries of the southern part of the Ticino River (12 on the right bank and 1 on the left bank) and, from June to September 2015, we collected three random sub-samples in each stream, using a modified net (950μm mesh) with a square frame (22×23 cm, which corresponds to an area of 0.0506 m^2). Then we identified and counted all the amphipod specimens in the laboratory.

The results show that the non-native species *G. roeselii* was present in ten out of thirteen streams, with the density varying from 6 ind/m^2 to 1329 ind/m^2. On the contrary, the presence of the native species *E. stammeri* was recorded in all thirteen streams, with density varying from 6 ind/m^2 to 2197 ind/m^2. During the identification process of the gammarid specimens, we also found a few adults of *G. roeselii* which did not have any ommatidia structures.

The high density of *G. roeselii* found in almost all the investigated streams indicates that the non-native amphipod is well established in the sub-lacustrine Ticino River basin, but it does not seem a real threat to the native species, which is still very abundant.

In conclusion, we can affirm that these two gammarid species can live in sympatry, sharing similar resources and habitats.

EGGSPOSURE: A PROMISING APPROACH TO ASSESS THE TOXICITY OF ENVIRONMENTAL POLLUTANTS

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Several studies have shown the presence of diverse pollutants in terrestrial ecosystems, but to date there is a dearth of information regarding their adverse effects on organisms. This is particularly true for the so-called emerging pollutants, which are new chemicals with no regulatory status, whose environmental and health effects are unknown. In terrestrial ecosystems, the eggs of birds have been used to monitor the contamination levels by different xenobiotics, but their usefulness as a tool to investigate their toxicity has been largely neglected. Thus, the present study was aimed at assessing the suitability of an *in ovo* manipulation of the levels of a focal emerging pollutant to explore its potential toxicity. In a within-clutch experimental design, we injected two environmentally relevant concentrations (100 ng/g egg weight and 200 ng/g egg weight) of perfluorooctane sulfonate (PFOS) into the eggs of the yellow-legged gull (*Larus michahellis*). We investigated the effects of PFOS treatment, laying order and sex on phenotypic traits of embryos, including morphometric and biochemical endpoints. Specifically, we assessed changes in embryo body mass and tarsus length, as well as in liver and brain mass. Moreover, imbalances of embryo oxidative status were assessed by measuring Total Antioxidant Capacity (TAC) and Total Oxidant Status (TOS) in both liver and brain, while protein carbonylation and DNA fragmentation were considered as oxidative and genetic damage endpoints, respectively. Our findings showed that both the injected PFOS concentrations did not significantly alter the investigated phenotypic traits, independently of laying order and sex. These results suggested that environmentally relevant concentrations of this emerging pollutant did not negatively affect the early development and oxidative status of embryos. In addition, we demonstrate that the yellow-legged gull can be considered a reliable model organism for terrestrial ecotoxicology and its eggs are an useful tool to monitor the toxicity of emerging pollutants.

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1

DROUGHTS INCREASE TURNOVER AND NICHE WIDTH OF DIATOM BENTHIC COMMUNITIES IN MEDITERRANEAN STREAMS

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Mediterranean streams are characterized by intermittent flow regime, with droughts in summer followed by floods in the colder season. In particular, droughts are currently exacerbated by human pressure and are expected to increase in a climate change scenario. With the creation of lentic habitats (i.e. lentification process) and dry stretches in the riverbeds (i.e. fragmentation process) droughts represent a strong selective pressure on biotic communities. Species not adapted to water scarcity may thus be filtered by both species replacement (i.e. turnover) and species gain or loss (i.e. nestedness). In this work we aim to test whether droughts in Mediterranean streams filter species in biotic communities and which process dominates, focusing on benthic diatoms as model organisms. Secondly, we also tested if niche assembly rules are the major force or if assembly rules are governed by dispersal-driven mechanisms behind the selective pressure. This work was conducted in the Ligurian Alps (NW-Italy) on five oligotrophic streams, similar in terms of physico-chemical parameters. Data were collected at least monthly from April to October 2014 in: 1) impacted sites, where the water scarcity was exacerbated by human pressure; 2) control sites. For each sampling site, we collected six benthic diatom samples from different microhabitats. We examined the temporal order of diatom communities by means of the nestedness analysis to check if the communities during droughts were a subset of communities collected during moderate flow. We further related the ecological niche width of species and the number of motile species to droughts to unravel if communities colonizing stretches during drought were composed of higher number of motile and/or tolerant taxa. Our results showed a lowering nestedness during drought, since the number of idiosyncratic species increased. Idiosyncratic species also resulted as more tolerant than nested species, while no differences were encountered in terms of number of motile taxa. According to our results, species replacement due to niche assembly rules seemed to be the dominating process in diatom benthic communities during drought. These results indicate that future climate change may drive the loss of specialist species, being replaced by more tolerant taxa.

They are among us: the European invasion of the alien brown marmorated stinkbugs *Halyomorpha halys* (Heteroptera, Pentatomidae).

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The brown marmorated stinkbug (BMSB), *Halyomorpha halys* is an invasive alien species native to eastern Asia. Its presence outside the original area of distribution has been recorded for the first time in North America (Canada and U.S.A.) and, more recently, in Europe (Switzerland, France, Hungary, Romania, Austria, Serbia and Italy), where *H. halys* is spreading rapidly. Other than being a household pest all over its introduced range, this stinkbug is causing great economic losses in the U.S.A and Italian orchards/crops due to its highly polyphagous nature and bivoltinism. Tracing back the pattern of introduction and monitoring the spread of BMSB in the European territory will be useful to implement better pest control strategies. The present study aimed to identify the potential pathways of entry of *H. halys* in Europe by detecting the genetic diversity of specimens collected all over Italy, and in Switzerland, Romania and Greece. The analyses of 1,175 bp of mitochondrial DNA cytochrome c oxidase I and II genes (*cox1* and *cox2*) of over 200 specimens led to the identification of 12 haplotypes never observed before (10 for *cox1* and 2 for *cox2*). Present data indicate a higher haplotype diversity of European specimens compared with the American ones; instead, the diversity is lower with regard to the Asian samples, except for the *cox2* marker. A clear-cut difference in haplotype distribution was found between North and South Europe: Switzerland and France share a similar haplotype pattern, whereas Italian, Hungarian, Romanian and Greek samples are more similar, with the Italian and Greek specimens showing the higher genetic diversity. In Italy, genetic diversity for both markers is higher in Piedmont, Lombardy and Veneto, while in the remaining regions of Northern Italy in which the species is spread, it is fairly low. Haplotype similarity with both Chinese and Korean samples led to hypothesize that the introduction of *H. halys* in Europe, and in Italy in particular, has occurred by means of multiple events from Asia and that the BMSB is currently expanding its range in the European continent.

An integrated view on the regeneration of the different skeletal elements in the arm of *Amphiura filiformis*

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Echinoderms are marine invertebrates that possess an elaborate calcite skeleton presenting a great diversity in morphology among classes. The arm skeleton of ophiuroids, for example, is jointed and metameric, each segment containing one aboral, one oral and two lateral shields, a variable number of spines, and a central "vertebra". Representatives of all classes can efficiently regenerate body parts after trauma; however, the skeleton has a crucial role in regaining protection and functionality.

In the present study *Amphiura filiformis* was used as experimental model to study at structural level the skeletal elements of the arm, their anatomy, and their development during regeneration.

Light and electron (TEM and SEM) microscopy approaches together with gene expression pattern analyses were used to assess this subject.

SEM and histological analyses on non-regenerating arms allowed to outline the anatomy of the skeletal elements in relation to the other organs. Microscopy results on regenerating arms instead showed that sclerocytes (*i.e.* skeletogenic cells) and developing spicules are mostly found in specific regions of the dermis and in two central rows on the aboral side of the radial water canal (RWC). These cells often presented cytoplasmic pockets containing collagen fibrils, thus suggesting a possible double function (*i.e.* collagen and calcite deposition).

The first sclerocytes were detected at stage 2 in the dermis of the future shield regions, in agreement with [1], whereas the spicules of the "vertebral" region were visible only at later stages in the regeneration process. Vertebral primordia seem to be spatially and temporally linked to the appearance of the tube feet on both sides of the RWC and only at late stages fuse together to form a single vertebra. The RWC-related development of vertebra primordia supports the extraxial–axial theory (EAT) proposed by [2].

Preliminary results of *in situ* hybridisation on regenerating arms of genes known to be specifically expressed in axial and extra-axial components seem also consistent with the EAT and the ocular plate rule described by [2] in other echinoderms.

Ultimately further ultrastructural and molecular investigations are needed to better understand how skeletogenic precursor cells can lead to such complex and diverse structures and to shed light on their origin.

[1] Czarkwiani et al 2016 *Front Zool* 13(18)
[2] Mooi et al 2005 *Evol Dev* 7(6): 542-555

Co-creating a decision support framework to ensure sustainable fish production in Europe under climate change (CLIMEFISH).

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ClimeFish is an international project, submitted by a consortium of 21 partners, and recently funded by EU within the context of the Horizon 2020 (BG2 15). The overall goal is to help ensuring that the increase in seafood production will take place in areas where there is a potential for sustainable growth of aquaculture and fishery industries, given the expected developments in climate, thus contributing to robust employment and sustainable development of rural and coastal communities. The underlying biological models are based on single species distribution and production, as well as multispecies interactions. Forecasting models will provide production scenarios that will serve as input to socio-economic analysis aimed at identifying risks and opportunities. . Strategies to mitigate risk and utilize opportunities will be identified in co-creation with stakeholders, and will serve to strengthen the scientific advice, to improve long term production planning and the policy making process. ClimeFish will address 3 production sectors (marine and freshwater fishery and marine aquaculture) through 16 case studies widely spread around Europe, involving 25 species, and study the predicted effects of 3 pre-defined climate scenarios. For 7 of these cases, ClimeFish will develop specific management plans (MPs) coherent with the ecosystem approach that will allow regulators, fishers and aquaculture operators to anticipate, prepare and adapt to climate change while minimizing economic losses and social consequences. A guideline for how to make climate enabled MPs will be produced, and published as a low-level, voluntary European standard after a consensus-based open consultation process. As a container for the models, scenarios and MPs ClimeFish will develop the ClimeFish Decision Support Framework (DSF) which also contains the ClimeFish Decision Support System (DSS).

Application of SMCE for sustainable development of shellfish farming along the Adriatic coast

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Available space for aquaculture activities becoming increasingly limited and a proper design of Allocated Zones for Aquaculture (AZAs) is necessary in order to avoid conflict, to promote a sustainable mariculture avoiding environmental degradation and negative interaction with other marine activities. The designation of new AZA should be considered in a context of Ecosystem Approach for Aquaculture, promoting sustainable development, equity and resilience of the social-ecological system in order to comply with the MSFD.

This work focuses on the selection of new areas to be allocated to shellfish farming along the coast of the Emilia-Romagna Italian region (Northern Adriatic Sea). Shellfish site suitability was assessed by means of a methodology based on a Spatial Multi-Criteria Evaluation (SMCE), which provided the framework to combine mathematical models and operational oceanography products. Intermediate level criteria considered were growth optimization, environmental interactions and socio-economic evaluation. Seven different scenarios of development of shellfish aquaculture industry were explored. The potential development of this activity in the study area is high, in fact the space with Suitability Index > 0.5 increases when prioritizing the optimal growth condition criteria. In the meantime, the socio-economic evaluation resulted the most restrictive intermediate level criteria.

Results showed that the coastal area comprised within 0 and 3 nm is highly suitable for mussel farming, while the area comprised between 3 and 12 nm is divided between a highly suitable northern part, and a less suitable southern one. The suitable area for shellfish aquaculture would not decrease dramatically with the introduction of a new farmed species (Pacific oyster).

The work shown the capabilities of Spatial Multi-Criteria Evaluation in aquaculture space allocation and the importance of integrate resources provided by remote sensing and operational oceanography by means of mathematical models. Results can support a science-based design of Allocated Zones for Aquaculture (AZAs) with an Ecosystem Approach for Aquaculture, promoting sustainable aquaculture in the Mediterranean Sea, where the space for these activities is becoming increasingly limited.

Spatio-temporal variations in virus and prokaryote abundance in the Antarctic Sea Ice

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Global warming is influencing the sea ice formation-melting dynamic cycle in polar seas. This is progressively fostering seawater freshening, as well as modifying oceanic circulation and mixing regimes. In the Antarctic, the sea ice formation-melting process represents one of the most impressive phenomena on our planet, and is a key engine of Earth climate. Therefore, the comprehension of how sea-ice processes influence marine biogeochemistry is essential to accurately predict past, present, and future climate change responses of marine ecosystems in both the polar and global oceans (Steiner et al 2016). During the Austral spring, the bottom and platelet layers of annual sea ice host a huge biomass of microorganisms (Thomas & Dieckmann 2002), which are known to interact each other and provide the energetic basis for the functioning of the under-ice ecosystem (Guglielmo et al. 2001). Marine viruses interact actively with the present climate change and are a key biotic component that is able to influence the oceans' feedback on climate change (Danovaro et al., 2011). Despite this, knowledge of virus-prokaryote interactions in the Antarctic sea ice are relatively still limited. During austral spring, we investigated spatial and temporal variations in virus and prokaryote abundance in both the "brown" and platelet sea ice of Terra Nova Bay (Ross Sea, Antarctica) and related them with the availability of organic substrates and light regimes. We show that: 1) light intensity and the availability of organic nitrogen influence either virus or prokaryote abundance and their relationships; 2) short-term variations of virus and prokaryote abundance, though with different patterns in brown and platelet ice, are related with the development of sympagic algae and the thickness of the sea ice. These results suggest that any major change in the thickness of Antarctic sea ice, as expected with global warming, not only could have consequences on the development of sympagic algal biomass but could also influence the sympagic microbial and viral loops.

TOWARDS AN ECO-FRIENDLY MANAGEMENT OF SEDIMENT FLUSHING FROM ALPINE RESERVOIRS

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Sustained long-term preservation of reservoir storage threatened by sedimentation is crucial to maintain the current hydropower production and other water uses in the Alps, where many reservoirs are more than fifty years old. To date, sediment flushing represents a feasible technique to recover the storage capacity of small-sized to medium-sized reservoirs. Nevertheless, its application has risen several concerns due to its downstream environmental impacts that are only marginally investigated in literature. This work reports on the main evidences provided by the biomonitoring of ten sediment flushing operations carried out between 2006 and 2012 from four hydropower reservoirs in the Lake Como catchment. For all of the operations, suspended sediment concentration (SSC) thresholds were established using a dose-response model on fish to limit the flushing impacts that were quantified through monitoring of benthic macroinvertebrates and brown trout (*Salmo trutta trutta*). Benthic communities were severely impaired by all of the monitored flushing operations in spite of their differences (e.g. duration, season, SSC, flow, evacuated sediment mass). A considerable density reduction and a change in community composition were observed immediately after flushing. However, benthic assemblages usually recovered to pre-flushing conditions within a year if the riverbed alteration due to sediment deposition was low. Trout density also decreased after almost all of the monitored flushing operations, with a selective pressure on juveniles. The results on trout populations demonstrated that the dose-response model on fish applied during the planning phase can be considered adequate for predicting the order of magnitude of the flushing impacts. These indications may support the development of ecosystem-based management criteria that could be considered, in addition to economical and technical criteria, for evaluating the feasibility of sediment flushing operations in the Alpine context.

Anthropogenic impact on microbial assemblages in the Lagoon of Venice revealed by functional metagenomics

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Coastal lagoons are highly productive ecosystems characterized by large chemical and physical gradients, that make them highly unstable and subjected to fluctuating conditions. Lagoons are extremely vulnerable to a variety of anthropogenic disturbances, and this holds especially true for those lagoons located in the Mediterranean Sea, which are increasingly impacted by the human population through a range of agricultural, industrial, mining and touristic activities. Nonetheless, these ecosystems still remain poorly studied in term of species and functional diversity of bacterioplankton assemblages, which are key ecological players and drive the biogeochemical cycling within lagoons. In this study, we collected water samples across the Lagoon of Venice, one of the largest Mediterranean lagoons, characterized by a high anthropogenic impact deriving from a multitude of stressors, that include large industrial plants, touristic and commercial harbors, agricultural and municipal wastes, and freshwater inputs. Samples from five stations, selected along a gradient of anthropogenic impact from the inner, more anthropized part of the lagoon to the open sea, were analyzed using shotgun sequencing to describe the microbial taxonomic and functional diversity. Functional profiles of the five metagenomes were explored to uncover and quantify the anthropogenic impact, by searching genes related to the following categories: i) chemical pollution (e.g., resistance to metals and hydrocarbons), ii) eutrophication (e.g., nitrogen and phosphorous metabolism) and iii) antibiotic resistance. Biodiversity of the assemblages revealed a complex microbial community with dominance of Proteobacteria, Actinobacteria, and Bacteroidetes. Functional reconstruction of the metagenomes revealed the presence of various metal and antibiotic resistance genes, and showed different community fingerprints according to the type of pollution, indicating that microbial communities responded to anthropogenic pressure. Our results expand the knowledge on the microbial functional and taxonomic composition in coastal lagoons under anthropogenic pressure, and highlights the power of metagenomics as a tool to measure anthropogenic impact in coastal lagoons.

Gilthead seabream juveniles migration dynamic in the Venice lagoon

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The role of transitional water ecosystems as nursery areas for many marine species has already been emphasized. A full description and understanding of the habitat use of juvenile marine migrant species within the estuarine systems is a crucial step in identifying, preserving and managing the functioning of transitional ecosystems. In this study, we focus on the gilthead seabream (*Sparus aurata*) in the Venice lagoon with the aim of describing the fry migration dynamic. Data on juvenile *S. aurata* densities in different shallow areas were obtained from several monitoring programmes conducted between 2004 and 2016. Sampling was carried out with a beach-seine net fortnightly between March and June in 2004, 2005 and 2008, and in two sampling occasion during spring from 2014 to 2016. In each station the main chemical/physical parameters of the water column (temperature, salinity, dissolved oxygen, turbidity), the presence of seaweed and seagrass and the characteristics of the substrate (percentage of sand and organic matter) were recorded. Negative binomial Generalized Linear Model (GLM) were used to test temporal changes in density and presence/absence binomial GLM were used to analyse the effects of environmental parameters on juveniles distribution. Results suggest that small (<20 mm S.L. - Standard Length) gilthead seabream juveniles continue to enter the lagoon from the sea until late April, while since mid-April, the fish show a quick size increase within the lagoon, due to the optimal growth conditions. The smaller gilthead seabream seem to prefer clear, warm and well oxygenated waters in areas with sandy bottom, while specimens with a standard length between 20 and 35 mm are more likely found in areas with a smaller share of sand in the bottom sediment. The larger seabreams (35-50 mm SL) are associated to areas with a higher organic matter content in the sediment. Relative high densities have been recorded in the lagoon, with a positive trend over time, suggesting the presence of demographic changes at population level, involving also the stages living at the sea. These results suggest that the management of marine estuary-related species, like *S. aurata*, should take into account of their habitat use in the different stages of their life.

GEOGRAPHIC VARIATION AND HABITAT PREFERENCE OF THE ITALIAN ENDEMIC BUTTERFLY MELANARGIA ARGE.

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Melanargia arge is an endemic Italian species occurring in fragmented habitats, ranging from Tuscany to Calabria and Apulia. As other endemic species requiring particular attention, *M. arge* is included in the Habitats Directive (92/43/EEC) Annex II and Annex IV.

Our study aims at elucidating the relationship between patterns of geographic distribution and genetic variation at the species level, and to identify the habitat of this species, as required by the Habitats Directive.

Using a phylogeographic approach, we have reconstructed the history of *M. arge* across the Mediterranean Basin, thus investigating its recent biogeographical past. We have used the bayesian inference to clarify the historical and present demographic trends as well as to estimate the time of the last expansion.

We used both a Species Distribution Model (SDM) approach and a fine scale, approach overlaying butterfly occurrence data and vegetation layers. The first was aimed at understanding the present, past and future potential distribution for this species, while the latter to determine the dominant vegetation systems in the localities of the presence of *Melanargia arge* in comparison with the composition of habitats over the entire Lazio region.

M. arge depends on the arid grasslands: this habitat is experiencing a strong reduction due to the abandonment of the rural areas, with deep changes in land use. The results of our study offer a genetic framework for planning future conservation strategy for *M. arge.*

GENETICS OF LOCAL ADAPTATION IN ATLANTIC BLUEFIN TUNA FROM THE MEDITERRANEAN SEA.

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The Atlantic Bluefin tuna (ABFT, *Thunnus thynnus*), one of the largest top-predator fish inhabiting the pelagic ecosystems of the North Atlantic Ocean and Mediterranean Sea, has been extensively overexploited in recent decades. However, in the Mediterranean Sea, the mixing rates between the eastern, central and western basins have not yet been resolved. Both electronic tagging, otolith and genetic markers cannot still depict e clear scenario of tuna movements and structuring, essential requirement for a proper management of ABFT fisheries. Here we used Expressed Sequence Tag-linked (EST-linked) microsatellites to explore the patterns of adaptive evolution of *T. thynnus* population and of its population dynamics in the Mediterranean Sea. For this purpose 16 EST-linked microsatellites were genotyped in 177 tuna individuals from the Mediterranean and several methods were used to explore population genetic structuring and estimate/detect signals of local adaptation. Bayesian clustering results indicated the presence of a single cluster, corroborated also by the Correspondence Analysis and pairwise F_{STs}. Similarly the two methods, used for the detection of F_{ST} outliers, did not reveal any pattern suggesting the presence of selective pressure. Our results advise that the low level of polymorphism detected in EST-SSR loci used in this study could be ascribed to the presence of relatively conserved regions flanking these microsatellites. These genomic regions are probably not involved in physiological responses to local adaptation and we were able to rule out action of divergent or balancing selection on EST-SSR polymorphism.

Ecotoxicological evaluation of a digestate for an agricultural use: effects on reproduction of *Folsomia candida* (Collembola: Hexapoda).

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The agricultural industry plays an important role in waste production with high amounts of CO_2 emitted. The organic fractions of agricultural and industrial wastes and residues are considered as possible renewable energy resources, and their potential can be exploited through an anaerobic digestion process with the production of methane-rich biogas and residual organic material, commonly called digestate, that could be used in agriculture as soil improver. To evaluate the effects of digestate on soil fauna and to improve the data obtained with the conventional chemical analysis, a standardized ecotoxicological test can be used. The International Organization for Standardization (ISO) has published 11267: 2014 guidelines that describe a standardized test system based on the determination of effects of soil contaminants on *Folsomia candida* Willem (Collembola). The ISO 11267 has reproduction as endpoint. The disadvantage of the test is that reproduction cannot be observed directly and effects on oogenesis cannot be separated from effects on embryonic development and/or on post-embryonic development. The aim of present study was to evaluate, according to ISO guideline, the effects of a digestate obtained from the anaerobic digestion of grape seed, plum stone and biological sludge, on reproduction of *F. candida* and in particular to highlight what traits of reproduction are sensitive to the addition of the digestate to soil. Firstly, a study to evaluate number of oviposition, hatching time, length increase of juveniles of *F. candida* during 28 days, corresponding to the duration of the toxicity test ISO 11267, was carried out and the obtained data were used to identify the size-age classes corresponding to the juveniles of the different oviposition made within 28 days of the test. The digestate was tested at four different concentrations added to standard artificial soil (OECD, 1984). We detected that the digestate has negative effects on reproduction of *F. candida* evident also at lower concentrations. These effects can be mainly attributed to the pH increase of experimental soils, caused by digestate added in lower concentrations, while, for the higher concentration, it is possible to assume a combined effect of pH and other digestate characteristics. Thanks to the measurement of all juveniles and their attribution to age size classes built previously, it was possible to attribute the adverse effects of the digestate on reproduction to effects on oogenesis.

Comparative embryotoxicity of Glyphosate-based herbicide and Glyphosate active ingredient on *Xenopus laevis*.

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Glyphosate (G) is an Active Ingredient (AI) largely employed in broad-spectrum herbicide commercial formulations and is among the most used in agriculture, domestic area and aquatic weed control worldwide. Moreover G-based herbicide growing market is due to the corresponding increase of G-tolerant transgenic crop cultivations in several countries.

In recent years a number of scientific studies have raised concerns about G safety since different adverse effects have been denounced in non-target organisms. Recently, ISPRA report (May 2016) evidenced measurable quantities of G in surface and ground waters of Lombardy region, highlighting the potential risk for human health and aquatic organisms. Amphibians are good indicators for aquatic pollution and represent indirect target of agrochemical exposure, which has been pointed out as one of the main factors involved in the worldwide phenomenon so-called *Amphibian decline* during last decade. In fact, a huge number of Amphibian species belong to the IUCN Red List.

In light of this, we focused our attention on the effects of Roundup Power 2.0® (RP) and G as AI on the embryonic development of *Xenopus laevis* using Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX). RP is a next-generation formulation characterized by potassium salt of G and etheralkylamine ethoxylate as surfactant that improve G penetration through plant surface to the site of action. Embryos were exposed to increasing concentrations of RP (1-50mg/L) or G (7.5-50mg/L) from midblastula to tadpole stage.

Our results point out that RP causes lethality at high concentrations (96 hpf LC50 24,78 mg/L), while sub-lethal doses are enough to induce dose-dependent malformations (96 hpf EC50 7,28 mg/L) in larvae, such as oedema, modified eye shape, craniofacial defects and abnormal gut coiling. Since the calculated Teratogenic Index is higher than 3, according to ASTM guide RP is classified as highly teratogen. On the contrary, G is not lethal at the tested concentrations and only at 50mg/L shows a malformation rate different from control. To better characterize RP- and G-induced malformations, histological and ultrastructural analysis were performed.

Since the G penetration through the cell membrane is facilitated by surfactants present in RP, the limited G embryotoxicity in *Xenopus* larvae could be explained by its low bio-availability. For this reason to ascribe the toxicity to each component of RP, it will be necessary to investigate the toxicity of adjuvant.

Distribution and diet of a recently-established population of *Lutra lutra* in the Valley of the River Ticino.

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During the 20th century, the European otter (*Lutra lutra*) has dramatically declined in central and southern Europe because of hunting, destruction of riparian habitat and chemical pollution. In Italy, few population have survived in the most remote areas of the southern Apennines, where recent studies have recorded an unexpected recovery. In the valley of the River Ticino, a pair of otters was reintroduced in 1997 from a breeding centre (Cameri) located in Piedmont. A further pair possibly escaped before the end of the century. Additionally, during floods in 1991 and 1993 respectively, a pair and a cub and a pair with two sub-adults escaped from their breeding enclosure "La Fagiana" on the Lombardy side of the river. In spring 2016 an otter survey was carried out with the aim of drawing a picture of the current distribution of the species in the area. By surveying 600 m long transects, otter spraints were found in 8 different stations, between Cameri and Parasacco towns, corresponding to about a 35 km section of the river. Sprainting sites were mainly found inside the weave of meanders, on streams, canals and oxbow lakes, rather than on the main course of the river. All collected otter spraints were stored in silver paper, labelled and frozen until diet analysis. Each spraint was soaked for 12 hours in a solution of hydrogen peroxide and then placed into sieves with 0.5 mm wide meshes, and washed by a water jet. Fish remains were identified from their vertebrae, jawbones and scales, using personal collections and the keys of different authors. Amphibians were identified by the keys of Di Palma and Massa (1981), whilst the telson, chelae and thoracopods were the main diagnostic features for crustaceans. Results were expressed as percent frequency of occurrence F%, percent relative frequency of occurrence FR%, estimated per cent volume V% and per cent mean volume Vm%. In the area, fish - mainly trout (Vm%=21.4), redfin perch (Vm%=17.3), Eurasian minnow (Vm%=13.6) and South European nase (Vm% =9.4) -, formed the bulk of otter diet. Fresh faecal samples were preserved in 96% ethanol and frozen for genetic analyses with the aim of assessing the size and kinship for the otter population in the valley.

LARVAL AND ADULT NERVOUS SYSTEMS IN *Rhabdopleura meronuda* (Hemichordata, Pterobranchia)

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Abstract

Rhabdopleurid pterobranchs are a small hemichordate taxon traced back to 536 Mya. Among them, *Rhabdopleura* species are regarded as living fossils of the otherwise extinct subclass Graptolithina (Mitchell *et al.*, 2013). As discussed elsewhere (Pennati et al., this meeting), the evolution of the deuterostome nervous system is still a debated question. So far, much information has been obtained mostly from Enteropneusta, including approximately 3/4 of the known hemichordate biodiversity. By *in vivo* and analytical observations (i.e. scanning electron microscopy, confocal laser scanning microscopy, immunohistochemistry, and histological sections), this study describes for the first time the neuroanatomies of the zooid and the "spotted bean" larva of a newly described species, *Rhabdopleura meronuda* from the Mediterranean Sea. In the zooid, a dorsal brain is identified at the base of the mesosomal tentacles, with four distinct neural subsets characterized by FMRF-, GABA-, serotonin-, and dopamine- IR cells. A serotoninergic peripheral nerve cord run all along the U-shaped gut. In the spotted bean larva, GABA IR-positive ectodermal cells can be identified along the whole larval body, with a higher concentration at posterior larval end. A few serotonin IR-positive cells were detected in correspondence to the ventral ridge of the larva. This information corroborates previous evidence from *Cephalodiscus gracilis* (Stach et al. 2011) and supports the hypothesis of the occurrence of a centralized nervous system in the last common ancestor of deuterostomes.

1. Mitchell et al. 2013.
6. Stach et al. 2011.

Distributional pattern of cold-adapted plants and arthropods in a peripheral mountain range: cold- and warm-stage refugia should be considered

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The geographical isolation due to the ice-sheets expansion in cold-climatic stages (e.g. Last Glacial Maximum, LGM) has been traditionally involved to explain the present patchy distribution of some cold-adapted plant and arthropod species on peripheral mountain ranges; more recently, the areal contraction during warm-climatic stages (e.g. Holocene Climate Optimum) was also called into question. Recent studies on the Inner Alps demonstrated the role of ice-related landforms (glaciers and rock glaciers) as habitat for cold-adapted species. Since such landform may represent potential warm-stage refugia, the comparison of their occurrence, that of cold-stage refugia and that of cold-adapted species may shed light on the events driving present species distribution.

In this work we aimed to explain the present distribution of cold-adapted plant and animal species on a peripheral mountain range (Orobian Alps: Italy). We focused on ten species: 5 plants (*Viola comollia*, *Androsace alpina*, *Artemisia genipi*, *Ranunculus glacialis* and *Saxifraga oppositifolia*); 3 ground beetles (*Oreonebria castanea*, *O. lombarda* and *O. soror tresignore*) and 2 spiders (*Coelotes pickardi tirolensis* and *Drassodes heeri*) living on debris-featured landforms above 2000 m a.s.l. Some of these species are endemic while others show wide distribution, but they share the same main ecological requirements.

We compared by GIS-approach 574 records of plants and arthropods (379 plants, 54 ground beetles and 141 spiders) with the distribution of cold-stage refugia (summits emerging from the LGM ice-sheet) and potential warm-stage refugia (highest summits and 80 glaciers and 27 intact rock glaciers).

The results highlighted two rather different distributional patterns: 1) plant species seems more linked to warm-stage refugia, being mainly distributed where highest peaks occur and around glaciers and intact rock glaciers, and absent even at favorable altitude where the overall elevation of the chain is lower; 2) arthropod species seem linked to cold-stage refugia, being widely distributed in most of the summit emerging from the LGM ice-sheet.

Our study suggests the potential role of both cold- and warm-stage refugia on the current distribution of cold-adapted plants and arthropods on peripheral mountain ranges. This result could help to a better interpretation of the results obtained by phylogeographical studies performed on taxa with distribution limited to peripheral mountain range.

Metagenomic analyses of bacterial mats from deep basaltic rocks

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In the present study, deep-sea microbial assemblages colonizing deep-sea basaltic rocks from Atlantic Ocean were investigated by using microscopic and molecular-based techniques. Microbial mats were characterized by white, long filaments, which in turn were colonised by heterotrophic microbes and metazoa, generating complex food webs. Biodiversity and putative metabolic pathways of microbial consortia were investigated by metagenetic and metagenomic analyses, which revealed the presence of highly diversified assemblages potentially represented by chemosynthetic and heterotrophic components. Our analyses also suggested that microbial consortia colonizing deep-sea basaltic rocks were characterized by a wide spectrum of alternative metabolic pathways for the exploitation of CO_2 coupled with sulphur oxidation. Overall, findings reported here provide new information for expanding our understanding of biodiversity and metabolic functions of complex ecosystems such as deep-sea microbial mats.

Microbial community dynamics during biodesulfurization process of Ground Tire Rubber.

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Millions of tons of waste rubber are discarded every year. The microbial desulfurization process offers the advantage of rendering the rubber suitable for reformulating and re-curing to usable articles. Although some articles about biodevulcanization were reported in literature, in these studies only a basic microbiological monitoring was applied (optical density or dried weight measure and estimation of bacterial attachment on GTR surface with the SEM). Nevertheless, in some preliminary studies we observed the persistence of autochthonous GTR bacterial community to sterilization treatment. For this reason, in this study ARISA and Illumina sequencing were used for monitoring the bacterial community structure and the persistence of the inocula. Furthermore, 16S rRNA gene and *dsz*A catabolic gene were quantified by qPCR. The molecular microbiological analysis were combined with usually physical analysis of tire blends.

The aim of the present study was to test bioreactor-based ground tire (GTR) biodesulfurization processes using two different strains: i) *Gordonia desulfuricans* 213E, a strain described in a biodesulfurization process patent and ii) *Rhodococcus* sp. AF21875, a strain isolated from tire factory wastewater.

On GTR before the treatment, 56% of bacterial community was represented *of Rhodococcus.* Moreover, an elevated value of *dsz*A number of copy was found on untreated GTR. In addition, ARISA characterization showed that there is a naturally community hosts on GTR ant it survived to thermal treatment. Indeed, it was observed a shift towards the GTR bacterial community in the two bioreactors.

At the final time the persistence of *G. desulfuricans* 213E was observed both by ARISA and Illumina sequencing. On the contrary, in the other bioreactor the strain AF21875 was confounded due to the presence of matching ARISA fragments in the untreated GTR. In addition, 41% of the community at the end of the treatment belonged to the *Rhodococcus* genus. Both bioreactors showed an increase of *dsz*A copy numbers over time.

The desulfurized GTR from each bioreactor was blended with fresh natural rubber in order to test if devulcanized rubber can be used for compounding and revulcanization. The results showed that the both biological process led to an increase of the mechanical and rheological properties of vulcanizates containing biodesulfurized GTRs compared to the untreated GRT.

Paranthura japonica Richardson, 1909 (Anthuridea, Isopoda): actual state of the invasion in the Mediterranean Sea.

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Paranthura japonica, firstly described from Hokkaido Island (Japan) by Richardson (1909), was recently discovered in the southern Bay of Biscay (Arcachon Bay, France) and was identified as NIS (non-indigenous species). Likely the process of colonisation started from atlantic coast of France, after the Lagoon of Venice reached La Spezia and Olbia harbours in Italy. Subsequently it was found in several mediterranean areas such as La Grande Motte marina (Camargue, France), Mar Piccolo lagoon (Taranto, Italy), in Heraklion (Crete, Greece) and El Kantaoui (Tunisia) harbours and, finally, into Marina of Rome (Lazio, Italy), Livorno harbour (Tuscany, Italy) and Tortolì pound (Sardinia, Italy). The main hypothesis for the arrival and spread of *P. japonica* was oysters and mussels trade and farming and the wrong attribution of the species as a local one could have been the cause of its late identification; however, seafood trading and processing was supposed to be the main cause of unintentional introduction. The present review documents the actual distribution of the species *P. japonica* in Mediterranean Sea basin, where it was found during samplings of fouling and soft bottom assemblages of mediterranean commercial and touristic harbours and marinas. *P. japonica* can be distinguished by the native, and often co-occurring *Paranthura nigropunctata* (Lucas, 1846) from several characteristics: pleonites 1-5 fused medially but not laterally, uropod exopods with distal concavity on mesial margin and pleotelson not reaching beyond tip of uropod endopods. It is interesting to note that *P. japonica* was found in the most protected area of several harbours, along the docks. Specimens fit clearly into the descriptions of *P. japonica* provided by Richardson (1909), Nunomura (1977) and Frutos (2011). This record supports the hypotesis that the species has a preference for both natural and artificial hard substrate but this does not exclude the possibility to find it on soft sediments as epibenthic macrofauna. This review within Mediterranean harbours, along with others already reported in the literature, can help to understand the dynamics of temporal and spatial distribution of the species and show signals of a quick range expansion in the Mediterranean Sea, facilitated by port activities and trades.

Sicilian endemic Amphibians and Reptiles of the Nature Reserve of Pergusa Lake (Enna, Italy).

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The Nature Reserve “Lago di Pergusa” is located in Central Sicily (667 m a.s.l.) and is managed by Libero Consorzio Comunale of Enna. This protected area covers about 140 ha and represents the unique natural Sicilian endorheic lake, also included in the network Natura 2000 as core breeding and resting site for rare and threatened species, and some rare natural habitat types which are protected in their own right. Pergusa Lake is an hotspot of herpetological diversity in the context of Central Sicily, including 12 species (4 Amphibians and 8 Reptiles), with three interesting Sicilian endemic taxa: *Discoglossus pictus pictus* Otth, 1837, *Bufo siculus* Stöck, Sicilia, Belfiore, Buckley, Lo Brutto, Lo Valvo, Arculeo, 2008, and *Emys trinacris* Fritz, Fattizzo, Guicking, Tripepi, Pennisi, Lenk, Joger, Wink, 2005. Based on a monitoring plan of about 20 years (1996-2016) the presence of these three endemisms has been evaluated in detail with regard to phenology, habitat preference, population size and breeding sites. The presence of the species has been detected through direct observations of specimens, eggs, larvae, clutches, and song signals. We highlighted regular explosive breeding events for *Bufo siculus*, which during May-August, for 1-6 weeks, shows very high population density around the lakeside (50-400 specimens/m^2), including mostly neo-metamorphosed and a weak part of specimens of *Discoglossus p. pictus*. The Lake is currently one of the most important breeding site for *Emys trinacris* in Central Sicily, with regular observations of specimens during basking, foraging and nesting. The first specimen was observed in 2005, after the increasing of the water level; from the late ‘80s to 2002, Pergusa Lake became almost drained, with very high degree of salinity, thus *Emys trinacris* abandoned this site to populate alternative neighbouring wet sites (e.g., one pond located on a south-west hill). The allochthonous *Trachemys scripta* (Schepff, 1792), represented by the two subspecies *T. s. elegans* (Wied, 1839) and *T. s. troosti* (Holbrook, 1836), has been detected several times, and its presence suggests careful monitoring actions for possible ecological impacts on indigenous communities. Our results emphasize the need for considering functional aspects of the ecological traits of the herpetological fauna, to improve management actions for conservation strategies, especially of the Sicilian endemic species, in order to preserve both diversity and ecosystem functioning.

Role of ecological scenarios in modelling accumulation of organic chemicals in vegetation

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Plants play an important role in influencing the environmental fate of organic chemicals which are taken up from both air and soil. A number of multimedia fate models, with different degrees of complexity, were developed to describe the uptake and accumulation of organic chemicals in vegetation and plants where also included in regulatory predictive approaches such as that recommended in the European Union for risk assessment of organic chemicals (EUSES, European Union System for the Evaluation of Substances); however, it has recently been pointed out that the current procedure employed for environmental risk assessment lacks of ecological realism to properly evaluate the dynamic of air/plant/litter/soil exchange, especially when environmental conditions are subject to sudden variations of meteorological or ecological parameters. This work focuses on the development of a fully dynamic scenario that considers the variability of exposure concentration, meteorological and ecological parameters. In order to develop the scenario, two different sampling campaigns were conducted to measure variations over time of 1) Polycyclic Aromatic Hydrocarbon (PAH) concentrations in air of a clearing and a forest site, as well as in leaves of two broadleaf species (*Acer pseudoplatanus*, maple and *Cornus mas*, cornel) and 2) two important leaf and canopy traits, Specific Leaf area (SLA) and Leaf area index (LAI); moreover, a number of meteorological parameters (temperature, solar radiation, wind speed and direction, rainfall and planet boundary layer height) were also obtained. This scenario was adopted in a dynamic bioaccumulation model (SoilPlusVeg) to predict the temporal uptake and release of some PAHs in a mixed broadleaf wood located in Northern Italy and to evaluate how the variability of meteorological and ecological parameters can influence these processes, as well as, air and leaf concentrations.

Acoustic survey for broad-scale studies on insect distribution: the case of the tree cricket *Oecanthus dulcisonans* Gorochov 1993

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Field studies on insect distribution frequently deal with a number of unavoidable problems associated with specimen detection and identification, commonly making species monitoring unfeasible at a large spatial scale. These difficulties become even tougher when considering small, nocturnal and poorly studied species. This is the case of the newly discovered tree cricket *Oecanthus dulcisonans* (Orthoptera: Oecanthidae), which has been recently distinguished from the well-known *O. pellucens* on the basis of calling features. In this regard, this study is aimed at testing an acoustic monitoring scheme in order to provide cues on major distributional patterns of *O. dulcisonans* in south-eastern Italy.

Data have been randomly collected along Apulia region, quickly identifying singing individuals in the field by means of their specific song. For each record, coordinates, elevation and weather conditions were assessed.

In total, 239 observations of *O. dulcisonans* were made across the region. Calling males were recorded through all the night, from mid-June to early November. Though higher variability in the song was found with reference to literature, calling features proved to be highly reliable in direct species identification. With regard to data distribution, species occurrence proved to be primarily driven by elevation. Indeed the species was found to be common and widespread in warmer lowlands, from sea level to 300 m.a.s.l., while only few observations were made above this range.

While underlining the effectiveness of the tested survey scheme for the investigation of insect distribution, these findings are preliminary to future studies on species behaviour and habitat selection. This information may also be of use for exploring the relationship with the sympatric populations of the sibling species *O. pellucens*. Moreover, monitoring macro-ecological patterns of insect distribution over time and space may also represent a helpful tool for the interpretation of the effects of climate change.

Aglaophenia octodonta (Cnidaria, Hydrozoa) and the associated microbial community: a cooperative alliance?

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In the last decades, the widespread application of genetic approaches has revealed a bacterial world astonishing in its ubiquity and diversity as well as a growing knowledge of the vast range of animal–bacterial interactions, which has altered our understanding of animal biology. The abundant and unique microbial diversity hosted by marine invertebrates plays a very important role in their biology and ecology. Cnidarians host an impressive number of microbial organisms and the study of prokaryotic assemblages associated with these invertebrates is potentially important to better understand the specificity of their interactions, their evolution and influence on the cnidarian health. In the present work we describe the diversity of the microorganisms associated to the hydroid *Aglaophenia octodonta*. Epifluorescence, optical and scanning electron microscopy observations evidenced the presence of different microorganisms, whereas 16s rDNA gene sequencing and taxon specific primers for the 18s rDNA were employed to identify the various microorganisms. All the specimens of *A. octodonta* observed under blue light excitation showed a clear green fluorescence on the external side of the perisarc (chitinous exoskeleton) around hydrocladia due to *Vibrio* sp. AO1 and a red fluorescence due to the presence of microalgae. Moreover, the presence of peritrich ciliates attached to the hydrothecal rims was observed. By the molecular sequencing the microalgae were assigned to *Symbiodinum* sp. AO1 belonging to the Clade A and the peritrich ciliates assigned to *Pseudovorticella* sp. AO1. To understand the potential relationships between the host hydroid, *Vibrio* sp. AO1, *Symbiodinium* sp. AO1 and *Pseudovorticella* specific treatments were conducted with the antibiotic ampicillin and other substances that interfere with bacterial and hydroid metabolism. Treatment of *A. octodonta* with the ampicillin resulted in a decrease of vibrios luminescence followed by a *Symbiodinium* expulsion and *Pseudovorticella* detachment suggesting an involvement of all these microorganisms in a *A. octodonta* consortium. To reinforce this hypothesis hydrogen peroxide that stimulates the bacterial metabolism and ascorbic acid that acts as an antioxidant were used. Thus a model was hypothesized in which there is a close alliance and mutual benefit of the system *A. octodonta*, *Vibrio* sp. AO1, and *Symbiodinium* sp. with microalgae that release oxygen during photosynthesis useful to bacteria for their metabolism.

Pheromone signalling in ciliates: insights into the evolution of the pheromone molecular structure and pheromone-gene organization in *Euplotes*

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As is common among many life forms, also ciliates communicate via diffusible pheromones that act as cell signals for a self/non-self recognition mechanism. In *Euplotes*, pheromone secretion appears to be a pervasive phenomenon, which provides a unique opportunity to characterize the structures of pheromones and pheromone genes from different species, and compare these structures in an evolutionary context. For long time, knowledge of the pheromone and pheromone-gene structures has been limited to only three species, namely *E. raikovi*, *E. nobilii* and *E. octocarinatus*, that are proxies of only two of the six, or seven clades that are usually recognized in the *Euplotes* phylogenetic tree. We recently determined the pheromone and pheromone-gene structures also from *E. petzi* which forms the earliest branch of the tree, and from *E. crassus* and *E. focardii* that cluster inside the latest branching clade of the tree. In the light of these new determinations, the following two overall comparative pictures come forth. (i) The evolution of the pheromone-gene structure essentially involves lengthening of both the coding region and the leader 5' non-coding region with a parallel insertion of intron sequences and, in *E. crassus*, gene duplication. (ii) The evolution of the pheromone molecular structure primarily involves an addition of random-coil domains that cause substantial modifications of the basic pheromone architecture based on a three-helix bundle core.

Eco-morphological patterns of sagittal otolith in European anchovy from northern and middle Adriatic Sea

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Teleost fishes are able to perceive their environment through the sense of hearing and particularly through the sagittal otoliths, calcareous structures in the inner ear, that are involved in the capture and transduction of sound. An eco-morphological study was carried out on the sagittal otolith of *E. encrasicolus* (L., 1758) from northern (Conero-Istria) and middle (Conero-Tremiti) Adriatic Sea in order to identified the relationship among otolith morphology, environmental factors and population structure for fisheries management strategy. 220 anchovies were collected by MEDITS trawl survey in 2015. A representative subsamples was analyzed by scanning electron microscopy (SEM). Crystalline arrangement of external proximal surface of otoliths showed a similar specie-specific profile but different features between sites. In all samples, along the *sulcus acusticus* linked to auditory system, a similar texture was found: bigger and more defined morphological units were shown in the posterior region (cauda) arranged in a circular pattern, while in the anterior area (ostium) the microstructures were smaller and grouped with preferential direction. The otoliths of two sites showed prismatic crystals of similar dimension, but morphological differences. All the northern sub-samples showed: 1) well defined acicular crystals in the caudal area (length ≅ 100 μm); 2) well defined crystallographic planes, crystal faces and habits (length ≅10-20 μm) in the ostial area. Instead middle samples showed: 1) a less evident morphological structures in the caudal area, with morphological units joined together; 2) roundish crystals with no well-defined habits, embedded in an amorphous substance, in the ostial area. These differences in otolith microstructures between sites, confirmed those suggested by shape analysis (Montanini *et al*., 2016) and could be related to metabolic and environmental influences. The results suggest that environmental conditions influences the morphology of fish otoliths, the efficiency of otolith morphology for stock identification and support management strategy.

Regenerative potential of *Echinaster sepositus* (Retzius, 1783) arm explants: macro- and microscopic analyses

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Although partial regeneration has been described in crinoid and ophiuroid arm explants, starfish are unique among the echinoderms in being able to regenerate a whole individual from single arm. The aim of this research was to provide a first description of the cell-tissue patterning occurring during regeneration of arm explants in the red starfish *Echinaster sepositus*.

Specimens of *E. sepositus* were collected in the Marine Protected Area of Bergeggi Island (SV, Liguria). All the five arms of each animal were double amputated to obtain isolated explants. The latter were left to regenerate in the aquaria and collected at prefixed time-points (24 hours, 1, 3, 6 and 10 weeks post-amputation (p.a.)). The proximal and the distal side of each explant were photographed under a stereomicroscope to evaluate the general external anatomy and then processed for standard histological analyses.

Our results indicate that, differently from crinoids and ophiuroids, in this species regenerative phenomena occur at both the proximal and the distal explant side, although differences in timing and extent of regeneration can be observed. Within the first week both sides accomplish the repair phase (wound healing). From 1 to 6 weeks p.a. early regenerative phenomena can be observed at the level of the terminal ossicle and tube foot (only drafted in the proximal side). After 10 weeks p.a. regeneration in the distal side has reached an advanced stage (miniaturized arm), whereas in the proximal side is delayed and partial (mainly aboral ossicles), the appearance of new metameric units (tube feet and associated ambulacral ossicles/muscles) being prevented. The distalization-intercalary regeneration model proposed for *E. sepositus* arm-tip regeneration [1] is appropriate to describe also explant regeneration in the distal side, whereas in the proximal one only distalization but not intercalation occurs.

As in other Asteroids, the regenerative mechanism is mainly morphallactic; however, differently from arm-tip regeneration [1], in explants a massive cell recruitment apparently occurs from pyloric caeca rather than muscle bundles, thus underlining the notable plasticity of tissue remodelling exhibited by starfish.

This work represents the cell-tissue basis for future studies addressed to analyse in detail explant regeneration and, in general, to shed light on the mechanisms underlining the remarkable regenerative abilities of starfish.

[1] Ben Khadra *et al.*, 2015. WRR 23(4), pp.623-634.

GENETIC AND PHENOTYPIC FEATURES OF BARBEL POPULATION (OSTEICHTHYES: CYPRINIDAE) IN OGLIO SUD REGIONAL PARK.

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Barbels (Osteichthyes: Cyprinidae) are bottom dweller fish adapted to a variety of habitats, ranging from small mountain brooks (rheophilic group) to large and slow-flowing rivers and lakes (fluvio-lacustrine group). The Padanian barbel *Barbus plebejus* (Bonaparte, 1839) is fluvio-lacustrine, endemic to the upper Adriatic Sea river drainages, specifically in the heavily regulated lowland rivers of the Padano-Venetian district. Since in the last decades the introduction of fluvio-lacustrine European barbel (*Barbus barbus*) has adversely impacted the endemic *B. plebejus*, favouring the loss of local genetic integrity through widespread introgressive hybridization, efforts useful to the conservation and protection of endemic species are more and more required. Here we present a genetic and morphologic survey on barbel from Oglio Sud regional park with the aim to provide a combined insight on the current ecological status of local population. We measured the genetic and morphologic diversity sequencing mitochondrial (cytochrome b) DNA and phenotyping the fish body shape using landmark-based geometric morphometric methods, respectively. All barbels collected in Oglio Sud regional park relied in *B. barbus* species, showing large genetic variation (12 haplotypes, with $H = 0.800$ and $\pi = 0.001$). Through the morphometric analyses small phenotype variation, based only on different age of the sampled specimens, was found. In conclusion, although this study confirm the consistent invasion of *B. barbus* in one of the main Po basin tributary, the fish community remain stable showing a potential co-occurrence of both barbel fluvio-lacustrine species.

Determination of labelled and unlabeled nitrate via combination of sediment slurries and MIMS

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The analytical determination of dissolved nitrate is generally very accurate and mostly based on chemical reduction of nitrate with cadmium and spectrophotometric determination of the produced nitrite. However, the increasing use of the stable isotope $^{15}N\text{-}NO_3^-$ for the quantification of ecological processes (from primary producers uptake to microbially mediated nitrification, denitrification or anammox) requires alternative analytical approaches able to distinguish between the labelled and unlabeled nitrate forms. Here we present a novel approach based on the microbial reduction of the mixed pool of ^{14} and $^{15}NO_3^-$, the determination of the produced ^{29} and $^{30}N_2$ via membrane inlet mass spectrometry (MIMS) and the back calculation of the original nitrate concentrations. The reduction is carried out in 12 ml exetainers containing 2 ml of sediments and 10 ml of water sample, under anoxic conditions. In order to validate such approach we prepared a wide range of standard solutions containing $^{15}NO_3^-$ alone or in various combinations with $^{14}NO_3^-$, with final concentrations varying from 1 to 1000 µM, in fresh as well as in marine waters. We used organic-enriched sediments exposed to elevated concentrations of nitrate in order to have a large pool of denitrifiers in the slurry. On average we recovered nearly 80% of the initial nitrate (^{14} or ^{15}N) concentration, regardless the ratios of the two isotopes in the standards. The recovered versus added nitrate relationship was linear within a 1-700 µM range, allowing a wide range of experimental applications. As we did not use any poison, the MIMS is a reagent-free machine and many samples can be processes in just a couple of days, we think this method has a high potentiality and many applications for ecological studies.

Improving runoff calculation in Soilplus model: implementing slope correction to predict chemical fate in mountain river basins.

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Evaluation of the environmental fate of pesticides in agricultural basins is the first step to understand the potential for a pesticide application to soil to reach off target compartments, such as surface waters. In this context, a modeling approach is a useful tool to predict contamination levels. To meet this goal, the estimation of correct water runoff fluxes is of primary importance for most chemicals. SoilPlus is a dynamic soil model, in which a GIS object is embedded, that allows to predict chemical contamination in layered soil and air compartments, employing input data related to chemical properties and applications of substances, soil characteristics and meteorological conditions. SoilPlus model implements the Curve Number (CN) method for the estimation of water runoff. This method is a widely used approach to predict direct runoff for given rainfall events in small agricultural, forest, and urban watershed. CN method was originally developed for soils with slopes generally smaller than 5%. Some equations were recently published in order to consider slope factor to modify the traditional CN approach and improve the predictions for slopes steeper than 5%. The objective of this work is to improve water runoff prediction of Soil Plus model using slope-adjusted CN methods. These equations were implemented and verified using published dataset. A preliminary validation of these equations was done on a mountain watershed, comparing runoff volumes obtained by the traditional and adjusted CN values with corresponding measured flow rates at the basin outlet. Runoff simulations were carried out, comparing events recognition of the traditional and slope-adjusted approaches. The results show that employing the slope-adjusted methods a number of events ignored by traditional approach are detected. In order to evaluate chemical runoff, simulations were run for several representative chemicals using realistic meteorological and application information.

Risks of forage in dumps: the case of White Stork (*Ciconia ciconia*) in Sicily.

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The White Stork (*Ciconia ciconia*) is an opportunistic species that feeds on whatever is available. Foraging on rubbish dumps is a very common practice (Tortosa *et al.*, 1995, 2002, 2003; Blanco, 1996; Peris, 2003). In the Plain of Gela, White Stork nestings are 50.6% -53.7% of Sicilian couples and the distance from the dump (Timpazzo) to the nearest nest is average 11.2 km (Zafarana, 2016). From 2011 we collected data in order to verify a relationships between dumps and White Storks and we assessed the risks (Zafarana, 2014). Weekly monitoring in 2015 have allowed the acquisition of information about colour-ringed birds. The study shows how dumps are the preferred foraging sites of storks. A greater number of adults were observed in the period between 1-2 weeks before fledging of juvenile storks, probably because of the increased demand for food by chicks. Between July and August, fledged juveniles used dumps frequently: 6 colour-ringed birds observed in at least two of the four dumps considered (Timpazzo - Gela; Chiaramonte Gulfi - Ragusa; Lentini - Syracuse; Campobello di Mazara - Trapani). The closure of Campobello di Mazara dump and the consequent displacement of storks in Lentini (Barbera, *com. pers.*) seems to confirm this close correlation. Moreover, independent juvenile storks often made daily trips from the Plain of Gela to Chiaramonte Gulfi dump, then return to the sites of birth. Probably, the ease of finding the food and the continuous daily intake of organic waste in dumps push storks to prefer them, rather than to hunt in natural and semi-natural areas (abundantly present in Plain of Gela) which is more wasteful. The forage in dumps can represent advantages, but also a serious threat especially for the ingestion of considerable quantities of plastic and hazardous materials. These risks should be added the danger of poisoning on site for the use of rat poison; this is the probable cause of death of White Stork "Nelo" (IABPA342), of which the carcass was found in Timpazzo dumps. Current studies have shown that the constant presence of storks in dumps leads to the increase the cases of electrocution in neighboring electricity poles (Zafarana & Barbera, *ined.*).

Zebrafish nutrition: zoological, ecological (and ethical) challenges of live feeding in the lab animal science field

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Zebrafish (*Danio rerio*) is one of the most used species in research as valuable model for studying human genetics and disease. Despite its importance has increased worldwide, no FELASA (Federation of European Laboratory Animal Science Associations) guidelines are available. Therefore, many aspects of its husbandry, including nutrition, are not standardised and left to *in house* procedures.

Live feeding is one important aspect of zebrafish husbandry, although nutritional requirements and feeding strategies differ according to the developmental stage (fry, juveniles and adults). *Artemia* sp., rotifers (*Brachionus plicatilis* and *Brachionus rotundiformis*) and *Paramecium* sp. are widely used as live feeding. The diet is chosen according to several aspects: developmental stage, growth and survival rates of the colony, type of aquaria (static or RAS -Recirculating Aquatic systems) and water quality.

We summarise main strategies of zebrafish live feeding and we address challenges of this method.

I- zoological question: evidence based data on best solutions for zebrafish feeding are still missing and this have an impact on zebrafish colony management as well on *Artemia*, rotifers and *Paramecium* cultures.

II- ecological question: most of *Artemia* on the market for zebrafish is coming from Great Salt Lake, Utah (US) and China. Since the demand is increasing, the exploitation of natural resources is increasing as well. Moreover, the massive and uncontrolled use of exotic invasive strains may alter the evolutionary trajectory of autochthon species and seriously threaten the local biodiversity.

III- ethical question: alternatives to zebrafish live feeding should be more exploited and implemented. Moreover, the quality control of *Artemia* production, as well for *Paramecium* and rotifers should be highly considered. In fact, the culture condition of live feeding has a huge impact also on zebrafish health and the possibility of infections or death is not so low.

Long term fish based ecological status evaluation in the Venice lagoon

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After 16 years from its emanation, the Water Framework Directive (2000/60/EC) still cannot be considered fully implemented. For instance, a new method (HFBI - Habitat Fish Bio-Indicator) for the evaluation of the ecological status based on the fish fauna has been recently proposed for the Italian transitional water bodies, and intercalibrated with the other Member States of the Mediterranean area. We took advantage of several fish assemblage monitoring programmes carried out in the Venice lagoon (Northern Adriatic Sea, Italy) with homogenous methodologies (small beach seine net, used during spring and autumn in shallow waters) between 2002 and 2014 to apply the HFBI and study the evolution of the ecological status in this ecosystem. The overall quality is good, but the analysis of the space-time dynamics highlights some interesting patterns: there is a negative temporal trend of the index in the sea-related areas, while an improvement of the more confined zones can be observed in the last years. In general, stations located on seagrass meadows have higher ecological status than sampling points on unvegetated sites, and the conditions observed in autumn are worst than in spring. In particular, the difference between the index values recorded in spring and in autumn tends to increase over time. This observation, together with the fact that water quality-related anthropogenic pressure indicators (Nutrients load, oxygen and chlorophyll content) are the ones that show the strongest negative correlation with the HFBI, indicates that the index is sensitive to the anoxic crises recorded in the last summers. The application of the HFBI suggests that the new method should be not only used under the monitoring activities of the environmental agencies, but also tested in well known systems (like the Venice lagoon), as well as in new ones, in order to fully understand its behaviour. The knowledge gathered from these exercises could help in better understanding the relationship between the evaluation outcomes and anthropogenic pressures, and hence better inform and support the decision maker in formulating management strategies.

Ambienti di transizione e marino costieri