



79° Congresso Nazionale
UZI2018
Unione Zoologica Italiana
Lecce 25-28 Settembre 2018

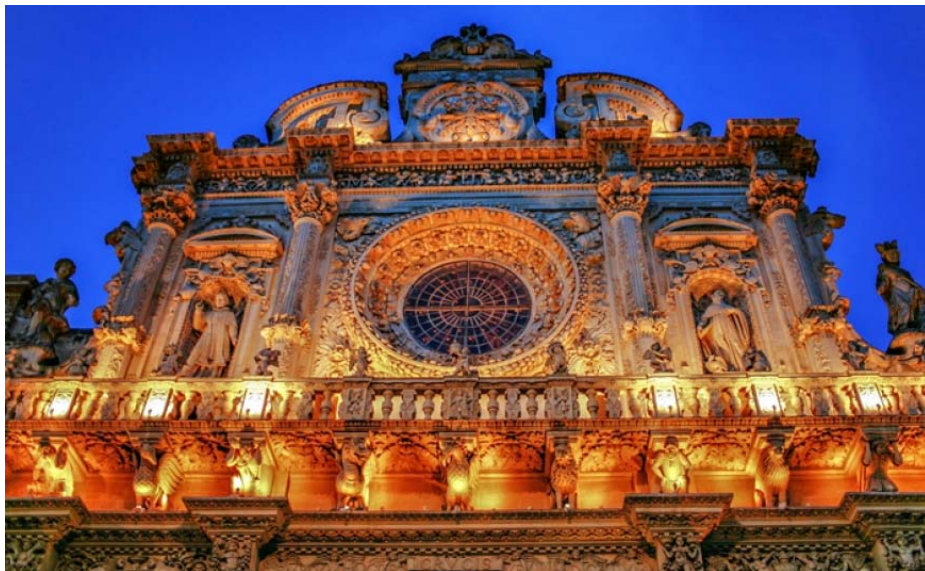


**UNIVERSITÀ
DEL SALENTO**

*Unione Zoologica Italiana
79° Congresso Nazionale*

Lecce, 25-28 Settembre 2018

Riassunti



Sede del Congresso

Grand Hotel Tiziano

Via Porta d'Europa, 73100 Lecce



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Unione Zoologica Italiana

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Cari Colleghi,

la nostra Società ritorna per la seconda volta nella città della “pietra leccese”, una calcarenite formatasi sul fondo del mare miocenico e ricca di resti di foraminiferi, invertebrati, rettili, pesci ossei e cartilaginei, delfini, balene e altri grandi mammiferi, degna scenografia per un convegno dell’Unione Zoologica Italiana. Oggi quella pietra morbida, finemente lavorata a punta di coltello dagli scalpellini del ‘500, costituisce il materiale alla base del “Barocco leccese” riscontrabile in innumerevoli palazzi e chiese presenti in tutto il centro storico.

Esattamente undici anni fa, dal 24 al 27 settembre 2007, molti dei presenti erano in questa stessa sala per inaugurare il 68° Congresso, dedicato al tema dell’evoluzione. Si trovava qui con noi allora il compianto Walter Gehring, la cui relazione di apertura - sull’origine ed evoluzione dell’occhio prototipico di Darwin - poi pubblicata sulla nostra rivista societaria, divenne uno degli articoli più letti dell’Italian Journal of Zoology.

Il 79° Congresso ha tra gli obiettivi il rinnovamento dei legami scientifici e culturali che uniscono le due anime fondatrici dell’Unione Zoologica, la Zoologia e l’Anatomia Comparata & Citologia, attraverso l’esplorazione di temi scientifici trasversali di grande attualità e a diversa scala di osservazione, temi adeguati ad una lettura moderna di quella Biologia Integrata e Comparata che da sempre caratterizza le attività di ricerca dei Soci dell’Unione Zoologica Italiana. Con oltre 150 partecipanti, 9 relazioni introduttive, 30 comunicazioni e 70 poster, di cui 37 concorrenti all’assegnazione dei premi UZI per Giovani Ricercatori, il Congresso annuale 2018 costituisce una fondamentale occasione di incontro per tutta la Comunità scientifica di riferimento, una tribuna di confronto e discussione tesa a promuovere le opportunità di collaborazione tra ricercatori giovani e meno giovani, ed a suggerire nuovi spunti di progettualità.

Il 79° Congresso di Lecce si articola in 4 simposi:

- 1) Riconoscimento e comunicazione nel mondo animale*
- 2) La valutazione della biodiversità a diversi livelli di organizzazione*
- 3) Le aree naturali protette per la gestione e protezione della fauna*
- 4) Cellule staminali, differenziamento e riprogrammazione cellulare: modelli tradizionali e modelli innovative.*

I primi tre simposi sono dedicati alla memoria di Soci recentemente scomparsi (Enzo Ottaviani; Susanna De Zio e Paolo Tongiorgi; Bernardino Ragni) che hanno ispirato e guidato numerosi ricercatori, alcuni dei quali oggi qui presenti. Il quarto simposio è ispirato dall’avvio di MARISTEM, una rete europea sulle cellule staminali da invertebrati acquatici, che darà vita (dal 1 al 6 ottobre p.v.) come appendice post-congressuale ad un breve corso teorico pratico presso il laboratorio di biologia marina dell’Università del Salento “Avamposto Mare”, nel borgo marittimo di Tricase Porto. Completerà l’agenda del Congresso una tavola rotonda sulla Didattica della Biologia Animale (nuove metodologie, nuovi corsi di laurea e nuove prospettive occupazionali). Un tema di grande attualità, anche alla luce del recente avviamento del processo di “manutenzione” delle classi di laurea.

Un doveroso ringraziamento è rivolto agli sponsor (Università del Salento, Banca Popolare Pugliese, Parco Nazionale Alta Murgia, UTET università) che con il loro contributo hanno favorito la realizzazione dei simposi e della Tavola Rotonda, ai relatori ed a tutti i partecipanti.

Ciò premesso, a nome di tutto il Comitato Organizzatore, ho il piacere di darVi il più caloroso benvenuto a Lecce, augurandoVi un piacevole soggiorno ed una fruttuosa partecipazione al 79° Congresso dell’Unione Zoologica Italiana.

Stefano Piraino

Presidente del Comitato Organizzatore

Simposio 1

Riconoscimento e comunicazione nel mondo animale

Coordinatori

Mario Pestarino, Stefano Piraino

Relazioni ad invito

(keynote lectures)

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MOLECULAR MECHANISMS AND BIOLOGICAL FUNCTIONS OF AUTOPHAGY ACROSS EUKARYOTES

Autophagy is a catabolic process targeting intracellular macromolecules and organelles to the lysosome for degradation to ensure both basal turnover and an efficient response to cell damage caused by a variety of stress.

Originally identified in yeast, core autophagy genes are highly conserved throughout the eukaryotes. Studies in mammals have expanded our understanding of the role of autophagy and underlined its important crosstalk with a variety of other processes, such as development, metabolism, immunity and ageing.

Studies in other multicellular organisms with specific types of cells in terms of composition, morphology, and organization, including invertebrate models, have contributed to elucidate how the autophagic machinery integrates signals from other cells and environmental conditions to maintain cell, tissue and organism homeostasis.

In particular, studies in flies and worms have provided insight on how context-specific regulation and function of autophagy has evolved to contribute to specific aspects of development, ageing and protection from infections.

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COMUNICARE LA FIDUCIA: STRATEGIE CHIMICHE ADOTTATE DAI NEMICI NATURALI PER ACCEDERE AI NIDI DI API E VESPE SCAVATRICI

Molte specie di api e vespe (Hymenoptera: Aculeata: Apoidea) scavano nidi nel suolo, dove depongono le uova e immagazzinano le risorse trofiche necessarie al completo sviluppo della prole. Sia le risorse che la prole immatura sono utilizzati a loro volta dai nemici naturali di questi insetti come cibo per la propria prole. È dunque necessario per questi nemici naturali evitare di essere riconosciuti dagli ospiti e rendere l'invasione dei nidi effettiva. Infatti, la presenza di un complesso sistema di riconoscimento, basato principalmente negli idrocarburi cuticolari, permette agli ospiti di discriminare conspecifici da eterospecifici e membri della stessa colonia da membri esterni alla colonia. I nemici naturali hanno evoluto tre strategie chimiche che sostanzialmente limitano tale riconoscimento: *mimetismo* (innato profilo chimico molto simile a quello dell'ospite), *camuffamento* (assorbimento graduale del profilo chimico dell'ospite dopo l'invasione del nido) e *insignificanza* (il profilo chimico è così semplice da rendersi invisibile all'ospite). Tali strategie sono state indagate in dettaglio per i parassiti sociali (femmine parassite vivono nelle colonie di specie sociali e sfruttano la forza lavoro delle operaie), ma quasi nulla è noto per i parassiti solitari. Studi recenti condotti da me e collaboratori si sono centrati sulle vespe cuculo del genere *Hedychrum* (Chrysididae) e le api cuculo del genere *Sphecodes* (Halictidae), i quali mostrano strategie diverse. I crisididi hanno evoluto mimetismo chimico, con un grado di perfezione che dipende dal tipo di profilo chimico dell'ospite. Al contrario, le api cuculo mostrano insignificanza chimica, con profili cuticolari estremamente semplici e composti sostanzialmente da alcani lineari, noti per essere poco importanti per il riconoscimento negli ospiti. Esperimenti comportamentali mostrano che entrambe le strategie effettivamente evitano le aggressioni da parte degli ospiti. L'insignificanza chimica trova congruenza con l'ampio rango di ospiti utilizzati dalle diverse specie di *Sphecodes*, mentre il mimetismo chimico implica l'impossibilità di attaccare molti ospiti, che ovviamente differiscono nel loro profilo cuticolare. Il camuffamento chimico, comune nei parassiti sociali, non si attende si sia evoluto nei parassiti solitari in quanto questi ultimi non passano lungo tempo nei nidi degli ospiti. Ampi studi comparativi possono fare luce sull'evoluzione di queste tre strategie negli imenotteri aculeati.

Simposio 1

Riconoscimento e comunicazione nel mondo animale

Coordinatori

Mario Pestarino, Stefano Piraino

Comunicazioni orali

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THE AUTOPHAGIC PROCESS IN LEPIDOPTERA: A MATTER OF LIFE AND (OR) DEATH

Metamorphosis represents a critical phase in the development of holometabolous insects. During the transition from the larval to the adult phase, several organs are remodeled or disappear as a result of apoptosis and autophagy. While apoptosis brings about cell death, the role of autophagy in this biological setting is quite controversial. In particular, although numerous studies have analyzed this self-eating process in Lepidoptera, none of them has demonstrated a causative role of autophagy in cell death in larval organs. In recent years we focused our attention on the degeneration of the larval midgut and posterior silk gland. Our data demonstrate that in the silk gland autophagy is necessary to produce the energy the gland cells need to stay alive and complete the spinning of the cocoon, while in the midgut the setting is more complex and autophagy acts in a coordinated manner with apoptosis. In fact, autophagy starts when the larva ceases to feed and not only permits larval midgut cells to survive starvation during early metamorphosis, but also allows for the catabolic degradation of the cell components that can be used by the newly forming pupal-adult epithelium; apoptosis intervenes later and actually drives the demise of the larval epithelium. In conclusion, our data suggest that autophagy plays a pro-survival rather than a pro-death role in the larval organs of Lepidoptera during metamorphosis.

CELL-CELL COMMUNICATION VIA WATER-BORNE SIGNALLING PHEROMONES IN CILIATES: INSIGHTS INTO THE PHEROMONE/RECEPTOR INTERACTIONS ON THE CELL SURFACE FROM THE DETERMINATION OF *EUPLOTES* PHEROMONE CRYSTAL STRUCTURES

Cell type-distinctive protein pheromones are constitutively secreted by species of *Euplotes* and used as autocrine (autologous, or self) signals that promote the cell vegetative (mitotic) growth and paracrine (heterologous, or non-self) inducers of cell-cell unions in mating (sexual) pairs. These activities require the pheromone binding to cell surface receptors, that have been identified with membrane-bound pheromone isoforms encoded through a phenomenon of intron splicing by the same genes which encode the soluble pheromones. As a consequence of this genetic origin in common, the soluble pheromone of each cell type finds a structural counterpart with the extracellular ligand binding domain of its twin membrane receptor, and this intermolecular structural identity has suggested to look at the pheromone/receptor interactions on the cell surface as mimicked by the protein/protein interactions that stabilize the pheromone molecules into a crystal.

We applied non-conventional *ab initio* crystal structure determination methods (that exploit the high-resolution of collected diffraction data) to highly homogeneous preparations of two *E. raikovi* pheromones, *Er-1* and *Er-13*, secreted by strongly mating compatible cell types. In spite of sharing more than 50% of amino acid sequence identity, the same disulphide bond pattern and the same up-down-up three-helix fold, *Er-1* and *Er-13* revealed a marked difference in the arrangement of the molecules in the crystals. The resulting protein-protein contacts that stabilize the pheromone molecules in the crystals assign *Er-1* to a C2 crystallographic space group (substantially confirming previous results), and *Er-13* to a P41 space group. In both patterns, however, helix 3 (which is the most conserved element among different pheromone structures) shows a common central role in securing the protein-protein crystal contacts. These results imply, first, that the autocrine (cell-growth promoting) pheromone/receptor interactions on the cell surface may result from the pheromone capability to undergo specific protein-protein homo-oligomerization patterns and, second, that helix 3 may function as the key structural element that ensures protein-protein hetero-oligomerization for the paracrine (mating inducing) pheromone/receptor interactions.

EXTRACELLULAR VESICLES AS CONSERVED MECHANISM OF CELL-CELL COMMUNICATION

Nanosized extracellular vesicles (EVs) have ignited much attention in the last decade. Initially reported in 1983 as a waste disposal mechanism, today they stand at the forefront of research into intercellular, interorganismal and interspecies communication. Size and origin classify EVs into 2 classes, exosomes (EXOs, 50-100 nm, produced via a lyso-endosomal pathway) and microvesicles (MVs, 100-1000 nm, formed by budding of the plasma membrane). Documented thus far eukaryotes, from microorganisms up to mammals, EVs offer a way for interaction derived from the sheer variety of content of carried molecules (nucleic acids, proteins, lipids), both as internal cargo and on their surface, capable to affect a multitude of processes simultaneously within a target cell. Invertebrate model organisms suggest their role in multicellular organisms: in *C. elegans* embryos, excessive EVs release affects development from gastrulation to larvae and adult phases; similarly, in *Drosophila* EVs carry morphogens that are important for nervous system and wing axes determination. Moreover, the presence of EVs has been demonstrated in the hemolymph of mollusks, suggesting a role in immune system response.

This multidimensional form of communication has special significance in human physiologic and pathologic conditions and their presence in a variety of fluids make them readily accessible. This renders EVs suitable for study and diagnostic tools, as it is possible to monitor their fluctuation in the circulation. The EVs are able to modify the phenotype and function of the target cells: this is particularly important in a number of human chronic disease, including metabolic disorders, and cancer.

For example, in cancer field it has been shown that several mechanisms of Glioblastoma multiforme (GBM) pathobiology are mediated through EVs that could contribute to the aggressive nature of GBM. In fact, GBM cells shed a high number of EVs, displaying a different protein and lipid profile able to indicate the status of the disease and to modulate macrophages activation towards an anti-inflammatory phenotype. Likewise, hyperglycemic condition is a strong inducer of EVs release by macrophages, suggesting a modulation and an amplification of immune responses. This indicates that EVs can be considered as a springboard for future advances, that can be used to better understand the mechanism by which EVs coordinate body function and dysfunction exploitable for therapeutic and diagnostic purposes.

**BSTLR: A NEW MEMBER OF THE TLR FAMILY OF RECOGNITION
PROTEINS FROM THE COLONIAL ASCIDIAN *BOTRYLLUS SCHLOSSERI***

Toll-like receptors (TLRs) represent a well-known family of conserved pattern recognition receptors the importance of which, in non-self recognition, was demonstrated in both vertebrates and invertebrates. Tunicates represent the vertebrate sister group and, as invertebrates, they rely only on innate immunity for their defense. As regards TLRs, two transcripts have been described and characterized in the solitary species *Ciona robusta*, referred to as CiTLR1 and CiTLR2. Using the *Ciona* TLR nucleotide sequences, we examined the available transcriptomes of *Botryllus schlosseri* looking for similar sequences. We were able to identify a sequence, with similarity to CiTLR2 and, through *in silico* transduction and subsequent sequence analysis, we studied the domain content of the putative protein. The sequence, called BsTLR, has a TIR and a transmembrane domain, four LLR and two LRR-CT domains. In addition, we analysed *bstlr* transcription *in vivo* and *in vitro*, under various experimental conditions and in different phases of the *Botryllus* blastogenetic cycle. Our data show that, in different phases, there is a change in gene transcription and mRNA location, according to the blastogenetic phase.

CHEMORECEPTION IN OCTOPUS VULGARIS (CUVIER, 1797), SENSORIAL PERCEPTION AND INTRASPECIFIC INTERACTION: SMELL BY TOUCH

In *Octopus vulgaris* as well as the other aquatic animals, chemical cues convey a remarkable amount of information critical to social interaction, habitat selection, defense, prey localization, courtship and mating, affecting not only individual behavior and population-level processes, but also community organization and ecosystem function. *Octopus vulgaris* possesses chemosensory systems that have anatomical similarities to the olfactory systems of land-based animals, but the molecules perceived from distance are different because their water solubility is of importance. Many insoluble molecules that are detected from distance on land must, in an aquatic system, be perceived by direct contact with their source. Most of the studies regarding olfaction in cephalopods have been performed considering only waterborne molecules detected by the “olfactory organs”. However, *O. vulgaris* is also equipped with “gustatory systems” consisting of receptors distributed on the arm suckers. Behavioral studies show that octopuses use their arms for different purposes, such as the distant chemoreception, the detection of the prey and the caring of the eggs. The recent study on the genome and transcriptome of the congeneric *O. bimaculoides* has shown that they are capable of editing about 60% of the RNA (in spite of the 1-2% observed in humans) and that they have a high number of genes (33,000 genes compared to 25,000 of humans), with the presence of expansions of few families of specific genes, such as receptors associated with G proteins, implicated in chemoreception. Here, using a behavioral, morphological and a molecular approach we show that octopus arms are able to detect, through the presence of specific receptors on their suckers, odorant molecules. In light of the fact, that many volatile odorant molecules are insoluble or have a very low solubility in water, octopuses exhibit a peculiar performance that can be provocatively described as “smell by touch” supported by the demonstration of the presence of olfactory receptors on their “gustatory systems”.

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COME TROVARE UN AGO IN UN PAGLIAIO? LO SQUALO BALENA POTREBBE RIUSCIRCI

Sebbene sia il pesce più grande del mondo, lo squalo balena è una specie molto elusiva e difficile da studiare, anche in virtù dei suoi costumi di vita strettamente pelagici. Molti aspetti della sua biologia ed ecologia rimangono ancora oggi in gran parte poco conosciuti, mentre invece sembra piuttosto chiaro lo stato di grave minaccia in cui vertono le sue popolazioni, soprattutto in conseguenza della pesca indiscriminata cui la specie è soggetta nei paesi asiatici e sudamericani. Nel mese di dicembre 2017 ha avuto luogo nell'isola di Nosy Be in Madagascar una spedizione scientifica coordinata da enti di ricerca italiani e malgasci proprio per studiare la locale popolazione di squali balena, con particolare attenzione all'ecologia alimentare della specie. Nello specifico, il primo obiettivo che si è voluto conseguire è stato quello di descrivere i comportamenti alimentari di questo gigante e correlarne la frequenza con la componente fitoplanctonica presente nei siti di studio: sebbene lo squalo balena si nutra di zooplancton, si è voluta verificare l'ipotesi che sia proprio grazie al fitoplancton che esso riesca ad individuare nella vastità dell'oceano il suo microscopico cibo. Sono stati raccolti 31 campioni di acqua di mare del volume di 2 litri nelle cinque seguenti situazioni comportamentali: 1. Assenza di squali balena, 2. Squali balena in nuoto orizzontale, 3. Squali balena in alimentazione orizzontale, 4. Squali balena in alimentazione verticale, 5. Squali balena in gulping verticale. I campioni di acqua sono stati immediatamente filtrati e quindi conservati a -20°C per la successiva analisi con HPLC. I comportamenti alimentari hanno dimostrato una maggiore frequenza in siti caratterizzati da maggiore biomassa fitoplanctonica, in particolare Criotofite, Aptofite e Cianobatteri. I risultati, inoltre, sembrerebbero evidenziare una certa correlazione tra il comportamento alimentare degli squali balena e la presenza di taxa fitoplanctonici che potrebbero essere responsabili del rilascio in acqua del solfuro dimetile (DMS). Questa sostanza è prodotta dalle piccole alghe in risposta a fattori di stress, come lo stress da pascolo esercitato su di esse dallo zooplancton. Poiché il DMS ha un forte odore, esso potrebbe essere la prova che sia proprio grazie all'olfatto che lo squalo balena riesca ad individuare agevolmente lo zooplancton di cui si nutre.

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CHEMICAL AND VIBROACOUSTICAL STRATEGIES OF AN INQUILINE ANT TO INTEGRATE INTO ITS HOST COLONIES

Social parasitism represents a peculiar type of interaction where a parasite exploits a whole society instead of a single organism. One of the most fascinating forms of social parasitism is the “inquilinism”. In this association a workerless parasitic queen coexists with the resident queen in the host colony and produces its own sexual offspring, which is raised by the host workers. To bypass the host nestmate recognition system, thus achieving a full integration within a host colony, inquilines have evolved a repertoire of deception strategies. In our study the chemical and vibroacoustic deception mechanisms employed by the inquiline *Myrmica karavajevi* (Arnoldi, 1930), which exploits the colonies of *Myrmica scabrinodis* Nylander, 1846, have been investigated. The analysis of cuticular hydrocarbon (CHC) profiles of the colony members showed that *M. karavajevi* employs chemical mimicry to integrate in the host colony. Indeed, *M. karavajevi* gynes possess CHC profiles similar to those of *M. scabrinodis* queens and the similarity increases if only the methylated alkanes are considered. The vibroacoustic signals produced by the inquiline gynes were also similar to those emitted by the host queens. Finally, in brood rescue experiments the white pupae of *M. karavajevi* gynes were saved prior to the other categories of the host offspring. Our results suggest that *M. karavajevi* has evolved sophisticated deception strategies involving multiple communication channels, to increase its social status in the colony hierarchy. This work has been carried out within the project VIBRANT (No. 2016/23/P/NZ8/04254) supported by the Polish National Science Center under POLONEZ programme which received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No.665778.

**PARASSITI SOCIALI: STRATEGIE CHIMICHE E MORFOLOGIA FUNZIONALE DEI
MICRODON SPP. (DIPTERA, SYRPHIDAE, MICRODONTINAE)**

Numerosi artropodi, conosciuti con il nome di "mirmecofili", hanno evoluto indipendentemente complesse interazioni con le formiche. Tra questi, 10.000 sono le specie stimate di parassiti sociali che, utilizzando varie strategie, riescono a sfruttare le risorse della colonia. *Microdon* Meigen, 1803 (Diptera, Syrphidae, Microdontinae) è un particolare genere di ditteri sirfidi che include più di 300 specie di cui solo 6 presenti in Europa. Durante tutta la vita larvale, queste mosche vivono all'interno dei formicai cibandosi delle larve e pupe dei loro ospiti e accrescendosi fino alla larva matura (terza età larvale). Poiché estremamente localizzate e di difficile reperibilità, le larve di questo dittero sono ancora poco studiate. Per ampliare le conoscenze sugli stadi di sviluppo di questo parassita sociale abbiamo voluto analizzare aspetti di morfologia funzionale di 4 specie europee di *Microdon* (*M. analis*, *M. devius*, *M. mutabilis* e *M. myrmicae*) integrando microscopia elettronica (FIB/SEM) e ottica. Inoltre abbiamo studiato la strategia utilizzata da questi parassiti sociali per eludere il sofisticato sistema di comunicazione ed identificazione chimica dell'ospite mediante analisi di gas cromatografia-spettrometria di massa. I risultati del presente lavoro mostrano che le larve di *Microdon* sviluppano una morfologia difensiva caratterizzata da una robusta cuticola multistratificata, assenza di appendici, testa retrattile e una forma del corpo a campana. Inoltre questo studio ha evidenziato numerose peculiari strutture come ad esempio la banda marginale, la reticolatura dorsale e i sensilli "flower-like". Gli studi di ecologia chimica hanno poi permesso di caratterizzare il profilo cuticolare medio degli stadi immaturi di *Microdon* e delle larve e operaie delle formiche ospiti. I *Microdon* esprimono un mix di idrocarburi cuticolari molto semplice, per lo più caratterizzato da alcani lineari che è quasi totalmente condiviso con larve delle formiche ospiti, suggerendo la presenza di mimetismo chimico. Siamo sicuri che questo tipo di studi possano aiutare a chiarire la tassonomia incerta di questo gruppo dovuta soprattutto alla presenza di specie criptiche oltre a comprenderne l'ecologia e le strategie mirmecofile.

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PERCEZIONE E GENERALIZZAZIONE DEL CONCETTO DI *RELATIVE PITCH* NEL TURSIOPE (*TURSIOPS TRUNCATUS*)

Nell'uomo, la capacità di riconoscere scale di toni si ritiene sia legata allo sviluppo della prosodia, ma le sue basi evolutive sono ancora largamente inesplorate. In generale, la capacità di percepire relazioni tra scale di suoni puri (*relative pitch*) sembra essere una caratteristica omoplasica parallela nei vertebrati, che andrebbe indagata in ottica comparata e in un crescente numero di *taxa*. Da un lato, evidenze sperimentali suggeriscono che i primati non umani siano parzialmente in grado di generalizzare il concetto di toni ascendenti e discendenti se trasposti di un'ottava. Dall'altro, gli uccelli sembrano non avere questa capacità di generalizzazione, processando prevalentemente l'informazione contenuta nelle singole componenti di frequenza (*absolute pitch*). I delfini possiedono abilità cognitive paragonabili a quelle delle scimmie antropomorfe, uno dei più efficienti apparati uditivi del regno animale, ed un complesso sistema di comunicazione vocale che include abilità di *vocal production learning* analoghe solo a quelle degli uccelli canori e psittaciformi. È quindi ipotizzabile che essi siano in grado di identificare i rapporti relativi che intercorrono tra scale di suoni in rapida successione. In questo studio, iniziato nel 2017 presso il parco Oltremare di Riccione (Costa Edutainment S.p.A.), due maschi adulti di tursiope (*Tursiops truncatus*) sono stati addestrati a discriminare tra scale ascendenti (A) e discendenti (D) di suoni puri, attraverso una procedura di condizionamento operante della durata di oltre 2000 sessioni. Gli stimoli sono stati costruiti combinando serie di tre suoni puri nel *range* di frequenza compreso tra 3400 Hz e 6000 Hz, trasposti tra loro di un fattore compreso tra 1,05 e 1,20 e separati da 250ms di silenzio. I risultati della fase di addestramento hanno evidenziato una fortissima differenza individuale nel numero di sessioni necessarie per associare le due scale ai rispettivi *target* di risposta, sebbene entrambe gli animali abbiano completato con successo il processo di associazione. Successivamente, ai delfini sono state proposte scale A e D al di fuori del *range* di frequenze utilizzato nella fase di addestramento, che hanno permesso di inferire sulla loro capacità di generalizzazione del concetto di *relative pitch*. Complessivamente, questo studio amplia le conoscenze sulla plasticità e complessità del sistema di comunicazione vocale dei tursiopi e contribuisce alla comprensione delle basi evolutive della musicalità.

Simposio 1

Riconoscimento e comunicazione nel mondo animale

Coordinatori

Mario Pestarino, Stefano Piraino

Sessione Poster

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DIFFERENT BUT COMPLEMENTARY ROLES OF RNASET2 AND AIF-1 IN THE MODULATION OF MEDICINAL LEECHES INNATE IMMUNE RESPONSE

Several studies demonstrated that RNASET2 (Ribonuclease T2) and AIF-1 (Allograft Inflammatory Factor-1) regulate the activation and modulation of innate immune response in both vertebrates and invertebrates. Even if it is known that these proteins are involved in macrophages recruitment and that their expression significantly increases after bacterial infections, the mechanisms by which they regulate the inflammatory response are still poorly defined. In order to explore the links between RNASET2 and AIF1 and their interrelation during innate immune response, here we focus our research on the effect of LPS (bacterial lipopolysaccharides) injection on their expression in the medicinal leech. This invertebrate is a well-established experimental model for studying innate immunity, given its very simple anatomy and its marked similarity with vertebrates in inflammatory processes.

Our results, obtained by prokaryotic-eukaryotic co-cultures and in vivo infection model, show that RNASET2 and AIF-1 play a crucial role in orchestrating a functional cross-talk between granulocytes and macrophages. RNASET2 is first released by granulocytes, playing an early antibacterial role. Subsequently, RNASET2-recruited macrophages expressing AIF-1, which, in turn, chemoattracts other macrophages to further sustain the anti-bacterial inflammatory response. The final result is the activation of a marked and effective response against pathogen infection.

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ARSENIC BIOACCUMULATION IN *SABELLA SPALLANZANII* (GMELIN,1791). EFFECTS AND ASSOCIATED RISKS ON ENZYMATIC ACTIVITIES AND BACTERIAL RECOGNITION

In the present study, we evaluated the risk associated with the exposure of the tissues of the polychaete *Sabella spallanzanii* to Arsenic, as hyper accumulation specifically in the branchial crown (Bio Accumulation Factor: 1,869) respect to sea water. Such elevated As concentrations found in the branchial crown of this sabellid would not reflect a gradual accumulation of this element with the organism age, avoiding variability as a result of the size effect. The physiological roles (such as antipredatory strategies) performed by As in the branchial crown make this tissue more suitable for biomonitoring studies specifically focused on As.

A galactose binding lectin (SsGBL) has been characterized and purified from the mucus of *S. spallanzanii*. The SsGBL was able to agglutinate bacteria. The strongest activity was observed towards *Vibrio alginolyticus* and *E. coli*. To a lesser extent SsGBL agglutinated the Gram positive *Micrococcus lysodeikticus*, suggesting its possible involvement in host pathogen recognition. We then investigated the arsenic effect and observed no effect against enzymatic activities (Phosphatase, Esterase and Peroxidase) but, according to the branchial crown accumulation, the mucus bacterial agglutinating activity results show that the presence of As determines inhibition only at the higher used concentration (BAF ranging from 2 to 20,000).

Probabilistic plots (cumulative probability against the agglutination activity) have been achieved respect to the As concentration and the season. An anomaly can be observed for the curve at the highest concentration compared to branchial crown BAF of As probably indicating the maximum tolerable As concentration.

ADULT-LARVA VIBRATIONAL COMMUNICATION: PLAYBACK EXPERIMENTS IN THE PAPER WASP *POLISTES DOMINULA*

Communication through substrate-borne vibration is widespread among social insects and regulates fundamental aspects of social life. Females of paper wasp, *Polistes dominula*, by performing an abdominal oscillatory behavior known as abdominal wagging, are able to produce vibrations which propagate through the nest. Because it is widely recognized that abdominal wagging is strictly associated with the presence of larvae in the comb, it has been suggested that abdominal wagging might represent an adult-brood vibrational signal. Indeed, substrate-borne vibrations would have short-term effects related to food and trophallactic exchanges between adult and larvae. According to this, two opposite hypotheses have been proposed: i) vibrations could prepare larvae to receive food by decreasing the amount of salivary secretion, or ii) they could be used by adult to stimulate the release of nutrient larval saliva. Here, we used an electro-magnetic shaker to play back the *P. dominula* vibrations on nests containing larvae. We assessed, for the first time, the short-term effect of abdominal wagging on larval behavior by recording larval response and by measuring the amount of saliva released immediately after abdominal wagging playback. Our results show that larvae i) are able to perceive the substrate-borne vibrations and ii) react to abdominal wagging by increasing the movement of their body, likely to attract the attention of adult females during feeding inspection. Nevertheless, vibrations neither increase nor decrease the release of larval saliva. Although our results support the alleged role of vibrations in adult-larvae communications, they do not support the hypothesis about salivary release modulation.

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A REGIONALISATION OF THE VISUAL CAPABILITIES IN THE EYES OF *TALITRUS SALTATOR* (CRUSTACEA, AMPHIPODA)?

Many insect species exhibit a typical regionalisation of their visual capabilities since specialized ommatidia are located in the dorsal margin of their compound eyes (Dorsal Rim Area). The supralittoral sandhopper *Talitrus saltator* (Montagu, 1808) during its zonal recovery along the sea-land axis of sandy shores relies on several celestial cues whose identification is dependent on the perception of skylight blue wavelengths. The aim of this work is to investigate the eventual regionalisation of the visual capabilities in the compound eye of this species. We conducted behavioural tests in a confined environment to assess the celestial orientation of individuals subjected to the black-painting of the dorsal (1/3) or the ventral (2/3) region of their eyes. Experiments have been carried out by using a grey (neutral density, transmittance = 23.5%) and a blue (transmittance = 73%, $\lambda_{max} = 450$ nm) gelatine filter in conditions of screened sun. Furthermore, we investigated the morphological features of ommatidia located in different areas of the compound eye of sandhoppers by means of light microscope observations and 3D reconstructions of their structure. Behavioural tests showed that individuals with the dorsal part of their eyes painted released under the blue filter were more dispersed or worse oriented than the other groups of individuals tested under the grey or the blue filter. Sandhoppers with the dorsal part of their eyes painted met also higher difficulties in their directional choices than the other individuals since the frequencies of radially-orientated animals were in both cases lower with respect those recorded in the other trials. Morphological observations revealed that ommatidia occurring in the dorsal margin of the eye of this species are shorter and straighter than those present in the rest of the eye. Therefore, this work suggests a regionalisation of the visual capabilities of *T. saltator* that appears related to the anatomical structure of ommatidia; in particular, the dorsal 1/3 region seems to be involved in the perception of the celestial orienting factors.

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THE DUTY IS ON ME: FEMALES DETERMINE THE RHYTHMIC STRUCTURE OF SONGS IN INDRIS (*INDRI INDRI*)

The coordination of vocal emission is a universal human ability. Singing behaviour is a critical example of coordination during vocal displays. In primates, singing is rare and is present only in tarsiers, gibbons, indris, and titi monkeys (the so-called "singing primates"). In these species, males and females of a mating pair perform coordinated duets or choruses where temporal characteristics play a central role to convey information to conspecifics. Among lemurs, *Indri indri* is the only species that produce songs, loud and powerful vocal displays that spread across eastern rainforests of Madagascar. Indris live in family groups in which all individuals emit their contribution, in the form of notes and phrases, in a precise and coordinated manner. Thus, the indri's vocal behaviour provides a good model for studying the production of complex rhythmic signals that require input from different individuals. We analysed 119 songs uttered by 17 indris (8 females and 9 males) in order to understand whether the characteristics of the individuals' contribution to the song may vary according to chorus size, the total duration of the song or the duration of the individual contribution, using the inter-onset intervals within a phrase and between phrases. We hypothesised that the coordination in the indris' song is obtained by the matching of an individual rhythm to the other's singing. We predicted that, in indris, the female could match male's singing, showing a higher degree of rhythmic variation when compared to males. Since previous studies showed that the number of singers per chorus might influence the own singing, we also predicted a change in the female's contribution according to the number of singers. We found that the rhythmic features of indri's song rely on factors that are different for males and females. We demonstrated that females show significantly higher variation in the rhythm of their contribution than males for both inter-onset intervals within phrases (paired t-test, $t = 5.786$, $df = 8$, $p\text{-value} < 0.001$) and between phrases (paired t-test, $t = 5.9627$, $df = 8$, $p\text{-value} < 0.001$) and we also found that only female singing is affected when the number of singers increases from 2 to 3. Our findings suggest that in this species, females sustain a higher cost of singing than males when the chorus' dimension arises.

FLUORESCENCE TO DETECT THE PRESENCE AND ACTIVITY OF XYLOPHAGOUS INSECTS WITHIN WOOD STRUCTURES AND ARTEFACTS: FIRST RESULTS ON *RETICULITERMES LUCIFUGUS*

The termites of the family Rhinotermitidae are obligatory xylophagous isopters that degrade the wood of human structures and artefacts. In addition, the "workers" of *Reticulitermes lucifugus* (commonly known as "underground termite") build walkways (until 80-90 meters long) with digested lignin. This work not only protects termites from light but also permits them to create a chemical trace that guarantees the connection between the nest and the foraging area. The degradation of lignin in termites occurs thanks to their intestinal microbiota, among which bacteria of the genus *Spirillum*. This agrees with several studies on natural mechanisms for anaerobic lignolysis, since a large part of the termite alimentary tract appears anaerobic.

The main problem to be solved is to identify both nest and foraging area before the insects have seriously degraded the wooden structures or artifacts in which they nest. Here, we propose to use fluorescence to identify the presence and the relative activity of *R. lucifugus* highlighting lignin walkways. Traces of *Spirillum* bacteria have been found along active walkways while there is no trace of bacteria in abandoned walkways.

The development of a monoclonal antibody that expresses its activity in the presence of *Spirillum* and to conjugate the antibody with the fluorescein, has two purposes: (i) to understand the complete life cycle of one of the most complex social insect; (ii) to highlight the presence of termites before damage caused becomes evident and dangerous on structures or artefacts.

ACOUSTIC MONITORING OF THE GOLDEN JACKAL (*CANIS AUREUS*) IN THE DANUBE DELTA: EVALUATING THE POTENTIAL FOR SPECIES RECOGNITION THROUGH SEMI-AUTOMATIC TECHNIQUES FOR BIO-ACOUSTIC ANALYSIS

The golden jackal (*Canis aureus*), although remarkably understudied, is one of the most widely distributed canid species. The species has expanded its European distribution range during this decades but the available data are still scanty and fragmented. We aimed to understand whether we could contribute to the development of new methods for the non-invasive monitoring of wild population and their census. We focused on a free-ranging population of golden jackal in the private reserve of Ultima Frontiera, district of Tulcea, Danube Delta (RO). We performed a similarity analysis of the vocal responses to the jackal howling activity recorded in the field in Romania, but also included the howls of other species of jackals and wolves. We used the fundamental frequencies of the first and second howl of golden jackal (*Canis aureus*), black-backed jackal (*Canis mesomelas*), side-striped jackal (*Canis adustus*) and gray wolf (*Canis lupus*). We collected 30 files of the first howl and 32 files of the second howl. Once calculated the acoustic distances, the resulting matrix was submitted to a clustering algorithm. We identified six and eight clusters depending on the clustering solution chosen. We found a dissimilarity in the first and second howls of animals of the same species, also showing that the golden jackals do not have a type of howl utterly distinct from the other species. Further analysis will investigate whether the increase in the number of howls used for the various species can allow a better prediction of the species of the emitter and of the area from which the recordings were collected.

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***HAPLOSYLLIS CHAMAELEON* LAUBIER, 1960 (ANNELIDA, SYLLIDAE). A NEGLECTED COMMENSAL SPECIES**

Symbiotic relationships are common strategies of life in many marine ecosystems and the members of the subclass Octocorallia are known for their capacity to establish different kinds of relationships with organisms belonging to numerous phyla. In the Mediterranean Sea, the purple/yellow gorgonian *Paramuricea clavata* Risso, 1826 is considered an important habitat-forming species where several taxa live on, such as sponges, hydroids, solenogasters, bivalves, nudibranchs, pycnogonids, amphipods and the syllid *Haplosyllis chamaeleon* Laubier, 1960. To date, this species has been rarely recorded in the Mediterranean Sea and its actual distribution and ecology are still unknown.

In the present study, a review of published papers has been performed to update the geographical distribution of this association and the records were plotted on a map. To clarify the seasonal cycle of *H. chamaeleon*, apical branches of *P. clavata* were sampled from different localities: Ligurian Sea, Central Tyrrhenian Sea, North Sardinia and Croatia. Additionally, others 20 apical branches of the gorgonian were seasonally collected, around 30 m depth, in the Ligurian Sea for morphological analyses and assess the reproductive maturity.

In all the studied localities the gorgonian hosted the polychaetes. These findings represent a new record for the North-Eastern Mediterranean Sea, this symbiosis being reported only for the Western Mediterranean basin. The analysis of the Ligurian specimens gave new information concerning the seasonal abundance (ind./cm), the frequency (% of findings) and the reproduction of this species. After one year of sampling the species appears less abundant during the winter season, but reproductive stolons, both male and female, are present all year round. *H. chamaeleon* is highly mimetic being identical in colour to the host colony, violet or yellow. If this species feeds on the tissue of its host and/or utilizes its pigments for camouflage, as hypothesised in literature, remains an open question.

USE OF THE ANIMAL MODEL *HIRUDO VERBANA* (LEECH) TO EVALUATE THE POSSIBLE CORRELATION BETWEEN IMMUNE RESPONSE, AMYLOIDOGENESIS AND CHRONIC INFLAMMATION

Toll-like receptors, essential pattern-recognition receptors (PRRs) of the innate immune system, recognize a range of conserved molecules of invading pathogens. Among them, TLR4 is expressed on the cell surface on both hematopoietic and non-hematopoietic cells, including cells of the central nervous system, playing a crucial role in both innate and neuroimmune responses. It is the receptor of LPS (Lipopolysaccharide) endotoxins, the major outer membrane components of Gram- bacteria and a potent activator of innate immunity and inflammatory response, inducing the expression of the pro-inflammatory molecules IL-18 and TNF- α .

Recently, amyloidogenesis has been identified as new player in innate immune responses and has been proposed as a detoxifying event to fight ROS (reactive oxygen species) increase, given that an excessive oxidative stress becomes harmful to cells when their antioxidant capacities result insufficient to retain the appropriate redox state. Thus, an uncontrolled activation of the innate immune system can lead to amyloid fibrils accumulation and chronic inflammation. In addition, it has been shown that LPS and TLR4 are associated with Alzheimer disease (AD), characterized by the accumulation of amyloid fibrils and neuroinflammation.

Within this context, microglia in the brain and monocytes/neutrophils in the periphery have a prominent role in initiating and regulating inflammation processes.

Here we propose the use of the medicinal leech, *Hirudo verbana*, as a powerful model system to understand the role of TLR4 in innate immune response and neuroimmune activation. The advantages of using this model is found in its innate immune system that is very similar to Vertebrate's one, but lacking the complex cross-talk typical of adaptive immunity. Our *in vivo* and *in vitro* approaches, by means of histological, ultrastructural, immunohistochemical and Western Blot techniques aim to correlate amyloid fibrils and ROS production with LPS treatment, clarifying the relationship between peripheral and central nervous system immune responses. Furthermore, by blocking TLR4 intracellular cascade we demonstrate that both macrophages and microglia cells undergo to a rescue process that implicate amyloid fibrils degradation and restoration of physiological conditions.

In conclusion, our study is promising to gain novel insight about the correlation between peripheral/neuro inflammation and amyloid accumulation.

**ADAPTIVE CROSS-TALKING BETWEEN BIPOLAR SPECIES OF THE
CILIAE *EUPLOTES* AND THEIR ENDOSYMBIONT
*FRANCISELLA BACTERIA***

In shallow waters of polar regions, microbial life blooms during the short summer period. A pre-requisite of this bloom is the adaptive evolution of very effective defense mechanisms against environmental hazards, represented in primis by non-ionizing irradiation such as ultraviolet B light. In two species of the ciliate *Euplotes*, *E. nobilii* and *E. petzi*, isolated from tidal pools of Antarctic and Arctic sites, this mechanism was observed to involve the activity of two essential enzymes, Methionine-Sulfoxide Reductase (MSR) A and B (that reduce protein oxidized R and S methionine sulfoxides back to methionine), and motivated the identification and functional characterization of the relevant coding genes. Screening of the *E. nobilii* and *E. petzi* genomes, fully sequenced from the sub-chromosomal and transcriptionally active cell somatic macronucleus, resulted in the identification only of *Euplotes*-specific MSR-B coding sequences. No *Euplotes*-specific MSR-A coding sequence was instead identified, raising the question of the source of this enzyme in the *Euplotes* cytoplasm. The answer was provided by isolating and sequencing the genome of *Francisella* gamma-proteobacteria from *E. nobilii* and *E. petzi*, in which these bacteria live as endosymbionts. In addition to revealing gene sequences encoding MSR-A proteins properly supplied with a secretory N-terminal signal peptide, the *Francisella* genome was found to contain gene sequences encoding the so-called 'Type-6 secretion system' responsible for the protein transport across the bacterial envelope into the host cytoplasm. On the other hand, it was found to lack gene sequences specific for enzymes necessary to the biosynthesis of four essential amino acids, cysteine, lysine, methionine and tyrosine. By implying that these amino acids are supplied to the *Francisella* endosymbionts by the host *Euplotes* cells, this *Francisella/Euplotes* genetic and physiological cross-talk provides an instructive example of prokaryotic/eukaryotic adaptive co-evolution that might not necessarily be limited to polar regions.

HOW BLUES VIBES TRICK ANTS? THE ROLE OF ACOUSTIC SIGNALS IN BUTTERFLY SYMBIOSIS

Acoustic signaling is a widespread form of communication among insects but our knowledge on the occurrence of this kind of communication between distantly related species is scarce. It has been hypothesised that the exchange of acoustic signals plays a fundamental role in butterfly-ant interactions (myrmecophilous systems). Although the vibroacoustic signalling has been investigated primarily on parasitic species of Lycaenids (Barbero *et al.* 2009 - *Science* 323: 782-785), butterfly associations with ants may also be mutualistic and range from facultative to strictly obligate. Therefore, a broader survey is crucial to disentangle how these communication cues could have contributed to foster myrmecophilous interactions in butterflies.

To assess if the variation in vibroacoustic signals emitted by blue butterflies (Lycaenidae: Polyommatainae) better reflects the level of myrmecophily, according to Fieldler's classification (Fieldler 1991 - *Bonn. Zool. Monogr.* 31: 1-210), compared to the phylogenetic relationships between species, we

- Compared the characteristics of vibro-acoustic signals emitted by Lycaenid (blues) larvae and Myrmicinae ant species and assessed the similarities between signals;
- Evaluated the degree of interaction between each Lycaenid larva and several ant species through behavioural observations of worker-larva attendance;
- Investigated the reaction of worker ants to the vibro-acoustic signals emitted by Lycaenid larvae in playback experiments.

Ant behaviors were analyzed using BORIS (Friard & Gamba 2016 - *Methods Ecol. Evol.* 7(11): 1324-1330).

Ants reacted differently to alive larvae and acoustic stimuli showing several neutral (e.g. antennating) or benevolent (e.g. staying) behaviors and scarce aggressive responses (e.g. biting), even towards non-myrmecophilous species. For ant species provided with stridulatory organs (Myrmicinae) signals were recorded and compared with Lycaenid signals, showing that to a higher degree of interaction corresponds a higher acoustic similarity.

We discuss our findings in both the evolutionary and the conservation framework as the survival of myrmecophilous species depends on the persistence of multiple factors, including the ant occurrence. The multifarious communication strategies in such symbioses are far from being fully disentangled and deserve to be further investigated in order to assure the long-term survival of these complex interspecific associations.

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ASSESSING SEX DIMORPHIC COMBINATORICS IN THE SONG OF THE INDRIS (*INDRI INDRI*) USING THE LEVENSHTTEIN DISTANCE

When seen in comparison with humans, animals showed a limited combinatory ability to concatenate vocal emissions in phrases, at least in the acoustic domain, but the information available on the variability within a species is very little. Moreover, few investigations on primate vocal sequences are currently available, and none of them is evaluating the stereotypy of song structure between sexes using easily comparable methods. The Levenshtein distance (LD) is a logical distance commonly used to quantify the difference between two strings of data (e.g., human words, sequences of visual movements or sequences of song themes). It calculates the minimum number of necessary changes (insertions, deletions, and substitutions) to transform one string into another. We investigated the phrase structure of the indris' song (*Indri indri*) and its potential for sex discrimination. We assumed that phrase combinatorics and variability in the number of elements within phrases set the basis for interactions between different groups. We hypothesized that song structure varied between males and females showing that sex differences should also reflect in the combination of song phrases. We analyzed 142 female and 119 male contributions to the song, recorded from 17 individuals of eight groups. We considered phrases consisting of two (DP2), three (DP3), four (DP4), five (DP5), and six (DP6) units. To understand whether there were differences in song structure between sexes, we investigated the DPs combinatorics in each contribution. We transformed each contribution in a string of labels separated by a break symbol (e.g., DP2|DP3|DP4|DP3...) and then we used LD to investigate how the descending phrases followed each other. We found an apparent sex dimorphism in phrase structure, also showing that female between-individual variability was much higher than males' one. In agreement with previous studies that reported sexual dimorphism in the overall timing and repertoire size, and the frequency modulation, duration and the rhythm, we found that male and female indris also differed in the phrase combinatorics of their songs. This result is in line with the hypothesis that song structure varied between male and female suggesting that songs may play a role in finding a partner and mediate pair formation, as it happens in birds.

Simposio 2

La valutazione della biodiversità a diversi livelli di organizzazione

Coordinatori

Maurizio Casiraghi, Adriana Giangrande

*Relazione ad invito
(keynote lecture)*

ESTINZIONI, SPECIAZIONI, NUOVE FRONTIERE DELLA SISTEMATICA

Non è certo capitato a tutti gli uomini di poter assistere a cambiamenti epocali. Per noi è così, dato che oggi la Terra entra in un nuovo capitolo della sua storia, l'Antropocene, un periodo segnato dal significativo impatto globale che gli umani stanno avendo sugli ecosistemi del pianeta. L'Antropocene porta con sé una dicotomia: da un lato è oramai accertato che le specie in tutto il mondo stiano scomparendo a tassi senza precedenti e che la causa principale di queste estinzioni sia l'azione dell'uomo, tanto che si parla addirittura di una sesta estinzione di massa. Dall'altro canto si sta assistendo ad un notevole aumento nel tasso di descrizione di nuove specie. Dopo oltre un secolo di convinzione che non è rimasto nulla di entusiasmante da scoprire e in un momento in cui la carriera di un tassonomo sembra poco allettante per i nostri giovani, ci troviamo in un periodo che viene paragonato al 18° e 19° secolo quando i naturalisti europei avventurandosi in nuovi territori riportarono un numero sorprendente di nuove piante e animali esotici. Le cause di questo aumento di biodiversità possono essere molteplici, dal rinnovato interesse negli studi sulla biodiversità, alla rapida espansione urbana e rurale in molte aree selvagge. Alcune voci critiche hanno anche supposto l'esistenza di una "inflazione tassonomica": un aumento eccessivo del numero di taxa riconosciuti, non a causa della loro "reale" esistenza, ma per via di cambiamenti nel modo in cui sono definite e riconosciute le specie.

La suddivisione di una specie in due è oggi in gran parte il risultato dell'applicazione (ormai diffusa) delle tecniche di indagine molecolare nel campo della tassonomia e della sistematica. Questi progressi, associati a nuove applicazioni di ambito statistico e informatico, hanno portato fino all'analisi di interi genomi per produrre filogenesi sempre più raffinate e ben risolte, mostrando in molti casi diversità criptiche. Ciò è ulteriormente amplificato dal fatto che molti ricercatori che oggi si occupano di sistematica, anche in ambito zoologico, hanno una limitata conoscenza dei concetti di specie, tanto da non conoscere neppure quale concetto stanno utilizzando per descrivere una specie. Questo è reso manifesto dalla proposta di un "concetto di specie molecolare", avanzato "con leggerezza" da alcuni ricercatori. L'utilizzo "disinvolto" del rango tassonomico di specie, secondo alcuni scienziati, ha portato ad un aumento del 50% del numero di specie di vertebrati descritte negli ultimi anni.

I professionisti della conservazione, come lo IUCN e la CITES, stanno sollevando preoccupazioni riguardo alla tassonomia in continua evoluzione che può ostacolare la conservazione. Ma non dobbiamo confondere il piano scientifico da quello gestionale: se vogliamo salvaguardare la biodiversità allora la scienza non può esimersi dall'apprendere di più sull'incredibile varietà della vita sulla Terra.

Rimanendo nel tema della conservazione, quando gli scienziati esaminano l'impatto degli umani sul pianeta, l'attenzione è rivolta principalmente all'estinzione delle specie. Ma sempre più ricercatori stanno arrivando alla convinzione che gli esseri umani sono diventati una forza trainante evolutiva che porta anche ad un aumento della biodiversità. Le continue storie di successo ecologico ed evolutivo dell'epoca antropocenica ci impongono di rivalutare la nostra relazione con il resto della natura. Il cambiamento operato dall'uomo è sicuramente troppo veloce e limita le capacità di adattamento di molte forme. Le specie stanno davvero morendo, ma forse non dovremmo spendere così tanto tempo lamentando solo le perdite che hanno già avuto luogo, cercando di capire come poter ricreare il passato. Non possiamo riavvolgere la storia e potrebbe non essere neppure così necessario farlo per valorizzare la ricerca tassonomica e proteggere la biodiversità anche se, così facendo, ci allontaniamo da come era un tempo il mondo.

Simposio 2

La valutazione della biodiversità a diversi livelli di organizzazione

Coordinatori

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Comunicazioni orali

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LA BIODIVERSITÀ PERDUTA: UNA TESTIMONIANZA AL MUSEO DI ZOOLOGIA “PIETRO DODERLEIN” DI PALERMO

Uno dei ruoli dei musei di storia naturale è quello di fungere da “*archivio della biodiversità*”, passata e attuale. Degno di nota a tal proposito è il Museo di Zoologia “Doderlein” del Sistema Museale dell’Università degli Studi di Palermo, che contiene una vasta collezione di esemplari del regno animale a secco o in liquido, molti dei quali raccolti nell’area siciliana. Il Museo, custode di migliaia di esemplari, dagli invertebrati marini ai primati, risalenti a più di un secolo fa, è oggi punto di incontro di competenze tassonomiche differenti e fonte di materiale per ricerche da sviluppare in diversi ambiti.

Riguardo la biodiversità passata, alcuni esempi tra i vertebrati marini sono rappresentati da due generi di elasmobranchi *Rhinobatos* e *Glaucostegus* e dallo storione *Acipenser sturio* Linnaeus, 1758, oggi rari in Mediterraneo. In ambito terrestre, per quanto riguarda l’avifauna non più presente in Sicilia, sono da annoverare invece la quaglia tridattila *Turnix sylvaticus* (Desfontaines, 1787), la Gallina prataiola (*Tetrax tetrax* (Linnaeus, 1758)) e il gufo reale *Bubo bubo* (Linnaeus, 1758); mentre il lupo (*Canis lupus* Linnaeus, 1758), è rappresentante della popolazione siciliana oramai estinta, per i mammiferi. Tra gli esapodi l’Elateridae *Agrypnus notodonta* (Latreille, 1827) (estinto in Sicilia), insieme a un cospicuo numero di scarabeidi coprofagi oggi largamente in declino nel territorio isolano. A questi si aggiunge la lucertola delle Eolie *Podarcis raffoneae* (Mertens, 1952) un endemita in pericolo critico di estinzione, o il paratipo del Rospo smeraldino siciliano *Bufo siculus* Stöck et al., 2008.

Un *assemblage* faunistico utile a verificare o dimostrare l’esistenza di specie in tempi passati: tra gli elasmobranchi è questo il caso del grande esemplare conservato a secco di *Dipturus batis* (precedentemente *Raja batis*) (Linnaeus, 1758) – di cui analisi genetiche future potranno costituire la conferma tangibile della presenza della specie in Mediterraneo – a cui si aggiungono anche altri gruppi tassonomici affini che oggi fungono da confronto teso a dimostrare la validità specifica per i mari italiani (vedasi *Centrophorus granulosus* (Bloch & Schneider, 1801) e *Centrophorus uyato* (Rafinesque, 1810), oppure *Raja montagui* Fowler, 1910, *R. polystigma* Regan, 1923 e *R. brachyura* Lafont, 1871).

Se da un lato le collezioni naturalistiche costituiscono un vero e proprio inventario della biodiversità, dall’altro rappresentano un importante strumento di studi più ampi. Questo è vero soprattutto per quei gruppi tassonomici alloctoni al territorio: per i mammiferi la collezione primatologica voluta proprio da Pietro Doderlein, seppur parzialmente rappresentativa, consente di avere una visione di insieme dei nostri parenti viventi più prossimi, oggi altamente minacciati di estinzione; mentre per l’erpetofauna è il caso dello scinco gigante capoverdiano, *Chioninia coctei* (Duméril & Bibron, 1839), oggi scomparso globalmente.

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***ANTIPATHELLA SUBPINNATA* AS A MODEL SPECIES: LIFE HISTORY AND AUTOECOLOGY**

The black coral fauna of the Mediterranean Sea includes seven species belonging to five different families distributed throughout the basin from 60 to 2000 m depth. For long time considered extremely rare species, in the last 10 years, remotely operated vehicle (ROV) explorations completely overturned this paradigm such that, now, at least 4 of these species are considered common or dominant components of the deep circalittoral and bathyal cold-water corals (CWC) assemblages. A large effort has been dedicated to map the distribution of populations, however very little information exists on the life history and ecological role of these paramount species. In particular, *Antipathella subpinnata*, a large arborescent species, is known to thrive from 60 to 600 m depth on exposed hardgrounds commonly forming dense aggregations. Over 70 populations have been currently mapped in the Mediterranean basin; therefore, this species may well represent a model for the entire order. A 360° approach (coupled to literature search) has been carried out to investigate, in the field and in controlled systems, numerous traits of the life history of this species and to elucidate its ecology. In particular: i) phylogenetic position, ii) morphological characteristics, iii) phenotypic plasticity, iv) geographic and bathymetric distribution, v) size frequency distribution, vi) habitat preferences, vii) associated fauna, viii) associated microbiome, ix), sexual reproductive cycle, x) asexual reproduction through fragmentation, xi) genetic structuring and connectivity among populations, xii) growth rates, xiii) isotopic diet, xiv) skeletal degradation, xv) 3D modelling and biomass, xvi) response to anthropic impact. Although various aspects have not been investigated yet (e.g. age, skeletal architecture, larval ecology), such extensive dataset is crucial when identifying the vulnerability traits of a species and of the ecosystem it sustains.

DISTRIBUZIONE E DENSITÀ DI *DISTICHOPORA VIOLACEA* (CNIDARIA, HYDROZOA) IN QUATTRO ATOLLI DELL'ARCIPELAGO DELLE MALDIVE

Distichopora violacea (Pallas, 1766) appartiene agli Stylasteridae, una delle poche famiglie di idrozoi caratterizzate dalla produzione di uno scheletro calcareo. Sebbene siano disponibili numerose informazioni dal punto di vista tassonomico, finora gli aspetti ecologici di questi idrozoi sono stati scarsamente investigati. Durante tre spedizioni scientifiche condotte a maggio 2016, 2017 e 2018, è stata studiata la distribuzione superficiale di *D. violacea* in quattro atolli dell'Arcipelago delle Maldive (Ari, Felidhoo, Malè Nord e Malè Sud). Il campionamento vivo in immersione subacquea è stato condotto nelle fasce batimetriche 4-6 m, 9-11 m e 14-16 m mediante la tecnica del belt transect (transetto a fascia di 5 m • 2 m), disposto parallelamente al margine del reef. I risultati mostrano che *D. violacea* è presente in tutti gli atolli indagati, ma la densità delle colonie non è omogenea. Infatti, i valori risultano più elevati nei reef oceanici rispetto a quelli lagunari. Inoltre, considerando i reef oceanici, si osserva una densità più alta in quelli localizzati sul lato orientale dell'arcipelago rispetto a quelli del lato occidentale. Infine, confrontando la densità di colonie nei reef oceanici dei tre atolli posizionati sul lato est, questa risulta inferiore nei reef di Felidhoo rispetto a quelli di Malè Nord e Malè Sud, dove si è registrata la densità massima di 234 ± 46 colonie/10 m².

Le informazioni idrologiche relative all'Arcipelago delle Maldive sono molto scarse e ciò rende difficile l'interpretazione di questo particolare pattern di distribuzione e densità. Tuttavia, è possibile ipotizzare che esso sia il risultato di una sinergia tra fattori abiotici (correnti, salinità, tasso di sedimentazione e pendenza del reef) e biotici (periodo di rilascio delle larve e tempo trascorso prima del loro insediamento e metamorfosi). Il severo fenomeno di sbiancamento dei coralli che ha recentemente interessato le scogliere coralline maldiviane non sembra aver coinvolto questi idrozoi che non sono associati a zooxantelle. Questo studio amplia le conoscenze sulla biodiversità delle scogliere coralline maldiviane e fornisce, per la prima volta, dati quantitativi sulla distribuzione e densità di *D. violacea*. Tali informazioni possono essere di interesse nell'ottica di una corretta gestione delle specie di questo gruppo soggette agli obblighi della Convenzione di Washington, e contribuiscono a produrre un riferimento essenziale per la valutazione del valore e dello stato di salute degli ecosistemi maldiviani.

MEGABENTHIC BIODIVERSITY AND ANTHROPOGENIC DISTURBANCE OF THE LIGURIAN DEEP CONTINENTAL SHELF

The Ligurian Sea is among the most studied Mediterranean basin due to the historical occurrence of numerous research groups that, since the beginning of the last century, have characterized the benthic and pelagic fauna mainly thanks to SCUBA dive and trawl surveys. Nevertheless, a large knowledge gap was left on the deep circalittoral communities thriving along the deepest part of the continental shelf. Recent reviews, however, suggest that this bathymetric zone may host the deepest extension of shallow-water animal forests as well as deep circalittoral assemblages thriving in the so-called *roche du large* ecosystems. Considering that hardgrounds at relatively short distance from the coast attract a considerable amount of professional and recreational fishermen, it resulted very important to carry out an extensive investigation in this area. A four-year ROV survey (2012, 2015, 2016 and 2017) was conducted along the Ligurian continental shelf and break between 50 to 220 m in order to characterize the benthic biocoenoses and quantify the anthropogenic disturbance. About 80 sites have been investigated and a through visual census was carried out in order to define bottom type, depth and slope variation, diversity and spatial distribution of megabenthic species as well as traces of impact and lost litter. A community analysis was carried out by means of R so to identify the dominant biocoenoses and the most relevant environmental parameters describing them. The most representative assemblages included gorgonian and black coral forests, horny sponge grounds, bryozoan beds, forests of *Dendrophyllia cornigera* as well as soft-bottom meadows of sabellids and alcyonaceans. A georeferenced database was then created and biocoenotic maps were overlapped with areas of high impact in order to identify a large network of the most sensitive habitats that may represent a basis for the identification of future Protected Areas.

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INTEGRATIVE TAXONOMY, EVOLUTION, AND ECOLOGY OF SYMBIOTIC ZANCLEIDA (HYDROZOA, CAPITATA)

Hydrozoans belonging to the superfamily Zancleida are characterised by a relatively simple anatomy and diverse ecological and reproductive traits. The taxonomy of most species is hampered by their rarity, the difficulty in observing their complete life cycles, and the recurrent presence of cryptic species. Several species, mostly belonging to the families Zancleidae, Cladocorynidae, Sphaerocorynidae, and Milleporidae, are involved in symbiotic relationships with other organisms, including scleractinian corals, octocorals, sponges, bryozoans, and algae. Little is known about the diversity of these symbiotic hydrozoans, and the relationships with their hosts or symbionts are largely understudied. Therefore, an integrative approach was used to characterise these enigmatic taxa. Specifically, detailed morphological and morphometric analyses of classical and newly discovered characters, together with DNA taxonomy techniques, phylogeny reconstructions, comparative phylogenetic analyses and ecological surveys, allowed to shed light on different aspects of symbiotic Zancleida. For instance, in different groups the genetic diversity is better explained by the host specificity rather than the classical morphological features, even though a detailed morphometrical analysis of nematocysts and other peculiar structures statistically supported the genetic distinction of some lineages. In other cases, the systematics of entire families was updated, thanks to phylogenetic assessments, description of previously unknown life stages, and discovery of new genera and species. Finally, certain taxa were investigated from an ecological point of view, describing their prevalence and preferences, and assessing their possible roles in the association in which they are involved. All together, these results suggest that the biodiversity of tropical symbiotic hydrozoans, as well as their ecological importance, are underestimated and require further attention.

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A REAL CAN OF WORMS: PSEUDOCRYPTIC DIVERSITY WITHIN THE *ARICIDEA CATHERINAE* (ANNELIDA, PARAONIDAE) SPECIES COMPLEX

Until recently, a large part of polychaete species were considered cosmopolitan and commonly reported from environmental monitoring programmes. However, studies employing fine morphological characterisation coupled with molecular markers highlighted the occurrence of cryptic and pseudocryptic species in a number of allegedly cosmopolitan species. The paraonid *Aricidea catherinae* Laubier, 1967, originally described from the Mediterranean Sea, has been reported from all over the world, but redescriptions often showed discrepancies with respect to the original description, suggesting that it might represent a species complex. Samples from several geographic areas was employed to test this hypothesis, and partial sequences of the genes coding for COI, 16S rDNA and 18S rDNA were analysed. Molecular data highlighted the occurrence of seven lineages within samples identified as *A. catherinae*. Among them, one lineage corresponds to *Aricidea catherinae sensu stricto*, another one corresponds to the Pacific *Aricidea eximia* Imajima, 1973, which was considered synonymous with *A. catherinae*, and the remaining five represent undescribed species. Our results suggest that *Aricidea catherinae s.s.* occurs only in the Mediterranean Sea, where two further pseudocryptic species were detected. Two undescribed species occur in the temperate and sub-arctic parts of the north-eastern Atlantic Ocean, respectively, whereas a third species in shallow environments of the north-western Atlantic. Different lineages are morphologically diagnosable based on the shape and length of the antenna and on fine characters of the modified neuropodial chaetae. At least Mediterranean lineages show different ecological requirements, especially with regard to the sediment grain. It is not unlikely, therefore, that a thorough re-examination of individuals identified as *A. catherinae* from other areas will show the occurrence of additional lineages. The lumping of different species within a single taxon is likely to reduce the resolution of environmental monitoring focusing on benthic assemblages, thus impinging the effectiveness of environmental management programmes; the resolution of species complexes, as well as the taxonomic revision of entire macrobenthic groups, and in a more general way a renewed effort on taxonomy, is expected to have an impact also on practical environmental issues.

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**AFTER SCILLA AND CARIDDI, ANOTHER UNCOMMON ANIMAL RECORDED IN
SICILIAN WATERS: THE DEEP-SEA CARNIVOROUS ASCIDIAN *DICOPIA*
ANTIRRHINUM C. MONNIOT, 1972**

Nowadays the knowledge about the Mediterranean deep-sea fauna is still scarce, especially concerning Ascidiacea. Unlike their shallow counterparts, deep-sea ascidians are rather uncommon to find. Visual technologies, such as Remotely Operated Vehicles (ROVs), represent a unique opportunity to observe several rare or uncommon species in their natural habitat. This study reports the occurrence of a rare deep-sea ascidian, *Dicopia antirrhinum* C. Monniot, 1972 (family Octanemidae), on the deep seabed off Aeolian Islands (Tyrrhenian Sea). This species was firstly found in 1975 off Malta (Sicily Channel) at 500 m depth, with only juvenile specimens. Afterwards, in 2011, few specimens of *D. antirrhinum* were observed and sampled in La Fonera canyon (Balearic Sea) at 1100 m depth. Our observation (23 specimens in total) represents the first record in Italian waters. In particular, *D. antirrhinum* was found at Eolo seamount (2 specimens; 760 m depth), at Filicudi bank (4 specimens; 643–870 m depth), off Secca del Capo (11 specimens; 568–761 m depth), around Lipari (1 specimen; 807 m depth) and off Stromboli (5 specimens; 662–812 m depth). The presence of macroscopic diagnostic characters, such as the body shape and the peculiar lip-like siphons, allowed the specific identification from ROV images. This unique morphology makes *D. antirrhinum* a macrophage carnivorous, adapted to cope with several feeding strategies in the deep-sea. This finding add one more tile to the still incomplete mosaic of distribution of this species in the Mediterranean Sea, extending its presence in the Tyrrhenian Sea. Deep-sea visual explorations are providing continuous new findings and information concerning this still little known environment and its increasingly rich fauna, being now far from the concept of a ‘desert of mud’.

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BIODIVERSITY AND DISTRIBUTION OF MEIOFAUNAL COMMUNITIES ALONG SOME REEF SLOPES OF MALDIVIAN ARCHIPELAGO (INDIAN OCEAN)

The importance of meiofauna in the marine ecosystem functioning has been documented in both temperate and tropical regions. After the 1998 bleaching episode, Maldivian reefs have been regarded as a vulnerable ecosystem that must be carefully monitored. Accordingly, an extensive investigation on meiofaunal status and distribution along the reef slopes of the Maldivian archipelago was carried out, taking into account position in the atolls, type of habitat (inner vs. outer slope), inclination and depth gradients.

Twenty-four taxa revealed the highest meiofaunal richness and diversity ever found in Maldives.

Nematoda, Copepoda and Polychaeta were the most abundant and frequent taxa in the study area, while Bivalvia, Gastropoda, Kinorhyncha, Isopoda, Cumacea, Amphipoda, Cnidaria, Thermosbenacea, Priapulida, Syncarida, Rotifera, Nemertea and Chaetognatha were found in half of the stations.

Noteworthy is the presence in the study area of taxa such as Thermosbaenacea and Syncarida considered until now as typical of freshwater ecosystems along with the discovery in our sediments of a taxon, Chaetognatha, considered to be exclusively planktonic.

The type of habitat, affected by different hydrodynamic conditions, was the main factor influencing the structure and diversity of meiofauna. Outer reefs showed the highest levels of diversity, confirming previous observations on the rate of coral reef growth and vitality and underlining the greatest vulnerability of the inner slopes. In contrast, the factor 'depth' significantly affected only the community structure, but not its density or diversity. Accordingly, community structure was more sensitive than abundance and diversity indices when it comes to detecting depth gradients. The 10° inclination of the inner slopes revealed the most different community structure with the highest dominance of nematodes and the lowest diversity levels.

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SYSTEMATICS AND PHYLOGEOGRAPHY OF *TIGRIOPUS FULVUS* (COPEPODA, HARPACTICOIDA, HARPACTICIDAE) IN THE MEDITERRANEAN SEA AND EASTERN ATLANTIC OCEAN

The copepod genus *Tigriopus* Norman, 1869 is worldwide distributed in coastal rock-pools and presently includes 12 valid species. In the Mediterranean and eastern Atlantic Ocean, the species *Tigriopus fulvus* (Fischer, 1860), its subspecies *T. f. adriaticus* Van Douwe, 1913 and *T. f. algericus* Monard, 1935, and possibly *Tigriopus minutus* Božić 1960 are reported to occur, but a sound revision of the group based on modern morphological and molecular standards is to date lacking.

We used phylogenetic and coalescence-based approaches to assess the diversity, distribution and phylogeography of *Tigriopus* populations throughout the study area, and to investigate the relationships among the aforementioned taxa, including in the analyses also a population of the closely-related North Atlantic *T. brevicornis* (Müller, 1776).

Our results indicated that all the studied Mediterranean and eastern Atlantic populations studied belong to *T. fulvus* s.s, and no support was found for the taxa of subspecific rank described for the species. Moreover, the high level of inter-populations mitochondrial DNA differentiation and the absence of shared haplotypes among different populations of *T. fulvus* revealed a pronounced molecular structuring on geographical basis even for small geographical distances.

***FISTULARIA COMMERSONII* RÜPPELL, 1838 (PISCES: FISTULARIIDAE) IN
THE SOUTHEASTERN COAST OF SICILY: HISTORY OF A LESSEPSIAN
MIGRANT EIGHT YEARS AFTER ITS FIRST RECORD**

The Bluespotted cornetfish, *Fistularia commersonii* Rüppell, 1838, is an Indo-Pacific species that has entered the Mediterranean Sea through the Suez Canal in 2000 (GOLANI, 2000). Three years after, a specimen was caught with trammel net in Lampedusa (AZZURRO *et al.*, 2004). In the subsequent years, the species undergoes rapid expansion in the whole basin (AZZURRO *et al.*, 2013). In 2010, two specimens were caught in the southeastern coast of Sicily (DEIDUN & GERMANÀ, 2011; record of the author FT published in ODDO, 2012). In 2011, in collaboration with Sicilian fishermen, a monitoring project (AlienFish) was started, in order to estimate the abundance and the increase of the species along the time. For each specimen caught, they were recorded: date, depth, fishing gear, location and bottom nature. During the years from 2011 to 2018, they were recorded a total of 472 specimens, in the area between Portopalo di Capo Passero (36.69672° N, 15.14884° E) and Siracusa (36.98838° N, 15.30634° E). Most of the catches occurred between October and March. All the specimens were caught with trammel nets, in the depth range 3–32 m, on mixed bottoms (sand and rocks), close to *Posidonia oceanica* meadows. Total length (56 specimens randomly measured) ranged from 67.8 to 122.3 cm. In the study period, a mean of 59 specimens per year were recorded. In the first year of study (2011) it was recorded the lowest number of specimens (22), while, between the years 2012 and 2017 (we excluded from analysis the 21 specimens of 2018 because of incomplete data collection), the registered catches showed no statistical differences in abundance ($X^2 = 2.14$ with 5 df, $p = 0.828$). This suggests that the species had become established in 2012. In conclusion, our data suggest that the species rapidly established itself in the south-east coast of Sicily. Although the species is considered overall rare by fishermen (usually no more than 2 specimens per boat were occasionally caught, and often only one), we should consider it as well-established and relatively abundant in the area. In Portopalo di Capo Passero, the species is also caught with trawl (Tiralongo, personal observation). Most of the adult specimens were caught during the cold months when they migrate close to the coast, as already noted in other studies (AZZURRO *et al.*, 2013 and references therein).

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ANCESTRAL CHARACTER-STATE RECONSTRUCTION OF *BACULUM* IN PRIMATES: WHY A CRITICAL LITERATURE REVIEW IS CRUCIAL

Selection pressures rapidly shape the anatomy of biological structures, usually affecting both their occurrence and form. This is the case of structures involved in copulatory system, as for example the penile bone (*i.e. baculum*), either detectable in the distal end of penis or covering its entire length. Recently, Schultz et al. (2016) reviewed this neglected topic attempting to map *baculum* evolution on mammalian phylogeny, using data from the literature. Among all species included in the analyses, there were also some primate species (N=102). However, they were not able to set the ancestral state of this character for the Primate order, probably due to both a low number of primate species included into the analysis, and several arbitrary choices in assessing some occurrence data. We accomplished a complete literature research and critical revision, collecting data about the occurrence of penile bone in 306 primate species. The resulting presence/absence binary matrix was mapped onto the most complete primate phylogeny by Springer et al. (2012) and counting 367 primate species. Starting from this species list, we matched our species list with tip labels of primate phylogeny by performing two analyses (1) including (N=367), and (2) excluding (N=255) *taxa* with missing data. By doing so we aimed at verifying whether a higher number of species could modify results about the ancestral state reconstruction of the character. No significative differences between analyses were found, rather a lower phylogenetic signal for the ancestral state of *baculum*. Main results show 8 independent losses of *baculum*, 2 of which need to be deepened: 1) in the Atelinae subfamily *baculum* losses observed in *Lagothrix* and *Ateles* genera might be interpreted as a phylogenetic parallelism (homoplasy); 2) in the genus *Tarsius*, only *T. syrichta* (Linnaeus, 1758) is reported to have a *baculum*, whereas remaining species apparently lost it, contrary to Schultz et al. (2016) who missed *T. syrichta*'s *baculum* presence, and therefore considered an absence of penile bone for the entire genus. Based on data analysed it appeared that the ancestor of primates had a *baculum*, which therefore is a symplesiomorphic trait for the entire order. Our study may finally clarify uncertainty reported by Schultz and colleagues, however further analyses including more primate species might help to disentangle both evolution and function of this neglected bone.

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BUTTERFLIES IN DIRE STRAITS – ECOLOGICAL CONSTRAINTS AND FUNCTIONAL TRAITS EXPLAIN HIGH GENETIC DIFFERENTIATION BETWEEN SICILY AND THE ITALIAN PENINSULA

Genetic lineages expand over continents following environmental changes, but when they meet they frequently form narrow areas of overlap. Understanding the mechanisms maintaining the boundaries between lineages requires relating genetic diversification, functional traits and dispersal constraints. We analysed the mitochondrial COI gene of 1249 representative specimens of the butterfly communities composed by 82 species which occur around the narrow Messina strait (3 km wide) separating Sicily from the Italian Peninsula. We compared them with 4680 specimens originating from the Palaearctic region. Forty percent of species showed a high genetic differentiation across the strait ($G_{st} > 0.5$) and 32.4% revealed Sicilian endemic lineages. Several Sicilian lineages (41.7%) had their closest relatives in distant Palaearctic areas, instead of the neighbouring Italian Peninsula, thus showing evidence for relict distributions. For each species, we evaluated functional traits determining species mobility, feeding ecology, phenology, eco-physiological constraints; moreover we evaluated ecological constraints calculated as dispersal costs based on the length of direct paths and on environmental permeability and as the impediment for dispersal represented by winds blowing over the strait during the flight period of each species. Phylogenetic regressions showed that haplotype diversity and non-standardized population differentiation (D_{st}) were mostly related to intrinsic phenological traits (length of flying period), while standardized differentiation (G_{st}) was also explained by local constraints (winds and climatic costs of dispersal). These results demonstrate that butterfly matriline do not easily cross and establish across the Messina strait but, given the unfavourable winds, only matrilines of more dispersive species did not evolve endemic variants probably because they frequently cross the strait and admix over it. These data confirm that strong genetic diversification and endemism can occur in mobile taxa even in the less isolated islands and that they are produced by a complex combination of ecological forces and historical events, acting differently on the species of a given community. Notably, we showed how understanding the pattern of genetic differentiation among two very close areas requires the use of different kinds of data (DNA sequences, climatic and occurrence data, species traits) and that the interpretation of some of these data also requires an assessment at a much larger scale.

Simposio 2

La valutazione della biodiversità a diversi livelli di organizzazione

Coordinatori

Maurizio Casiraghi, Adriana Giangrande

Sessione poster

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SIZE MATTERS: A TINY KEY ADDITION TO THE MEDITERRANEAN MARINE INVERTEBRATE FAUNA

Inconspicuous species are less attractive to public at large but – apparently – are overlooked also by scientific world. The distribution of entire groups of species seems influenced by sampling effort and by the availability of specific taxonomic expertise. The microscopic observation of seabed communities, however, may be rewarded by surprises. While looking for microscopic hydrozoans on samples from coralligenous habitats along the Salento peninsula (Ionian Sea, and Adriatic Sea, South Italy), colonies of a rare hemichordate group - Graptolithina pterobranchs – were found. This is a previously considered extinct taxon with an old history that goes back to more than 500 million years ago, in the Cambrian period. Indeed, morphological and molecular analyses disclosed *Rhabdopleura recondita* Beli, Cameron & Piraino 2018 from the Salento as a new species endemic of the Mediterranean Sea. Its colonies are few millimetres large and high, and each zooid member is less than a millimetre long and it is visible to the naked eye only as a tiny black dot. However, given their large distribution over the area, it is surprising how they have been overlooked so long. Morphological and genetic investigation revealed the existence of a living graptolite new species in the Mediterranean Sea. Graptolites are guide fossils known primarily for the traces left by their colonies, whereas very rare testimonies remain of fossil zooids, they are mainly unknown. The discovery of *R. recondita* in the Mediterranean Sea allows easy access to a group of organisms that few scientists in their life have had the opportunity to observe. The simplicity with which they can be found allows to study in detail their life and thus read the stone pages in the fossil record with a new key.

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ENTOMOFAUNA ON BURIED CARRIONS IN CALABRIA, SOUTHERN ITALY

Murderers often try to conceal evidence of their violent crimes. Several cases involving buried corpses have been reported in Italy but studies of insects associated to buried remains are still limited. A simulation of a case involving a buried human corpse was carried out using pig carcasses. Six pigs weighing 110 kg each were used: five carcasses were buried at a depth of 60 cm and one was left on the surface as control (“S”). In order to evaluate short- and long-term effects of burial on decay and insect succession, four of the five buried carcasses were excavated every 45 days and one of these was used as second control (“B”). Carcass B was then excavated every 10 days over the sampling period until its complete decay. Daily controls on insect presence were performed on carcass S and on the burial surface. Air and soil temperature, humidity and rain rates were recorded throughout the sampling period. Insects observed visiting the carcass S along the decay process were *Calliphora* spp, *Hydrotaea dentipes*, *Necrodes littoralis*, *Necrobia* spp, *Creophilus maxillosus*, *Dermestes frischii*. Diptera such as *Calliphora vomitoria* and *Musca domestica* were observed over the burial carcass B one day after the first excavation. At the time of second excavation (10 days later) *C. vicina* maggots and adults of *Philonthus* sp. were collected inside the ear of carcass B. At the time of eleventh excavation of carcass B, maggots of *Lucilia sericata*, *C. vomitoria*, *Hydrotaea aenescens*, *Hydrotaea capensis*, *Hydrotaea ignava*, *Muscina prolapsa* and *Fannia canicularis* and adults of *C. maxillosus*, *Hyster cadaverinus*, *Saprinus semistriatus*, *D. frischii*, *Tanatophilus rugosus*, *Necrobia violacea* and *Necrobia ruficollis* were collected. Since the beginning of the study, larvae of *H. aenescens*, *H. capensis*, *Muscina stabulans* and *F. canicularis* and adults of Staphylinidae Aleocharinae and Oxytelinae (*Platystethus* spp), *Margarinotus brunneus*, *S. semistriatus* and *D. frischii* were collected from day 109 to 171 on carcass B. At each excavation control, no insects on the grave and on other buried carcasses were collected. Comparing the results of buried and exposed carcasses, more species were collected on buried remains in comparison to exposed ones. This experiment provides the first data on insect colonization patterns of buried carcasses in Italy and indicates that burial depth (60 cm) and soil cohesion are relevant factors preventing the access of insects to the buried carcass.

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Soft bottom deep-sea sponges from the Central Mediterranean Sea: new data and perspectives of conservation

Deep-sea soft bottom are considered paucispecific unattractive habitat inhabited mainly by cryptic fossorials communities and, to a lesser extent, by macro and mega benthonic organisms with burrowing habitus. Unlike more appealing habitat such as those to Cold Water Corals, very few attention was placed on the investigation of the biological and ecological aspects of this ecosystem. As consequents, the inventory of its associate fauna, is still scant and fragmented, often generated almost incidentally as a by-product of scientific projects and Environmental Impact Assessments or by fisher trawling activities. In particular for the sponge fauna correlated to muddy/detritic bottoms, the unique information come from few studies restricted to limited spatial and temporal scales. The systematic collection of data on different species and their abundance through European MEDITS survey programme (International bottom trawl survey in the Mediterranean) provides a unique opportunity to gain more spatio-temporal detailed informations about benthic and demersal assemblages, included sponges. In the present study we utilized data generated through the MEDITS programme to characterize the deep sea sponge assemblages of Geographical Sub-Area (GSA) 18 (Adriatic Sea). Samples were collected during bottom trawl surveys undertaken from 2011 to 2016, at the bathymetric range between 70 – 540 m. We censused a total of 28 taxa all belonging to the class Demospongiae, shared mainly among the orders Poecilosclerida (21%) and Axinellida (14%). The most common species (*Rhizaxinella pyrifer*, *Suberites ficus* and *Thenia muricata*) are characteristic soft bottom dwellers, while the remaining sponge fauna seems to be linked to the presence of small patches of hard substrate scattered on these bottoms indicating a remarkably affect of the neighboring hard bottom biocoenoses on the fauna composition. Most of the species showed eurybathic distribution; only few were restricted to the bathyal zone. The most notable result was the finding of widespread *T. muricata/Desmacella annexa* grounds in the bathyal sector of the southern Adriatic Sea. Contrary to what was previously believed, our results highlights a conspicuous sponge assemblages associate to soft bottom, characterized by rare sponge-dominated systems of ecological and functional relevance. Our recommendation is to intensify the research efforts in order to develop a correct legislative framework for protection and conservation of this overlooked habitat.

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ANALISI FAUNISTICA E BIOGEOGRAFICA DELLE COMUNITÀ DI TARDIGRADI DI FORESTE NORVEGESI DI AREE PROTETTE

La conoscenza dei tardigradi norvegesi è scarsa e la loro diversità, distribuzione ed ecologia nelle foreste norvegesi è ancora sconosciuta. È stato quindi avviato un progetto di ricerca internazionale per studiare la diversità a tardigradi associata a svariati tipi di substrati raccolti in diverse tipologie di foreste norvegesi e per valutare l'impatto delle pratiche di gestione forestale sulla biodiversità per future politiche di conservazione.

Nel 2017 sono stati raccolti 300 campioni di licheni, briofite e lettiera di foglie in varie foreste di latifoglie e conifere di aree naturali protette localizzate nella Norvegia meridionale. I tardigradi (animali e uova) sono stati estratti da quantità standardizzate di campioni mediante vaglio a umido, raccolti con l'utilizzo di una micropipetta e osservati allo stereomicroscopio. Successivamente, gli esemplari estratti sono stati montati su vetrino in una goccia di Hoyer ed osservati al microscopio ottico (con interferenza e contrasto di fase, fino ai massimi ingrandimenti) allo scopo di individuare la specie grazie alla consultazione di chiavi dicotomiche. Inoltre, una parte degli esemplari è stata preparata (fissazione in etanolo, disidratazione e metallizzazione) per essere analizzata con il microscopio elettronico a scansione (SEM) al fine di effettuare un'analisi più approfondita dei caratteri tassonomici che consentono l'identificazione più accurata delle specie.

Le analisi preliminari hanno evidenziato differenze nell'abbondanza e nella composizione delle comunità a tardigradi tra i tipi di substrato, così come fra i tipi di foresta considerati. In alcune aree geografiche sono state infatti ritrovate comunità costituite da un numero limitato di esemplari, ma con una notevole diversità specifica, mentre in altre aree vi era una notevole abbondanza di esemplari, ma una bassa biodiversità. In generale, nei campioni di lettiera di foglie e licheni erano presenti meno tardigradi rispetto ai campioni di briofite, ma con una maggiore diversità di taxa. Nonostante lo studio dei campioni raccolti non sia ancora terminato, è stato possibile osservare che i substrati e i tipi di foresta campionati e analizzati ospitano comunità di tardigradi differenti.

ANDAMENTO A SCALA MILLENARIA DELLA BIODIVERSITA' NELLE COMUNITA' A SPUGNE DEL CORALLIGENO

Le biocostruzioni coralligene, dovute all'accumulo di alghe calcaree in ambienti scarsamente illuminati, sono tra le comunità bentoniche tipiche del Mediterraneo. Tali strutture hanno cominciato a svilupparsi circa 8000 anni fa, quando il livello del mare era 13-16 m inferiore rispetto ad oggi. Circa 2000 anni fa, il loro sviluppo sembra essersi sostanzialmente bloccato in diversi punti del Mediterraneo, per cause non ancora completamente chiarite. Con oltre 300 specie presenti, i poriferi sono oggi uno tra i gruppi più diversificati dell'intera comunità coralligena. Grazie all'analisi tassonomica delle spicole silicee intrappolate nei sedimenti che si accumulano nel tempo nelle cavità del conglomerato, è possibile valutare la variazione dinamica della ricchezza specifica di questa comunità durante l'intera storia evolutiva delle biocostruzioni.

Analisi condotte su diverse strutture coralligene in varie località del Mediterraneo, hanno mostrato una sostanziale stabilità temporale della comunità a spugne: la maggior parte delle spicole antiche osservate fanno parte di generi ancor oggi presenti nelle e sulle biocostruzioni. Un'importante eccezione è rappresentata da *Alveospongia*, un tempo diffusa in tutte le comunità studiate e oggi totalmente assente dall'intero bacino del Mediterraneo. Altri generi, un tempo variamente rappresentati, oggi sono riscontrabili solo in faune profonde come quelle legate ai coralli bianchi. Nonostante questa stabilità di base, la ricchezza della comunità a poriferi, mostra evidenti variazioni nel corso dei millenni che sembrano in accordo con le variazioni climatiche note per l'emisfero settentrionale. In particolare, i più elevati livelli di biodiversità si sono riscontrati durante l'optimum climatico dell'Olocene. L'andamento temporale mostra una repentina riduzione durante la crisi dell'età del bronzo per risalire ancora durante l'optimum dell'impero romano, tendendo ancora a ridursi durante la piccola glaciazione medioevale. Da un punto di vista spaziale, queste comunità sembrano siano state molto più simili tra loro nei millenni passati, quando tutte le biocostruzioni erano in una fase di crescita attiva, mentre oggi mostrano un maggior grado di differenziamento, probabilmente in relazione alle diverse condizioni di vitalità del coralligeno stesso.

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**DNA SEQUENCING AND PHYLOGENY OF AN
UNDESCRIBED *SALENTINELLA*
SPECIES (AMPHIPODA, SALENTINELLIDAE): CAVE ISOLATION AND IN
SITU SPECIATION?**

The species of the genus *Salentinella* Ruffo, 1947 (Amphipoda, Bogidiellida, Salentinellidae) are known from the Mediterranean region and generally were found in groundwater environments and cave habitats. In this study, we report on a new *Salentinella* species collected in 2016 and 2018 from the anchialine water of a cave, 85 m asl, in Peloponnese, Greece. The cave develops with a gallery long about 70 m and ends with a large circular lake 4,5 m depth (23° French, pH = 8.2), which is connected with the sea. Morphological analysis indicated specimens (ca. 1.6 mm) belonging to an undescribed *Salentinella* species, but related to *S. angelieri* Ruffo et Delamare–Deboutteville 1952. We carried out a molecular analysis of this putative new taxon using mitochondrial (mt) and nuclear gene sequences, cytochrome oxidase I (COI), and histone H3 (H3), respectively. For the first time in amphipod phylogeny, we deal with the Bayesian analysis of multiple molecular sequences from a *Salentinella* species. DNA sequences of mt and nuclear genes from other families of Bogidiellida and infraorders (Talitrida, Hadziida, Corophiida, Gammarida) of the suborder Senticaudata were included in the analysis. Evolutionary questions relative to anchialine cave isolation and in situ speciation are here discussed.

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**PHYSIOLOGICAL RESPONSE OF THE INVASIVE POLYCHAETE
BRANCHIOMMA BOHOLENSE (ANNELIDA: SABELLIDAE) TO OCEAN
ACIDIFICATION: RESULTS OF A TRANSPLANT EXPERIMENT AT A CO₂
VENT SYSTEM**

Branchiomma boholense is a sabellid polychaete considered highly invasive in the Mediterranean Sea as in few years it was able to expand its distribution range across the entire basin. Along the Italian coasts, this species has also been reported in venting areas near Castello Aragonese (Ischia Island) where CO₂ emissions decrease the seawater pH up to 6.0 units. The aim of this study was to test the physiological response of *B. boholense* to the decrease of seawater pH, measuring the activity of carbonic anhydrase (CA), a zinc metalloenzyme known to be affected by low pH conditions in other taxa. A transplant experiment in the CO₂ vents of Ischia was carried out. Worms were collected from a control area (normal pH) in the Mar Grande of Taranto and transplanted in two areas off Ischia: the control (~ 4 km from the vents) and the acidified zone of Castello. Three sites per area were utilized. Thirty individuals per site were exposed in plastic cages for 30 days. CA activity was measured electrometrically on the tissue homogenate of each specimen. Results of the ANOVA analysis on translocated worms showed no significant differences in CA activity either between individuals exposed to control and acidified conditions, nor among specimens transplanted in different sites within the same area. However, organisms exposed to high *p*CO₂ showed a decrease of their wet weight of about 20% compared to animals translocated in control sites. Moreover, CA activity was also investigated in specimens native from normal (Taranto) and acidified environments (Castello vents). The analysis of their wet weight revealed a marked decrease (~ 70%) in worms from the acidified area with respect to control animals. Interestingly, protein tissue content showed a significant (50%) increase in specimens native from the vents with respect to the Taranto area. Therefore, significant differences in CA activity (U/g wet weight) between specimens naturally exposed to normal and acidified conditions were also observed, with individuals living in acidified environments showing doubled values of enzymatic activity. The results of this study demonstrated that *B. boholense* is inclined to maintain a great homeostatic capacity when exposed to low pH. However, a marked decrease in body weight was observed under chronic exposure to high *p*CO₂, suggesting the need of further studies to understand if the maintenance of a homeostatic balance occurs at the energetic expense of other physiological processes.

THE DYING ART OF NAMING

Morphological description is the base of Taxonomy, the science of naming, describing and classifying organisms. A science that is losing its place in the scientific landscape today, because of the scarcity of resources and loss of expertise due both to retirements and lack of new generations training. The absence of grants specifically devoted to pure taxonomy compels many taxonomists to move their studies towards more “appealing” branches of science. An evidence of this decline is the low Impact Factor of taxonomic journals. This scenario is placed in a period of increasing of biodiversity loss and, consequently, when the necessity of expertise to assess and understand it is essential.

The loss of taxonomists is fast replaced by molecular taxonomy, a faster tool for species identification often considered the only valuable one, in spite of the contrasting results it yields. Therefore, most species remain undescribed, distancing and delaying the knowing of biodiversity.

A particularly niche field of study is larval biology. The knowledge of the morphology of developmental stages, which allow a just hatched larva to become a young adult, provides very important information about phylogeny, often not detectable in the adult. Among decapod crustaceans, the description of morphological characters of the larvae has confirmed or not the systematic position of species known as adult. Recently e.g., it has been confirmed with a clear evidence the separation of the Caridea *Periclimenes aegylios* Grippa & d'Udekem d'Acoz, 1996 from *P. sagittifer* (Norman, 1861), species separated only because of different adults coloration pattern: larvae of the two species show numerous and remarkable differences in every larval stage.

The importance of traditional taxonomy does not mean ignoring all the tools available to investigate biodiversity. A huge amount of alike but unidentifiable zoeae was found in planktonic samples collected in the Ligurian Sea: only through DNA barcoding the larvae were identified as belonging to the Brachyura *Palicus caronii* (Roux, 1830), known only as adult. The specimens collected are now under morphological description, in order to fill the literature gap.

Today the morphological description, in some cases supported by DNA barcoding, remains the irreplaceable tool to study crustacean larvae as regards identification, comparison among species, taxonomy position, environmental distribution and larval dispersal, a key element of the population dynamics.

WHO'S NEXT? NON-INDIGENOUS CNIDARIAN AND CTENOPHORAN SPECIES APPROACHING TO THE ITALIAN WATERS

The EC Regulation 1143/14 tackles the problem of invasive alien species (IAS) driving a coordinated effort across EU Member States. IAS can easily spread across borders but some of them can cause the most damage to native biodiversity, and for which concerted measures are required across the EU. The list of IAS of Union Concern (the Union list) contains 49 species so far, but none of them are marine. However, about 1000 marine non-indigenous species (NIS) are reported in the Mediterranean Sea, and over 60% out of them are established (i.e. reproducing) in the basin. The biodiversity of Cnidaria and Ctenophora (CC) of the Mediterranean Sea has changed considerably in the last two decades and 26 out of 89 Mediterranean CC NIS are present in Italian waters.

Most of the Mediterranean CC NIS (56%) are of Indo-Pacific origin, 38% of Atlantic origin, and the remaining 6% is classified as cryptogenic (uncertain origin). The main pathways of introduction in the basin are migration through the Suez Canal (38%) and the shipping (18%) with a small quota deriving from the aquaculture activities (3%). Uncertainties remain for many NIS (21%), whereas the remaining quota (20%) refers to natural expansion of distribution from the Atlantic waters. Some of the successful NIS also have the ability to remain dormant to survive adverse conditions, both seasonal and during long journeys into ballast waters.

Among CC IAS, the comb jelly *Mnemiopsis leidyi* A. Agassiz, 1865 and the jellyfish *Rhopilema nomadica* Galil, 1990 have been already reported in Italian waters. However, fourteen additional CC NIS, some equipped with high invasive potential, already established across different areas of the Mediterranean basin, should be regarded as good candidates to become future immigrants of Italian waters. Anticipatory NIS forecast based on biogeographical and eco-physiological analyses may provide a useful tool for a targeted management of the NIS/IAS issue and for the monitoring and assessment of the second descriptor of Good Environmental Status. Unfortunately, the lack of historical (i.e. long-term reference) databases, the difficulties inherent to regular conduction of biodiversity surveys, and the increasing paucity of taxonomists hinder our knowledge of NIS throughout the Mediterranean Sea.

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“WHO SEEKS, FINDS”: NEW RECORDS OF POLYCHAETE SPECIES FROM THE WESTERN MEDITERRANEAN BASIN (TYRRHENIAN SEA, ITALY)

Since the beginning of the 20th century, the Mediterranean Sea has been widely considered as a hot-spot of marine species diversity, with a uniquely high percentage of endemic species. The evaluation of such high levels of species biodiversity has been associated to different driving factors, including the geological history of the basin, its east-west orientation as well as higher levels of scientific investigations compared to other geographical area worldwide. However, despite years of studies, biodiversity data concerning the Mediterranean Sea are almost confined to coastal environment, and various regions remains poorly investigated. This still entails a lack of a complete knowledge on Mediterranean species biodiversity, especially to what concerns invertebrate taxa and in particular annelid polychaete species. In this study we report a list of 53 new polychaete records chiefly from the northern and central Tyrrhenian Sea and from the Ionian Sea, with the aim to integrate the checklist of polychaete species recorded in the Italian seas. Newly reported taxa identified belong to 20 polychaete families, among which the most represented in terms of new records were Syllidae (20.8%), Lumbrineridae (13.2%) and Spionidae (9.4%). Among the reported species, six (11.3%) are reported for the first time in the Mediterranean Sea, while another 15 (28.3%) are reported for the first time in Italian waters. All newly reported species are considered native, and their presence has been most likely overlooked due to the scarcity of studies and/or the uncertain taxonomy of several groups. This study contributes to integrate the previous knowledge on Mediterranean polychaete species reported in the “Checklist of polychaetes from the Italian coasts”, with the addition of 21 species new for the Italian Seas, allowing as well to re-assess the distribution of several species in the Mediterranean Sea.

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ASSESSING CONSERVATION STATUS OF THE MEDITERRANEAN SEAHORSES: DISTRIBUTION, POPULATIONS' CONSISTENCY, HABITAT CHOICE AND DIET PREFERENCES

The unique life history features of seahorses (low mobility, site fidelity, low fecundity, high parental care) make them vulnerable to the environmental changes and anthropic pressures. Despite increasing concern over their conservation status, many biological and ecological aspects remain poorly documented therefore disabling and limiting management strategies. Most of the Mediterranean populations are threatened by human activities and a recent document drawn up by the Italian Committee of IUCN for marine fish of the Italian coasts defined the two species as Near Threatened (NT). During the last 15 years, despite the absence of specific funding projects and thanks to the large group of volunteers working alongside researchers, a considerable research group has been created, allowing investigation of several aspects of seahorse life cycle in an area that has never been studied before. In this period, we have conducted several studies on their spatial and temporal persistence, population structure, habitat preferences, and diet composition.

Hippocampus guttulatus and *H. hippocampus* are two sympatric species distributed throughout the entire Mediterranean Sea. Thau lagoon was considered as the area with the highest seahorse density but, thanks to this research group, the importance of Taranto Mar Piccolo as a site of the extraordinary conservation interest has been highlighted.

Both species display patchy distribution and a high spatial demographic heterogeneity despite the fact that the most abundant populations are unbalanced in terms of size classes, presumably indicating problems in the local recruitment or due to the anomalous mortality.

They show a tendency for covered substrates (e.g. *Corallina* sp. and *Cladophora* sp. facies) offering complex and holdfast-rich microhabitats. Both species, however, can also exploit uncovered substrates as well as infralittoral habitats.

The diet shows strong differences in diversity and abundance of preyed taxa related to the habitat of occurrence. Overall, amphipods, copepods and isopods are the major contributors to the diet.

Although temporal persistence on a large scale was observed, indicating the capacity to resist and persist in time and space, seahorses are in a state of distress because of patchy distribution and unstructured populations. This, even more, highlights the need for suitable strategies to protect foraging habitats and ecosystems where there are numerous populations, like in Mar Piccolo of Taranto.

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THE HITCH-HIKERS OF THE SEA: FIRST RECORD OF THE NON-INDIGENOUS ASCIDIAN *SYMPLEGMA BRAKENHIELMI* (MICHAELSEN, 1904) IN THE WESTERN MEDITERRANEAN

Ascidians are important indicators of invasions, especially since the short-lived non-feeding larvae do not usually disperse very far from the parental area. The presence of only few ascidian-specialized taxonomists and the consequent inaccurate specific identification heavily limited the possibility to detect non-indigenous ascidians in the Mediterranean Sea. This study reports the presence of several colonies of the alien colonial ascidian *Symplegma brakenhielmi* (Michaelson, 1904) along the North-eastern coasts of Sardinia (Western Tyrrhenian Sea) and in the Gulf of Taranto (Northern Ionian Sea). Both areas are strongly influenced by human activities such as commercial shipping and import/export of marine species for aquaculture purposes, being all human-mediated pathways for the introduction of alien species. *S. brakenhielmi* has a pantropical distribution, having been recorded almost in all tropical region of both Atlantic and Pacific Oceans. Based on actual knowledge, the presence of this species in the Mediterranean Sea was limited to the eastern basin, along the coasts of Turkey, Lebanon and Israel. This study reports the first record of this species in the western Mediterranean Sea. In both the areas, i.e. off Olbia and off Taranto, the colonies presented two different colorations, the yellow and the red type, but morphological analysis and DNA barcoding analyses show that all these colonies belong to the same species. Species delimitation analyses for the subfamily Polyzoinae based on the COI (Cytochrome c Oxidase subunit I) barcode also suggest to further investigate a possible synonymy between the species *Symplegma brakenhielmi* and *Symplegma rubra*. The Target 5 of the EU Biodiversity Strategy (2011) states that European countries are committed to identifying Invasive Alien Species and their pathways by 2020, in order to develop management strategies able to control and prevent introduction and establishment of new invasive species. This study allows updating the knowledge about a Mediterranean non-indigenous ascidian, as well as the most likely invasion pathway driving the arrival and spread of these “aquaculture hitch-hikers”.

LA SPONGOFAUNA DEI FONDI A RODOLITI DELL'ISOLA DI USTICA (PA)

In Mediterraneo numerosi studi confermano l'importanza ecologica delle biocenosi associate ai letti a rodoliti, substrati biogeni considerati analoghi per valore ecologico alle foreste di kelp e alle praterie di fanerogame marine, e in grado come queste di ospitare una ricca fauna bentonica incrementando la biodiversità locale. Di recente, l'inserimento dei fondi a rodoliti nell'Allegato I della direttiva Habitat e l'attenzione riservata a questo ambiente dal Mediterranean Action Plan hanno fatto sì che l'interesse della comunità scientifica aumentasse progressivamente. In tale contesto questa indagine si è occupata di studiare il popolamento a poriferi associato ai letti a rodoliti circostanti l'isola di Ustica (PA), AMP dal 1986.

Ustica occupa una posizione privilegiata nel basso Tirreno lungo il flusso della Corrente Atlantica e i suoi fondali ospitano una grande ricchezza biologica. Il materiale oggetto di studio è stato campionato nel periodo 1995-97 in due stazioni localizzate rispettivamente al largo della costa occidentale e meridionale dell'isola, nelle zone B e C dell'AMP. I campioni, raccolti fra 70 e 100 m di profondità durante diverse campagne di pesca effettuate da operatori locali regolarmente autorizzati, sono stati rinvenuti intrappolati all'interno delle reti. Di seguito, nelle stesse aree di pesca sono stati realizzati transetti video subacquei la cui analisi ha fornito ulteriori dati.

Sono stati rinvenuti 71 esemplari, appartenenti a 25 taxa, 22 dei quali (88%) identificati a livello di specie. *Haliclona (Reniera) sp.*, è risultato il taxon più abbondante (14% degli esemplari campionati), insieme a *Jaspis johnstonii*, *Haliclona (Halichocona) fulva*, *Axinella cannabina*, *Siphonochalina coriacea* e *Hymerhabdia typica*, che costituiscono il 40% degli esemplari campionati. La stazione 1, soggetta ad un regime di protezione più severo, ha mostrato valori più elevati di ricchezza specifica rispetto alla 2 (24 e 14 taxa, rispettivamente). Va sottolineato che 12 dei 25 taxa individuati (48%) non sono mai stati segnalati su altri substrati dell'isola, e ben 19 (76%) costituiscono nuove segnalazioni per la check-list dei poriferi censiti su rodoliti in Mediterraneo, che in tal modo arriva a contare 87 specie. Le notevoli differenze riscontrate con i popolamenti di diversi siti del Mediterraneo Occidentale rimarcano come sia tuttora fondamentale ampliare le conoscenze sulle biocenosi associate ai fondi a rodoliti mediterranei.

I TAXA DEI CHIRONOMIDI COSTITUENTI GLI SCIAMI ATTRATTI DALLE LUCI ARTIFICIALI AL LAGO TRASIMENO

Negli ultimi anni si è assistito ad un eccezionale proliferare di Ditteri Chironomidi nell'area del Lago Trasimeno. Gli intensi sciame di questi insetti, attratti dalle luci artificiali, sono fonte di disagio per turisti e abitanti interferendo con le attività antropiche. Per far fronte a questo problema sono state messe in atto una serie di iniziative dai vari enti pubblici e recentemente è stato finanziato dalla Fondazione Brunello e Federica Cucinelli uno specifico progetto di ricerca per il contenimento dei popolamenti dei Chironomidi al Trasimeno. In anni precedenti (2007-2009), su ca. 41000 esemplari adulti monitorati in varie fonti luminose attorno al lago, si era osservato che le specie costituenti gli sciame attratti dalle luci sono essenzialmente *Chironomus plumosus* (67%), *Tanytus punctipennis* (22%) e *Procladius* sp. (probabilmente *P. choreus*, 9%). Scopo dello studio è di analizzare la consistenza di queste specie nei popolamenti larvali. A tale proposito, durante il 2017, è stata condotta un'indagine bentonica nell'area litorale (estesa 350 m e profonda 1,50 m) presso Castiglione del Lago attraverso l'uso di una draga manuale (14x8 cm) per bassi fondali a granulometria fine. Nel periodo primaverile-autunnale sono state esaminate ca. 4000 larve prelevate mediante 17 sopralluoghi (ognuno costituito da ca. 35 sub-campioni) che hanno permesso di identificare 14 taxa di Chironomidi e le relative abbondanze: *C. plumosus* (32%), *T. punctipennis* (22%), *Cladotanytus* sp. (11%), *Cryptochironomus* sp. (8%), *Procladius* sp. (7,5%), *Stictochironomus* sp. (6,3%), *Cladopelma* sp. (5,8%), *Microchironomus* sp. (4,5%), *Polypedilum* sp. (1,7%), *Dicrotendipes* sp. (0,7%), *Tanytus* sp. (0,5%), *Cryptotendipes* sp. (0,2%), *Pseudochironomus* sp. (0,2%), *Microtendipes* sp. (0,03%). I risultati dell'indagine mostrano che i 3 taxa responsabili degli intensi sciame attratti dalle luci ammontano al 61% delle larve rappresentando la componente predominante della comunità a Chironomidi. Inoltre, prendendo in considerazione solo questi taxa, si rileva una ripartizione delle popolazioni larvali simile alle popolazioni costituenti gli sciame, infatti *C. plumosus*, *T. punctipennis* e *Procladius* sp. nei popolamenti larvali ammontano rispettivamente al 66%, 25% e 9%. In conclusione, la composizione specifica degli sciame di Chironomidi attratti dalle luci è caratterizzata dalla struttura quali-quantitativa della comunità larvale a Chironomidi colonizzanti il Lago Trasimeno.

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OFF-SHORE CORALLIGENOUS BIOCECENOSIS ALONG THE IONIAN APULIAN COAST

The coralligenous is a unique tridimensional biogenic habitat representing a 'hot spot' of biodiversity in the Mediterranean Sea. The coralligenous biocoenosis up to 40m depth has received higher attention respect the mesophotic one, which remain still poorly studied. The present work investigates the coralligenous banks at 60m depth in front of Santa Caterina (Lecce) along the Ionic Apulian coast by technical scuba diving. During the survey, photos and videos were taken to characterize the coralligenous assemblages. The coralligenous is composed by several blocks laying on a horizontal substrate built by organogenous debris. The studied blocks were up to 2m high and, when characterised by an erect layer, it is dominated by *Paramuricea clavata* or by the gold coral *Savalia savaglia*. Where the *P. clavata* was absent, the octocoral *Eunicella cavolini* was more abundant. The analyses of the photos showed a quite similar assemblage both with and without the erect layer of big anthozoans, suggesting that the typical forest effect recorded in the shallower coralligenous is not detectable here. Anyway, here we found an unexpected abundance of the sea urchin *Centrostephanus longispinus* suggesting a key role of this species in deep coralligenous assemblages.

Fishing lines were occasionally found entangled among *P. clavata* and *S. savaglia* colonies. In these cases, epibiotic organisms settle on the bare skeletons. In the case of *S. savaglia* the bivalves *Neopycnodonte cochlear* and *Pteria hirundo* start to colonise the apical/middle portion of the colony, triggering the development of a complex epibiotic community. The analysis of the community settled on the shell aggregation showed a high biodiversity, composed by 112 species belonging to 7 phyla: Foraminifera (12), Porifera (15), Cnidaria (3), Anellidae (20), Briozoa (59), Brachiopoda (1), Crustacea (1) and Ascidiacea (1). The ascidian *Cystodytes* sp. was particularly abundant and among the bryozoan 4 new species were identified.

The incredible development of these epibiotic assemblages increased excessively the weight of the colonies branches leading, in some extreme case, to the detachment of the whole colonies. The present study gave new information about the mesophotic coralligenous habitats and described an interesting epibiosis involving the cnidarian *S. savaglia*. The analysis of the biodiversity at different scales showed an incredible number of species in this habitat including the discovery of new species.

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A FIRST ASSESSMENT OF THE MEIOBENTHIC COMMUNITY OF PUNTA CAMPANELLA (MARINE PROTECTED AREA), CAMPANIA, ITALY

Meiofauna represents the most abundant group among metazoans in marine benthos and it is considered a good biological indicator.

Punta Campanella is a Marine Protected Area in South Italy, which extends from the Gulf of Naples to the Gulf of Salerno, divided in three protection regimes (A, B, and C zones).

The strategic position and the favorable climate allow the formation of an ideal habitat for a huge variety of animal and vegetal species, however there are no data about the meiobenthic community composition of this area. The purpose of this study is to investigate, for the first time the meiofaunal communities inhabiting the coastal sediments of the Marine Protected Area of Punta Campanella.

Samples from the three protection regimes, with three replicates for each of them, have been collected during a spring-summer campaign (May - June, 2016). In order to analyze the meiofauna community structure and abundance we identified and counted individuals, to evaluate the biodiversity or pollution indexes (i.e Nematoda/Copepoda ratio) of the area.

Results show a rich community structure: a total of 19 taxa in the zone A, 16 in the zone B and 17 in the zone C.

Nematodes and Copepods are the dominant taxa and their abundance is inversely proportional. Nematodes show a positive trend, increasing from the zone A to the zone C. Opposite situation happens for the Copepods, which are the most abundant in the zone A and decrease in the zone C.

Total meiofauna abundance characterizing the zone A ($6,410 \pm 1,195$ ind/10 cm²) is extremely high, also zone B ($3,474 \pm 195$ ind/10 cm²) and zone C ($6,833 \pm 427$ ind/10 cm²) show a high number of individuals.

This study shows that Punta Campanella hosts a rich and copious diversified meiobenthic community. There is a higher level of biodiversity and meiofaunal individuals in the more protected zone A, of the Marine Area. However, also the other two zones B and C show a good level of biodiversity, indicating the good management of the whole area.

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A PRELIMINARY MAP OF THREATS TO BREEDING POPULATION OF KENTISH PLOVER *CHARADRIUS ALEXANDRINUS* IN THE GULF OF GELA

Direct threats are the proximate human activities or processes that have caused, are causing, or may cause the destruction, degradation and/or impairment of biodiversity targets (SALAFSKY *et al.*, 2008). The Kentish plover *Charadrius alexandrinus* L., 1758 is a shorebird breeder in coastal areas of W and S Europe, threatened by the disturbance of coastal habitats (LAFFERTY *et al.*, 2006), the degradation and loss of wetland through environmental pollution (KELIN and QIANG, 2006), land reclamation (DEL HOYO *et al.*, 1996; BARTER, 2006), urbanisation (DEL HOYO *et al.*, 1996) and reduction of coastal sediment (BARTER, 2006). From 2016 to 2018 we collected data at the Gulf of Gela, between Randello beach and Licata's port, Southern Sicily. In the period recommended by CNCF (Italian Committee for the Conservation of the Kentish Plover) a census of the breeding population on 63 1x1 UTM squares was carried out; the presence/absence of threats was noted during 3 years of census (from 0 = never observed to 3 = observed each year). An overall threat score was calculated for each 1x1 km square. In order to explore spatial patterns of threat we matched our threat scores to species distribution data for the Kentish Plover: a) nest-site fidelity on a 3-year scale (from 0 = never observed to 3 = observed each year); b) max. No. of breeding pairs in that square (0= none; 1= 1 pair; 2= 2 pairs; etc.). The data are divided in class of value using Sturges rule (GERARDI and SILVA, 1981). Through the data of matrix is generated a colour-coded map of geographical variation in threats to the Kentish plover in order to define areas where priority conservation measures are needed. The 19% of 1x1 squares are included in a threatened category, which indicate an increasing risk of extinction in the short or medium term (the criteria adopted are similar to the IUCN but in a local scale). Even the 27% of squares is in Least Concern. Thanks to the threat map it is possible to set some localized direct strategies. This map will enable to tally the frequency of threats or actions across projects at various organizational or spatial scales to help set priorities and allocate resources.

The European project Life CHOO-NA! by LIPU is training 310 young people to save 11 bird species and their habitats. Kentish Plover is one of these biodiversity targets since the breeding population of the Gulf of Gela is 30-50 pairs and it represents one of the most important and numerous in Sicily (ZAFARANA, 2017).

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POLYP EXPANSION IN PASSIVE SUSPENSION FEEDERS: THE RED CORAL (*CORALLIUM RUBRUM*) CASE STUDY

Climate variability will change physiological, energetic, and ecological trends in most passive suspension feeders. Heterotrophic octocorals obtain their food from dissolved organic matter, detrital particles, and zooplankton, transported by water movement. One of the most neglected issues in the trophic ecology of these cnidarians is the polyp expansion. In these organisms, the polyp expansion is related to several environmental and biological factors such as water current, temperature and food availability. The Mediterranean octocoral *Corallium rubrum* polyp expansion was investigated as a case study, following two approaches: 1) high frequency in-situ observations (five days, four times per day) testing different environmental and biological variables of the water column, and 2) laboratory video-recording flume controlled experiments in different environmental-biological conditions (i.e., water temperature, flow speed, and nutritional stimuli). On the field, the polyp expansion of *C. rubrum* was related to changes in particle composition (seston and zooplankton) and water movement. Laboratory experiments showed differences in the polyp expansion not only due to the environmental changes (water temperature and current speed), but mostly in response to nutritional stimuli. Food availability and water movement appeared to be crucial factors controlling polyp expansion. The results suggest that the energy budget of passive suspension feeders (and probably for the overall benthic community) may rely on their ability to maximize prey capture during food pulses, described as discontinuous seston availability for suspension feeders. These non-continuous food input may be the key to better understand benthic-pelagic coupling processes and trophic impact in the marine animal forests, composed by sessile suspension feeders.

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A BIOGEOGRAPHIC ANALYSIS OF LOSS OF PLANKTOTROPHY IN CAENOGASTROPOD MOLLUSCS (GASTROPODA, NASSARIIDAE)

In marine invertebrates the larval development is a key feature for evolution and ecology of species. In planktotrophic development (P), the larvae spend from a few days up to one year in the plankton feeding actively. In non-planktotrophic development (NP), larvae spend very little or no time in the plankton feeding almost exclusively on yolk supplies. In the Caenogastropoda, NP is mostly considered as a derived condition that arises in response to conditions that counterselect P, allowing independence from trophic environmental availability. It is suggested that NP represents an advantage in phytoplankton-poor regions as the Mediterranean Sea, in areas where food availability is strictly limited by seasonality as in Antarctica, or in response to major environmental changes occurred in the past.

We have tried to detect and analyse, in a phylogenetic framework, the distribution of events of loss of planktotrophy in a group of marine gastropods, aiming at identifying eco-evolutionary patterns. We used a comprehensive robust phylogeny of the family Nassariidae (Buccinoidea) to identify pairs of sibling species, or group of species, that differ in larval development thus representing independent losses of P in the tree. The fossil-calibrated phylogeny allowed dating events of loss of P by using a relaxed molecular clock model. We found at least 15 P-NP switches in the Nassariidae and most of them were dated to the Miocene (23-10 Mya) and the Pliocene (4-2 Mya), and most occurred in Caribbean bioregion. Statistical analyses were performed to evaluate the presence of significant variation differences between different geological epochs and between different bioregions. Then a plausible paleoceanographic scenarios were reconstructed to explain the environmental conditions that may have favored the loss of planktotrophy.

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OCCURRENCE OF *BOTRYLLUS SCHLOSSERI* “SPECIES COMPLEX” (ASCIDIACEA) ALONG THE ITALIAN COASTS

The colonial ascidian *Botryllus schlosseri* is a model system for processes such as development, aging, and regeneration. In 2012 Bock et al suggested that this is a “species complex” consisting of five genetically divergent clades, named from A to E, each corresponding to a cryptic species. The geographic range of these clades, although biased by an uneven sampling around the world, shows that clades A and E are widespread, globally and only along the European coasts, respectively, while clades B, C and D are geographically restricted. Indeed, so far clade B was found only in Vilanova (Balearic Sea, Western Mediterranean); clade C in Vilanova and Fornelos (Galizia, NorthEast Atlantic); clade D in Fornelos and the English Channel. These clades were identified using the standard “COI DNA barcode”, i.e., a 600 bp-region of the mitochondrial COI gene, however the entire mitogenome (mtDNA) suggests the existence of further cryptic species within clade A.

Given the usage of *B. schlosseri* as model system, additional data on the geographic range, the morphology and the taxonomic status of these clades are required.

Here we have molecularly characterized 77 colonies of *B. schlosseri* accidentally collected since 2013 in the North and South Adriatic Sea and in the Ionian Sea (Mediterranean Sea). As barcode, we sequenced a longest COI region of 860 bp (860-COI) in order to attempt to increase the phylogenetic signal for the downstream analyses. As expected, most of the collected colonies belong to clade A but, surprisingly, we also found: 9 clade E and 2 clade C colonies in the Venice Lagoon; 2 clade E colonies in the South Adriatic and 2 in the Ionian Sea; 9 clade D colonies in the South Adriatic Sea. This is the first report of the presence along the Italian coasts of the rare C and D clades, and of the “European” clade E: given the non-systematic nature of our sampling, we hypothesize an even wider distribution of these clades in Italy. Remarkably, phylogenetic reconstructions with our 860-COI discriminate two groups within clade A, roughly corresponding to those identified by the entire mtDNA: most Italian colonies belong to group A2, to which also belongs the recently published *B. schlosseri* neotype (Brunetti et al 2017), while the published nuclear genome of a Californian colony belongs to group A1.

Our results underline the need of further studies on the distribution, the morphology and the genetic features of the various *B. schlosseri* clades.

ISOPOD (CRUSTACEA: EUMALACOSTRACA) AND ARGULOID (CRUSTACEA: BRANCHIURA) PARASITES OF FISH AND CEPHALOPODS FROM CENTRAL MEDITERRANEAN SEA

Parasitic crustaceans belonging to the orders Isopoda and Arguloidea are known to affect wild and farmed fish in freshwater, brackish and marine environments. In aquaculture, infections are responsible for severe losses as a result of inadequate growth and mortality.

Information regarding the distribution of parasitic isopods and arguloids in wild marine fauna is still scarce and fragmentary, therefore the present work is aimed at characterizing their distribution in different fish and cephalopod species from a previously uninvestigated area of the Mediterranean Sea.

Specimens of marine fish and cephalopods were caught by trammel nets in the Marine Protected Area of Porto Cesareo (Ionian Sea, Southern Italy) and examined for the presence of isopod and arguloid parasites, with particular attention to their site of attachment on the host.

Based on morphological analyses of parasitic stages we compiled a list of host-parasite associations, including species of isopods belonging to several genera (chiefly *Aega*, *Anilocra*, *Ceratothoa*, *Emetha* and *Nerocila*) and one species of arguloid in 14 species of fish hosts (*Bothus podas*, *Diplodus annularis*, *D. vulgaris*, *Epinephelus costae*, *Mullus surmuletus*, *Pagellus erythrinus*, *Serranus scriba*, *Scorpaena notata*, *S. porcus*, *S. scrofa*, *Spicara maena*, *Symphodus tinca*, *Trigloporus lastoviza*, *Xyrichthys novacula*) and one species of cephalopod host (*Octopus vulgaris*).

Our results provide information on the distribution of cymothoid species in demersal fish from Mediterranean Sea and include records of new host-parasite associations. Moreover we report a rare occurrence of isopod infection in cephalopods. Collected data also suggest a low host specificity of cymothoid species in demersal fish fauna.

OSSERVAZIONI SULLA COMUNITÀ ITTICA PRESENTE ALLA FOCE DEL FIUME CHIDRO (RISERVA NATURALE REGIONALE ORIENTATA DEL LITORALE TARANTINO ORIENTALE – TA)

Il Fiume Chidro è un breve corso d'acqua alimentato da sorgenti carsiche che sfocia sul versante ionico salentino dopo un percorso di circa 300 m. In corrispondenza delle sorgenti vi sono due specchi d'acqua denominati “vasca grande” e “vasca piccola”, profondi rispettivamente 12 e 8 m, che formano un habitat caratteristico, unico a livello regionale. A parte sporadiche informazioni sulle principali specie vegetali igrofile presenti, non sono disponibili in letteratura dati sulla fauna acquatica del bacino. Con l'intento di contribuire a colmare tale gap, è stato condotto uno studio della fauna ittica presente nella foce del Chidro, mediante censimento visivo subacqueo (Underwater Visual Census, UVC) supportato da riprese video ad alta definizione (High-Definition, HD). I campionamenti sono stati condotti in immersione con autorespiratore ad aria, durante la stagione primaverile ed estiva in entrambe le vasche. Sono state individuate cinque stazioni ad intervalli batimetrici di quattro metri. In ogni stazione sono state registrate presenza ed abbondanza delle specie ittiche. Sono state complessivamente rinvenute cinque specie di pesci eurialini: l'anguilla europea *Anguilla anguilla* (Linnaeus, 1758), il latterino *Atherina boyeri* (Risso, 1810), la spigola *Dicentrarchus labrax* (Linnaeus, 1758), il cefalo comune *Mugil cephalus* (Linnaeus, 1758) e la bavosa di fiume *Salaria fluviatilis* (Asso, 1801). Tra queste, *A. anguilla* rientra nella categoria Critically Endangered (CR) della Lista Rossa delle specie minacciate IUCN (International Union for Conservation of Nature) sia a livello italiano che globale, mentre *S. fluviatilis* rientra nella categoria Least Concern (LC) a livello globale, ma Data Deficient (DD) a livello italiano. Dall'analisi dei risultati ottenuti, non sono state osservate differenze nella composizione in specie tra le due vasche. Le specie più abbondanti sono risultate *A. boyeri*, *M. cephalus* e *D. labrax*. Inoltre, per queste ultime è stato osservato un aumento dell'abbondanza con la profondità. In particolare, *A. boyeri* è risultata la specie più abbondante raggiungendo valori di oltre 200 individui durante la stagione estiva. *S. fluviatilis* e *A. anguilla* sono state osservate occasionalmente sia in primavera sia in estate, probabilmente anche in relazione al loro comportamento criptico ed alle abitudini spiccatamente notturne di *A. anguilla*.

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CARATTERIZZAZIONE MORFOMETRICA DI DIVERSE POPOLAZIONI DI *ACERENTOMON ITALICUM* (PROTURA: ACERENTOMIDAE)

Acerentomon italicum Nosek, 1969 è specie molto comune nell'ambito della proturofauna italiana, diffusa in Italia settentrionale, Toscana, Svizzera, Austria, Corsica e Slovenia. In un recente lavoro di ridescrizione della specie (Galli et al, 2016) è emersa una piccola, ma potenzialmente significativa differenza a livello di protassi tra due blocchi di popolazioni: quello delle Alpi Centro-Orientali (Svizzera, Austria, Lombardia nord-orientale e Alto Adige) e quello relativo alla restante parte dell'areale (includente la località tipica – Colli Euganei, PD, Veneto). Contestualmente, il DNA barcoding evidenziava una rilevante distanza tra tre popolazioni liguri (secondo blocco) ed una austriaca (primo blocco). Detti elementi hanno spinto gli autori ad approfondire la questione, cercando di individuare ulteriori caratteristiche distintive tra le popolazioni delle due aree sulla base di analisi morfometriche mirate e attraverso un approccio di morfometria geometrica assolutamente innovativo per i Proturi. Dall'analisi di 54 esemplari adulti di entrambi i blocchi sono emerse differenze significative sia in termini di morfometrie tradizionali che geometriche tali da ipotizzare l'esistenza di due specie differenti.

Simposio 3

Le aree naturali protette per la gestione e protezione della fauna

Coordinatori

Marzio Zapparoli, Genuario Belmonte

Relazioni ad invito
(keynote lecture)

GIAMPIERO SAMMURI

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LE AREE PROTETTE PER LA GESTIONE E PROTEZIONE DELLA FAUNA

In Italia oggi ci sono 24 parchi nazionali, 134 parchi regionali, 147 riserve naturali statali e 365 regionali e 27 aree marine protette oltre al santuario dei cetacei Pelagos. La superficie protetta a terra copre 31.635 kmq a terra pari al 10,5 % del territorio nazionale e 2.955 Kmq a mare, (escludendo il Santuario Pelagos). Inoltre le aree “Natura 2000” (SIC, ZPS, ZSC) hanno complessivamente una superficie a terra di 58.368 kmq, dei quali 30.963 esterni alle aree protette. Sommando quest’ultima superficie a quella delle aree protette abbiamo 62.598 Kmq destinati alla conservazione della biodiversità pari al 20,78% del territorio nazionale, un quinto del totale.

Però una problematica evidente è che non tutta questa importante superficie è gestita nello stesso modo ed anzi ci sono aree protette e ancora di più aree Natura 2000 che esistono sulla carta.

Nonostante questi limiti le aree protette hanno contribuito alla conservazione di numerose specie animali e vegetali e il fatto che l’Italia sia il primo paese europeo per numero di specie animali e vegetali, oltre che alla indubbia varietà morfologica e climatica del nostro paese, è dovuto anche al lavoro di conservazione svolto negli anni.

Bisogna però riconoscere che non sempre il lavoro delle aree protette italiane si concentra sui taxa più meritevoli di azione di conservazione: un contributo in questo senso possono dare le liste rosse IUCN per l’Italia redatte per alcuni gruppi da Federparchi per conto del Ministero dell’Ambiente. È uno strumento opportuno per meglio indirizzare la gestione dei parchi e delle aree natura 2000.

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FROM MARINE PROTECTED AREAS TO NETWORKS OF MPAs

Biodiversity protection and conservation are the first and most important objectives of Marine Protected Areas. The institution of MPAs is based on the distribution patterns of important benthic habitats and obeys an exquisitely terrestrial perspective. In the marine domain, in fact, space is not measured as a surface: space is a volume, and the water column is the most widespread habitat of the planet. The processes that take place in the water column are the main drivers of the biodiversity distribution patterns also in the benthos and, in the last decade, it became apparent that effective marine conservation must link patterns to processes, in a three dimensional space. The dynamics of marine ecosystem functioning, furthermore, is mostly based on plankton processes and, hence, a fourth dimension (time) is also to be considered. Marine systems, in fact, are highly dynamic and it is rarely the case that they maintain a set of features for a long time, especially in a period of global change as the present one. The shift from MPAs to MPA Networks requires the identification of coherent management and conservation units in which Networks are to be nested. Connectivity is the main feature that allows for the identification of such units.

Contrary to terrestrial systems, where vegetation plays a structural role in defining habitats, and animals play an exquisitely functional role, in marine systems animals are play important structural and functional roles. Sessile animals, in fact, have the same structural functions of land plants, and many are even photosynthetic due to the presence of symbiotic algae. Animals are the only visible portion of biodiversity in the water column!

Biodiversity and ecosystem functioning are the pillars of Good Environmental Status in the Marine Strategy Framework Directive of the European Union and zoology has an important role in defining the structure of biodiversity and its role in ecosystem functioning, translating basic knowledge into management options leading to Maritime Spatial Planning, Integrated Coastal Zone Management, and the application of the Ecosystem Approach.

Simposio 3

Le aree naturali protette per la gestione e protezione della fauna

Coordinatori

Marzio Zapparoli, Genuario Belmonte

Comunicazioni orali

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THE ITALIAN BUTTERFLY MONITORING SCHEME: AN OPPORTUNITY FOR BIODIVERSITY CONSERVATION

The European Butterfly Monitoring Scheme (eBMS) is a partnership to promote and develop butterfly monitoring through a representative network across Europe aiming to:

- improve butterfly conservation
- produce an effective database to set up a suite of indicators
- provide tools to estimate supra-national population trends
- develop an online data entry system to grow the network - allowing new Countries to submit data efficiently and in a standard format.

Since butterflies and their habitats are increasingly threatened by multiple anthropogenic pressures and conservation needs sound evidence to protect and mitigate impacts, Butterfly Monitoring has proven to be highly cost effective, with impact for research and conservation. 14 European Countries have already joined the eBMS and more than 6000 transects are walked every year across Europe. Projections, like Grassland butterfly indicator, are available thanks to monitoring schemes (van Swaay & van Strien 2005). Italy hosts 2/3 of European butterfly species and 60% of protected species, yet no butterfly monitoring scheme has never been conducted. Thanks to a partnership agreement (Consortium) among Europarc, University of Turin, University of Florence and Crea Research Centre of Forestry and Wood of Calabria, Italy is building up, for the first time, the Italian BMS (iBMS). Europarc has already supported the Italian Butterfly Red List project (Bonelli et al. 2018), and the same group of researchers is also responsible for the Barcoding of Italian Butterflies (www.barcodingitalianbutterflies.eu).

To ensure the long-term persistence and sustainability of iBMS, all the 23 National Protected Areas will be involved and coordinated by Europarc. Each Protected Area will be responsible of a 500m-long transect where butterflies will be monitored twice per month by rangers or volunteer citizens, according to the Pollard method. Several regional Parks have already joined the project and others expressed their intention to participate. The Consortium for iBMS will (i) develop an input system for transect data following the European guidelines, (ii) organize local training on butterfly identification, (iii) collect detailed spatial information and habitat categorization for transects, (iv) validate the data collected. In addition, the Consortium established a formal data sharing agreement with existing European monitoring schemes to clarify intellectual property and access-to-data rights.

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HOST PLANT SELECTION AND DIFFERENTIAL SURVIVAL ON TWO ARISTOLOCHIA L. SPECIES IN AN INSULAR POPULATION OF *ZERYNTHIA CASSANDRA* GEYER (LEPIDOPTERA, PAPILIONIDAE)

Understanding host plant preference and the relative quality of resource provided by co-occurring host plants is a key step to predict butterfly species abundance and responses to environmental changes, and, consequently, to plan management measures. *Zerynthia polyxena* is an European butterfly included in the annex IV of Habitat Directive since its habitat, where the host plant *Aristolochia* spp. occurs, is declining. The populations inhabiting the Italian peninsula represent an endemic species, *Zerynthia cassandra*. The insular population occurring on Elba island (Tuscan Archipelago), is highly threatened, and has two host plant species: *Aristolochia rotunda* and *A. lutea*. In 2017, we carried out field surveys and rearing experiments to i) identify the characteristics of the host plants (vegetative status) and the site characteristics (aspect, irradiation, distance from other patches) correlated with the number of eggs occurring on individual plants, ii) compare larval growth, food-conversions rate and larval and adult survivorship on the two host plants species. Egg occurrence depends on patch irradiation, the number of leaves and flowers occurring on individual plants and the occurrence of nearby patches. These findings allowed to identify the optimal *Aristolochia* patch features for egg laying and development. Laboratory rearing success was higher than 50% and although plant species did not show a significant effect on oviposition, we found that larval and adult survival was higher on *A. rotunda*. Our results suggest habitat management plans aimed at increasing resource availability for *Z. cassandra* and possible ex-situ conservation actions aimed at recovering the population after possible catastrophic events.

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**MONITORAGGIO SPECIE IN DIRETTIVA HABITAT E PROTOCOLLO
CMR: UN CASO DI STUDIO SU *COENAGRION MERCURIALE*
(CHARPENTIER, 1840)**

Coenagrion mercuriale è uno dei cinque membri del genere *Coenagrion* presenti in Italia Centrale ed è una delle 9 specie di odonati italiani inclusi negli allegati della Direttiva Habitat (DH). Queste specie, ai sensi dell'art. 11 della DH, richiedono che venga effettuato un monitoraggio periodico delle popolazioni al fine di valutare il loro stato di conservazione e l'efficacia delle misure di conservazione adottate nelle aree di presenza. Scopo del lavoro è stato quello di sperimentare sul campo la metodologia di cattura marcatura e ricattura (CMR) su questa specie, al fine di valutare la dimensione della popolazione, stimare i parametri di catturabilità e sopravvivenza e definirne lo stato di conservazione. Le indagini sono state eseguite nei mesi di giugno e luglio 2017 in un piccolo corpo idrico soleggiato e con ricca vegetazione dell'Italia Centrale, in una zona di confine tra la regione biogeografica mediterranea, dove la specie di trova in uno stato di conservazione favorevole (FV), e la regione continentale dove lo stato di conservazione è sfavorevole-inadeguato (U1). Complessivamente sono stati catturati e marcati 1296 esemplari, 1/4 dei quali sono stati ricatturati nel corso delle 12 sessioni di attività. Per la stima dei parametri di popolazione è stata utilizzata la metodologia POPAN e sono stati testati 7 diversi modelli in condizioni costanti e variabili nel tempo, successivamente confrontati con tecnica AICc. È stato riscontrato un significativo bias della *sex ratio* a favore dei maschi (2.88:1), con un coefficiente di catturabilità medio dei maschi doppio rispetto a quello delle femmine. Il miglior modello estratto ha evidenziato tuttavia una probabilità di sopravvivenza giornaliera maggiore nelle femmine rispetto ai maschi. Il volume stimato della popolazione supera i 3500 esemplari con una dimensione media giornaliera di circa 450 individui. Questi valori sono molto superiori a quanto precedentemente ipotizzato attraverso indagini eseguite con il metodo del transetto lineare. Dai risultati della sperimentazione è stato inoltre possibile desumere la progressione della stima della popolazione e valutarne le implicazioni ai fini della programmazione del numero di visite da effettuare per l'ottimizzazione dello sforzo di campionamento.

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MONITORAGGIO DI *PATELLA FERRUGINEA* NELL'AREA MARINA PROTETTA DI TAVOLARA-PUNTA CODA CAVALLO: LE BASI PER UN PIANO DI GESTIONE E RIPOPOLAMENTO DI ALTRE AREE

Obiettivo dello studio è stato quello di analizzare la variabilità genetica della specie protetta e a rischio di estinzione *Patella ferruginea* Gmelin, 1791 (Mollusca: Gastropoda) all'interno dell'Area Marina Protetta di Tavolara-Punta Coda Cavallo, per valutarne lo status di conservazione e fornire le basi per un ripopolamento mirato legato al progetto Re-Life (Re-establishment of *Patella ferruginea* in Liguria). Basandoci sui risultati di un nostro precedente monitoraggio di *P. ferruginea* (censimento e analisi genetiche) nell'AMP (2013), è stata individuata la migliore strategia di campionamento degli individui da utilizzare come fondatori per massimizzare la diversità genetica nei siti di ricezione. Sono state identificate come aree donatrici le isole di Molarà e Molarotto, che ospitano circa il 95% di tutti gli individui della AMP. Sono state scelte due diverse classi di taglia (<3 cm e >3 cm), stabilite adattando la taglia media degli individui presenti nell'AMP alle taglie proposte in letteratura, in accordo con l'obiettivo principale del progetto. Mediante le analisi eseguite con 8 marcatori microsatelliti sono state stimate le dimensioni effettive della popolazione e la presenza di divergenza genetica tra le due classi. Sono stati genotipizzati 200 individui di *P. ferruginea*, suddivisi fra le due classi di taglia, i cui tessuti sono stati prelevati attraverso un metodo non letale da noi standardizzato e autorizzato dal Ministero dell'Ambiente. I risultati ottenuti suggeriscono che non vi è una differenza significativa nella variabilità genetica tra le due classi di taglia. Perciò la scelta degli individui da prelevare deve essere basata sulla numerosità di *P. ferruginea* nelle microaree di raccolta e sulla collocazione degli individui che meglio si prestano alla manipolazione legata al distacco dal substrato. Dalle analisi è emerso che 100 individui rappresentano un numero sufficiente di fondatori da utilizzare a fini di ripopolamento per garantire livelli di diversità genetica adeguati nell'area ricevente. Infine si conferma l'assenza di barriere di dispersione all'interno dell'AMP di Tavolara-Punta Coda Cavallo. In conclusione l'AMP sembra svolgere un ruolo di protezione efficace per la salvaguardia della specie. Questi risultati indicano inoltre quanto sia importante non solo il monitoraggio numerico ma anche il monitoraggio genetico nella pianificazione della gestione e nei progetti di reintroduzione di *P. ferruginea*.

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Le aree naturali protette per la gestione e protezione della fauna

Coordinatori

Marzio Zapparoli, Genuario Belmonte

Sessione Poster

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FEEDING CHOICES OF TWO SAPROXYLIC BEETLES *CUCUJUS CINNABERINUS* AND *C. HAEMATODES* (COLEOPTERA, CUCUJIDAE) LARVAE IN LABORATORY CONDITIONS

Inside the saproxilic arthropods community the diet of different taxa is unknown. Many studies have focused to identify the organisms (invertebrates and fungi) associated with *Cucujus cinnaberinus* and *H. haematodes* in order to demonstrate their role in the food chain. The food preferences of both adults and larvae of the genus *Cucujus* are still scarce and not fully clear. Smith and Sears (1982), examining the gut contents of *C. clavipes*, reported for the first time on predatory feeding habit of larvae, Ślipiński (1982) found that adults of *Cucujus* spp are facultative predators, while Lawrence (1991) considers the larvae of *Cucujus* feeding generalists on insect, plant and fungal material. Mamaev et al. (1977) found that *Cucujus* species are scavengers, preying only occasionally on larvae and pupae of other beetles. We investigated the feeding choices of larvae of two saproxilic beetles, *C. cinnaberinus* Scopoli and *C. haematodes* Erichson, 1845, examining the behavior in laboratory conditions. These species are elements of high conservation value and share their habitat with other red listed beetles. The *Cucujus* larvae considered for our test was kept in the arena for 10 minutes before trial. Each larva was tested once by offering one individual of the hereby listed preys: larvae of *Lasius* sp., *Ips paraconfusus*, *Rhagium inquisitor* and *R. inquisitor* (pieces), *Acanthocinus aedilis*, *Tenebrio molitor*. first age, and *Calliphora vicina*, first age, at the same time. Minced meat has been used as control. The trial began when all preys were put into the moistened arena, until the first prey was ingested. With no predation, the trial lasted for 30 minutes. During the trial, the behaviour of each larva was recorded using a digital camcorder (Nikon, mod. Coolpix P610). Differences among attacking occurrences of different prey species were evaluated using the Chi-square test, using the SPSS v.22.0 statistical package. When offered a choice to naïve third/fourth instar larvae, they more frequently attacked and ate *Ips* and *Lasius* and the pieces of *Rhagium* larvae than immature larvae of *Rhagium* and *Acanthocinus*. These have been attached but never consumed. This study indicates that *Cucujus* larvae show a feeding preference for small and soft preys that move poorly. Moreover, they refused large and more sclerotized preys (*Rhagium* and *Acanthocinus*) even if their saprophagous habits should not be excluded.

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MONITORING HABITATS DIRECTIVE ENTITIES IN TUSCANY: AN OVERVIEW ON ANIMAL SPECIES FROM THE PROJECT MONITO-RARE

The Project MONITO-RARE, underwritten by the Tuscan regional government in 2018 with the Universities of Florence, Pisa and Siena (Reg. Res. n°1047, 02/10/17), envisages a monitoring program for animals, plants and habitats protected by Council *Directive 92/43/EEC* of 21 May 1992 within the regional territory. The Project is in two stages: a preliminary updating of the distribution maps of the species and habitats included in the Directive that occur in Tuscany; a following field trial of the monitoring protocols suggested by ISPRA and MATTM in Manuals 140/16, 141/16 and 142/16. MONITO-RARE is an innovative experiment to establish interdisciplinary cooperative monitoring for Directive species and habitats, involving academics specialized in zoology, botany and ecology from the different Universities.

Concerning animal species, we updated their distribution maps for the years 2011-17 based on conventional and grey literature, unpublished data of the working group, and recent museum records. The data was mapped on a 10x10 km grid with QGIS software. We updated the previous maps with records of 61 species in a total of 752 grid squares (10 amphibian, 10 arthropod, 6 fish, 23 mammal, 2 mollusc and 10 reptile species), mainly from central and southern Tuscany, including two records for species whose occurrence has not been reported until 2010 in the Region [*Saga pedo* (Pallas, 1771), and *Vespertilio murinus* Linnaeus, 1758].

The second on-going stage aims to test on a selection of species and Natura 2000 Sites whether the monitoring protocols suggested by ISPRA/MATTM can be successfully put into practice, and at the same time to provide data useful for updating the Standard Data Forms of the selected Sites. The main aim in this first year is to test the cost-effectiveness and timing of a potential regional monitoring plan, currently applied to the selected species and Sites. Target species and Sites were selected by expert opinion to include species with highly differentiated ecological features, distribution and abundance, and to cover the entire regional territory. We selected 4 amphibian, 6 arthropod, 5 fish, 6 mammal, 2 mollusc and 3 reptile species and more than 30 Special Areas of Conservation, corresponding to about 25% of the SACs in the Region; each species is currently being monitored in a subset of the selected SACs.

L'AVIFAUNA DELLA ZSC IT9120002 MURGIA DEI TRULLI (PUGLIA – BARI/BRINDISI). ANALISI PRELIMINARE

Le conoscenze circa l'avifauna della ZSC IT9120002 sono abbastanza scarse e consistono essenzialmente sui dati riportati nel Piano di Gestione del Sito (D.G.R. n. 1615/2009). Solo di recente sono stati o sono in via di pubblicazione contributi che mirano a chiarirne lo status locale (cfr. Chiatante & Todisco 2006; Todisco & Liuzzi 2008; Todisco, in stampa).

Il Sito si estende tra le province di Bari e Brindisi per 5457 ha ed è inserito in un sistema collinare carsico prevalentemente caratterizzato da coltivazioni estensive (in particolare uliveti e seminativi arborati) e boschi cedui a *Quercus trojana* e *Q. pubescens*.

A partire dal 2005 è stata effettuata un'indagine di tipo qualitativo, visitando (minimo 4 uscite mensili) tramite transetti lineari a piedi e in auto e punti fissi di osservazione/ascolto, tutti i principali habitat. Per le specie notturne sono state effettuate uscite in orari idonei. Per le identificazioni in campo sono stati utilizzati cannocchiali 20-50x e binocoli 10x50. Sono infine stati considerati anche alcuni dati pregressi forniti da osservatori terzi di fiducia. L'analisi fenologica delle specie è stata effettuata analizzando circa 14700 dati. Per le categorie fenologiche e la sistematica si è fatto riferimento a Bricchetti & Fracasso (2015).

Sono state censite 163 specie pari al 42,7% di quelle note a livello regionale (Liuzzi *et al.*, 2013). 27 specie sono migratrici regolari e 13 irregolari (di cui una nidificante possibile). 14 specie sono migratrici e nidificanti (una specie, *Oenanthe hispanica*, non più riscontrata dal 2011), mentre 18 specie sono migratrici e svernanti (regolari o irregolari). 15 specie sono migratrici, svernanti (reg. o irr.), stanziali e nidificanti (certe, probabili o possibili). 23 specie sono stanziali e nidificanti. Delle specie migratrici e/o svernanti, 8 risultano anche estivanti. 45 specie sono accidentali (di cui 2 nidificanti possibili), e tra queste, 5 lo sono anche a livello regionale (Liuzzi *et al.*, 2013).

Nel complesso 54 specie sono attualmente nidificanti (pari al 33% del totale), certe (43), probabili (4) e possibili (7). 8 sono incluse nella Lista Rossa dei Vertebrati Italiani (Rondinini *et al.*, 2013) e 2 nell'All. I della Dir. 2009/147 CE. Tra le specie nidificanti sono da citare *Sylvia crassirostris* (nidificante possibile), specie di recente comparsa in Italia (Janni & Fracasso 2012, Todisco & Liuzzi 2015) e *Strix aluco*, raro e localizzato in Puglia centro meridionale (Todisco & Liuzzi 2008).

Questi dati preliminari vogliono rappresentare la base per future ricerche mirate a chiarire e approfondire lo status dell'avifauna del Sito, anche al fine di accertare la presenza di specie nidificanti di interesse conservazionistico, che per prossimità di areale e/o presenza di habitat idoneo sono da considerarsi nidificanti potenziali (ad es. *Pernis apivorus*, *Circaetus gallicus*, *Burhinus oedicephalus*, *Caprimulgus europaeus*; *Coracias garrulus*; *Sylvia conspicillata*, *Anthus campestris*).

Simposio 4

*Cellule staminali, differenziamento e riprogrammazione cellulare:
modelli tradizionali e modelli innovativi*

Coordinatori

Loriano Ballarin, Luciana Dini

*Introduzione e relazioni ad invito
(keynote lectures)*

LORIANO BALLARIN

Dipartimento di Biologia, Università di Padova

INTRODUCTION TO MARISTEM - STEM CELLS OF MARINE/AQUATIC INVERTEBRATES: FROM BASIC RESEARCH TO INNOVATIVE APPLICATIONS

Marine/aquatic invertebrates constitute the largest biodiversity and the widest phylogenetic radiation on Earth, from morphologically simple organisms (e.g., sponges, cnidarians), to the more complex mollusks, crustaceans, echinoderms, and protochordates. Today, adult marine/aquatic invertebrate stem cell (MISC) biology is of prime research and medical interest. However, studies on stem cells from organisms outside the classical vertebrate (e.g., human, mouse, and zebrafish) and invertebrate (e.g., *Drosophila*, *Caenorhabditis*) models have not been pursued vigorously. These organisms contain a variety of MISC-types that allow the production of a large number of novel bioactive-molecules, many of which are of significant potential interest for human health. MISCs further participate in aging and regeneration phenomena, including whole-body regeneration.

For years, the European MISC-community has been highly fragmented and has established scarce ties with biomedical industries in an attempt to harness MISCs for human welfare. Thus, it is important to (i) consolidate the European community of researchers working on MISCs; (ii) promote and coordinate European research on MISC biology; (iii) stimulate young researchers to embark on research in MISC-biology; (iv) develop, validate, and share novel MISC tools and methodologies; (v) establish the MISC discipline as a forefront interest of biomedical disciplines, including nanobiomedicine; and (vi) establish collaborations with industries to exploit MISCs as sources of bioactive molecules. In order to fill the recognized gaps, the EC-COST Action 16203 “MARISTEM” has recently been launched.

At its initial stage, the consortium unites scientists from 24 EC countries, Cooperating countries, and Near Neighbor Countries.

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MOLECULAR MECHANISMS OF MOUSE NEURAL STEM CELL AGING

Adult neurogenesis declines with aging due to the depletion and functional impairment of neural stem/progenitor cells (NSPCs). An improved understanding of the underlying mechanisms that drive age-associated neurogenic deficiency could lead to the development of strategies to alleviate cognitive impairment and facilitate neuroregeneration. An essential step towards this aim is to investigate the molecular changes that occur in NSPC aging on a genome-wide scale. We compared the transcriptional, histone methylation and DNA methylation signatures of NSPCs derived from the subventricular zone (SVZ) of young adult (3 months old) and aged (18 months old) mice. Surprisingly, the transcriptional and epigenomic profiles of SVZ-derived NSPCs were largely unchanged in aged cells. Despite the global similarities, we detected age-dependent changes at several hundred genes and regulatory elements, thereby identifying putative regulators of neurogenic decline. In particular, the homeobox gene *Dbx2* was upregulated in aged NSPCs. Elevated *Dbx2* expression in young adult NSPCs promoted age-related phenotypes, including the reduced proliferation of NSPC cultures and the altered transcript levels of age-associated regulators of NSPC proliferation and differentiation. Depleting *Dbx2* in aged NSPCs caused the reverse gene expression changes. These results provide new insights into the molecular programmes that are affected during mouse NSPC aging, and uncover a new functional role for *Dbx2* in promoting age-related neurogenic decline.

STEM CELLS CELL IN SPONGES (PORIFERA): AN UPDATE

Sponges (Porifera) are thought to be the sister group of all other animals and the earliest branching multicellular lineage of extant animals and as such a key group for understanding of the evolutionary history of animal stem cells and their regulation. Sponges are known to possess remarkable reconstitutive and regenerative abilities and high cell dynamic. There is a widespread opinion that all sponges cells are capable of transdifferentiation and under certain conditions exhibit properties of pluripotency. However, in experiments on the regeneration and reaggregation of dissociated cells, it was shown that not all cells exhibit the properties of stem cells. I will review some classical and recent data from morphology, experimental manipulations and from molecular biology on the history and current state of our knowledge about stem cells in Porifera. Sponges do not have well-established stem cell lineages. Furthermore, presumable stem cells differ between four sponge classes. The most consistent model of the stem cell system is elaborated for fresh-water Demospongiae. According to this model demosponges have two stem cell lineages: archaeocytes and choanocytes. Both express the ortholog of the stem cell marker *Piwi* and show the proliferation activity in the intact sponges. During regeneration in demosponges these cell types play an important role: they give rise to the new exopinacoderm and participate in the restoration of the choanosome structures. Additionally, the archaeocytes and choanocytes have ability for (trans)differentiation to various cell types during the restoration processes after sponge tissue dissociation. Despite the importance of archaeocytes as stem cells of demosponges, there is still no ultrastructural characterization of this type of cell; moreover, there are contradictory and unclear interpretations of the morphology of this cell type. However, both Calcarea and some Homoscleromorpha do not have mesohyl cells similar to demosponges archaeocytes. In these sponge clades choanocytes and pinacocytes exhibit properties of polypotentiality, as follow from gametogenesis, experiments on regeneration, and cell dissociation. These cells can directly transdifferentiate into other cell types without archaeocytes-like stage. Finally, I will emphasize the importance of models diversification: the comparison between different sponge taxa may help to shed light on the diversity of stem cells in Porifera and their properties. Financial support by Russian Foundation for Basic Research n° 16-04-00084 and the Russian Science Foundation n° 17-14-01089 is gratefully acknowledged.

**TISSUES REGENERATION AND *IN HOSPITE* MICROALGAE
PROLIFERATION IN THE PHOTOSYMBIOTIC MARINE FLATWORM
SYMSAGITTIFERA ROSCOFFENSIS (XENACOELOMORPHA, ACOELA)**

Some marine animals evolved long-term functional partnership with photosynthetic micro-algae they reared inside the animal tissues. The biology and physiology of the green flatworm *Symsagittifera roscoffensis* show how a population of around 100.000 photosynthetically active green unicellular algae (*Tetraselmis convolutae*) is controlled beneath the epidermis of this animal. The non-photosymbiotic juvenile animal must ingest (not digest) micro-algae found in the surrounding environment or die (after 15 days in the lab) if no ingestion occurred. Once ingested, algae divide, confers the green color of the animal and supply energy, releasing in the tissues various photosynthetates, the unique source of food for the animal. Controlling the life cycle in captivity of this flatworm – including the induction of photosymbiosis (i.e culture of the free living photosynthetic partner) - allows having access to any developmental stages in order to explore the intimate trophic relationship and other features. In the animal tissues algae are fully dedicated to photosynthesis as suggested by the absence of typical structures expressed in the algae free-living state for which lot of energy is allocated, such as the synthesis of body-wall (complex polysaccharides) and the movement flagella. The *in hospite* algae take advantage of the animal nitrogen wastes and recycle them. At the very beginning of the establishment of the symbiosis algae also recycle the uric acid crystals that accumulate in the non-photosymbiotic juveniles flatworm. Intertidal natural *S. roscoffensis* colonies submitted to submarine groundwater discharge, enriched in nitrogen also show that the flatworms are nitrate interceptors. In the natural environment, animals are exposed, several hours each day, to sun rays and are adapted to overcome the excess of sun (including UVs) and high oxygen concentration (from photosynthesis) in their tissues. Beside coping with putative detrimental physiological conditions (oxidative stress) *S. roscoffensis* also exhibits strong capacities of tissue regeneration including brain. After more than a decade of functional exploration related to *S. roscoffensis*, - Tuning the techniques for completing life cycle in captivity - Investing huge efforts in genomics/transcriptomics, - Morphological (cell types) characterization advances, many conditions are met for starting a formal exploration of the molecular and cellular mechanisms underlying brain and peripheral nervous system regeneration.

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EVOLUTION OF THE REGULATORY PROGRAM FOR SKELETON DEVELOPMENT IN ECHINODERMS

One of the biggest challenges of modern biology is to understand how the diversity of animal forms has been generated throughout evolution. We use echinoderms and the development of endoskeleton to study principles of regulatory program evolution underlying cell type specification. The wealth of knowledge on developmental gene regulatory networks and the evolutionary context for larval skeleton development provide an excellent system to study mechanisms of cooption and evolution of gene regulatory networks (GRN) underlying developmental processes.

Only two of the five extant classes of echinoderms develop skeleton in the larva: the echinoids (sea urchins) and the ophiuroids (brittle stars), although all adult echinoderms have complex endoskeleton in the form of various calcium carbonate ossicles. Our studies focus on analyzing the development of skeleton in ophiuroid embryos and adults regenerating arms to identify general features of skeletogenesis and specific embryonic developmental genes. High-resolution spatio-temporal gene expression data are coupled with disruption of signaling pathways (*i.e.* Fgf and Notch signaling) and transcriptomic data. The resulting data are then compared side-by-side to the well-characterized sea urchin mesoderm regulatory network. We identified a high degree of rewiring between larval skeleton formation in ophiuroids and echinoids. For instance, some nodes are absent in ophiuroids (*i.e.* *foxb* and *dri*), while others have heterochronic expression (*e.g.* *nk7*) or are not functionally similar in the two species (*e.g.* *pmar1* and *pplx*). Finally, at the level of differentiation, class specific genes are identified. Our results together with recent studies done in pencil sea urchins (cidaroids) shed light into the evolution of echinoderm GRN governing larval skeleton development. On the contrary, a comparison between developmental program in larvae and regenerating arms of the ophiuroid species *Amphira filiformis* shows a remarkable similarity of the GRN in these two developmental contexts.

Simposio 4

*Cellule staminali, differenziamento e riprogrammazione cellulare:
modelli tradizionali e modelli innovativi*

Coordinatori

Loriano Ballarin, Luciana Dini

Comunicazioni orali

IDENTIFICATION OF GENES EXPRESSED IN DIFFERENTIATED CELLS INVOLVED IN STEM CELL REPOPULATION FOLLOWING LOW-DOSE X-RAY TREATMENT IN PLANARIAN

A major challenge in stem cell research is the comprehension of the extrinsic control exerted in vivo by the niche. Despite the significant advances derived from the successful application of molecular, cellular, and genomic approaches in planarians, the nature of extrinsic signals regulating stem cell biology remains to be understood. With the aim to identify genes, preferentially enriched in differentiated cells, whose expression is involved in the regulation of stem cells, we took advantage from the stem cell repopulation process that follows low-dose X-ray treatment in planarians. Due to its awfulness, our idea is that this process might represent a context in which the release of signaling molecules by differentiated tissues is highly emphasized.

We searched for differentially expressed tags in a DGE library obtained from low-dose treated animals sacrificed between 4 and 7 days after irradiation compared to a DGE library from control animals and we selected some upregulated genes on the basis of their fold change. Expression analysis of the selected genes in intact un-irradiated planarians by WISH experiments revealed that these genes were preferentially expressed in differentiated tissues such as the gut and in the cephalic ganglia and their expression strongly increased in low-dose irradiated animals respect to not-irradiated animals.

Genetic silencing of some of them by RNA interference impaired the stem cell repopulation, suggesting a tight Extrinsic control of stem cell activity.

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THE REVERSE DEVELOPMENT OF *TURRITOPSIS DOHRNII* (CNIDARIA): NOVEL MORPHOLOGICAL AND MOLECULAR ASPECTS

Turritopsis dohrnii is a small hydrozoan attracting the interest of the scientific community for its renowned capacity to undergo reverse development (RD) even at the adult medusa stage. Through RD and in response to different environmental stressors or senescence, the medusa of *T. dohrnii* can revert back to the polyp stage. It has been suggested that different cellular processes such as trans/dedifferentiation, apoptosis and stem cell proliferation are involved in RD. Hydrozoans have numerous pluripotent cells, some of which are known as interstitial cells (i-cells) and are involved in the astonishing regenerative capability of these animals. Moreover, transdifferentiation have been suggested to play a crucial role. To shed light on the mechanisms controlling this peculiar process, we deeply characterized RD morphological aspects by confocal, transmission and scanning electron microscopy. Particularly, we observed an increase of programmed cell death and degeneration events during the first stages of RD, while the presence of metabolic active cells, rich of mitochondria and RER, were found in the cyst stage. To clarify the role played by stem cells and transdifferentiation, we focused on the homologs of Yamanaka's factors, the core genes governing pluripotency induction in mammal cells. We found putative homologs of *SoxB1*, *Klf4*, *c-Myc*, *Oct 3/4* by blast search in the draft transcriptomes of polyp and medusa stages. These genes are mainly expressed in tissues rich in stem cells in medusae while they have a dynamic expression profile during the RD stages. These results suggest that RD is a complex process in which both stem cells and trans/dedifferentiation events play a pivotal role. Further studies will clarify which reprogramming factors control cell differentiation and fate.

PLURIPOTENT *VERSUS* REPROGRAMMED CELLS: CELL PLASTICITY IN ECHINODERM REGENERATION

Echinoderms display remarkable regenerative capabilities and offer a variety of models to study this phenomenon widely distributed throughout the Phylum. Although their regenerative phenomena have been traditionally attributed to two different mechanisms (i.e. epimorphosis and morphallaxis), the true origin and fate of the involved cells are still unclear. An up-to-date overview of cell recruitment processes in the different echinoderm classes is here provided in order to define the state of the art, including the main unsolved issues, as well as the necessary future steps to cover the knowledge gap. Among stellate echinoderms, crinoids are the only group clearly displaying the recruitment of morphologically undifferentiated cells stocked in the stump tissues (i.e. coelomic canals and brachial nerve): these undifferentiated cells actively migrate to form a true blastema where they undergo proliferation and differentiation up to regenerate the lost tissues. Reprogramming of differentiated cells occurs only in stress conditions. In contrast, a true regenerative blastema is missing in brittle stars and starfish, which apparently mainly rely on recruitment of dedifferentiated cells from mature tissues. In starfish, dedifferentiation is massively employed at the level of muscle tissues, also in location far from the wound site. In both these classes progenitor-like cells are provided and recruited via epithelial-mesenchymal transition (EMT) from coelomic epithelium. In sea cucumbers neural and intestinal regeneration are the main process under investigation. In the former, the absence of “stemness” marker in the transcriptome suggests that radial nerve cord regeneration depends on dedifferentiation of the supporting glial cells that re-differentiate in both the same cytotype and new neurons. Myocyte dedifferentiation markedly occurs during gut regeneration. In sea urchins, damaged test and broken spines are reformed through dedifferentiation of stump cells with only minor local cell proliferation, whereas totally removed spines are regenerated via undifferentiated (pluripotent) cells. Overall, echinoderm regeneration appears mainly to rely on dedifferentiation phenomena rather than recruitment of pluripotent cells already stocked in the stump tissues, the exact origin, identity and fate of the involved cells being still unknown in most cases. Echinoderm tissues, especially coelomic epithelium and muscles, show a high potential of plasticity in terms of cell differentiation/dedifferentiation and activity (proliferation, migration), and EMT plays key roles in this plasticity. Cell tracking coupled to molecular and microscopy approaches will be strongly needed to unravel in detail the strikingly effective cellular mechanisms and pathways (from cell origin to fate) employed by echinoderms in their regeneration processes.

Simposio 4

*Cellule staminali, differenziamento e riprogrammazione cellulare:
modelli tradizionali e modelli innovativi*

Coordinatori

Loriano Ballarin, Luciana Dini

Sessione Poster

**EXPRESSION STUDY OF MOLECULAR MARKERS INVOLVED IN STEMNESS
MAINTENANCE AND DIFFERENTIATION IN THE COLONIAL ASCIDIAN
*BOTRYLLUS SCHLOSSERI***

Ascidians are invertebrate chordates, members of the subphylum Tunicata, and represent the sister group of vertebrates. They offer the opportunity to investigate and compare the behaviour of both embryonic and adult stem cells. Morphological data suggest the presence of undifferentiated haemocytes (haemoblasts) able to proliferate and give rise to terminally differentiated cells. Relevant studies were also carried out in the neural lineage, in which neural progenitor cells regenerate the brain after extirpation. In *B. schlosseri*, during the cyclical generation change, bud primordial cells, probably deriving from a pool of long-living stem cells, are able to give rise to the neural complex. We screened the *B. schlosseri* genome and transcriptome, looking for transcripts/genes showing similarity to vertebrate molecular markers of haematopoietic and neural stem cells. Four sequences, orthologous to mammalian transcripts considered markers of haematopoietic progenitor cells, were identified in *B. schlosseri*. They are: bsabcg2, bscd133, bsgata1/2/3 and bsgata4/5/6. ISH on haemocyte monolayers and colony sections, resulted in labelling of cells in the sub-endostylar haemolymph lacunae. This results matches previously morphological data that identified the endostyle as a stem cell niche. Quantitative real time PCR (qRT-PCR) highlighted the overexpression of the considered genes in the mid-cycle phase of the blastogenetic cycle. During this phase, there is the formation of new secondary buds emerging from the primary buds. The high expression levels of bsabcg2, bscd133, bsgata1/2/3 and bsgata4/5/6 genes in the mid-cycle phase reflect the presence of undifferentiated cells involved in proliferative and differentiation events required for the new blastogenetic generation.

For the neural lineage, we identified two transcripts orthologues of vertebrate neural stem cell markers (BsSox2 and BsMsi2). ISH with riboprobes for BsSox2 and BsMsi2 revealed a common labelling in the endostyle niche. The presence of bssox2 and bsmsi2 transcripts in the cells of the region known to be a stem cell niche, led us to conclude that in *B. schlosseri* a single population of pluripotent stem cells is probably present that could differentiate into haematopoietic or neural cells.

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EFFECTS OF ALTERED GRAVITY ON STEM CELL BIOLOGY AND TISSUE REGENERATION IN THE PRESENCE OF CERIUM OXIDE NANOPARTICLES

The gravitational force of 1 g is the oldest environmental factor and life on Earth has adapted perfectly to this nearly constant force. It is well known that isolated cells in culture can sense altered gravity and modify important biological processes such as proliferation and differentiation, cytoskeleton organization and signal transduction responses. Altered gravity also induces reactive oxygen species (ROS) production and cellular oxidative stress with lipid peroxidation and DNA damage and this effect is more pronounced after long-duration space flights and lasts for several weeks after landing. Recently, the use of particles with redox-reactive properties, such as cerium oxide nanoparticles (nanoceria), has been proposed, due to their self-regenerating capability as free radical scavengers to contrast ROS production in altered gravity.

With the aim to analyse the effect of altered gravity on stem cell biology and tissue regeneration, as well as to assess the ability of nanoceria to protect a living organism, we exposed planarians (*Dugesia japonica*), to simulated micro-gravity, reduced gravity paradigm and hyper-gravity in the presence or absence of nanoceria. Preliminary results suggest an influence of hyper-gravity on stem cell determination and a protective activity of nanoceria.

TAVOLA ROTONDA

LA DIDATTICA DELLE SCIENZE ANIMALI: NUOVE METODOLOGIE, NUOVI CORSI E PROSPETTIVE OCCUPAZIONALI

*Moderatrice
Maria Agnese Sabatini*

Interventi

BIANCA MARIA LOMBARDO

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L'ESPERIENZA DEL PIANO NAZIONALE LAUREE SCIENTIFICHE NELLA DIDATTICA DELLA BIOLOGIA ANIMALE

Il Piano Lauree Scientifiche (PLS) è stato istituito, a partire dal 2004, per iniziativa del MIUR, della Conferenza dei Presidi di Scienze e Tecnologie e di Confindustria, con la motivazione iniziale di incrementare il numero di iscritti ai corsi di laurea in Chimica, Fisica, Matematica e Scienza dei Materiali. Nel 2009, visti i risultati raggiunti, è stato confermato per mettere a sistema le pratiche migliori e per rafforzare i rapporti tra scuola e università, da un lato, e tra università e mondo del lavoro, dall'altro. Nel 2014 è stato avviato il terzo ciclo, con un consistente allargamento delle classi di laurea coinvolte (Statistica, Biotecnologie, Scienze Biologiche e Scienze Geologiche) e un ampliamento degli obiettivi, con particolare attenzione alla riduzione del tasso di abbandono fra primo e secondo anno, a cui è stata dedicata la quarta azione di intervento. La riduzione del tasso di abbandono nei corsi di laurea a carattere scientifico dovrebbe, in realtà, essere il risultato sia delle prime tre azioni in cui si articola il progetto (laboratori di orientamento, autovalutazione e aggiornamento degli insegnanti), sia del miglioramento della didattica universitaria del primo ciclo, in particolare degli insegnamenti di primo anno, e dell'intervento diffuso di attività di tutorato. Ciò ha comportato la sperimentazione di modalità innovative di svolgimento delle attività anche nelle discipline biologiche, le quali pur non essendo spesso alla base di ritardi e abbandoni, possono anch'esse migliorare l'efficienza dei corsi di laurea di area biologica. Le esperienze laboratoriali messe a punto per le attività nelle scuole dai docenti universitari PLS, con la collaborazione preziosa dei tutor, le metodologie didattiche innovative, basate sull'apprendimento attivo e sulla valutazione formativa, più vicine al modello di insegnamento scolastico, possono e devono essere integrate negli insegnamenti universitari di primo anno, rafforzando così il ponte fra scuola e università. Il Piano Nazionale Lauree Scientifiche di Biologia e Biotecnologie ha coinvolto 44 sedi universitarie e ottenuto importanti risultati, raggiungendo tutti i target previsti per gli indicatori scelti in fase di progettazione.

JACOPO VIZIOLI

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PRODUCTION OF E-LEARNING TOOLS FOR ZOOLOGY TEACHING

At the University of Lille (France) we recently developed a student-centred didactical project aimed to improve the attractiveness of animal biology using innovative e-Learning tools. The use of multimedia supports, available on different devices, can increase the motivation and promote individual involvement in learning in young generations. We produced movies and interactive e-books describing the body plan and the anatomy of organisms representative of main Metazoans phyla. We are also using an innovative 3D photo approach to produce high resolution images of samples from our naturalist collections. This approach permits the digitalization and the virtual handling of historically important specimens. In addition, the 3D photos and animations are used for the production of interactive technical records on different fields (i.e. vertebrate skeleton evolution or mammal diets). All these tools are used by students to prepare and revise lab courses. The integration of such multimedia tools, either in lab courses or in lectures, contributes to give an innovative approach in zoology teaching. The learning feedback on the use of such ICTE tools indicates that this approach has to be complemented by a solid and regular inductive instruction of the discipline.

ATTIVITÀ DI EDUCAZIONE AMBIENTALE NELL'AREA MARINA PROTETTA "PORTO CESAREO"

L'Area Marina Protetta (AMP) Porto Cesareo è stata istituita ufficialmente il 12 dicembre 1997, con il Decreto del Ministro dell'Ambiente pubblicato sulla "GAZZETTA UFFICIALE N. 045 SERIE GENERALE PARTE PRIMA DEL 24 02 1998. L'Ente gestore è un Consorzio tra la Provincia di Lecce e i Comuni di Porto Cesareo e Nardò. L'AMP ha una superficie di 16.654 ha con una linea di costa di circa 32 km, limitata a nord da Punta Prosciutto e a sud da Torre Inserraglio: un litorale frastagliato, in cui si alternano tratti sabbiosi e scogliere basse, che si distende all'interno dei territori dei comuni di Porto Cesareo e Nardò. L'AMP ospita specie ed habitat di straordinaria valenza conservazionistica tanto che dal 2011 è inserita nella lista delle Aree Specialmente Protette di Importanza Mediterranea (ASPIM). Il comune di Porto Cesareo (LE), nel cui territorio ricade gran parte del litorale dell'AMP, ha una popolazione residente di circa 6.000 abitanti, ma ospita fino ad 1.400.000 di visitatori estivi, gran parte dei quali, legati al turismo delle spiagge o diportistico. Porto Cesareo ha un'economia tradizionalmente legata al mare poiché qui le attività prevalenti sono la piccola pesca professionale, e le attività di ricezione ed accoglienza turistica.

In un contesto così fortemente esposto alla pressione antropica, l'obiettivo primario di istituzione dell'Area Marina protetta, che è la tutela ambientale, deve necessariamente trovare conciliazione con le necessità economiche e sociali della comunità umana residente. La tutela ambientale, pertanto, diviene "Gestione sostenibile del territorio" con l'obiettivo di promuovere forme alternative di sviluppo ambientalmente e socialmente sostenibili.

In quest'ottica, l'Area Marina Protetta costituisce un laboratorio privilegiato di educazione ambientale, ossia un contesto geografico, economico ed ambientale concreto nel quale è possibile conoscere l'ambiente naturale ed "esercitarsi" in pratiche di fruizione sostenibile della biodiversità protetta.

È per questo che, sin dall'istituzione, l'AMP Porto Cesareo ha individuato tre filoni di educazione ambientale:

- un filone di formazione-informazione-aggiornamento indirizzato agli operatori turistici, che rappresentano il primo interfaccia con i fruitori finali dell'ambiente. Agli operatori turistici sono stati dedicati mediante corsi di aggiornamento di materiale informativo, percorsi di condivisione di obiettivi di sviluppo;
- un filone di informazione rivolto ai fruitori finali, mettendo in atto progetti di coinvolgimento attivo della cittadinanza e dei turisti, esperienze laboratoriali lungo le spiagge eventi tematici estivi;
- un filone di educazione ambientale rivolto alle scuole, realizzando progetti didattici teorico-pratici-esperienziali in collaborazione con strutture deputate quali il Museo di Biologia Marina di Porto Cesareo.

LA COMUNICAZIONE DELLA RICERCA: STRATEGIE PER IL PUBBLICO

Il Museo di Biologia Marina “Pietro Parenzan” dell’Università del Salento nasce nel 1966 da una collezione del fondatore, cui successivamente è stato intitolato. E’ gestito attraverso una convenzione fra Unisalento, Comune di Porto Cesareo, Area Marina Protetta e Provincia di Lecce.

Essendo un museo universitario, il Museo di Biologia Marina ha alle spalle la ricerca sul campo condotta da un gruppo di ricercatori che fanno capo al laboratorio di Zoologia e Biologia Marina del Dipartimento di Scienze e Tecnologie Biologiche e Ambientali. Gli studi nel campo hanno una forte valenza scientifica e sono riconosciuti a livello internazionale.

In relazione alla moderna visione di museo che lo identifica a servizio della comunità, una delle responsabilità (forse la principale) che il Museo di Biologia Marina sente di avere nei confronti del territorio è proprio legata all’informazione in ambito ecologico marino, anche in concomitanza della sua localizzazione in un’area a vocazione fortemente turistica e quindi molto antropizzata.

Con i suoi allestimenti e le sue attività educative per i diversi target di pubblico, il Museo provvede a disseminare i risultati della ricerca, con lo scopo di far conoscere la bellezza del nostro mare per incoraggiarne la difesa e la protezione.

Nel Museo si svolgono visite guidate semplici e approfondite, laboratori didattici, conferenze, convegni, spettacoli. Il pubblico del Museo è molto variegato da tutti i punti di vista (età, livello culturale, motivazioni...) ed è costituito da studenti di ogni ordine e grado (dalla materna all’università), famiglie con bambini, anziani, gruppi di adulti, diversamente abili, studiosi. Per questo motivo, particolare attenzione viene posta alle metodologie di comunicazione, dal pannello all’apparato multimediale, allo scopo di rendere comprensibili alla maggior parte del pubblico i contenuti e i messaggi.

In particolare, si conducono studi di valutazione sia sulle attività educative (visite guidate e laboratori), sia sull’attrattività e comprensibilità degli apparati di comunicazione (audioguide, pannelli, apparati multimediali) che il visitatore adopera autonomamente visitando il museo. E’ stato anche studiato il cosiddetto “Visitors book”, ed è stata proposta di una chiave di lettura unitaria dei variegatissimi messaggi lasciati dai visitatori, allo scopo di comprendere e, ove possibile, soddisfare le esigenze espresse nei messaggi.

Particolare attenzione viene posta al pubblico dei più piccini, perché possano assumere fin da subito atteggiamenti corretti nei confronti dell’ambiente marino: una baby area, attrezzata con giochi, letture e attività incentrate sul mare, è risultato l’ambiente ideale allo scopo.

ANTONIO DURANTE

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MUSEO E DIDATTICA: IL VIAGGIO E L'AVVENTURA

I nuovi metodi della didattica a tutti i livelli (musei, scuole e università) tendono al coinvolgimento di studenti che appaiono sempre meno motivati. Se ne ricercano le cause in aspetti socio-economici che tendono a distorcere i fini del sapere e del saper fare e in approcci didattici spesso obsoleti rispetto a sistemi di assimilazione culturale più accattivanti anche se spesso fuorvianti. Si fornisce una possibile soluzione per mezzo di metodi coinvolgenti e partecipati di accostamento alla cultura, tali che i fruitori siano essi stessi autori della propria istruzione creativa.

“ZOOLOGO”: PROFESSIONE ANTICA E MODERNA, IMPORTANTE DENTRO E FUORI L’ACCADEMIA

Il 90% dei laureati in Zoologia non trova uno sbocco lavorativo all’interno dell’Accademia o delle altre istituzioni Statali legate al mondo della ricerca. A quale futuro lavorativo può adire un giovane laureato o dottore di ricerca? Attualmente sta aumentando la domanda, da parte di aziende produttive, di supporto alla progettazione di attività strategiche in cui sono coinvolti gli animali in maniera diretta ed indiretta. Sempre di più è richiesta la competenza zoologica non solo per affrontare il tema principale del progetto ma, molto spesso, per analizzare e sviluppare modelli e prototipi, concreti o virtuali, in cui può essere rappresentato e monitorato l’intero “biotopo produttivo” al fine di prevedere e, eventualmente, prevenire ogni variazione.

Iniziano a moltiplicarsi le “nuove/antiche” professioni e specializzazioni che coinvolgono competenze zoologiche di alto livello e che prevedono una propensione all’interdisciplinarietà; esempi attuali spaziano dall’identificazione di insetti xilofagi e tricofagi nei beni culturali alla progettazione e posa in opera di impianti per la produzione di insetti da cui ricavare sfarinati proteici, dalla gestione genetica delle acquacolture all’estrazione e produzione in vitro delle cellule dell’apparato tegumentario dei rettili gekkonidi.

Dal punto di vista economico/finanziario sicuramente la professione di zoologo, svolta in ambito privato, ha indubbiamente una maggiore fragilità rispetto al posto pubblico. Come contraltare, il lavoro privato ha un grande vantaggio: la continua possibilità di potersi rimettere in gioco per la propria crescita all’interno dell’azienda stessa oppure al di fuori per affrontare nuove sfide anche a livello internazionale. La presenza di zoologi nel complicato mondo produttivo attuale è sempre più richiesta, sia per la loro importanza strategica decisionale che per la propensione all’interdisciplinarietà.

LA CURA ED IL BENESSERE DEGLI ANIMALI: UN NUOVO AMBITO PROFESSIONALE PER LA “ZOOLOGIA”

Il decreto legislativo 26/2014 in attuazione della direttiva 2010/63/UE sulla protezione degli animali utilizzati a fini scientifici stabilisce che tutto il personale operante nelle strutture in cui vengono allevati animali vivi deve essere professionalmente abilitato alla cura e all'assistenza degli animali.

Lo stesso decreto prevede inoltre l'obbligo di istituire l'Organismo Preposto al Benessere degli Animali (OPBA) all'interno di tutti gli stabilimenti di allevamento e di utilizzo. L'OPBA è composto almeno dal Responsabile del Benessere degli Animali (RBA), dal Medico veterinario e, nel caso di uno stabilimento utilizzatore, da un membro scientifico.

L'acquisizione di un livello di istruzione adeguato e dimostrato risulta oggi ancor più cogente per tali figure data l'imminente pubblicazione del decreto che disciplina la formazione del personale abilitato a tutelare gli animali sulla base degli elementi già elencati nell'allegato V del vigente D. Lgs. 26/2014. In base a tale allegato, è necessario possedere una profonda conoscenza del comportamento animale e della biologia di base e propria delle singole specie in relazione all'anatomia, alle caratteristiche fisiologiche, alla riproduzione, alla genetica e all'alterazione genetica. A nostro avviso risulta fondamentale in questo contesto una comprovata competenza sulle esigenze ecologiche ed etologiche delle specie animali in una corretta prospettiva evolutiva ed in un appropriato contesto sistematico.

Deve essere inoltre ricordato che il decreto legislativo 26/2014 ha incluso tra gli animali da tutelare un'intera classe di invertebrati, i cefalopodi e che sta aumentando sempre più la richiesta di promuovere lo sviluppo di approcci sperimentali alternativi come l'impiego di invertebrati quali nuovi organismi modello.

In tale contesto e per soddisfare la nuova e sempre più sentita necessità di formare figure professionali in tali ambiti, l'UZI ha recentemente dato l'appoggio per l'istituzione di un corso professionalizzante che dovrebbe prevedere un discreto numero di crediti per gli S.S.D. BIO/05 e BIO/06. La nuova Classe di Laurea riferita alle “Scienze e tecnologie della cura e del benessere animale” avrebbe promettenti prospettive occupazionali per i futuri laureati in un settore in espansione, quale quello della gestione e assistenza degli animali negli allevamenti, nei parchi, nei centri faunistico venatori e nella ricerca biomedica.

Sessione Poster a Tema libero

**These authors contributed equally to this work*

MORPHOFUNCTIONAL CHARACTERIZATION OF *HERMETIA ILLUCENS* LARVAL MIDGUT

The use of insects as a primary agent for organic waste reduction and bioconversion is a promising and sustainable strategy to produce protein that can be used for feed production. The non-pest black soldier fly (BSF), *Hermetia illucens*, is among the most promising insect species to this purpose because of the ability of the larvae to grow on a wide variety of organic substrates and their efficiency in the bioconversion process. Moreover, the high nutritional value of the larvae and pupae makes them an interesting alternative protein source for the production of fish feed.

Although the literature provides information on the rearing methods for BSF and indications on its use for waste treatment, little is known about *H. illucens* biology. In particular, a deep understanding of the physiology of the midgut, which is implicated in food digestion and nutrient absorption, is essential to better comprehend the extraordinary dietary plasticity of the larva, which is able to grow on different food substrates.

In the present study we performed a structural and functional characterization of the midgut of last instar larvae grown on two different food substrates, a standard diet for Diptera and a vegetable mix, by using morphological, biochemical and molecular approaches.

Our results demonstrate that the larval midgut is composed of three distinct anatomical regions with different luminal pH. They are characterized by different cell types that accomplish digestion and absorption activities (columnar cells), acidification of the middle midgut lumen (cuprophilic cells), and growth of the epithelium (stem cells). The proteolytic activity is particularly high in the posterior region and the major activities are due to chymotrypsin-like enzymes.

Larvae reared on the vegetable mix do not show alterations in the general morphology of the midgut, but a significant variation in digestive enzyme activities can be observed, especially for those involved in protein digestion.

This work represents the first attempt to characterize from a morphofunctional point of view the larval midgut of *H. illucens* and evaluate the effects of different diets on the features of this organ. Moreover, it sets the stage for the best exploitation of the bioconversion ability of this insect.

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GENOMIC INVESTIGATIONS ON MIDGUT BACTERIAL SYMBIONTS OF THE PHYTOPHAGOUS STINK BUG *ACROSTERNUM ARABICUM*

Symbiotic partners in natural associations engage a wide and mutable spectrum of interactions. Some well characterised cases are the nutritional symbioses, in which mutualistic bacterial symbionts provide to their animal hosts essential metabolites (e.g. amino acids, vitamins), absent from their diet, such as the partnership of sap-feeding aphids and the intracellular *Buchnera* symbionts. Similar cases were identified among the stink bugs (Hemiptera: Pentatomidae), which may harbour several different gut symbionts that are necessary for complete host development, including bacteria from genus *Pantoea* (Erwiniaceae, Enterobacteriales). *Pantoea* spp. are widely distributed, and can be found in free-living or host-associated forms, including plant and opportunistic human pathogens. Previously characterised *Pantoea* stink bug symbionts are not strictly phylogenetically related, suggesting a recurrent independent establishment of similar symbiotic partnerships. In this work, we report the discovery and genome analysis of a novel *Pantoea* sp. and a coexisting *Serratia marcescens* (Yersiniaceae, Enterobacteriales), midgut symbionts of *Acrosternum arabicum*, a phytophagous agricultural pest of Iranian pistachio plants. Shotgun Illumina sequencing of a dissected host midgut was performed, and reads were processed bioinformatically in order to isolate the genome of each symbiont respect to host sequences. The draft genome of *Pantoea* sp. (2,853,304 bp, 58.7% GC, 27 contigs, N50:343,358 bp) is significantly smaller in size respect to free-living *Pantoea*, and bigger than most other obligate symbionts of stink bugs. The predicted genome content includes the biosynthetic pathways for several essential amino-acids, vitamins and other metabolites, which suggests that this bacterium could supply such molecules to its host, supporting its role as a nutritional mutualist. The draft genome of *Serratia marcescens* (5,347,236 bp, 59.4% GC, 36 contigs, N50:303,229 bp) presented consistent features with free-living conspecifics. Two alternative hypotheses on the role of this second bacterium can be formulated, either it is an opportunist, or it is a recently acquired secondary symbiont, in cooperation with *Pantoea* for the benefit of the host.

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SPERMATOOZOA ULTRASTRUCTURE AND SPERMATOGENESIS OF AN UNDESCRIBED SPECIES OF *ACANTHODASYIS* (GASTROTRICHA, MACRODASYIDA)

The spermatozoon morphology and the spermatogenesis of an undescribed species of *Acanthodasyis* (Thaumastodermatidae) from Brazil were studied at ultrastructural level. The spermatozoon is a filiform cell formed by a cork-screw-shaped acrosome, a helical nuclear-mitochondrial complex and a long flagellum. The acrosome contains an axial tubular structure composed of a pile of electron-dense rings. The helical nucleus is thin and wraps a single, giant mitochondrion. A large and thick perinuclear helix surrounds the nuclear-mitochondrial complex extending for its whole length, except the two most basal coils.

The flagellum with typical $9 \times 2 + 2$ axoneme is surrounded by a monolayered obliquely striated cylinder, in the terminal part of which the axoneme disappears. The general architecture of the spermatozoon of *Acanthodasyis* species agrees with the general sperm model described for the family Thaumastodermatidae, and perfectly matches that observed in *A. aculeatus* and *Diplodasyis ankei*, so confirming the great uniformity of the sperm fine morphology within the subfamily Diplodasyinae. Spermatogenesis includes the early and simultaneous development of acrosome and flagellum, the lengthening of nucleus and the fusion of single mitochondria into a single giant mitochondrion which later sinks into the nucleus, and the final appearance of a perinuclear helix and a peraxonemal striated cylinder. The process takes place through the same main steps already observed in all Macrodasyida species studied so far.

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NEW IMPORTANT STOP-OVER SITES FOR RUDDY SHELDUCK *TADORNA FERRUGINEA* PALLAS, 1764 (AVES, ANATIDAE) IN SICILY

The Ruddy Shelduck *Tadorna ferruginea* PALLAS, 1764 is a species widely distributed in Southeast Europe and central Asia (MACKINNON *et al.*, 2000). Records in N and W Europe (including Northern Italy) mainly involve escapes from captivity since this species of duck is commonly kept in wildfowl collection, private gardens and parks. Despite this, actually, in Sicily the Ruddy Shelduck is considered an irregular migratory and wintering species and the observations must be attributed to the presence of wild individuals (CORSO, 2005). From 2009 to 2017, 67 observations were collected and compared with those included in the available bibliography (CORSO, 2005). The species was observed in 7 different locations. Compared to the last 20 years, the observations of the species are increased; previously, they are located mainly in the three most important known locations: Vendicari (SR), Simeto river (CT) and Lentini lake (SR) (CORSO, 2005), but, in addition to these, three new different International stop-over sites for migratory waders and ducks are added: Salt-pits of Trapani (TP), the Plain of Gela (CL) and the Pantani of South-Eastern Sicily (RG-SR). The observation in Ganzirri Lake (ME) (2014-2015) was instead discarded because about an ind. with confidence behaviour, probably escaped from captivity. In the last 9 years the observations of the species were mainly made in these three new sites, where, moreover, there are also unconfirmed reports of possible breeding pairs (L. Barraco, A. Corso, personal communications). The Plain of Gela is the site with more observations, in which this species has been reported regularly for 8 consecutive years; in the Pantani of S-E Sicily and salt-pits of Trapani the species was observed more regularly in the last 10 years than in the '90s.

Freshwater pools, flooded grasslands, marshes, brackish or saline lakes and artificial reservoirs nearby to agricultural lands (agroecosystems) result the habitats most frequented by this species.

The observations are referred mainly to the migration period, with peaks in March, in November and in December/January, with established wintering cases. This pattern of observations could be related to the increase and the possible expansion of the breeding and wintering Algerian population (BOULKHSSAÏM *et al.*, 2013).

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I COLLEMBOLI E I TEST ECOTOSSICOLOGICI: STUDIO E VALUTAZIONE DELL'UTILIZZO DI *PARISOTOMA NOTABILIS* (SCHÄFFER, 1896)

Tra i test ecotossicologici standardizzati utilizzati per la valutazione degli effetti di sostanze contaminanti nei suoli, grande rilievo hanno quelli che utilizzano i collemboli, tra questi il test ISO 11267 utilizza il collembolo *Folsomia candida* Willem, 1902. Recenti studi da noi condotti hanno messo in evidenza che nella valutazione di sostanze che alterano i parametri del suolo, come i rifiuti organici, questa specie risponde diversamente nei differenti terreni standard previsti per i test ISO (artificiale OECD e naturale LUFA 2.2) portando a valutazioni contrastanti del rischio ecotossicologico e mettendo in discussione l'attendibilità del test standardizzato. Inoltre, molti autori sono critici sulla validità dell'utilizzo di *F. candida*, specie con bassa densità in natura, praticamente assente dai terreni agrari, con preferenza per terreni a pH 5 e quindi poco adatta per la valutazione del rischio ecotossicologico in terreni alcalini e l'aumento del numero di specie da utilizzare nei test viene auspicato da più autori, anche nell'ambito delle linee guida ISO. Lo scopo di questo studio è stato la ricerca di una specie di collembolo da studiare, valutare e proporre per lo svolgimento di test ecotossicologici, in particolare per la valutazione degli effetti di sostanze organiche. La specie scelta è stata *Parisotoma notabilis* (Schäffer, 1896), caratterizzata da partenogenesi telitoca, ciclo vitale relativamente breve e ben rappresentata nei terreni agrari compresi quelli alcalini. Di questa specie sono stati studiati i tratti del ciclo vitale a 20°C necessari per determinare le modalità e i tempi del test ecotossicologico. In base a ciò è stato allestito un test con *P. notabilis* condotto nei due terreni standard OECD e LUFA 2.2; in aggiunta è stato allestito un test in terreno OECD a pH 7 per valutarne la risposta all'innalzamento di pH. In tutti i terreni utilizzati è stata rilevata una sopravvivenza superiore all'80%, parametro richiesto per la validazione del test ISO. Per quanto riguarda la riproduzione i risultati in terreno LUFA e OECD a pH 6 sono paragonabili, mentre nel terreno OECD a pH 7 il numero dei figli è risultato significativamente inferiore. Per comparazione, in parallelo sono stati allestiti test con *F. candida* utilizzando gli stessi terreni. La specie *P. notabilis* ha caratteristiche che potrebbero essere idonee ad un suo utilizzo nei test, ma maggiori considerazioni possono essere fatte solo in base ai risultati di test con contaminanti.

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DISTRIBUTION OF THYROSCYPHIIDAE (CNIDARIA, HYDROZOA, LEPTOTHECATA) AT THE CONTINENTAL SHELF OF SERGIPE AND SOUTH OF ALAGOAS, NORTHEAST, BRAZIL

Thyroscyphiidae is a Leptothecata family composed by six genus and 18 species of which *Sertularella cylindritheca*, *Thyroscyphus marginatus*, *Thyroscyphus ramosus*, *Thyroscyphus torresii* and *Thyroscyphus vitiensis* were registered to the Brazilian coast. Regarding the Northeast of Brazil, *S. cylindritheca* was recorded to the State of Bahia, *T. marginatus*, *T. vittensis* and *T. torresii* to Pernambuco, and *T. ramosus* is the only species registered to Sergipe (plus 4 States). Several checklists includes those species but distributional data are scarce, so this study aimed to verify the Thyroscyphiidae species distribution at the continental shelf of Sergipe (CSS) and South Alagoas (SA) related to environmental parameters.

Samplings were done by fishery trawls in 18 stations (-36.6 longitude; -10.6 latitude and -37.1 longitude; -11.35 latitude), at depths of 10, 20 and 30m, between 2001 and 2003 (four sampling campaigns). At each station, water and sediment were collected. We used general linear models (binomial distribution) to verify if time (year and month), space (depth and station) and environmental parameters influenced the distribution.

The family was represented by three species recorded in 13 stations, *S. cylindritheca* appeared in 11 stations, followed by *T. ramosus* (9) and *T. marginatus* (5). The distributional patterns differs: *T. marginatus* occurred in restricted stations in the middle of CSS and was the only species sampled in 30m at CSS and SA (only occurrence in SA); *T. ramosus* besides be sampled in all the CSS was prevalent in 10m, and was found in all depths at SA; *S. cylindritheca* occurred in all the CSS equally distributed in 10 and 20m and in all depths of SA. Time and space didn't influence the species distribution. Sedimentary selection (standard deviation) was the only environmental parameter related with the species distribution ($p=0,03$), showing that different species can prefer bottoms with different size grains and moderate to high hydrodynamism. It is known the preference of *T. marginatus* from Bermudas for places with tidal currents and limestone seaweed and barnacles. *S. cylindritheca* and *T. ramosus* were related to rock bottoms, differing from this study since they occurred at different bottoms, including mud. This results increase the knowledge of Thyroscyphiidae to CSS and SA and to Brazil.

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**CARETTERIZZAZIONE DELLA RISPOSTA CELLULARE ED UMORALE IN *HARPALUS (PSEUDOOPHONUS) RUFIPES* (DE GEER, 1774)
(COLEOPTERA, CARABIDAE)**

I Coleotteri Carabidi sono uno tra i maggiori gruppi di artropodi benefici delle reti trofiche degli agroecosistemi in quanto predatori di molte specie infestanti come afidi, lepidotteri, ditteri e semi di piante erbacee. Recenti studi hanno evidenziato che sono anche dei buoni modelli di studio per monitorare, su specie non target, gli effetti di sostanze agrochimiche comunemente usate in agricoltura per il controllo di specie infestanti. Nonostante la loro rilevanza ecologica, rare sono le informazioni riguardanti le loro risposte immunitarie. In questo studio, abbiamo caratterizzato la risposta cellulare (fagocitosi *in vivo*) ed umorale (attività dell'enzima fenolossidasi) in *H. rufipes*, una specie polifaga e mesofila, comune degli agroecosistemi che può essere adottata come modello per valutare gli effetti negativi che l'esposizione a pesticidi può causare su specie benefiche. L'attività di fagocitosi indotta in vivo è stata valutata inoculando a singoli individui particelle di lattice carbossilato dal diametro di 0.9 µm e fissando mediante protocolli di microscopia elettronica a trasmissione, dopo due ore dall'inoculo, gli emociti per l'analisi ultrastrutturale. Sono state identificate tre tipologie cellulari, proemociti, plasmacociti e granulociti, con un coinvolgimento specifico nella fagocitosi dei granulociti che presentano particelle di lattice all'interno dei fagosomi. L'attività della fenolossidasi plasmatica (PO) è stata valutata misurando fotometricamente la formazione di dopacromo, usando come substrato L-DOPA, in individui di controllo ed in individui trattati con LPS (3mg/mL). Dopo 24 ore dal trattamento in vivo con LPS è stato registrato un significativo (Wilcoxon rank sum test, p=0.018) aumento dell'attività della PO basale (0.0073±0.0022 A492/min/µL n=11) rispetto ai campioni di controllo (0.0023±0.00046 A492/min/µL n=18).

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COINVOLGIMENTO DI MOLECOLE BIOPROTETTRICI NELL'ANIDROBIOSI: IL CASO STUDIO DEI TARDIGRADI

Il coinvolgimento di alcuni enzimi antiossidanti, di alcune acquaporine e del disaccaride trealosio nel processo di anidrobiosi dei tardigradi è stato verificato applicando la tecnica dell'RNA interference nella specie *Paramacrobiotus richtersi*. In particolare, animali attivi sono stati iniettati con gli RNA a doppio filamento costruiti sui geni di interesse in modo da silenziare tali geni, poi seccati sperimentalmente ed infine la loro motilità è stata valutata durante i vari momenti della reidratazione. La corretta applicazione dell'RNA interference è stata verificata effettuando una retro trascrizione PCR sia dei geni silenziati che del gene di controllo della *DNA polimerasi II (DNA pol II)*. Come atteso, per ogni PCR è stata rilevata una riduzione nel livello di espressione dei geni di interesse e non della *DNA pol II*.

All'inizio delle reidratazione (t0), i tardigradi iniettati con gli RNA a doppio filamento per i geni della catalasi (*cat*), della glutatione reduttasi (*gr*) e della superossido dismutasi (*sod*) hanno mostrato una motilità significativamente più bassa di quella degli animali di controllo non iniettati (*cat* e *sod*: p<0,05; *gr*: p<0,01). Il silenziamento del gene codificante per la glutatione perossidasi (*gpx*) ha determinato una riduzione statisticamente significativa della motilità dei tardigradi a t0, dopo un'ora (t1), 24 ore (t24) e 48 ore (t48) dall'inizio della reidratazione (t0: p<0,001; t1, t24, t48: p<0,01). La motilità a t0 degli esemplari silenziati per il gene delle acquaporine 3 (*aqp3*) e 9 (*aqp9*) è risultata inferiore a quella dei controlli (*aqp 3*: p<0,05; *aqp 9*: p<0,01), mentre non sono state osservate differenze significative dopo il silenziamento del gene codificante per l'acquaporina 10. Anche per il gene della trealosio-6-fosfato sintasi (*tps*) non sono state registrate differenze tra la motilità dei controlli e quella degli animali iniettati. I risultati ottenuti dimostrano l'importanza delle acquaporine 3 e 9 e degli enzimi antiossidanti quali *cat*, *gr* e *sod* nella fase iniziale della reidratazione, mentre il gene *gpx* sembra essere coinvolto in tutte le fasi del processo di essiccamento e reidratazione di *P. richtersi*. Nei tardigradi la tolleranza all'essiccamento è quindi dovuta all'interazione di diverse molecole che agiscono tra loro in modo sinergico.

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RACCOLTA DEI NAUPLII DI *ARTEMIA*: UN METODO FACILE, VELOCE ED ECONOMICO PER LA SOMMINISTRAZIONE DI QUESTA INSOSTITUIBILE FONTE DI CIBO VIVO PER ZEBRAFISH

I nauplii di *Artemia* Leach 1819 (Crustacea, Anostraca) costituiscono la fonte di cibo vivo maggiormente impiegato in acquacoltura per svezzare ed accrescere le larve di *Danio rerio* Hamilton 1882 (Zebrafish) e, secessivamente, per incrementarne la fecondità durante lo stadio adulto. Tale alimento non solo costituisce una dieta con ottime qualità nutrizionali perché ricca in acidi grassi essenziali (EFA) per la crescita e lo sviluppo dei pesci e per la produzione di uova di alta qualità, ma risulta anche di facile conservazione quando si trova nello stadio di cisti (uovo duraturo) in condizioni di disidratazione. Inoltre l'impiego di cibo vivo rappresenta un vero e proprio stimolo visivo essenziale per soddisfare l'attitudine predatoria che caratterizza Zebrafish sin dai primi giorni di vita. Sebbene le cisti di *Artemia* attualmente disponibili sul mercato siano caratterizzate da un'elevata qualità di schiusa, risultano ancora molto laboriose e poco efficaci le tecniche utilizzate per separare i nauplii dai residui non commestibili dell'intero processo.

Il metodo che qui proponiamo permette di ottenere un'ottima efficienza di schiusa (HE = 268,000 nauplii/g) in condizioni standard e di raccogliere i nauplii di *Artemia* in quantità molto concentrate (media di 536 ± 79.38 nauplii/ml) ed estremamente pure. In particolare, il procedimento garantisce la totale eliminazione di gusci (corion) e di cisti non schiuse che, se ingeriti dagli avannotti e dagli adulti di Zebrafish durante l'assunzione quotidiana di cibo vivo, provocano gravi problemi metabolici ai pesci. Tale metodo è indicato per gli allevamenti di piccole dimensioni (fino a 1500 pesci) in cui si esegue la somministrazione manuale di nauplii con frequenza giornaliera.

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A HIDDEN MICROSCOPIC WORLD: THE MICROBIOME OF THE SESSILE CILIATE *STENTOR COERULEUS*

The study of microbiomes of multicellular organisms, especially mammals, became one of the trends of the last years. Numerous studies investigated human pathologies and animal behavior through screening of microorganisms forming the microbiota. However, few studies dealt with microbiomes of protists. Protists are widespread in all environments and can be colonized by many different bacteria. Among the plenty of bacterial-protists associations, some pathogenic bacteria like *Legionella*, *Francisella*, *Chlamydia*, *Mycobacterium*, and *Vibrio cholerae* have been retrieved in amoebae and ciliates. The role of protists as “Trojan horses” is well known, first of all amoebae serve as natural reservoirs of pathogens. The aim of this pilot study was to characterize the microbiome of the sessile ciliate *Stentor coeruleus* (Ciliophora), and to investigate if also ciliates may harbour some pathogenic bacteria in low numbers. Herein, we isolated several cells of the *S. coeruleus* from a wastewater stream in Russia. 16S rRNA gene metabarcoding was performed on single cells of *S. coeruleus* and on their environment (wastewater from the same sampling locality). Illumina sequencing was carried out and bioinformatic and statistical analysis were performed. Our results showed that the bacterial community composition differed significantly between *S. coeruleus* cells and the environment, and this result was strongly supported by statistical analysis. The microbiome of *S. coeruleus* cells was similar among all specimen analyzed, and it was composed by a number of different bacteria, including some pathogens, such as *Neisseria*, *Streptococcus*, and human commensals, like *Porphyromonas*. However, some slight differences in bacterial composition were observed among *Stentor* cells, such as the presence of *Holospora obtusa* in one of them. This bacterium is known as a host-specific symbiont colonizing the nucleus of *Paramecium*. Most of the 16S rRNA gene reads belonged to a cosmopolitan endosymbiont called “*Candidatus Megaira polyxenophila*” from the family Rickettsiaceae. Interestingly, this endosymbiont was never found in association to *Stentor*, despite it is very widespread in association with ciliates. Indeed, this bacterium was found as a symbiont also in multicellular organisms, such as cnidarians and algae, and it was detected in animals and plants metagenomes. Our results show that also ciliates can host and shelter potential pathogens, thus facilitating their proliferation and transmission in environment.

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INVESTIGATION ABOUT THE PRESENCE OF 20-HYDROXYECDYSONE AND FLAVONOIDS IN SEVERAL PLANT SPECIES AND IN A TERRESTRIAL ISOPOD TARGET SPECIES, *ARMADILLO OFFICINALIS* (DUMERÏL, 1816) (CRUSTACEA, ISOPODA, ONISCIDEA)

Ecdysteroids are used by insects, crustaceans and other arthropods to control a variety of important physiological events such as moulting, reproduction, as well as metamorphosis. They are unable to synthesize steroids on their own, so they need precursors from plants. Phytoecdysteroids are widely distributed in plant kingdom and 20-hydroxyecdysone (20-E) is the most common amongst them (Dinan, 2001). Compared to aquatic crustaceans, literature lacks information about the presence, the distribution and the role of ecdysteroids in Isopoda Oniscidea, the only Crustaceans taxon to become completely terrestrial (Caruso *et al.*, 1987). Rarely has been discussed about the isolation of ecdysteroids from Isopods, in which, the levels of ecdysteroids greatly exceeded in the hemolymph and they are actively concentrated in the hepatopancreas prior to ecdysis (Suzuki, 1996). The purpose of the present study is to investigate, using HPLC methodic, about the presence of ecdysteroids and flavonoids in *A. officinalis* and in 15 plant species, belonging to different families, sampled in environments in which *A. officinalis* is typically distributed.

Several ecdysteroids and 20-E, in association with flavonoids, were detected in 6 of the 15-species of plants and for the first time in *A. officinalis*, since its original description, the species is well known for its pharmaceutical properties and its use in folk medicine (Duméril, 1816). These results suggest that terrestrial isopods probably take 20-E, or precursors of it, from plants that they fed on. Further investigations would aim to study the relationship between plants and animals and pharmacological applications.

ALTERAZIONI MORFOFUNZIONALI DELL'APPARATO BRANCHIALE DI *DANIO RERIO* DOPO ESPOSIZIONE A TEBUCONAZOLO

Negli ultimi decenni l'uso di pesticidi a fini agricoli è esponenzialmente aumentato e negli agroecosistemi la contaminazione ambientale rappresenta una delle principali minacce per la fauna selvatica. I fungicidi sono una classe di pesticidi ampiamente utilizzata nelle moderne pratiche agricole. Essi possono essere rinvenuti in tutti i comparti ambientali a concentrazioni molto superiori rispetto alle altre classi di agrochimici, anche in relazione alla loro ripetuta applicazione durante l'anno a scopi profilattici. Nei pesci, una delle principali vie di assorbimento dei fungicidi è rappresentata dall'apparato branchiale, un organo che svolge diverse e fondamentali funzioni quali la respirazione, l'osmoregolazione, l'escrezione ed il mantenimento del bilancio idrico e ionico. Il tebuconazolo (TBZ), un fungicida triazolico ad ampio spettro, è uno dei fungicidi più venduti in tutto il mondo ed in Europa esso è stato rinvenuto in numerosi ecosistemi d'acqua dolce raggiungendo una concentrazione pari a 175-200 µg/L in alcune acque superficiali. I dati bibliografici mettono in evidenza le proprietà di distruttore endocrino del TBZ e questo rappresenta l'aspetto maggiormente indagato dagli studi sinora condotti; per contro le informazioni relative ad altri meccanismi di tossicità sono estremamente scarse. Nel presente studio, usando zebrafish (*Danio rerio*) come modello sperimentale, sono state analizzate per la prima volta le alterazioni morfologiche ed ultrastrutturali indotte dal TBZ sull'apparato branchiale. L'esposizione - per 48, 96 e 192 ore - ad una concentrazione di TBZ estremamente bassa e riscontrabile in natura ha indotto severe alterazioni e l'intensità delle lesioni è stata tempo-dipendente. Le principali alterazioni osservate sono state: proliferazione dell'epitelio primario, ectopia delle Chloride cell e riduzione delle lamelle secondarie; inoltre estesi fenomeni apoptotici e necrotici sono stati riscontrati sia nel filamento che nelle lamelle. Tali alterazioni sono in grado di compromettere le funzioni respiratorie ed osmoregatorie dell'apparato branchiale come dimostrato dalla ridotta espressione della Na⁺/K⁺-ATPasi e dell'acquaporina 3. L'esposizione al fungicida ha inoltre indotto un severo aumento nell'espressione della superossido dismutasi, un enzima di importanza fondamentale per il corretto mantenimento dello stato ossidoriduttivo cellulare.

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AQUATIC ACOUSTIC NOISE: BEHAVIORAL AND MOLECULAR RESPONSES IN ECHINODERMS, THE CASE OF *A. LIXULA* (LINNAEUS, 1758) SEA URCHINS

Anthropic noise is considered a real pollutant, in particular the submarine noise. The impact on biodiversity is not yet sufficiently understood. Further research is needed to evaluate any negative effects. The noises associated with anthropogenic activities are increasing: shipments, seismic surveys, sonar, recreational rowing and future mineral extraction activities from ocean depths (DSM). These noises are having an impact on the welfare of many marine species. The understanding of the effects on biodiversity could be important for the creation of guidelines, laws or rules for the most environmentally sustainable exploitation of natural resources. Our study aims to investigate the motility, biochemical and molecular responses of *Arbacia lixula* exposed to an acoustic stimulus produced by anthropogenic activities and perhaps perceptible by invertebrates. The animals were divided into a control tank and experimental tank. The specimens were exposed to sonic stress for 3 hours after which biological sampling was performed. The sonic stress used was a linear sweep from 100 to 200 kHz emitted every 1 second. The Sound Pressure Level ranges between 173 and 181 dBrms (re 1µPa), with a peak at 150 kHz. To measure the motility, on the top of the tanks two cameras were placed to make photo each 8 seconds during acoustic stimulus. The bottom of the tanks were divided virtually by squares and the vertical walls were divided by two quote. At each photo we assessed the position of the animals and count the number of squares/levels crossed comparing to the precedent photo. In this way we obtained the motility of the specimens in the three directions (cm/s). After three hours of stimulus projection, the sea urchins were captured and the coelomatic fluid was extracted. The protein concentration and the enzymatic activities of esterase, phosphatase, catalase and peroxidase were measured on the celomocytes and on the supernatant of the celomatic fluid. The gene expression of HSP70 and HSP90 with RT-PCR was evaluated on celomocytes. Exposure to this type of noise produced a significant changing in motility and an increase in the expression of HSPs gene, more so for HSP70. No statistical difference was observed in the extent of enzymatic activities and protein concentration. The results obtained indicate that this type of acoustic stimulus has effects on the behavior and on the gene expression of HSPs of individuals of *Arbacia lixula*.

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EVALUATION OF DIMETHOATE TOXICITY IN *ARMADILLIDIUM VULGARE* (LATREILLE, 1804) (CRUSTACEA, ISOPODA, ONISCIDEA)

In the soil compartment, terrestrial isopods play a key role in decomposition processes, vegetal litter fragmentation and nutrients recycling processes. Agricultural and industrial practices lead to soil contamination by xenobiotics. Isopods exposure to xenobiotics may cause a decrease in soil quality and soil ecosystem services. One of the most used insecticide in agricultural practices is dimethoate, an organophosphate, which successfully combines a selective toxicity to insects through a systemic action. It is known that this insecticide inhibits AChE (acetylcholinesterase) activity like other organophosphates, resulting in nerve damage which may lead to death. It was observed that dimethoate has a significant adverse impact on many organisms. Several studies have been conducted on the isopods locomotor behavior effects caused by dimethoate, but none of them has investigated the bioaccumulative potential of this substance. The aim of this study is to evaluate the toxicity of dimethoate in terms of mortality, in isopods belonging to the species *Armadillidium vulgare*, and to evaluate the expression of specific exposure biomarkers, such as HSP70 and CYP1A, by immunohistochemical analysis. The organisms used in this experiment were obtained from laboratory cultures; they were maintained for 30 days in plastic boxes containing 3 cm of soil. Scalar concentrations of dimethoate were used (4×10^{-3} , 4×10^{-4} , 4×10^{-5} mg/10 ml) and, in food exposure assays, the insecticide (20 μ l) was applied on every potato slice. No lethal effects, changes in locomotor behavior or damage to the hepatopancreas cells were observed, although immunohistochemical analysis revealed a marked positivity to antibodies anti-CYP1A and HSP70.

EFFECTS OF BISPHENOL A ON PIGMENTED ORGAN DEVELOPMENT IN ASCIDIANS

Bisphenol A (BPA, 2,2-bis-(4-hydroxyphenyl)-propane) is an organic compound used in the manufacturing of polycarbonate plastic and epoxy resins, the former employed in the realization of food and beverage containers, the latter in dental materials, for the lining of food and beverage containers and water supply pipes. After degradation of these products, BPA can be released into the environment from sewage treatment effluents and landfill leachates. It can act both as an endocrine disruptor by binding to different nuclear receptors and as teratogenic molecule.

Recently BPA-induced impairment of brain and sensory organs were reported in zebrafish and *Xenopus laevis*, with alteration of otolith formation and eye dysplasia respectively. In zebrafish, the malformations were demonstrated to be due to the BPA interaction with Estrogen Related Receptor (ERR). Ascidian larvae present two sensory pigmented organs in the trunk, otolith and ocellus. We analyzed the effects of BPA exposure during embryonic development on sensory organs formation in two ascidian species, *Ciona robusta* and *Phallusia mammilata*. To elucidate if BPA effects are exerted through ERR binding also in ascidian, a co-exposure with a ERR antagonist (4-OHT) was performed. After treatment with BPA, we reported the presence of mildly affected phenotypes, characterized by the presence of two pigmented organs with altered deposition of pigments, and severe affected phenotypes, characterized by the absence of one or both pigmented organs or extranumerary pigmented organs, in both species. With immunostaining and in situ hybridization techniques we demonstrated that either two otoliths or two ocelli can differentiate in treated larvae. Precursors of pigmented organs resulted altered in number and position as early as tailbud stage, indicating that BPA acts during the first stages of development. In *P. mammillata* the co-exposure to 5 μ M BPA and 1 μ M 4-OHT resulted in a reduction of abnormal phenotypes comparing to exposure to 5 μ M BPA alone. The rescue of normal phenotypes after co-exposure to 4-OHT suggests that BPA acts through binding to ERR also in ascidians, revealing a similar mode of action in vertebrates and invertebrates. These results highlight that ascidians are valuable invertebrate models for testing pollutants and investigating their way of action.

I NUOVI CONTAMINANTI: EFFETTI DELL'IBUPROFENE SULLO SVILUPPO DEL RICCIO DI MARE *PARACENTROTUS LIVIDUS*

Si definiscono “contaminati emergenti” quelli non inclusi nei programmi di monitoraggio di routine e per i quali non si ha una conoscenza sufficiente del rischio sanitario e ambientale associato alla loro presenza. La loro presenza nelle acque è considerata uno dei problemi ambientali più rilevanti dell'ultimo decennio.

Tra queste sostanze troviamo farmaci e ormoni, micotossine e fitofarmaci che a concentrazioni ambientali rilevanti sono in grado di indurre effetti in organismi non bersaglio. La valutazione del rischio ecologico è ancora agli inizi e spesso l'analisi della loro azione viene trascurata nella valutazione del rischio ambientale. Esistono esempi eclatanti come quello del ritrovamento nelle carni dei salmoni di 81 farmaci e prodotti per la cura della persona. In particolare, la presenza di farmaci commerciali, che possono alterare le vie chimiche degli organismi con effetti negativi sulla salute dell'uomo e degli organismi acquatici, può determinare importanti effetti sulla riproduzione, inclusa l'infertilità, come già osservato in alcuni pesci.

In questo lavoro, sono stati valutati gli effetti dell'Ibuprofene sullo sviluppo del riccio di mare *Paracentrotus lividus*. L'Ibuprofene è un farmaco antinfiammatorio non steroideo (FANS) largamente impiegato nel trattamento delle malattie reumatiche. La fertilità dei gameti dopo esposizione per 1h a tre diverse concentrazioni è stata valutata verificando la loro capacità di fecondare (spermi) o di essere fecondati (uova). In tutti i casi analizzati la percentuale di fecondazione era uguale a quella dei gameti non trattati. Tuttavia, dalle uova pre-trattate con Ibuprofene si sviluppavano embrioni anomali: 20% a 5, 50 µg/L, 100% a 500 µg/L. Lo sviluppo degli embrioni derivanti da spermi trattati prosegue invece correttamente fino alle 24h, mentre il pluteo mostra numerose anomalie sia scheletriche che agli organi interni. Inoltre, l'esposizione degli zigoti alle stesse concentrazioni di Ibuprofene ha determinato effetti sullo sviluppo con anomalie osservabili già allo stadio di blastula e alla concentrazione di 5 µg/L. Ritardo dello sviluppo e deformazioni dello scheletro si registravano negli stadi successivi.

Questo studio ha dimostrato il potenziale effetto tossico dell'ibuprofene sullo sviluppo del riccio di mare, ma rappresenta solo un punto di partenza per meglio valutare i rischi ambientali che possono derivare dalla presenza anche concomitante di tali contaminanti negli ambienti acquatici.

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KILLING EFFECT OF RHEINHEIMERA SP. EPRS3 (GAMMAPROTEOBACTERIA) AGAINST EUPLOTES AEDICULATUS (CILIOPHORA, SPIROTRICHEA): DISCOVERING THE ECOLOGICAL ROLE OF ANTIMICROBIAL COMPOUNDS FROM ENVIRONMENTAL BACTERIAL STRAINS

Bacterial endophytes are widely studied for their ability to produce antimicrobial compounds. *Rheinheimera* sp. strain EpRS3 isolated from the rhizospheric soil of the medicinal plant *Echinacea purpurea*, is known for its ability to produce antimicrobial compounds inhibiting the growth of other bacterial species, both environmental and opportunistic human pathogens. *In vivo* experiments demonstrated a killing effect of EpRS3 strain against the ciliated protist *Euplotes aediculatus* strain EASCc1, which is known by FISH and TEM analyses to harbour in the cytoplasm the obligate bacterial endosymbiont *Polynucleobacter necessarius* (*Betaproteobacteria*) and the *Francisella*-related endosymbiont "*Candidatus* *Nebulobacter yamunensis*." (*Gammaproteobacteria*). When the ciliate is treated with supernatant of *Rheinheimera* sp. culture (liquid broth free of bacteria), the number of living eukaryotic cells decreases with respect to control cells. TEM analysis, aimed at revealing the induced ultrastructural ciliate cell damages, showed that inside these ciliates *P. necessarius* endosymbionts went into degradation and vacuolization. When the ciliate culture is inoculated with bacteria plus medium, the number of living eukaryotic cells decreases until disappearance within six hours, and many damaged or highly degraded ciliate mitochondria and a decrement in number of *P. necessarius* endosymbionts are recorded. Additionally, in these ciliates both TEM observation and FISH experiments performed using a specific molecular probe disclosed the presence of *Rheinheimera* sp. both inside phagosomes and free in the cytoplasm, suggesting that it is somehow capable to escape from food vacuoles avoiding ciliate digestion. The obtained results suggest that *Rheinheimera* sp. EpRS3 produces and releases in liquid culture one or more compounds affecting *E. aediculatus* survival. Analyses are now focused on clarifying the molecular mechanisms behind the observed *Rheinheimera*'s killing effect.

FAUNA OF SUBMARINE CAVES OF THE SALENTO PENINSULA

A review of available literature has been realized to assess the biodiversity of the important E.U. habitat (code 8330: “submerged or partially submerged sea caves”) represented by more than 100 submarine caves around the Salento Peninsula (SE Italy). Information came from 18 degree thesis, 3 PhD thesis, and 43 papers resulting from researches explicitly carried out in Salento submarine caves in the last 50 years (1966-2016). The number of taxa reported (650) is interestingly high notwithstanding the exclusion of plant species (algae, present only at the entrance and not in the dark of the caves) and the limited space available (if compared with neighboring open sea habitats) approximately evaluated as 0.2 Km². The taxa list of Salento submarine caves share 254 and adds 392 taxa to the pioneer and extended study of Riedl (1966) on 277 Mediterranean submarine caves (181 in Italy, with a total of 779 taxa reported). A comparison has also been carried out with the adjacent marine habitats of *Posidonia* meadows and coralligenous. The relatively high number of animal taxa is not due to the habitat surface (Salentine submarine caves are represented by about 0.2 Km² of substratum, against 65.0 and 127.0 Km² of Salentine *Posidonia* and coralligenous, respectively). Although some important taxa (e.g., Mollusca) have not been studied in detail, the contribution to the species richness is due for sure to the communities considered, rich of small species as the zooplankton or the meiobenthos, but also to the rarely investigated taxa (e.g., Tardigrada, Gastrotricha, and Facetotecta). In addition, the peculiarity of the environment (with enhanced environmental gradients and co-presence of rocky, sandy, and muddy habitats in small spaces), the particular bio-geographic position of the Salento Peninsula, and the attention paid by many scientists, are probably co-responsible of the report of uncommon high number of faunal novelties, with 62 taxa new for Italian marine fauna, and 27 new for Science.

RED MARK SYNDROME OF RAINBOW TROUT: COULD THE PARASITIC CILIATE *ICHTHYOPHTHIRIUS MULTIFILIIS* BE THE DISEASE VECTOR?

Red Mark Syndrome (RMS) is a skin condition affecting farm-reared rainbow trout *Oncorhynchus mykiss*. Although RMS is non-debilitating for fish, it causes serious economic losses to fish farm.

Previous studies suggested a bacterium from the family “*Candidatus* Midichloriaceae”, indicated as *Midichloria*-like organism (MLO), as a possible etiological agent for RMS, but this hypothesis has not been confirmed yet.

In the ciliate fish parasite *Ichthyophthirius multifiliis*, some bacteria highly similar to RMS-MLO were found as endosymbionts, thus it can be hypothesized that *I. multifiliis*, or other ciliates, may act as vector or facilitate the transmission of RMS.

The main purpose of this study was to verify if bacteria from “*Candidatus* Midichloriaceae” can infect *I. multifiliis* when exposed to RMS diseased fish. Specific Pathogen Free (SPF) fish were employed for *I. multifiliis* production, adding two SPF fish in two tanks. Once these trouts were ready to shed *I. multifiliis*, SPF rainbow trout was euthanized and transferred to a small aquarium. During maturation of *I. multifiliis*, fish started to present the typical white spots caused by parasitic ciliate infection, and at this stage, *I. multifiliis* jumped out from the fish epithelia (T0). A part of these ciliates was kept in the aquarium until theronts were released, then a suitable amount was added together with water in tanks containing large SPF and RMS fish. The rest of *I. multifiliis* was fixed for different analysis aimed to verify if these ciliates hosted the MLO.

The previous steps, necessary to prepare *I. multifiliis* infection, were repeated and two separate infection experiments were carried out (T1=first infection experiment; and T2=second infection experiment). *I. multifiliis* cells were checked by fluorescence in situ hybridization, and the presence of MLO was detected in *I. multifiliis* both in T1 and T2.

Different combination of specific MLO primers were used to characterize the MLO organisms observed in *I. multifiliis* infected cells from T1 and T2. The sequence obtained was 1008 bp long and 99.14% identical with *Rickettsia*-like organism from rainbow trout (EU555284, Lloyd et al. 2008) and 99.12% identical with bacterium associated to *I. multifiliis* (KT851863, Zaila et al. 2017).

In conclusion, RMS-MLO can infect *I. multifiliis* when this ciliate is exposed to RMS diseased fish, thus opening new interesting perspectives aimed to study the development of RMS thanks to the future genomic analysis.

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TOXICITY EVALUATION OF ENGINEERED TITANIUM DIOXIDE NANOPARTICLES IN *APIS MELLIFERA* L., 1758 (HYMENOPTERA, APIDAE)

The aim of this study was to evaluate the toxicity of titanium dioxide nanoparticles (TiO₂NPs) by short-term toxicity tests on *Apis mellifera*. Literature data on TiO₂NPs toxicity on this species are lacking, and it was an incentive to investigate the potential toxic effects of TiO₂NPs on *A. mellifera*, considered a bioindicator organism mainly due to its sensitivity. The research of exposure biomarkers like metallothioneins 1 (MT1) and Heat Shock Protein 70 (HSP70) was performed to verify if a detoxification mechanism has been activated in the exposed animals, as already demonstrated by Salvaggio *et al.* (2017). The honey bees used for experiments were provided by local beekeepers. The TiO₂ nanopowders were supplied and characterized by the CNR-IMM of Catania. Starting from a TiO₂NPs solution (1 mg TiO₂/10 ml distilled water), three concentrations (0.1 mg/10 ml; 0.01 mg/10 ml; 0.001 mg/10 ml) were obtained. The bees were housed in plastic boxes in which a sugar solution called “*candito*” was placed, obtained by mixing the different TiO₂NPs solutions to the powdered sugar. In addition, the same TiO₂NPs solutions were sprayed on the flowers introduced into the boxes. The “*candito*” made only with distilled water and powdered sugar was provided to the controls. At the end of the treatment (10 days) the bees were sacrificed and gut and honey sac from each sample were collected. No histological alteration on the epithelium of the honey sac was observed in exposed organisms. A significant positivity for anti-MT1 antibody was observed only in the honey sac; no positivity was observed in the intestinal epithelium. A weak positivity for HSP70 was observed both in honey sac and gut. While in other studies (Scuderi *et al.*, 2014; Brundo *et al.*, 2016) was shown the non-toxicity of TiO₂NPs on other model organisms, in our study, titanium dioxide nanoparticles was proven to be highly toxic at the highest concentration tested and moderately toxic at lower concentrations. This study represents an effort to demonstrate the effects of nanocomposites on the bees and, because of the massive use of nanocomposites in the last decade, it is important to monitor the environment in which these xenobiotics are released.

FIRST RECORD OF PREDATION BY *PLUTONIUM ZWIERLEINI* CAVANNA, 1881 (CHILOPODA SCOLOPENDROMORPHA) ON *SPELEOMANTES SUPRAMONTIS* (LANZA, NASCETTI & BULLINI, 1986) (AMPHIBIA PLETHODONTIDAE) IN SARDINIA, ITALY

Plutonium zwierleini Cavanna, 1881 is the largest scolopendromorph centipede of Sardinia, discovered for the first time in the island over 130 years ago. Recently this species has been found in karst cavities of Central-East Sardinia (Supramonte karst massif). Extremely difficult to observe in natural conditions, the geographical distribution and ecology of *P. zwierleini* are still little known.

During some subterranean environment monitoring, on 12 May 2018 the first case of predation by this centipede on the vertebrate species *Speleomantes supramontis* (Lanza, Nascetti & Bullini, 1986) was accidentally documented into Istirzili Cave (Baunei, SA/NU 050). This cavity develops within Jurassic dolostones at the south-western margin of the outer Supramonte. Its entrance opens on the left side of the Bacu Stirzili valley (at 515 m a.s.l.). Cave atmosphere is characterised by a temperature of 14.4 °C and water vapour saturation (100% of relative humidity). The existence of an abundant invertebrate fauna and of cave salamanders and bats has been reported in previous biospeleological researches. Also the presence of *P. zwierleini* has lately been confirmed for this cave.

The centipede was observed in the western side of the cave, in an area where sediments and organic matter periodically enter from outside. It was moving quickly toward the entrance on a gently sloping flowstone floor and had already captured its prey. *P. zwierleini* was an unsexed specimen of about 13 cm long while the prey cave salamander was a young one, being only 5 cm long. The centipede was holding the cave salamander tightly by the belly with its last pair of legs and lifting the hind legs of its prey from the ground. Despite the cave salamander was still living, the predator dragged it sensing the ground with its antennae. The vertebrate was resisting, trying to adhere to the surface with its front legs still free. The activity of the centipede has been documented for about 15 minutes, covering about 5 m, but it is unknown whether the prey has been consumed or not. The predation of large centipedes on vertebrates is known only for tropical caves and this record provides the first evidence for Italy and Europe. Although previously considered doubtful, the use of ultimate legs of *Plutonium* in prey capture is also confirmed. This data is of particular interest as nothing is known about the *Plutonium* diet and the possible predation to which cave salamanders are exposed during their stay in cave.

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TERATOGENIC EFFECTS OF MOLYBDENUM DISULFIDE IN *GALLUS GALLUS DOMESTICUS*

The potential offered by the use of nanocompounds is impressive and attractive, they have applications in various sectors, but despite this the increase in their production has generated various doubts about the possible effects that their release may have on the ecosystem and on human health. Recently two-dimensional (2D) nanomaterials, such as graphene and molybdenum disulfide (MoS₂), have received much attention as adsorbent materials for the effective removal of organic contaminants. MoS₂ is attracting increasing attention, not only for its chemical-physical properties, but also for its wide availability in nature as a constituent of molybdenite. MoS₂ has a crystal structure consisting of weakly coupled layers of S-Mo-S, where a layer of Mo (molybdenum) atoms is covered by two layers of S (sulfur) atoms. In the environmental field, MoS₂ is used as a semiconductor for the disinfection of water, in addition its photocatalytic properties are also useful for the removal of organic dyes from wastewater. The aim of this investigation was to assess the effects of different MoS₂ concentrations on the embryonated eggs of *Gallus gallus domesticus*. We evaluated the toxic effect of the MoS₂ powder purchased at sigma-aldrich indicated as "received" and MoS₂ powder treated via mechanical milling indicated as "ball mille". According to the Beck method 100 µl of each concentration (0.5 mg / ml, 0.05 mg / ml, 0.005 mg / ml) were inoculated in the embryonated eggs which then were placed in an incubator at 37.8 °C for the incubation period. Subsequently, the embryos were sacrificed at different times of embryonic development (11th, 15th and 19th day after incubation) in order to evaluate their embryotoxic and teratogenic effects. The alterations of the embryonic development were studied through a morphological analysis of the tissues with histological and histochemical techniques, instead the bioaccumulation was evaluated by ICP-MS. The results obtained from our investigation have shown the toxicity of both powders of MoS₂ with a higher percentage of deaths and growth delays for the powders of ball milled MoS₂ at the maximum and intermediate concentrations, while for the minimum concentration the histological analysis of the embryos sacrificed on the last day showed alterations with related hemorrhages in the liver and lungs. Moreover embryos's livers exposed to MoS₂ have shown a response to antibodies anti-metallothionein 1.

**THE WATER FROG *PELOPHYLAX KL. ESCULENTUS* AS AN
EXPERIMENTAL EMBRYOLOGICAL MODEL FOR
NANOTOXICOLOGICAL STUDIES**

Nanomaterials have unique properties in respect to their larger counterparts because of quantum size effects and relatively large surface area to volume ratio. The interactions among nanomaterials, living organisms and the environment are complex. Because of the increasing development of engineered nanomaterials in industrial, medical and home applications, it is important to evaluate their effect on living organisms and identify models for research. In our laboratory we are testing the effects of engineered metallic nanoparticles of iron, nickel and cobalt on the embryogenesis of the water frog *Pelophylax kl. esculentus*. In respect to other model amphibians, such as *Xenopus laevis* it presents the advantage of being autochthonous in Italy and it has already been used as a bioindicator in ecotoxicological studies. We sampled a population of frogs living in the Botanical Garden of the University of Bari. Embryos at Gosner's developmental stage 10-11 were treated with increasing concentrations of iron, cobalt and nickel nanoparticles (LC50/2, LC50, and 2xLC50) in native water and the effects on the development were observed after 10 days. Treatments showed significant retard in growth and developmental stages, that in most cases did not reach Gosner's stage 21 whereas most controls reached Gosner's stage 23 (= hatching). In several treatments malformations were observed such as abnormally large ventral mass, bent body axe, and underdeveloped eye. The epidermis was analyzed at microscopical level. There are four cell types, i.e. ciliated cells, muciparous cells and two types of ionocytes, indicated as I and II, respectively. The cells of treatments showed signs of oxidative stress indicating an increased volume and mitochondrial swelling. Ultrastructural analyses indicated aggregates of nanoparticles out of the cell membranes and in endocytotic vesicles in the cytoplasm. These data indicate that nanoparticles can have massive effect on the development of the water frogs and confirm that they can be good models in nanotoxicology.

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**“MITOCHONDRION, SWEET MITOCHONDRION”: THE ENDOSYMBIONT
“*CANDIDATUS BANDIELLA WOODRUFFII*” AND ITS POSSIBLE
LOCALIZATION INSIDE *EUPLOTES WOODRUFFI* RESPIRATORY
ORGANELLE**

Euplotes species (Ciliophora, Spirotrichea) display a certain attitude to harbor microbial consortia, especially if they present the obligate symbiosis with *Polynucleobacter necessarius* (Heckmann et al., 1983; Boscaro et al., 2012, 2013; Schrallhammer et al., 2013; Vannini et al., 2010, 2012, 2014). Herein we describe the case of *Euplotes woodruffi* strain NDG2, sampled in an inner channel of Visakhapatnam harbor (Andhra Pradesh, India), in which three endosymbiotic bacteria have been detected by means of FISH analysis: *Polynucleobacter necessarius* (*Burkholderiales*, *Burkholderiaceae*), “*Candidatus* (*Ca.*) *Megaira polyxenophyla*” (*Rickettsiales*, *Rickettsiaceae*), and “*Ca.* *Bandiella woodruffii*” (*Rickettsiales*, *Midichloriaceae*). Among those three endosymbionts, *P. necessarius*, is known to be an obligate endosymbiont of several *Euplotes* species (Vannini et al., 2012) and “*Ca.* *Megaira polyxenophyla*” has been repeatedly found in several ciliate hosts, belonging to different genera and even classes (Schrallhammer et al., 2013) and in metazoans as well (Penn et al., 2006; Fraune & Bosch, 2007; Sunagawa et al., 2009; Murakami et al., 2017). “*Ca.* *Bandiella woodruffii*”, on the contrary, has been described from an *E. woodruffi* strain from Brazil (Senra et al., 2015) and, so far, detected exclusively in this ciliate species. *P. necessarius* and “*Ca.* *Megaira polyxenophyla*” are cytoplasmic endosymbionts, and, similarly, “*Ca.* *Bandiella woodruffii*” has been supposed to be harbored in the host cytoplasm according to FISH analysis in the original description of the species (Senra et al., 2015); however, to date, no data concerning its morphology and ultrastructure are available. Preliminary TEM analysis carried out in the present work on *E. woodruffi* strain NDG2 consortium, suggest that “*Ca.* *Bandiella woodruffii*” could occupy the mitochondrion of the ciliate. Interestingly, this feature has been previously described in other *Midichloriaceae*, such as “*Ca.* *Midichloria mitochondrii*”. Indeed, this close relative of “*Ca.* *Bandiella woodruffii*”, is specifically adapted for colonizing these cellular compartments in the ovarian cells of the tick *Ixodes ricinus* (Sassera et al., 2006). Further analyses with imaging techniques are currently undergoing with the aim to validate these ultrastructural observations.

IL MUSEO DI ZOOLOGIA DELL'UNIVERSITÀ DELLA CALABRIA: METODOLOGIE PER LE ATTIVITÀ DIDATTICHE E FORMATIVE NELL'AMBITO DEL PROGETTO "IL MUSEO SI APRE AI PICCOLI"

Il Museo di Zoologia dell'Università della Calabria, dopo un periodo iniziale, destinato al recupero delle collezioni ed alla loro sistemazione, ha iniziato ad operare volgendo particolare attenzione a tutte quelle attività rivolte alla divulgazione della cultura scientifica, sia a favore degli studenti che frequentano i corsi di laurea che alle scuole del territorio. Il Museo ha avvicinato gradualmente studenti e famiglie attraverso diverse iniziative con l'obiettivo di far conoscere sia la nuova struttura che l'attività svolta. La funzione di una istituzione museale può essere di ausilio alla didattica frontale svolta nelle classi, offrendo la possibilità di utilizzare strumenti normalmente non disponibili nelle scuole e che derivano dalla conservazione di materiale zoologico all'interno di collezioni scientifiche; questo materiale è in parte reso fruibile ad un vasto pubblico attraverso l'allestimento di settori espositivi. Parallelamente a questo aspetto, che ha a che fare con la natura stessa del museo, la realizzazione di laboratori didattici e la disponibilità di personale specializzato, consente al Museo una partecipazione ancora più attiva all'insegnamento delle Scienze Naturali nelle scuole di ogni ordine e grado. Una volta consolidato il rapporto con le scuole, l'attenzione è stata rivolta alle famiglie. Il Museo ha dunque promosso progetti educativi ed eventi pomeridiani dedicati ai ragazzi e alle famiglie offrendo cultura scientifica e intrattenimento. Tutte le attività hanno privilegiato l'uso delle risorse museali come i reperti naturalistici e le sale espositive, organizzate come spazi di contatto e sperimentazione. Le attività educative proposte, diversificate per livello scolastico nelle tematiche affrontate, nell'approccio, nel linguaggio e nelle strategie educative, sono esperienze coinvolgenti che favoriscono la partecipazione attiva degli studenti, il confronto di idee e la promozione di un pensiero critico. La didattica della Zoologia si è rivelata un potente elemento di attrazione di una vasta utenza scolastica che va dai bambini delle scuole dell'infanzia agli studenti delle scuole secondarie di primo grado. Visite guidate, laboratori ed eventi incentrati sulla zoologia, in base alle esperienze raccolte direttamente e tramite questionario, hanno suscitato un riscontro molto positivo da parte dei ragazzi, degli insegnanti e delle famiglie. Tutte le attività proposte hanno richiamato grande partecipazione ed entusiasmo.

THE LEGACY OF QUATERNARY COLONIZATION WAVES ON THE CURRENT MTDNA DIVERSITY OF TWO SICILIAN DIAPTOMIDS

Diaptomid copepods are known to be sound biogeographical indicators at a regional scale, and the central Mediterranean area is a renowned biodiversity hotspot for this taxon. Diaptomid regional molecular diversity was thus investigated with the aim of contributing to shed light on the origin and affinities of Sicilian inland water biota.

Two taxa with sharply different distribution areas, i.e the north-eastern graviting *Diaptomus serbicus* (Gjorgjevic, 1907) and the southern-graviting *Metadiaptomus chevreuxi* (Guerne & Richard, 1890), were analysed through the sequencing of a fragment of the mitochondrial DNA cytochrome b from different populations covering their whole distribution ranges. Obtained results shows that *D. serbicus* presents a private haplogroup in Sicily, which is possibly related to its ancient colonisation of the island and its subsequent differentiation *in loco* through independent evolution and lineage sorting. Conversely, *M. chevreuxi* Sicilian populations are genetically homogeneous and their single observed haplotype is shared with a central Tunisian population, which suggests its recent colonisation of the island from the Maghreb. These evidences are concordant with the phylogeographic patterns reported for other Sicilian diaptomid species and suggest the existence of at least two allochronic “colonization waves” constituting the current Sicilian freshwater crustacean fauna: an older one (possibly dating back to late Pleistocene) originating from the Balkan Peninsula, and a more recent, post-glacial one, which is currently underway, originating from the Maghreb.

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CLIMATE EVENTS RELATED TO BREEDING PERFORMANCE OF WHITE STORK *CICONIA CICONIA* L., 1758

Weather is an important regulator factor of the breeding success of birds (MOSS *et al.*, 2001).

The largest White Stork population in Sicily breeds in the Special Protection Area (SPA) “Plain of Gela” at the top of Medium Voltage electricity poles. During our study we have collected data on the breeding failures due to adverse weather conditions.

From 2016 to 2018, 29 nest failures due to three different causes were documented.

In 2016 there were 19 breeding failures (44.2% of the total nesting) due to the torrid and dry climate, persistent from April to June. The late-breeding has played a decisive role. In fact, the early breeding pairs have a greater productivity, according to the literature (TRYJANOWSKI *et al.*, 2009) and to the theories on the reproductive chronology of the birds (SVENSSON, 1997). It is assumed that the pairs in better condition breed earlier (TRYJANOWSKI *et al.*, 2009). Drought conditions can reduce food availability and consequently increase hunger and weakening of chicks. In the Plain of Gela 7 dead chicks under 20 days of age were found; according to other authors they not have already thermoregulatory ability (TORTOSA and CASTRO, 2003) and they probably suffered from stressful conditions (JOVANI and TELLA, 2004). In addition, extreme heat weather conditions can favour parasitic infections, which increase chick mortality (NEWTON, 1998). In 2017, a strong storm caused the greatest number of failures (19, that is the 50% of the total annual number of nests); punctual rainy springs could have a negative effect on the reproduction of White storks (JOVANI and TELLA, 2004). In 2018, furthermore, 5 nests were knocked out of the pylons by a windstorm (50-70 km/h), killing chicks and breaking eggs. A long-term study in progress will clarify the effects of wheatear on the overall mortality rate of young storks, already high due to electrocution (ZAFARANA and BARBERA, 2016).

For windstorms, it may be useful to pre-install nest platforms to protect future built nests.

This solution would also help the power companies to have no problems with branches that, moved by the wind or by white storks, can make short-circuits, malfunctions and, consequently, higher costs for electric companies and inconvenience for human communities.

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THE ENVIRONMENTAL IMPACT OF POWER LINES ON BIRDS IN SICILY

Electrocution is a serious conservation problem worldwide for a large number of bird species (BEVANGER, 1994, 1998; BIRDLIFE INTERNATIONAL, 2004; PRINSEN *et al.*, 2011). Due to its wide extension, it is necessary to seek methods that optimize the identification of the most dangerous pylons (JANSS and FERRER, 2001; MANOSA, 2001), lines and the highest risk areas (TINTÓ *et al.*, 2010; GUIL *et al.*, 2011). Actually, the data on bird mortality caused by electrocution and collision in Sicily are deficient, however, a preliminary study suggests that the White Stork (*Ciconia ciconia* L., 1758) is one of the most threatened species inside two Special Protection Areas (SPA) (ZAFARANA and BARBERA, 2016). The project C.L.E.S.A. aims at making a complete checklist of the species at risk, collecting information from published articles and personal reports. Unpublished data were collected through a request for information widely circulated among professional and dabbler ornithologists, local sections of bird conservation ONG and wildlife services. A standard monitoring method was used to collect data, monthly during the entire sampling period. First, we selected different transects randomly, and, subsequently, we counted and removed all the birds found dead. A total of 152 cases were collected from 1996 to 2017, of which 85 caused by electrocution and 67 by collision. The 55.5% of the founded species are considered as “Birds of Community Importance” (included in Annex I of the Council Directive 2009/147/EC on the conservation of wild birds). *C. ciconia* is the most species killed by electrocution and *Phoenicopterus roseus* Pallas, 1811 by collision. These preliminary results suggest that this problem, as well as having serious consequences in terms of conservation, could have serious economic repercussions for human societies, as power failures, loss of revenue, necessity of repairs infrastructures and cost of legal compliance (LEHMAN *et al.*, 2007). The monitoring activities of the C.L.E.S.A. volunteers have the purpose of implementing direct conservation actions for endangered species. The synergy between power companies and C.L.E.S.A. will be basic to adopt the measures suggested by the Conference of the Parties in Resolution 7.4 "Electrocution of migratory birds" (BONN, 2002) and the current management plans for SPAs. This partnership will improve the effective field actions in Sicily.

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