UNIVERSITÀ DI PISA

bio
DIPARTIMENTO di BIOLOGIA

CONGRESSO CONGIUNTO

83° CONGRESSO
UNIONE ZOOLOGICA
ITALIANA

34° CONGRESSO
SOCIETÀ ITALIANA DI
PROTISTOLOGIA

BOOK OF ABSTRACTS
POSTERS

11-14 settembre 2024 Pisa

I contributi evidenziati in verde e gli autori contrassegnati con (*) partecipano al premio UZI Giovani Ricercatori 2024.

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Simposio 1
Zoologia Molecolare

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ORIGINS AND IMPLICATIONS OF MITOCHONDRIAL GENES EXPANSION IN BIVALVES

Bivalvia, a class of molluscs, exhibit unique features in their mitochondrial genomes, only partly due to the distinct mitochondrial segregation mechanisms present in this group. These peculiarities include an abundance of introns and gene elongations that are rare in other animal mitochondria. To reassess mitochondrial protein annotations in bivalves, we employed a comparative approach focusing on gene boundaries. We investigated the genesis of gene extensions by examining the presence or absence of start and stop codons, as mutations in crucial nucleotides can significantly alter gene sizes. Our study aimed to provide a comprehensive evaluation of mitochondrial gene features in bivalves and to understand the causes, effects, and conservation of mitochondrial expansion across metazoans. We found that, while mitochondrial genes in bivalves generally exhibit greater length variability compared to other metazoans, this variability is primarily due to elongations in the COX2 gene within the Venerida order. The phylogenetic consistency of the elongations and the well-defined start and stop codons suggest accurate annotation of these genes. In contrast to the accurate annotations of COX2, ATP8—often believed to be absent in bivalves—was recovered in half of the species previously thought to lack it. Finally, we recommend caution when working with bivalve mitochondrial genomes due to their intrinsic complexities, which can impact the reliability of gene annotations.

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OUTLINING THE MATURATIONAL PATHWAY OF MITOCHONDRIAL SHORT NON-CODING RNAS USING *IN SILICO* AND *IN VITRO* APPROACHES

The network of regulatory systems that has arisen from the genomic interactions between the nuclear and mitochondrial genomes is, for the most part, poorly known. A new class of short non-coding RNA was recently discovered in four phylogenetically distant species. These small RNAs are transcribed in the mitochondrial genome and, according to in silico and in vivo investigations, appear to target nuclear transcripts. However, how these Small MITochondrial Highly transcribed RNA (smithRNA) can regulate different cellular processes is not clear yet. In the present work we identified a possible smithRNAs maturational pathway by analyzing which proteins interact with this new class of small RNAs.

Analyzing publicly available RNA ImmunoPrecipitation (RIP) and Cross-Linking ImmunoPrecipitation (CLIP) libraries revealed that in *Homo sapiens*, proteins involved in miRNA maturation (Ago2, Drosha, and DGCR8) interact with mitochondrial tRNAs, where the majority of smithRNAs are located. Furthermore, the protein-mitochondrial tRNA interaction was also observed in *Drosophila melanogaster* and *Mus musculus* Ago2, and *Caenorhabditis elegans* ERGO1, all of which are members of the Argonaute family and typically interact with nuclear short RNAs.

According to our results, it is likely that proteins involved in the maturation of nuclear small RNAs have been coopted for the smithRNA maturational pathway. Lastly, we conducted in vitro experiments in which biotinylated smithRNA sequences were immunoprecipitated alongside the interacting proteins. We aim to outline a potential smithRNA pathway thanks to the mass spectrometry identification of these proteins.

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INTEGRATING GEOMETRIC MORPHOMETRICS AND ddRADSEQ DATA TOWARDS THE CONSERVATION OF THE APENNINE ENDEMIC GENUS *ITALOPODISMA* HARZ, 1973 (ORTHOPTERA: ACRIDIDAE)

The grasshopper genus *Italopodisma* is strictly endemic to the central Appennines and includes nine species and five subspecies with scattered populations distributed from Sibillini Mountains to the Matese Massif. All populations are mainly present in ecosystems at high elevation (1600-2800 m-asl), such as grasslands, alpine meadows and rocky areas. For this reason, this genus should receive particular attention aimed at its conservation and, indeed, some species are classified by the IUCN as 'Critically endangered' (*I. ebneri*, *I. lagrecai* and *I. lucianae*), 'Endangered' (*I. fiscellana*, *I. samnitica* and *I. trapezoidalis*) and 'Critically endangered (Possibly extinct)' (*I. baccetti*). Despite that, a detailed taxonomic revision of the species is needed, considering the lack of detailed morphological characters and of molecular data supporting the species boundaries. In this study, we integrate molecular data (ddRADseq, >4000 loci) and a geometric morphometric analysis of aedeagus dorsal stylet shape to clarify the taxonomic status and the systematics of the putative species and subspecies, and to assess the phylogenetic relationships within the genus. Additionally, we investigate the potential existence of hybrids and/or co-distributed species in the same or nearby areas. The geometric morphometric analysis summarizing the shape of male external genitalia showed four main clusters that are in part confirmed by the phylogenetic assessment. Population structure did not support the co-existence of different lineages in the same localities and showed a hierarchical genetic structure congruent with phylogenetic inferences. Divergence time estimation and species delimitation analyses are underway to better understand the evolution of *Italopodisma*.

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SOIL BIODIVERSITY: FIRST DATA ON ITALIAN PAUROPODA BY INTEGRATIVE TAXONOMY

The edaphic ecosystem is characterized by a high biodiversity. Microarthropods are well represented and include abundant and extensively studied taxa such as mites and springtails and rare taxa such as pauropods. In the past, pauropods were systematically studied thanks to the extensive classification work carried out by Ulf Scheller between 1973 and 2014, who morphologically analysed several specimens collected by other researchers and provided to him from all over the world. Only recently, few molecular data on this group are available, and none relating to Italian species.

In Italy, the current checklist of pauropods totals 46 species (Minelli, 2021), 11 of which were retrieved in Emilia-Romagna. Within the Spoke 3 of the National Biodiversity Future Center, a study of Italian pauropods was undertaken to increase knowledge of soil biodiversity using an integrative taxonomy approach for species identification and systematic study of this group.

Pauropoda were collected from 8 localities in Emilia-Romagna. An appropriate DNA extraction method was developed for working on single specimens, allowing the recovery of exoskeletons at the end of the extraction, which were prepared for taxonomic identification and then used as a morphological voucher. Thanks to this protocol, both morphological and molecular information can be obtained from the same specimen that can also be kept in a zoological collection.

Pauropods belonging to 6 species of 4 genera (*Allopauropus*, *Decapauropus*, *Pauropus*, *Stylopauropus*) were identified. One species (*S. peduncolatus*) collected in Ravarino (MO) is reported for the first time in Emilia-Romagna. The first molecular data on 18S and COI genes were obtained for Italian specimens.

The developed methodology was successful in pauropods, allowing to obtain integrative information on these small-sized, rare soil animals, which so far, have not been successfully reared unlike other microarthropod groups. The first application of the integrative taxonomy approach in the study of Italian Pauropoda appears to be an effective strategy for monitoring little-known and understudied animals and it is valuable for increasing knowledge of soil biodiversity.

Project funded under NRRP, Mission 4 Component 2 Investment 1.4 funded by NextGenerationEU; Project code CN_00000033, Project title NBFC

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THE STICK INSECT GENUS *BACILLUS* AND THE ROLE OF SMALL MITOCHONDRIAL HIGHLY TRANSCRIBED RNAS (SMITHRNAS) IN HYBRIDIZATION AND SPECIATION

The stick insect genus *Bacillus* displays complex reproductive dynamics, with species such as *B. grandii*, *B. rossius*, and *B. atticus* engaging in various modes of reproduction, including bisexual and facultative parthenogenetic forms. Hybridization among these species has given rise to novel hybrids like *B. whitei* and *B. lynceorum*. Notably, mitochondrial DNA analysis has revealed asymmetrical hybridization events, with *B. rossius* consistently acting as the maternal parent. Recent research focused on a class of small non-coding RNAs called smithRNAs, encoded by the mitochondrial genome and implicated in regulating nuclear gene expression. The distribution and characteristics of smithRNAs were examined across parental *Bacillus* species. Results indicated a significant presence of smithRNA in *B. grandii* and *B. atticus*, while *B. rossius*, the mitochondrial donor species for hybrids, exhibited minimal quantities of smithRNA. These findings suggest a potential role for smithRNAs in cyto-nuclear interactions and the evolution of isolating mechanisms driving speciation within the *Bacillus* genus. The study underscores the importance of understanding the molecular mechanisms underlying hybridization and speciation processes, particularly in taxa exhibiting reticulate evolution. Bacillus stick insects serve as an experimental model for exploring the intricate relationships between sexual and clonal taxa, shedding light on the evolution of reproductive strategies and species boundaries.

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CARATTERIZZAZIONE GENETICA DEL DELFINO DI RISSO (CETACEA, ODONTOCETI, DELPHINIDAE) NEL MEDITERRANEO

Il delfino di Risso (*Grampus griseus*, Cuvier 1812) è una specie con un'ampia distribuzione geografica. Data la sua elusività, nel Mediterraneo viene avvistato raramente sebbene la sua presenza sia considerata regolare. Le informazioni sulla sua ecologia, distribuzione e struttura delle popolazioni sono limitate. Durante gli ultimi anni, si è registrato tuttavia un declino demografico, probabilmente causato da un maggiore sfruttamento della risorsa trofica sia da parte dei pescatori che dal cambiamento di alcuni parametri ambientali legati alla produttività primaria. In genere, la diminuzione delle dimensioni di una popolazione comporta anche una riduzione in termini di variabilità genetica, con un conseguente maggiore rischio di estinzione. Precedenti studi sulla caratterizzazione genetica delle popolazioni di grampo (GASPARI *et al.,* 2007; CHEN *et al*., 2018) hanno evidenziato una differenziazione tra quella mediterranea e quella atlantica. Tuttavia i campioni mediterranei analizzati erano particolarmente esigui e con una scarsa copertura geografica, rendendo questi primi risultati parziali. Obiettivo del presente lavoro è stato perciò quello di approfondire lo studio genetico di *G. griseus* in Mediterraneo e definirne meglio lo stato di conservazione. Attingendo sia da campioni museali italiani che da campioni moderni provenienti da diverse istituzioni italiane ed europee, è stato possibile ampliare il dataset iniziale derivante dal lavoro di GASPARI *et al.* (2007) coprendo tutto il bacino del Mediterraneo. Estrazione del DNA, amplificazione tramite PCR e sequenziamento Sanger sono state le procedure alla base delle analisi genetiche condotte. 473 bp della regione di controllo del DNA mitocondriale sono state amplificate e confrontate con sequenze già presenti in GenBank. I primi risultati hanno evidenziato sia la presenza di alcuni aplotipi già descritti per l'area mediterranea sia di altri finora inediti. Tutti gli aplotipi mediterranei sono risultati strettamente correlati fra loro e non sono stati osservati nell'area atlantica, sottolineando la differenziazione genetica tra i due bacini (Atlantico vs. Mediterraneo) ed evidenziando la necessità di approfondire le conoscenze genetiche di questa specie al fine di implementare piani di gestione e conservazione.

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ACTIVE VS. PASSIVE: UNVEILING VERTEBRATES BIODIVERSITY IN ACQUATIC ENVIRONMENTS THROUGH EDNA METABARCODING

Environmental DNA (eDNA) metabarcoding has become a powerful tool for non-invasive assessment of biodiversity in aquatic environments, fostering an in-depth understanding of ecological dynamics by targeting the genetic materials shed by organisms into the environment. The 12S-V5 primer pair used in this study for eDNA metabarcoding targets a short DNA fragment (~100 bp) of the mitochondrial 12S rRNA gene marker, to compare effectiveness between active and passive sampling methods. To unravel the biodiversity of the study area, five sites were sampled in the Serchio River basin. Active sampling was performed with cellulose filters, and passive sampling was carried out using granular activated carbon passive eDNA samplers (GAC-PEDS). Despite the short length of the amplified DNA fragment, the selected marker proved to be highly effective in the detection of a total of 60 OTUs with high taxonomic resolution, discriminating between three pairs of congeneric species: *Padogobius martensii* e *Padogobius nigricans*, *Rana italica* and *Rana dalmatina*, *Thymallus thymallus* and *Thymallus aeliani*. Statistical analyses, based on the comparison of the two methods, exposed important dissimilarity between PEDS and filters. One way of showing the particular strength of each method was through exclusive species detections. The ANOSIM analysis showed a moderate level of dissimilarity between systems, highlighting unique ecological characteristics. Vertebrate groups examined showed complex distribution patterns and pointed out the specificity of each system. This study provided key insights into primer pair efficiency, sampling method details, and highly variable ecological patterns in riverine systems.

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A MULTILOCUS APPROACH TO INVESTIGATE THE PHYLOGENY, PHYLOGEOGRAPHY AND POPULATION GENETICS OF *MYLABRIS OBSOLETA* NOWICKI, 1872, AN ENDEMIC BLISTER BEETLE (COLEOPTERA: MELOIDAE) OF THE ITALIAN PENINSULA

Mylabris obsoleta Nowicki, 1872 is a species endemic to central and southern Italy. It inhabits dry steppe habitats with sparse floral resources, but it can be also found in dry riverbeds, ranging from sea level up to 1500 m asl. The species is distributed mostly in southern regions of Italy, but scattered populations are also documented in Abruzzo and Latium (central Italy). *M. obsoleta* belongs to the *Micrabris* subgenus, which includes about 50 species distributed from the Mediterranean region to central Asia. Due to the ongoing soil conversion for cultivation, suitable habitats for this species have become increasingly rare and fragmented. Given the lack of genetic information, which can be critical for its conservation, this study aims to assess the phylogenetic position of *M. obsoleta* within the subgenus *Micrabris* and to reconstruct its phylogeographic history and population genetic structure using both mitochondrial (COI, 16S) and nuclear (CAD, ITS2, Rpp0) genes. Preliminary results showed a very low genetic diversity among populations of *M. obsoleta* for all the analyzed genes: this could suggest either the presence of extensive gene flow among individuals, which seems not realistic considering the widely fragmented range, or, more likely, a recent dispersion of the species from a refugium. Further analyses are in progress to better understand this genetic pattern.

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MITOCHONDRIAL PHYLOGENOMICS SUPPORTS A CARBONIFEROUS ORIGIN AND DIVERSIFICATION OF XENONOMIA (INSECTA, POLYNEOPTERA)

Polyneoptera includes some of the most known insect species, such as grasshoppers and cockroaches. While the evolutionary history of many Polyneoptera orders has been thoroughly explored, other clades have been partially or completely overlooked. This is the case of Xenonomia, a clade consisting of two species-poor insect orders with relatively recent taxonomic history: Mantophasmatodea and Grylloblattodea. Here, we provide a temporal framework for their origin and diversification, using a mitochondrial phylogenomics approach which encompasses all Polyneoptera orders. To strengthen the confidence in their origination and diversification timing, we specifically focused on the possible impact of phylogenetic biases, such as long branch attraction, nucleotide or amino acids datasets, the influence of specific fossil priors, and different clock models. Our results consistently support a Carboniferous origin of Xenonomia, and the divergence between the two orders is inferred to have happened before the Permian. While Grylloblattodea diversification is inferred to have occurred earlier than the Mantophasmatodea one, extant species of both orders most likely diversified after the Permian/Triassic and Triassic/Jurassic mass extinction. Our molecular divergence time analyses complement the fossil record and support the ancient relict status of these two polyneopteran orders.

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HIGH-RESOLUTION MELTING (HRM) AS A NOVEL TOOL TO IMPROVE CETACEAN SPECIES IDENTIFICATION IN THE MEDITERRANEAN SEA

Eight regular species of cetaceans live in the Mediterranean Sea: the bottlenose dolphin (*Tursiops truncatus*), the striped dolphin (*Stenella coeruleoalba*), the common dolphin (*Delphinus delphis*), the grampus (*Grampus griseus*), the pilot whale (*Globicephala melas*), the beaked whale (*Ziphius cavirostris*), the sperm whale (*Physeter macrocephalus*) and the fin whale (*Balaenoptera physalus*). Surveys on the cetacean species that inhabit the Mediterranean Sea are conducted principally with visual and passive acoustic monitoring during research or human activities, whose efficiency will depend essentially on animal behavior and environmental conditions. In this context, molecular approaches can contribute to perform better species identification and monitoring. For this purpose, we developed a new protocol using the High-Resolution Melting (HRM) method to identify marine mammal individuals at the species level.

We focused on the four cetacean species that alone make up more than 90% of all sightings: the bottlenose dolphin, the striped dolphin, the sperm whale, and the fin whale. In the Mediterranean Sea, striped and bottlenose dolphins are listed as Low Concern in the IUCN Red List, sperm and fin whales are considered Endangered. In order to identify species using HRM analysis, we designed specific mtDNA primers in the most hypervariable regions considering cytochrome b, D-loop, and control regions. We used muscles as positive controls, and on those, we successfully extracted DNA from samples, carried out PCR, and sequenced them to confirm species assignation. Subsequently, we performed the HRM analysis that revealed a success rate of 100% in terms of discrimination for all species included in this study, resulting in ranges of accuracy of 80% and 90% in terms of precision. This approach is not only less time-consuming but can also produce results quickly. It can be applied during necroscopy in stranded marine mammals, biopsy when suitable, recognition of putative hybrids, or alternative non-invasive methods such as fecal sampling.

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DNA BARCODING AND PROGRESSIVE SEX CHROMOSOME DIVERSIFICATION IN FURCIFER (SQUAMATA: CHAMAELEONIDAE)

Karyotype diversification is an important driver of evolution. Chromosome changes can promote population divergence and trigger speciation (MEZZASALMA et al., 2024). Squamate reptiles are characterized by a high taxonomic diversity which is reflected at the karyotype level in terms of chromosome number and morphology, localization of particular chromosome markers and insurgence of variably differentiated simple or multiple-sex-chromosome systems with either male (XY or X_1X_2Y) or female heterogamety (ZW or Z_1Z_2W) (MEZZASALMA et al., 2024). In this study, we performed a DNA barcoding using a trait of the mitochondrial gene coding for the Cytochrome oxidase I (COI) following the protocol described by NAGY et al. (2012), in order to provide a reliable taxonomic identification of 15 samples of the genus *Furcifer*. Then, we performed on the same samples a comparative cytogenetic analysis with standard karyotyping (5% Giemsa solution at pH 7), silver staining (Ag-NOR staining) following HOWELL and BLACK (1980) and sequential C-banding (C-banding + Giemsa, + fluorochromes) as described by ODIERNA et al. (2007), to assess the karyotype variability and the sex chromosome diversification of the studied taxa. Our molecular results highlight that the studied samples belong to 6 different species of the genus *Furcifer* (*F. balteatus*, *F. pardalis*, *F. petteri*, *F. major*, *F. lateralis* and *F. minor*) which are characterized by intra- and interspecific sequence diversity values comparable to those previously observed in NAGY et al. (2012). The comparative cytogenetic analysis highlighted that the chromosome diversity of the study species encompasses chromosome number (2n = 21-34, with a variable number pf micro- and macrochromosome pairs), morphology, and a variable differentiation of heteromorphic sex chromosome systems with female heterogamety. In particular, our results evidence that sex chromosome systems in *Furcifer* range from undifferentiated (mostly autosomal) pairs (in *F. balteatus*) to progressively differentiated ZW sex chromosomes in terms of dimensions and heterochromatin content. These findings support the hypothesized model of sex chromosome evolution by differential heterochromatinization followed by morphological divergence (see MEZZASALMA et al., 2021).

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DNA AMBIENTALE PER LA CARATTERIZZAZIONE DELLA BIODIVERSITÀ NEL SUOLO DI RISAIA IN AGROFORESTRY

Lo studio del DNA ambientale (eDNA) è ad oggi una delle tecniche più innovative impiegate per la rapida caratterizzazione degli organismi presenti in un ambiente (TABERLET *et al.,* 2012). L'eDNA applicato agli agroecosistemi offre la possibilità di monitorare la biodiversità in un ecosistema sotto forte pressione antropica. Inoltre, il suolo è tra gli ambienti terrestri meno studiati, nonostante ospiti una considerevole percentuale dell'intera biodiversità mondiale e fornisca numerosi servizi ecosistemici (KESTEL *et al*., 2022). In questo contesto, le indicazioni europee mirano ad un'agricoltura che favorisca l'eterogeneità ambientale, come la pratica dell'*agroforestry*, che mette insieme la coltivazione di piante erbacee produttive inframmezzate a piante arboree (MOSQUERA-LOSA *et al*., 2023).

Lo studio presentato vuole caratterizzare le comunità di Protisti e Metazoi tramite metabarcoding su eDNA da suolo di risaia coltivata in *agroforestry* e senza input di fitofarmaci. È stato estratto eDNA da campioni di suolo raccolti in diverse fasi produttive del riso. Il sequenziamento si è concentrato su due geni (18S rRNA e COI) ed è stato effettuato tramite tecnologia Illumina. Le sequenze ottenute sono state analizzate con diversi software dedicati. I risultati preliminari mostrano per i Protisti una comunità dominata da consumatori come Cercozoa, Ciliophora e Amoebozoa, dove l'α-diversità tende a diminuire nelle diverse fasi, per ristabilirsi ai valori iniziali alla fase di maturazione. Per le comunità di Metazoi invece, l'andamento dell'α-diversità è stabile lungo il periodo di coltivazione, con i taxa di Annelida e Arthropoda maggiormente rappresentati. Le analisi di β-diversità su Protisti e Metazoi mostrano situazioni differenti, con una specializzazione nelle diverse fasi per il primo gruppo e comunità stabili per il secondo. Una possibile spiegazione risiede nel diretto impatto sui Protisti della sommersione durante le fasi di coltivazione, che permette la sopravvivenza di meno specie con adattabilità alle nuove condizioni instaurate, comportamento noto anche nel regno Bacteria (NASUELLI *et al., in prep*). Tra i Metazoi troviamo organismi più influenzati dalla stagionalità (e.g. *Lissorhoptrus oryzophilus*) e adattati alle condizioni ambientali imposte dalla risaia (e.g. *Isotomurus palustris, Rhopalosiphum rufiabdominale*). Ulteriori approfondimenti sono necessari, ampliando ad esempio ad aziende con protocolli colturali diversi (e.g. agricoltura convenzionale).

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ANALYSIS OF STRESS INDUCED BY FISHING IN COMMON OCTOPUS (*OCTOPUS VULGARIS*)

The common octopus (*Octopus vulgaris*) is the most important commercially harvested octopus species in the Mediterranean Sea. Every year, millions of octopuses of different species are caught using artisanal fishing pots and the industrial trammel nests that are not compliant with the current European law relating to the protection of cephalopods (Regulation EU 2019/1241). The fishing method used impacts animal welfare, but it is generally evaluated considering macroscopic index and vitality, mantle excoriation, or arm mutilation. In this contribution, we used a molecular approach to evaluate fishing stress in *O. vulgaris* by measuring the differential gene expression of three gene markers: heat shock proteins (HSP70), Catalase (CAT), and Estrogen. CAT and HSP70 are principal parameters of oxidative stress, and their transcriptional expression is known to be altered by environmental stressors. Moreover, we determined the influence of the fishing method on hormone production, focusing on estrogen levels, considering animals in the same lifecycle stage and age (female, 1 year 800±50g). We analyzed 10 individuals captured with nests and traps compared to 5 control octopuses, which are wild animals housed in the facility for at least 10 days, to acclimatize and reduce capture stress. Transcriptional levels suggest alterations of gene marker expressions in fished animals compared to controls. The dissemination of the study's results and the desirable legislative support for the octopus fishing management plan are supported by our work. The improvement in the fishing behavior is essential for sustainable use of this iconic resource and improve the palatability of octopus as food.

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HOW DOES DOUBLY UNIPARENTAL INHERITANCE OF MITOCHONDRIA IN BIVALVES WORKS? PERSPECTIVES FROM COMPARATIVE MOLECULAR EVOLUTION AND TRANSCRIPTOMIC DATA

Bivalves represent powerful testing grounds for evolutionary convergence, due to the independent occurrence of traits such as freshwater colonization and extended longevity. Another interesting and overlooked feature that occurs in several bivalves is the Doubly Uniparental Inheritance of mitochondria (DUI). In these species, two mitogenomes are differentially transmitted across generations: oocytes transmit only female-type mitogenomes, while spermatozoa transmit exclusively male-type ones. The co-presence of these two evolutionarily independent mitochondrial lineages is the only stable exception to strict maternal inheritance in Metazoa, and the coevolutionary pathways of mitochondrial and nuclear genomes, as well as the mechanisms involved in the selective inheritance, are far from being understood.

In our project we investigated omics-derived data from 36 bivalves covering most representative families and including 6 occurrences of DUI. Comparing nearly 9000 single-copy orthologues, we identified a set of ~200 genes showing stronger purifying selection in DUI species and enriched for evolutionary correlations with mitochondrial proteins. Many of these genes were associated to microtubule-based transport processes, and we identified some candidates that might be involved in the peculiar pattern of mitochondrial aggregation that occurs specifically during male embryogenesis. Crossing these results with RNA-Seq data of eggs we obtained from *Mytilus galloprovincialis* females that produced strong sex-biased progenies (up to >90% same-sex offspring), allowed us to focus on more promising candidate pahtways that might be involved in the sex-specific mitochondrial inheritance. During the next mussel spawning season, candidate genes and their associated pathways will be investigated *in situ* and in the light of a Single-Cell RNA-Seq time series of early embryo stages.

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PHYLOGENETICS AND TAXONOMIC ASSESSMENT OF THE ITALIAN ENDEMIC GRASSHOPPER *ITALOHIPPUS* FONTANA & LA GRECA, 1999 USING DDRADSEQ DATA

The genus *Italohippus* includes three species: *I. albicornis* (La Greca, 1948), *I. modestus* (Ebner, 1915), respectively endemic to the Matese Massif and Monte Terminillo, and *I. monticola* (Ebner, 1915), with a wider distribution in several localities along the central Apennines. All the species inhabits rocky habitats and dwarf juniper formations at high elevation (1500 – 2200 m asl) and, due to their range restricted to threatened ecosystems, *I. albicornis* and *I. modestus* are classified as “Endangered” by the IUCN. Differences in forewing length and in courtship song are the main drivers that lead to the description of the three species, but evident distinctive morphological characters are lacking. In this study we combined a geometric morphometric approach on the forewings of males and females and genomic data obtained with ddRADseq to assess the taxonomic boundaries among the three species as well as their phylogenetic relationships. Preliminary results showed subtle differences in the forewings of the three species and revealed a paraphyletic condition for *I. monticola* which seems to also include *I. albicornis.* Analysis of population genetic structure seems congruent with the results obtained from phylogenetic reconstructions, with most populations assigned to different genetic clusters. Further analyses are ongoing for divergence time estimations and species delimitation, with relevant implications on the conservation of *Italohippus*.

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HIDDEN DIVERSITY AND PARAPHYLY IN *LEPIDODERMELLA* (CHAETONOTIDA: GASTROTRICHA) INFERRED FROM A MULTI-GENE PHYLOGENETIC APPROACH

Gastrotrichs are microscopic benthic metazoans virtually found in all water bodies, playing a crucial role in the food web of marine and freshwater systems. To date, over 890 species distributed in 72 genera, 18 families, and two orders are known. The classification of many of these taxa is based mainly on morphology and is often challenged after applying molecular data. Notably, several genera of the family Chaetonotidae appear non-monophyletic based on molecular phylogenetic analysis. One such genus is *Lepidodermella* Blake, 1933, which includes the well-known, cosmopolitan *L. squamata* (Dujardin, 1841) and 16 other species. All of these share the main diagnostic character of a dorsal cuticular covering consisting of flat, polygonal scales. However, mounting evidence shows that the current *Lepidodermella* may not be a natural grouping. To better understand the status and evolutionary relationships of genus-bearing species we gathered molecular data from species surveyed in previous studies and from *L. minor chaetifer,* recently discovered for the first time in Italy (Modena) and for which molecular data was previously unavailable. Additional information was mined from recent genomic/transcriptomic research. Overall, we built a concatenated matrix based on three genes sequence data from 130 terminals including 14 *Lepidodermella* specimens belonging to four putative species and subspecies and inferred their evolutionary relationships using Maximum Likelihood and Bayesian methods. Our phylogenetic analysis shows that *Lepidodermella* taxa separate into two unrelated clades. One clade includes *L. squamata* and *L. polaris*, and the other *L. intermedia and L. minor chaetifer.* From a morphological point of view, a column of rectangular scales covering the anterior portion of the ventral interciliary field characterizes members of the first clade, and this feature may be used as an additional trait to identify 'true' *Lepidodermella* species. Members of the other clade appear in sister-taxon relationships with a group composed of *Bifidochaetus* + *Lepidochaetus* species. Due to their striking morphological differences with the sister taxa, they may constitute a distinct lineage. Moreover, uncorrected pairwise distances based on an extended set of the COI gene indicate that the widely studied *L. squamata*, recently proposed as the model species for the phylum, includes specimens from at least three distinct lineages. This finding suggests caution when naming the model species.

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THE SEA-SLUG *MELIBE VIRIDIS* (KELAART, 1858): A WIDESPREAD SPECIES OR ANOTHER CASE OF CRYPTIC DIVERSITY?

Nudibranchia molluscs are a group of highly specialized gastropods characterized by the complete loss of the shell in the adult stage. These shell-less molluscs show an extreme variability in the body shape and colour pattern, with most of the species characterized by aposematic as well as cryptic colourations that reflect their specialized defensive strategies. In this framework, species belonging to *Melibe* genus are one of the most specialized groups with adaptation involving swimming ability, symbiotic relationships and feeding behaviour. In the Mediterranean Sea only *Melibe viridis* (Keelart, 1858), one of the biggest species known so far and one of the few capable of actively swimming and feeding through sucking and filtering, has been reported. The body shape is, in this species, functional for both these latter biological aspects: the cerata are located laterally and cooperate with the buccal veil to generate an active movement and the buccal apparatus is modified and perfectly adapted to the sucking habit. Additionally, the radula – the chitinous structure commonly used in nudibranch for feeding – is lacking and the oral veil is expanded and full of elongated and thin papillae in its external border. This species has a scattered distribution in the Mediterranean Sea with a stable population being reported from the Mar Piccolo of Taranto in Southern Italy (Ionian Sea). To fill some gaps of knowledge on its ecology, *in situ* observations were carried out between 2020 and 2024 and underwater pictures and samples collected. Moreover, considering the lack of molecular data currently available on Mediterranean specimens, genetic analysis was carried out on two collected individuals. Preliminary phylogenetic analysis performed on the COI molecular marker and involving newly obtained sequences and others, already available in Genbank, from extra Mediterranean *M. viridis*, revealed a more complex scenario than what expected. *Melibe viridis* resulted a complex of cryptic species that will need further in-depth molecular and morphological analyses, including specimens from the type locality (Sri Lanka), to unravel the correct systematic status of this species complex, and to finally determine if the identification of the mediterranean records must be updated.

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ADVANCING ALPHA DIVERSITY WITH MODERN APPROACHES: DNA BARCODING AND INTEGRATIVE TAXONOMY OF DECAPODA LATREILLE, 1802 IN THE MEDITERRANEAN SEA

The north-eastern Atlantic/Mediterranean region hosts a wide biodiversity, with each of its provinces displaying endemisms. Despite this, the majority of the Mediterranean biota lacks a revision based on molecular tools. We investigated the alpha diversity of Mediterranean Decapoda using a combination of DNA-barcoding and integrative taxonomy. The study area was located in the Gulf of Naples (GoN, Tyrrhenian Sea), topotypical for many species and synonyms. Samplings (2017–2024) were performed in all substrates, from the tide level to 700 m depth. After morphological identifications, ~5 specimens *per* morphospecies (from different areas/depths/substrates) were selected for the molecular work. The standard DNA barcode (Folmer) region of the cytochrome c oxidase subunit I (COI) gene was amplified (and a similarity value of 3–4% was used as intraspecific threshold). In case of cryptic diversity or to compare/generate bridge sequences between present data and those in GenBank, additional fragments of the COI (Palumbi region) or the 16S rRNA genes were obtained. Phylogenetic trees and delimitation analyses were performed to settle species boundaries. A total of 914 individuals, ascribed to 181 morphospecies, were barcoded, corresponding to ⅔ of the Italian biota. Our results yielded 198 molecular taxonomic units. Molecular data for the species or the COI region were first produced for 37 taxa, whereas 41 taxa were first barcoded from the Mediterranean. Cryptic diversity within the GoN was highlighted for 16 species, living in sympatry or syntopy. Sequences of >30 additional taxa showed mismatches with GenBank data, suggesting further cryptic diversity, new synonyms, and/or misidentifications by other authors. The taxonomic validity of *Calappa rosea* Jarocki, 1825 was questioned and a new species, even distinguishable through morphology, was found in *Pilumnus* Leach, 1816. Finally, 4 species were new for Italy, 4 thermophilic taxa were spreading north, and 3 rare species were found again after one century. The present study accounts for the first DNA barcoding dataset of Mediterranean decapods and contributes to re-assess and advance the alpha taxonomy of this order with molecular methods. It also sheds light on speciation patterns within the north-eastern Atlantic/Mediterranean and provides novel and useful data for phylogenetic, phylogeographic, and e-DNA studies. Last but not least, it represents a *continuum* of the tradition of zoological studies in the GoN.

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PHYLOGENETIC ALLIANCES OF THE CEPHALODASYIDAE (GASTROTRICHA, MACRODASYIDA) INFERRED THROUGH A MULTI-GENE APPROACH

Gastrotrichs are microscopic, free-living invertebrates found in aquatic ecosystems worldwide and are particularly diversified and abundant in marine sandy beaches. The phylum comprises more than 890 species, divided into two orders: Chaetonotida (483 species) and Macrodasyida (377 species). The internal phylogeny of both orders is in a state of flux as new species are discovered and novel information is acquired based on molecular data analysis. Regarding the marine Macrodasyida, the status of the family Cephalodasyidae Hummon & Todaro, 2010 is particularly puzzling. Currently, it includes five genera, *Cephalodasys*, *Dolichodasys*, *Mesodasys*, *Paradasys*, and *Pleurodasys*, that are mainly grouped on negative traits (e.g., absence of scales or spines) or characters bearing a poor phylogenetic signal (e.g., vermiform body). Moreover, recent phylogenetic studies based on the 18S rDNA gene have shown the family polyphyletic, with the five genera variably distributed across the order Macrodasyida. However, the specific alliances need more support at nodes. In order to shed new light on the status of the family and the phylogenetic alliances of its members, we used a bioinformatics pipeline based on whole-genome amplification and sequencing to obtain 57 new sequences from 19 species belonging to 11 gastrotrich genera. The new information was integrated with sequences available on GenBank and used to build a concatenated matrix of tree gene sequence data (18S rDNA, 28S rDNA, COI mtDNA) from 37 gastrotrich taxa to infer the evolutionary relationships using Maximum Likelihood and Bayesian approaches. Our results confirm that the family Cephalodasyidae is polyphyletic, with members of the genera *Dolichodasys* and *Paradasys* clustering, with high statistical support, with representatives of the family Redudasyidae. The species of the genera *Cephalodays* and *Mesodasys* form two distinct lineages. However, the positions of *Cephalodasys* and *Mesodasys* taxa along the macrodasyidan phylogenetic branch appear slightly different in the BI and ML analyses. The phylogenetic alliances of the genus *Pleurodasys* remain uncertain. Interestingly, in our analyses *Cephalodasys mahoae* Yamauchi & Kajihara, 2018 nests within *Paradasys*, suggesting a possible misidentification. While our analyses expand the knowledge on Cephalodasyidae, results indicate the need to improve the taxonomic sampling further to obtain a clearer picture of the phylogenetic alliances of its five genera.

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PRELIMINARY INFERENCE OF PATTERNS OF LOGGERHEAD SEA TURTLE COLONIZATION OF THE TYRRHENIAN COASTLINE USING MITOCHONDRIAL GENOME ANALYSIS

The loggerhead sea turtle (*Caretta caretta*) is a globally distributed species and commonly found in the Mediterranean Sea. The main nesting beaches (*rookeries*) have been described in the Levantine Basin, northern Africa and Ionian Sea. However, in the last decade, an increasing number of nests have been recorded along the western basin and particularly along the coast of the Tyrrhenian Sea, a pattern most likely due to environmental changes caused by global warming. The origin of these pioneer turtles has yet to be determined. In this study, we used mitochondrial DNA (mtDNA) analysis of samples from dead embryos collected in *C. caretta* nests and from stranded individuals in Tuscany and Latium to provide a preliminary description of the ongoing colonization of the Tyrrhenian coast. DNA was extracted from samples collected between 2012 and 2023. We obtained whole mitochondrial genome sequences for four dead embryos from different nests and eight stranded turtle samples which yielded high-quality DNA, while the mtDNA control region only was sequenced for low-quality DNA samples from 16 dead embryos from different nests and 36 stranded turtles of different life stages. The mtDNA genome sequences obtained in this study were aligned along with previously published sequences from our research group. Haplotypes and haplogroups were assigned based on networks reconstruction and published literature.

Mitogenomic haplotypic diversity was higher than control region haplotypic diversity. A single widespread mtDNA control region haplotype was split in five mitogenomic variants. All the haplotypes recorded in individuals from nests belonged to the haplogroup already known in the literature as the only one represented in Mediterranean rookeries, while half of the haplotypes recorded in stranded animals belonged to the Atlantic haplogroup.

Our results indicate that attempts to colonize Tuscany and Latium seashores are most probably made by turtles from the Mediterranean, while Atlantic juveniles reach the Mediterranean to forage and for development but not for reproduction. Calabria might be a possible source of Tyrrhenian colonizers, although other rookeries cannot be excluded at this time. Whole mitogenome sequence analysis also revealed a higher number of maternal lineages in the Tyrrhenian Sea with respect to single genetic markers, advocating the potential of mitogenomic analysis in population ecology studies.

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INTEGRATIVE TAXONOMY OF THE FAMILY PYURIDAE (ASCIDIACEA: STOLIDOBRANCHIA) OF EUROPEAN WATERS

The ascidian family Pyuridae Hartmeyer, 1908 includes around 250 species worldwide, among which important ecosystem engineers and invasive species. Of these, at least 36 species are reported from the North-Eastern Atlantic and the Mediterranean Sea. Although the ascidian fauna of this area is considered well-characterised, the taxonomy of Pyuridae is still widely debated. In fact, this group was barely touched by modern taxonomic approaches, and, when applied, they revealed taxonomic issues and cryptic diversity. In addition, Pyuridae resulted to be polyphyletic in the most recent phylogenetic reconstructions, calling for the necessity of a re-evaluation of the taxon.

In this context, we have carried out a first integrative taxonomic study of Pyuridae in the North-Eastern Atlantic/Mediterranean in order to clarify the taxonomy and systematic position of its members within the unsettled stolidobranch phylogeny.

Morphological examination and single or multi-locus phylogenetic reconstructions were done on more than 200 newly collected specimens from the entire investigated area. Comparative and type materials were gathered from international museums. As a first screening, a partial region of the cytochrome c oxidase subunit I (COI) gene was chosen as the marker of election for the DNA barcoding. In addition, an additional mitochondrial gene (16S rRNA) and two nuclear genes (28S rRNA and 18S rRNA) were sequenced from selected specimens to reconstruct the phylogeny. Distance-based methods and different species delimitation analyses were used to evaluate species boundaries and marker performances within Pyuridae.

As result, 16 nominal species have been sampled, corresponding to the ~60% of the total diversity of this family in the Mediterranean Sea, and ~44 % of the total reported for the North-Eastern Atlantic. The preliminary results on the COI dataset showed an average barcode gap of 4.9%, retrieving ~21 different molecular taxonomic units (MOTUs) and suggesting potential cryptic diversity in some genera. Results were corroborated by the multi-locus analyses.

The present study provides the first integrative taxonomy on the family Pyuridae of European waters, unveiling an unprecedented cryptic diversity in the area. Among the MOTUs retrieved, new species in the genera *Microcosmus* Heller, 1877 and *Heterostigma* Ärnbäck-Christie-Linde, 1924 will be the subject of future formal descriptions.

Simposio 2
Variabilità, Plasticità e Resilienza in un mondo in rapido cambiamento

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EFFECTS OF POLYETHYLENE NANOPLASTICS (NPET) ON THE PHYSIOLOGY OF THE COLONIAL ASCIDIAN *BOTRYLLUS SCHLOSSERI*

Plastics are persistent large-scale pollutants that adversely affect humans, wildlife and ecosystems. Every year, more than 20 million tons of plastic waste leaks into aquatic ecosystems, polluting lakes, rivers and seas. Plastics less than 5 mm and 1 µm in diameter are defined as microplastics (MPs) and nanoplastics (NPs) respectively: they derive from the degradation and fragmentation of large plastic objects. NPs are able to enter cells and cross the blood-brain barrier, accumulating in vital organs of organisms and potentially influencing their physiology over long periods of exposure and accumulation. Ascidians are invertebrate chordates closely related to vertebrates. Their phylogenetic position render them ideal organisms to study the evolution of various biological processes, with particular focus on the invertebrate-vertebrate transition. *Botryllus schlosseri* is a colonial ascidian widely used in studies of innate immune responses and to analyse the effects of pollutants on physiology. In this species, we are studying the behaviour of immunocytes once exposed to polyethylene NPs (nPET). Haemocytes were directly exposed to nPET, or the latter were directly microinjected in the colonial circulatory system. Preliminary results indicate that the exposure of haemocytes to NPs negatively influences the phagocytosis of yeast (Saccharomyces cerevisiae) cells. This is accompanied by a modification of phagocyte morphology, probably related to cytoskeletal alterations. In addition, NPs have a negative effect also on the degranulation of morula cells, the immunocytes with cytotoxic activity that trigger the inflammatory response. To support the results of degranulation assays, the enzymatic activity of phenoloxidase was spectrophotometric analysed. Furthermore, studies to evaluate the filtration rate of B. schlosseri after various times of exposure to nPET showed lower capacity of filtration after incubations with the pollutant.

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WARMING SEAWATER IMPAIRS IMMUNE RESPONSES AFTER BACTERIAL CHALLENGE OF A THERMOPHILIC CORAL SPECIES, *ASTROIDES CALYCULARIS* (PALLAS, 1766)

Increasing coral diseases and mortality has recently been linked to worldwide ocean warming, due to changes in pathogen occurrence and coral immune functions. The alarming temperature trends in last decads over the Mediterranean region were particularly worried, where recurrent marine heat wave has caused several mass-mortality events. To evaluate how elevated temperature affects the immune responses of corals, colonies of *Astroides calycularis* (Pallas, 1766) were exposed to environmental (23°C) or elevated (28°C) temperatures, and subsequently challenged with *Escherichia coli* lipopolysaccharide (LPS). *A. calycularis* is Mediterranean endemic coral, commonly found in the central-southern part of the basin and covers vertical rocky reefs, overhangs, and caves below the intertidal fringe. This azooxanthellate species occupies both well-lit and dark habitats and is considered a thermophilic species, thriving at relatively high temperatures. Several enzyme activities, which included phenoloxidase, glutathione peroxidase, lysozyme, alkaline phosphatase, and esterase, were assessed after 6 hours of LPS balneation and over time (0-, 12-, 48-, and 120-h). Enzymatic patterns showed upregulations immediately after the LPS challenge under environmental conditions, demonstrating an immune response, while warmer seawater impaired coral responses, delaying it over time. Moreover, using immunolabeling techniques with specific antibodies, was detected the regulation of Toll-like receptor 4 (TLR4), nuclear factor kappa B (NF-kB), and heat shock protein 70 (HSP70) activity. The expressions of this markers 6 hours after the LPS treatment revealed a significative over expression under control temperature. Warmer conditions and LPS almost suppressed TLR4-NF-kB activity, while HSP70 up-regulation appeared in both control and LPS treatments. These findings indicate that elevated temperature affects coral immune responses and, in thermophilic *A. calycularis*, results in an energy trade-off to health-state maintenance through sub-optimal conditions during multiple perturbations, such as diseases during warming periods. Such an integrated approach is useful for understanding the pathogen-defense mechanisms in Mediterranean corals, and disentangle the complex interactive effects linked to future climate change scenarios.

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COMPARISON OF FATTY ACID DESATURASES EXPRESSION IN THE ANTARCTIC CILIATE *EUPLOTES FOCARDII* AND THE MESOPHILIC *E. CRASSUS* TREATED WITH THE PLASTIC DERIVATIVE BISPHENOL A: IS THE ANTARCTIC SPECIES MORE VULNERABLE TO THE RAPID INCREASE IN PLASTIC POLLUTION?

Fatty acid desaturases (FADs) catalyse desaturation reactions, inserting double bonds into fatty acyl chains and increasing membrane fluidity. This process aids cellular acclimation to low temperatures. Several studies have shown that Antarctic algae and fishes upregulate FADs expression under low temperature (He et al. Biotech, 2019; Lu et al. Extremophiles, 2009; Palmerini et al. J Membr Biol, 2009). In ciliates, the incorporation of unsaturated fatty acids into the lipid bilayer increases its fluidity to allow different membrane functions, including regulation of ciliary motility. In *Tetrahymena thermophila* the importance of FADs in the regulation of cold adaptation processes has been demonstrated (Sanchez Granel et al. Biochimica et Biophysica Acta, 2019). The Antarctic *Euplotes focardii* is a validated model organism to study adaptation to the cold (Mozzicafreddo et al. Scientific Reports, 2021). Our interest in the E. focardii FADs emerged by an analysis of its response to plastic pollutants such as polystyrene nanoparticles (PS-NPs) and derivatives like bisphenol A (BPA), that were assayed at concentrations comparable to those found in the environment, including the Antarctic seawater. *E. focardii* appeared more sensitive to the pollutants with respect to the evolutionary closed mesophilic *E. crassus* by both toxicity tests and microscopical observations, showing a higher level of cell damages, probably due to a higher penetration of pollutants at membrane level. We analysed FADs gene expression in *E. focardii* and *E. crassus* treated with BPA by two approaches, a transcriptomic study and the real time quantitative PCR of the FADs genes that were identified from the available genomes of both organisms. Specifically, three fatty acid desaturase (FAD1-FAD3) genes and one polyunsaturated fatty acid delta-5-desaturase (PFAD) gene were annotated in both organisms. The RT-PCR suggested the involvement of PFAD and FAD1 in the stressor response in both ciliates, which were found to be upregulated at 24 hours from the stressor. PFAD seems to respond to temperature changes in both organisms, but the expression of the three FADs under BPA treatment is different. Our working hypothesis is that changes in FADs' gene expression and structure protect *E. focardii* at low temperature by increasing the membrane fluidity, but this condition may expose the ciliate to a higher vulnerability in the presence of new organic pollutants such as BPA.

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THE EFFECTS OF LEAD CONTAMINATION ON BLACK SOLDIER FLY LARVAE

Hermetia illucens (Diptera: Stratiomyidae), also known as black soldier fly (BSF), is a saprophagous insect with significant potential as a source of protein and fat to produce animal feed, as well as a wide array of valuable bioproducts. BSF larvae can be reared on organic waste and are thus potentially exposed to pathogens and contaminants, such as heavy metals. It has been reported that heavy metals, including lead, cadmium, and arsenic, have the potential to impact the health status of insect species used as food and feed. Moreover, heavy metals could affect the quality of the derived insect-based products, due to their bioaccumulative properties, although information on this aspect is still fragmentary and controversial.

To investigate the impact of heavy metals on their development and health status, BSF larvae were grown for 19 days on a rearing substrate mimicking the Organic Fraction of Municipal Solid Waste (OFMSW) spiked with varying concentrations of $PbCl_2$ (i.e., 15, 500, and 1000 mg/kg). Following a preliminary analysis of the insect growth performance and survival, markers of the cellular (encapsulation and phagocytosis) and humoral (proPO system) branches of the immune system were evaluated. Moreover, the concentration of lead in the rearing residue (the so-called frass) and in insects at different larval developmental stage was measured with Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Finally, the potential effects of lead on the midgut integrity, which is responsible for nutrient digestion and absorption, were analysed through morphological analysis.

Our results indicate that the bioaccumulation rate of the metal appeared to be correlated to the dose administered to the substrate as the concentration detected in larval samples increased with the amount of $PbCl_2$ administered. Indeed, providing $PbCl_2$ at high concentrations negatively affected larval growth and survival. The presence of heavy metals had a significant impact on the immunological markers, too: in fact, proPO system, encapsulation, and phagocytosis were activated by the intake of $PbCl_2$ into the larvae, indicating, as for the larval growth, a negative impact on the health status of the larvae.

This study provides new insights into the adverse effects of heavy metals on BSF health and contributes to delineate a species-specific risk profile for BSF in feedstuff.

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THE IMPACT OF TRANSPOSABLE ELEMENTS IN HEPATOPANCREAS AND MANTLE TRANSCRIPTOMES OF *CHAMELEA GALLINA*

Transposable elements (TEs), also known as “jumping genes”, despite their potential negative effects, are crucial in genomic evolution and gene regulation. They can move within genomes, affecting their size, generating new genes, and causing chromosomal rearrangements. Through this action, they increase genetic and epigenetic diversity and provide a foundation for natural selection, potentially resulting in beneficial traits that help organisms to adapt to new environmental conditions. While knowledges are available for species belonging to higher evolutionary lineages, mollusks represent a substantial understudied phylum.

The striped venus (*Chamelea gallina*) is a bivalve mollusk, belonging to the Veneridae family, widely distributed along the eastern Atlantic coast, from Norway to the Canary Islands in addition to the Black Sea, Mediterranean waters and especially in the Adriatic Sea where it represents one of the most important economic resource. From an ecological point of view, this species plays an important role in the ecosystems affecting nutrient cycling, habitat creation and food webs, and acting as biosensors for pollution in coastal waters. However, *C. gallina* populations are threatened by factors such as temperature changes, salinity, dissolved oxygen levels, toxic substances, and overfishing. Recently, several studies reported a correlation between environmental changes and TEs activity, therefore it is very interesting to evaluate this issue in *C. gallina*. Analyzing RNA-sequencing data, we investigated the transcriptional contribution of TEs in hepatopancreas and mantle of *C. gallina* specimens collected in two different sites of the Italian middle Adriatic coast (Senigallia and Silvi Marina), in two different sampling periods (spring and autumn). The former tissue was chosen since it is a target organ of main metabolic activities while the latter tissue is involved in shell formation, a structure important for protection against predators and quality of catches. The evaluation of TEs activity in these two tissues highlighted a different behaviour in relation to sampling site condition and seasonality. These findings might suggest a role of TEs in modulating *C. gallina* adaptability in response to environmental changes.

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MARINE GASTROPOD *PHORCUS TURBINATUS* PERSISTENTLY AVOID CLIFF AREAS IMPACTED BY ARTIFICIAL LIGHT AT NIGHT (ALAN)

The constant expansion of artificial light at night (ALAN) in coastal environments, closely related to human urbanization, is one of the major threats to species and biodiversity (MARANGONI et al. 2023). *Phorcus turbinatus* (Born, 1788) is one of the most abundant grazer gastropods on Mediterranean rocky shores and has a central role in the coastal trophic web. Considering individual's circadian movements and negative phototaxis behavior (CHELAZZI et al., 1983), we hypothesize that *P. turbinatus* populations could be significantly affected by ALAN. We investigated if specimens of *P. turbinatus* avoid areas impacted by artificial light only during nightly hours or throughout the day, and if this behavior changed depending on the moon phase. At the start of August, we selected four areas along a shore near Quercianella (LI), two affected by ALAN (LIT) and two as control (UNLIT). In each area, we sampled two boulders both landward and seaward, by registering the number and vertical position of *P. turbinatus* specimens in a 20x40cm plot. Data were collected in four periods (Morning: 09-10 am, Sunset: 07-08 pm, Night1: 10-11 pm, and Night2: 00-01 am) during a total of six days. We observed a lower number of individuals in LIT areas both during nocturnal and daily hours. Despite an increase in the number of individuals under the full moon in comparison to new moon conditions, the presence of full moon and ALAN negatively affected the density of *P. turbinatus* during the first nocturnal hours. In our results, we didn’t find an increased number of individuals under the water level or on the lower edge of the plot in response to the artificial light stimulus as well as any evidence of a circadian vertical movement. Our results suggest that ALAN may alter *P. turbinatus* behavior and results in sections of the boulders avoided by individuals also during diurnal hours, with potential cascading effects on the ecology of rocky shore environments, through trophic and non-trophic relationships.

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STRESS COPING STYLES INFLUENCE PHYSIOLOGICAL AND IMMUNOLOGICAL RESPONSES IN *SPARUS AURATA* LINNAEUS, 1758, AFTER BACTERIAL INOCULATION: SERUM PROTEINS BIOINDICATORS

Stress coping styles (SCS), a coherent set of individual physiological and behavioral differences in stress responses, have been described in various animal species, including fish, as a continuum between different phenotypes, termed proactive and reactive. Proactive are generally bolder and more aggressive, explore their environment more quickly, exhibiting less flexible behavior compared to reactive animals. In animal farming, the discussion of SCSs in relation to animal welfare is of considerable importance. The correlation between behavior and physiological response to external stimuli could be useful in selecting the most suitable behavioral traits to address the typical challenges of a breeding environment. *Sparus aurata*, one of the widely farmed species, was used as a model for the present study, which aims to evaluate the variations in physiological and immunological responses among different SCSs after *Vibrio anguillarum* inoculation, a pathogen that causes vibriosis in fish stocks. For this purpose, two different immunological and biochemical approaches were used to test the effectiveness the SCS approach on fish specimens classified into three groups (bold, intermediate and shy), after they were subjected to group risk-taking tests to screen their behavioral phenotype. The test was conducted in a rearing tank divided into two equal areas by a panel with a hole. After acclimation, the hole was opened, allowing fish to pass freely from the safe zone to the unknown zone. Fish passing data were recorded, attributing a behavioral score to each fish. The modulation in the concentration of, SauFBP32, an immunity lectin protein, and of the electrophoretic pattern of the serum proteins investigated by capillary electrophoresis were evaluated. Specimens showed increased lectin expression over time, but bold individuals exhibited higher expression than shy ones. Capillary electrophoresis revealed an increase in the percentage protein concentration of the β2 globulin fraction, presumably associated with immunoglobulins and molecules involved in the innate immune response against the bacterial infection. In contrast, the ϒ fraction decreased over time. This trend, particularly pronounced in the bold group than in the shy group. Therefore, the results suggest that bold individuals demonstrate a more decisive response to pathogen stimulation than shy ones, and that the parameters used effectively served as sensitive and immediate bioindicators.

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MULTIPLE APPROACH TO INVESTIGATE RESPONSES TO THERMAL STRESS DURING REGENERATION IN *ANEMONIA VIRIDIS*

This study investigates the molecular and transcriptomic mechanisms underpinning the regenerative process in *Anemonia viridis*, which has emerged as a useful animal model for the study of regeneration. The research adopts an integrated approach to examine the regeneration events occurring under natural conditions and in scenarios of increased temperatures, reflection of the ongoing rise in seawater temperatures caused by global climate change. The multiple approach consisted in a primary focus on assessing oxidative stress markers (protein carbonylation and total antioxidant capacity), in tentacle length measurement in the two conditions, on the study of the symbionts photosynthetic efficiency using Pulse Amplitude Modulation (PAM), on the evaluation of immunity genes (expression) and protein (presence and modulation). Key proteins associated with inflammation and tissue regeneration, such as toll-like receptor 4 (TLR-4), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), heat shock proteins (HSP70, HSC70), and interleukin-1ß, were analyzed using blotting techniques.

To address the question of whether an anthozoan subjected to thermal stress regenerates similarly to an organism merely experiencing a wound, phenotypic observations were made over a period of 7 days, measuring the tentacular length during regeneration.

The results indicate that *A. viridis* exhibits higher antioxidant capacity at 20 °C after 6- and 24-hours following tentacle removal compared to 27 °C. This redox state imbalance was further corroborated by a decrease in the photosynthetic efficiency of the symbionts, consistent with previous histological findings. Protein expression and modulation, as determined through blotting and q/RT-PCR analyses of markers such as proliferating cell nuclear antigen (Pcna), heat shock protein 90 (Hsp90), co-chaperone of Hsp90 (Cdc37), and collagen type XXIV α1 chain (Col24α1), suggest an activation and production of compensatory molecules aimed at restoring internal homeostasis despite the critical oxidative stress conditions.

Overall, this study provides significant insights into the complex molecular responses and regenerative mechanisms of *Anemonia viridis* under thermal stress, contributing to a better understanding of how global climate change impacts the physiological and immunological responses of marine organisms.

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THE IMPACT OF POLYETHYLENE TEREPHTHALATE (PET)-CONTAMINATED SUBSTRATES ON BLACK SOLDIER FLY LARVAE PERFORMANCE

The larvae of *Hermetia illucens*, also known as black soldier fly (BSF), have revealed to be an efficient system for the bioconversion of organic waste into valuable insect biomass. The contamination of organic fractions and by-products derived from food processing, agrifood chain, or municipal solid waste by microplastics (MPs) could potentially hamper the insect-mediated bioconversion of these substrates. However, the effects of MPs exposure on BSF larvae performance are scarcely known. To address this aspect, BSF larvae were reared on the Organic Fraction of Municipal Solid Waste (OFMSW) spiked with different amounts of polyethylene terephthalate (PET) microparticles (4 and 20% w/w), and their impact on larval growth and emerging rate, as well as on gut physiology and morphology, were investigated.
Larval growth performance demonstrated not to be influenced by PET exposure, although the analysis of the bioconversion indexes indicated a lower efficiency in substrate reduction for the 20% PET treatment compared to control. To investigate potential mechanical damages of the midgut due to MPs transit, a morphological analysis of this organ was carried out. No alterations in the gut epithelium or in the associated peritrophic matrix were observed, demonstrating that MPs ingestion does not affect midgut integrity. Moreover, measurements of endo- and exopeptidase activity in the posterior midgut and the evaluation of oxidative stress and detoxification markers as Reactive Oxygen Species, Glutathione S-transferase, and Catalase in the midgut, fat body, and Malpighian tubules were performed. Since in all the experiments no variation was recorded between treatments and control, it is possible to conclude that PET ingestion neither impairs protein digestion nor induces oxidative stress in the larvae.
The results herein presented provide insights into the bioconversion of PET-contaminated organic waste using BSF larvae, helping to expand our knowledge on the potential of insect-mediated valorisation of a challenging substrate as the OFMSW.
The study was funded by Fondazione Cariplo (2020-0900) and MUR (2020ENH3NZ).

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ANATOMICAL CHARACTERIZATION OF THE MALE REPRODUCTIVE SYSTEM IN CARTILAGINOUS FISHES: MORPHOLOGICAL AND MICROSTRUCTURAL DIVERGENCES

The structure of male reproductive system (RS) in chondrichthyans is of interest to reproductive and evolutionary biologists. It permits to study archetypal traits, as this group has an evolutionary line separate from other vertebrates that extends back to some 400 million yrs and includes many conserved features (Jones and Hamlett, 2006) with a variety of RS' specializations linked to different reproductive modes (oviparity vs viviparity) (Jordan et al., 2021). Given that, the focus of this work is to carry out a comparative study of male RSs among species belonging to different infraorders showing different reproductive modalities, to improve knowledge on their reproductive strategies.

A total of 111 mature male specimens (belonging to 27 species), caught around Sardinian seas, was examined. For each individual, the main biometric measurements were recorded, as well as body and gonad mass, to estimate the Gonado-somatic Index. In addition, the morphometric measurements of the different parts of their RS were registered throughout an imaging software and expressed as a % of body length. A histological analysis of testis, epididymis, deferens duct and seminal vesicle was conducted for selected species to describe their microstructure. Fresh ejaculates were also processed at field sites, treated following Rowley et al. (2019) and the sperm components were observed from digital images.

The results showed that the function and organization of the RS were maintained in all species examined. The main differences were found at the macroscopic level in the shape, position and size of the testes, morphology of the claspers, dimensions of the epididymis and deferens ducts. At the microscopic level, the main differences were attributable to the type of testicular organization and the shape of the mature spermatozoa. Only the rajid species showed a compound testis type, while the diametric type was the most common among analysed species. Sperm morphology was functional to the internal fertilization typical of the cartilaginous fish. Spermatozoa showed a variability in shape with head from elongated to helical favoring the diffusion and movement in the sperm fluid. Considering the high sperm competition characterizing the cartilaginous fish, and the high number of species with multiple paternity, a morphometric analysis of the different components of the spermatozoa should be conducted to evaluate whether this competition influences their shape.

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TRANSPOSABLE ELEMENTS IN ZEBRAFISH: INSIGHTS FROM EMBRYONIC DEVELOPMENT

Transposable elements (TEs) are genetic mobile elements that play a key role in speciation and adaptation processes. The transcriptional activity of TEs is controlled by Kruppel box-associated zinc finger proteins (KRAB-ZFPs) through the recruitment of the NuRD complex in sarcopterygians. Despite the absence of key genes encoding proteins like TRIM28 and KRAB-ZNF in actinopterygians, the TE transcriptional regulation system is active. Carotti and colleagues (2022) proposed that TRIM33 could be a potential substitute for TRIM28 in *Danio rerio* (zebrafish) that, together with a KRAB-like ZFPs specific of actinopterygians, allows the recruitment of the NuRD complex also in fish lineage. Therefore, the interaction of TRIM33 with KRAB-like ZFPs, and also with FiNZ ZFPs, specific of zebrafish, was tested by co-immunoprecipitation analyses that confirmed the binding between the domains contained at N-terminal of these zinc finger proteins and TRIM33. Moreover, the evolutionary relationship of KRAB-like, FiNZ and KRAB ZFPs was evaluated through phylogenetic analysis demonstrating their common origin. RNA-seq data on early zebrafish development revealed high TE expression at the 1-cell stage, attributed to mother-deposited RNA, which decreased at the 128-cell stage due to zygotic genome activation. Higher transcriptional levels of TEs were observed during the blastula/gastrula stages, consistent with increased activity of TRIM33, KRAB-like ZNFs and FZNFs, suggesting their role in controlling TEs. In addition, analyses conducted on zebrafish embryos treated with 5-Aza-2'-deoxycytidine, a DNA methyltransferase inhibitor, showed de-repression of TEs, particularly LTR retroelements. This was associated with increased transcription of genes involved in TE silencing, indicating a responsiveness of the system to TE activation and, in particular, to young LTR retroelements copies as demonstrated by the Kimura distance analyses. In conclusion, our results support the functioning of the TE controlling system against young LTR retroelements copies in actinopterygians.

Simposio 3
Biodiversità del Territorio e Governance condivisa: monitoraggi e casi studio

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A PUBLIC DATABASE TO TRACE FALCONS HOSTED IN ITALIAN WILDLIFE RESCUE CENTERS AS A TOOL TO DETECT POPULATION TRENDS AND ANTHROPIC IMPACTS

The five-year LIFE Lanner project "Urgent Conservation Actions for the Lanner Falcon" (https://www.lifelanner.eu/it/), funded by the European Community, began in 2020 with the main aim of protecting and increase the population of lanner falcon (*Falco biarmicus feldeggii*) inhabiting Central Italy, through multiple targeted actions. Among the partners of the Project, *Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri* (IZSLT) is responsible for the genetic characterization of species, subspecies and individuals of lanners and for the creation of a public database (https://lifelanner.izslt.it/app_index.php) to collect data on this and four other species of falcons that can be hosted, in difficulty, in Italian Wild Animal Recovery Centers (CRAS). To date, 38 Centers throughout the country have joined the initiative, recording on the web portal the findings of birds over a 30-years period from 33 of the 110 Italian provinces. Three different falcon species have been recorded: the lanner (*Falco biarmicus,* n=4), the gyrfalcon (*Falco rusticolus*, n=1), the peregrin (*Falco peregrinus*, n=300). Data derived from the portal strengthen the observation that lanner falcon populations were in decline in recent decades and that the species is now dramatically sporadic in Italy. Conversely, the peregrin represents by far the most frequently hospitalized species and thus probably the most common falcon in all regions. Overall, 71.8% of birds hosted in CRAS survived, while 28.2% died. Gunshot wounds (22.3%) and trauma (39.7%) were the most frequent causes of injury and/or death for falcons hospitalized in CRAS, highlighting how the anthropic impact (direct and indirect) still threatens Italian avifauna. In particular, poaching focused mainly on adult individuals, while subadults and chicks were impacted more by traumas, especially in the first phases of spring fledging. Wild Animal Recovery Centers, in addition to taking care of wild animals in difficulty for the purpose of their reintroduction into nature, also represent a fundamental outpost for indirect monitoring of populations, public health surveillance (for example, through the early detection of avian influenza) and for pointing up crimes against wildlife. The web portal developed by IZSLT as part of the EU LIFE Lanner Project is an attempt to build, for the first time, a network of Italian CRAS with the possibility of future extensions of action goals and territorial/species coverage.

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RESOURCE COMPETITION BETWEEN MANAGED HONEY BEES AND WILD BEES: A SYSTEMATIC LITERATURE REVIEW HIGHLIGHTS METHODOLOGICAL BIASES AND KNOWLEDGE GAPS

While some managed bee species (e.g. *Apis mellifera* L. 1758) are thriving around the world, other Apoidea members are dramatically declining. Among the factors driving this decline, there is concern that wild bees may be threatened also by the strong pressure exerted by honey bee by competition for pollen and nectar. This topic has been repeatedly investigated over the last decades but a clear picture has not yet emerged, with recent analyses highlighting the potential for competition but with a significant variability in study outcome (MALLINGER et al., 2017; IWASAKI and HOGENDOORN, 2022).

Following PRISMA guidelines (O'DEA et al., 2021), a systematic review focusing on interspecific trophic competition between managed *Apis mellifera* and wild bees was performed. The aim was to understand which factors best detected the presence of competition and explained the variability in the study outcome. These factors included biological feature, such as the geographical area and the habitat where the study was performed and the wild bee species examined, as well as methodological aspects, i.e. the experimental approach (correlative vs manipulative, data collection methodology) and the resource investigated (pollen, nectar or both).

The literature research led to more than 80 studies, of which the majority reported evidence of interspecific trophic competition. Unfortunately, most studies did not explicitly report the kind of trophic resource investigated, which prevents from safely concluding whether competition might occur over pollen, nectar, or both. For this reason, further studies that investigate on the Apoidea diet and their overlaps of trophic niches, mainly through quali-quantitative analyses on pollen and nectar, are desirable.

No significant association between evidence of competition and the research approach used, or geographical area was recorded. Conversely, the method used (flower visitation rate, or palynological analysis, or other) seems to affect the presence of competition. A strong bias was found among wild bee taxa investigated, with the genus *Bombus* being the most representative in the dataset, probably due to its long flight period, its high visibility and its strong contribution in pollination that garner an unbalanced attention.

This systematic review provides insights into interspecific trophic competition among managed honey bees and wild Apoidea and highlights the main gaps which must be tackled by future researches.

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A REGIONAL PLAN FOR THE MANAGEMENT OF THE ALIEN HORNET *VESPA VELUTINA* (HYMENOPTERA: VESPIDAE) IN TUSCANY

Biological invasions are one of the leading causes of biodiversity loss all over the world. Here we present the regional plan adopted in Tuscany for the management of an alien invasive hornet, *Vespa velutina nigrithorax* (Lepeletier, 1836; HYMENOPTERA: VESPIDAE). *V. velutina*, commonly known as the yellow-legged hornet, has been accidentally introduced in Europe from the south-East Asian in 2004. Since then, *V. velutina* has colonized numerous European countries and, from 2012, also Italy, with the first reports in Tuscany in 2017 (www.stopvelutina.it). The presence of *V. velutina* on the European territory has generated particular concern because of its multiple impacts: first of all, on the beekeeping sector, as it massively preys on honey bees; secondly on biodiversity, as it preys also on wild pollinators and other insects; finally on public health, since its sting is potentially lethal for allergic people.

The eradication of invasive social wasps and hornets is not an easy task. As eradication is no longer an option, it is necessary to develop management projects aimed at limiting the spread and the impact of this alien species.

It is in this context that the implementation of the management plan of the yellow-legged hornet *V. velutina* of the Tuscany Region was carried out with state funds allocated for the invasive alien species of Union interest, according to the Legislative Decree 15 December 2017 n. 230 (www.gazzettaufficiale.it). The plan involves the Department of Biology of the University of Florence, the Departments of Veterinary and Biology of the University of Pisa and the three Tuscan beekeeping associations: ARPAT, AAPT and Toscana Miele.

The plan, which was launched in 2023 and will run for three years, has set up a monitoring network of 369 stations spread over the entire regional territory, coinciding with apiaries of the members of the three beekeeping associations. The aim is to have a rapid alert system on the presence of the species in order to neutralize the colonies of *V. velutina*. This action currently represents the most effective countermeasure to contain the expansion of the hornet. The regional plan allowed, during the last year, to neutralize 247 colonies in the provinces of Massa Carrara, Lucca, Pisa and Pistoia. This contribution will present the regional plan aims, methods and the results of the first year, as well as it will raise awareness about this ongoing biological invasion of great interest.

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FAUNA ASSOCIATA ALLE BIOCOSTRUZIONI A VERMETIDI DELLA COSTA ADRIATICA PUGLIESE

Le biocostruzioni a vermetidi, caratteristiche delle coste rocciose poco profonde, svolgono un ruolo cruciale poichè migliorano la complessità dell'habitat e forniscono riparo a molte specie. Queste strutture, inoltre, formate dal Mollusco Gasteropode *Dendropoma cristatum* (Biondi, 1859) in associazione con alghe coralline come *Neogoniolithon brassica-florida* (Harvey) Setchell & Mason, 1943 e *Lithophyllum byssoides* (Lamarck) Foslie, 1900, prevengono e rallentano l'erosione costiera e sono indicatori di buono stato ecologico.

Questo lavoro descrive per la prima volta la fauna associata alle biocostruzioni a vermetidi lungo la costa adriatica pugliese in provincia di Brindisi. Per la caratterizzazione della biocostruzione, il tratto di costa compreso tra le località di Rosa Marina e Specchiolla (BR), lungo 19 km, è stato diviso in tre siti di 6,3 km ciascuno (siti RSM, TPZ e SPC) e in ogni sito sono stati fissati 3 transetti di 20 metri, per ciascuno dei quali sono stati effettuati 2 campionamenti quantitativi (A e B). I campioni sono poi stati analizzati in laboratorio per l'identificazione degli invertebrati macrobentonici. Sono stati poi calcolati alcuni parametri biotici (numero di taxa, abbondanza, diversità di Shannon, equitabilità di Pielou) per il confronto tra i siti di campionamento.

In totale, sono stati identificati 1837 individui appartenenti a 45 taxa; le specie più abbondanti appartenevano ai Phyla degli Anellidi, Molluschi e Artropodi. Sono state ritrovate specie rappresentative delle biocostruzioni a vermetidi in Mediterraneo come *Mytilaster minimum*, *Lepidochitona caprearum* e *Patella ulyssiponensis*; tra i policheti e i crostacei, quattro specie di Syllidae e due specie di Anfipodi, 1 Tanaidacea e 1 Isopoda, sono state segnalate per la prima volta in Italia in associazione con le biocostruzioni a vermetidi. La cluster analysis ha evidenziato una maggiore somiglianza tra i siti TPZ e SPC (69%) rispetto al sito RSM. I maggiori valori di abbondanza e ricchezza specifica sono stati osservati nel sito SPC, probabilmente in rapporto alla maggiore complessità topografica che caratterizza la biocostruzione in questo sito.

La presenza di una fauna associata ricca e diversificata mostra il buono stato di conservazione di queste biocostruzioni e contribuisce in maniera significativa ad accrescere le conoscenze su questo habitat negletto ma che richiede particolare attenzione nelle politiche gestionali per la sua importanza nel paesaggio del mesolitorale inferiore.

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BIODIVERSITY IN LIMESTONE QUARRIES: A REVIEW AND FUTURE PERSPECTIVES

The demand for cement and other building materials is increasing due to the growth of the human population and will continue to grow. Consequently, there will be an increase in the areas dedicated to the extraction of limestone and other materials needed to produce cement. Mining through quarrying has significant impacts on the environment (SOUZA and SANCHEZ 2018). In addition to large-scale habitat destruction, dust, noise and vibration emissions further exacerbate the environmental impact (KUMARASINGHE *et al*., 2013) Limestone quarries mostly occur in calcareous and karst environments, which has an outstanding biodiversity value and are in rapid, as a result of mining activity (CLEMENTS *et al*., 2006). These environments host a huge variety of endemic species, both vertebrates (GRISMER *et al*., 2013) and invertebrates (OSIELE *et al*., 2022). However, the knowledge about biodiversity and the impact from quarrying is still scarce and targeted to few taxa. Here, we report the results of a systematic bibliographic review addressed to highlight the state of knowledge in this field. Through a keyword search in WOS we extracted 795 articles related to biodiversity. Only 135 of these have been assessed for eligibility and included in the review. The 46% of the studies were focused on “Quarry rehabilitation”, considering especially habitat restoration at abandoned mining sites, but most of them concerned vegetation and only nine studies focused on fauna. Only 12 papers focused on analysing quarrying impacts, with a particular attention to the effects of dust pollution on lichens. About Quarry rehabilitation studies, the 69% of them analysed the management and dynamics during the process of recovery in the quarry through the plant community. Only 14% of these studies used animals, all invertebrates, as bioindicators to assess the success and trajectory of the revegetation process during extraction site recovery. Six of them (66%) used terrestrial arthropods, mostly at community level. Regarding spatial distribution, the outcome shows that Europe dominates 69% of studies, with the Czech Republic (22 papers) having the highest number of scientific works followed by Spain (17 papers). Our results show the presence of a wide knowledge gap, at the expense of active sites and their impacts on biodiversity, and a generalized lack of data regarding animal biodiversity for those sites where vegetation successional stages have been monitored during restoration activities.

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MONITORING *PARACENTROTUS LIVIDUS* (LAMARCK, 1816) REPOPULATION ACTIONS IN SARDINIA USING MOLECULAR TOOLS

A general population decline of *Paracentrotus lividus* was observed in its whole distribution area mainly due to overexploitation. Indeed, so far consumption and demand have increased, while the management measures have been not effective in slowing down the decline. In recent years, restocking actions were carried out by releasing hatched specimens in natural environment. However, these practices can have negative effects, which can be identified in genetic diversity and fitness loss as well as changes in population composition and structure.

Similar repopulation measures were planned/realized for the South Sardinian Sea urchin populations with the production of hatchery-reared sea urchins. In this context, prior to the release at sea, we used molecular tools to monitor the first pilot restocking action realised in the area. Molecular analyses were carried out using two mitochondrial (COI and Cyt-b) and nine nuclear (microsatellites) markers to compare the levels and patterns of genetic variation between sea urchins from three natural populations (n=144) vs hatchery-raised specimens (n=48).

Using the COI/Cyt-b concatenated sequences (1641 bp), the hatched sea urchins showed lower values of haplotype and nucleotide diversity with respect to the wild population (Cagliari) from which the breeders were originally sampled and to the other two nearby wild populations here analysed (Malfatano and Villasimius).

Similarly, the analyses performed using microsatellite data confirmed the low genetic diversity of the hatchery sample, characterized by very low values of Allelic richness, effective population size, and a high degree of relatedness between individuals.

In brief, our data indicate that hatchery-reared juveniles experimented a substantial reduction in genetic diversity and strong differentiation from wild populations possibly because of bottleneck effects. Therefore, our results confirmed the importance of correctly choose the number of breeders to be used in restocking actions to avoid the Ryman-Laikre effect (increase in inbreeding and a reduction in total effective population size).

Lastly, our outputs reconfirm the need 1) to characterize the wild populations to identify where to release ‘genetically compatible’ hatched juveniles and 2) to monitor over time the resettled areas to measure management actions' effectiveness and genetic effects, once the hatched individuals are eventually released.

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KIDS THE DINNER IS READY. THE ROLE OF MEDIUM-SIZE MAMMALS IN THE DIET OF WOLF PUPS OF A PACK LIVING IN A HUMAN-MODIFIED LANDSCAPE IN ITALY

Adult wolf diet in Italy has been widely investigated. Studies performed showed that wolf diet is usually mainly based on wild ungulates but show a great adaptability to different ecological conditions (MATTIOLI, *et al.* 2011; FERRETTI, *et al.* 2019). Nevertheless, to the best of our knowledge no data are yet available on wolf pups diets composition in Italy. In this work the diet of wolf pups of one pack living in a highly anthropic area was then investigated for the first time to collect preliminary data on wolf pups diet composition and assess if adults operate a selective provision of food resources to developing pups. Overall, 55 pup scats and 132 adult wolf scats were tested from samples collected from 2018 to 2021 in a suburban area in the lower Pisan hills in Tuscany, Italy. Standard laboratory procedures were used for scat analysis (KRUUK and PARISH, 1981; TEERIK, 1991; MERIGGI, *et al.* 2015; MYSLAJEK, *et al.* 2019) and percentages of frequency of occurrence (FO%), medium volume (MV%) and the Chi-square test were calculated to evaluate the composition of the diet and see difference between the diet of adults and that of pups. Prey categories found in wolf pups diet were the same of those found in adults diet. However, significant differences were recorded in the frequency of occurrence of prey categories in pups diet compared to adult ones. Wild ungulates resulted to be the main prey category in pups diet as for adults. However, a selective provision of medium-size mammals and birds prey categories for pups by adults occurred. Hare and coypu resulted to play an important role in the diet of pups and further investigation to assess the importance of these prey species for pups survival are desirable.

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VARIATIONS IN HUNTING PATTERNS AND SPATIAL BEHAVIOUR OF GRAY WOLF IN RELATION TO SOCIAL STRUCTURE IN A MEDITERRANEAN AREA

The wolf's social structure significantly influences predator-prey dynamics. Initial colonization typically involves a solitary individual establishing itself in a new area. Subsequent arrival of an opposite-sex individual can lead to the formation of a reproductive unit. We examined changes from the initial colonization phase of San Rossore Estate characterized by a stable solitary wolf (2016-2020; DEL FRATE *et al.*, 2023), to a subsequent phase (2021-2024), where the same wolf became the reproductive male of a pack in the area. Environmental characteristics of killing sites were evaluated, comparing conditions during solitary versus pack presence. The body condition of prey was evaluated by comparing bone marrow fat percentages with samples from both preyed and culled deer. Spatial behaviour was analysed using camera trapping data. Analysis of killing site characteristics revealed that 95.1% of lone wolf predations occurred in open areas near fences. In this phase body conditions of fallow deer preyed were better than randomly culled individuals (T-Student: $t=2.159$, $p=0.033$). Conversely, when a pack was established, open areas in killing sites decreased to 65.4%, possibly as a well-structured pack can successfully hunt in obstacle-free areas. As consequence, with a change in hunting areas and hunting tactic, the body condition of the prey was found to be poorer compared to the population average (T-Student: $t = -3.1153$, $p=0.0021$). Spatial behaviour evaluated with 37 camera traps revealed differences between the initial colonization phase, when lone wolf avoided areas with higher human presence, and the second phase, when pack members utilized the entire area, as shown by heatmaps. Summer home ranges were larger before pack formation compared to the second phase (Mann-Whitney U-test: $Z = -2.066$; $P = 0.039$). Daily activity rhythms recorded before reproductive events showed lower daytime activity than after pack formation (ANOVA: $F=39.074$; $p<0.001$) due to the increased daytime activity of the pups (ANOVA: $F=18.219$; $p<0.001$). In conclusion, social structure significantly influenced wolf behaviour in terms of spatial utilization, activity rhythms, and hunting patterns. With the formation of the pack, predatory activity spread across all environments of the Estate, and prey exhibited more debilitated body conditions. This might result in a higher number of predations to satisfy the predators' energy needs, which future studies could further investigate.

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CAMBIAMENTI ED ESTINZIONI DEGLI INVERTEBRATI MARINI DEL MEDITERRANEO NEGLI ULTIMI DECENNI

La necessità di identificare le specie indicatrici del cambiamento ambientale ha aumentato l'importanza dell'esistenza di database storici di biodiversità marina. Esistono, tuttavia, delle difficoltà oggettive che limitano l'utilizzo di questi database, primo tra tutti l'aggiornamento tassonomico. Per questa ragione i cambiamenti della biodiversità si basano soprattutto sull'introduzione di nuove specie, mentre la stabilità della biodiversità presente in un'area è raramente studiata. Negli anellidi marini, notoriamente buoni indicatori, un confronto temporale è praticamente impossibile, anche prendendo in considerazione famiglie molto ben conosciute come Syllidae e/o Sabellidae, a causa dei cambiamenti avvenuti nella loro tassonomia. Per contro, l'analisi della diversità dei sabellidi registrati attraverso *visual census*, rileva un notevole aumento di specie negli ultimi 40 anni dovuto alle introduzioni e alla descrizione di nuove specie. Un gruppo tassonomico dove la fauna del Mediterraneo è stata analizzata a livelli fini è quello degli Hydrozoa, in questo gruppo è stata analizzata la distribuzione spazio-temporale registrata dalla letteratura scientifica entro una soglia di circa 40 anni, adattando un metodo paleobiologico per analizzare casi di presunta estinzione di specie. Per questo gruppo, inoltre, è disponibile un'analisi spazio-temporale degli epifiti delle praterie di *Posidonia oceanica*. L'analisi degli Hydrozoa ha rivelato un elevato numero di specie scomparse. Molte delle specie segnalate in passato non sono infatti registrate negli ultimi decenni, inoltre l'utilizzo del Confidence of Extinction Index ha permesso di identificare casi di probabile estinzione. Il contingente più numeroso delle 53 specie non più segnalate è endemico del Mediterraneo (34%), seguito da quello boreale (19%), il 15% è mediterraneo-atlantico; i contingenti indopacifico e circumtropicale rappresentano l'11%, segue il 4% cosmopolita, il 2% è tropicale-atlantico, il 4% non classificabile. L'analisi delle specie di idroidi epifiti di *P. oceanica* ha mostrato invece un andamento opposto, con un aumento del 30% delle specie rispetto alle checklist precedenti. Ciò è probabilmente dovuto al contributo dei dati recenti ottenuti su praterie di *Posidonia* in prossimità di 'vents' di CO_2 dove gli Hydrozoa hanno mostrato una chiara tolleranza ai bassi livelli di pH ed in grado di sopravvivere in rapporto al futuro scenario di acidificazione degli oceani.

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DISTRIBUTION, ECOLOGICAL CHARACTERISTICS, AND HABITAT PREFERENCES OF *ERIOGASTER CATAX* IN UMBRIA (ITALY)

Eriogaster catax (Linnaeus, 1758) was studied to enhance the understanding of its distribution, ecological characteristics, and habitat requirements in the Umbria region (Italy). These findings directly apply to the conservation interventions planned for this moth species under the LIFE IMAGINE UMBRIA project (LIFE19 IPE/IT/000015). To achieve this, we updated the species distribution in the region through bibliographical research and field investigations. We also analysed the oviposition site selection by identifying the chosen host plant species, the oviposition height, the selected plants' total height, and the chosen branches' diameter. The database up to 2018 (1994-2017; IV Habitat Directive Report and SUN LIFE project) consisted of only 17 records; by 2024, the records have increased to 123 (1994-2024). Of these, 17 come from scientific literature or citizen science, and 106 from our specific surveys. In terms of data type, 31 refer to egg batches, 82 to larval nests (recorded from March 24 to May 14) and 10 records to adults (recorded from October 19 to November 14). The updated presence of *E. catax* in the Umbria region covers 17 squares 10x10 km, with an 89% increase compared to previous knowledge. Its updated presence in terms of Natura 2000 sites is 12, making a 71% increase compared to 2018 data. As for habitat characteristics, we observed almost exclusively egg batches and larval nests on *Prunus spinosa* (only in one case, *Crataegus* sp. was the larval host plant). The mean height of *E. catax* oviposition on *P. spinosa* was 74 cm, with a range of 20-175 cm. Analysing the distribution of the nests on 20 cm intervals, *E. catax* displayed a preference for the 41-60 cm interval, with a frequency of 34%. The mean height of *P. spinosa* shrubs was 124 cm, with a range of 60-300 cm. Analysing the distribution of *P. spinosa* heights on 50 cm intervals, the shrubs mainly belonged to the 51-100 cm interval, with a frequency of 46%. The mean diameter of *P. spinosa* branches on which *E. catax* egg batches or larval nests were found was 5 mm, with a range of 2-10 mm. Most recorded plants had only a single larval nest per plant, with only two instances of two larval nests on the same plant. Our study provides valuable data for the conservation of *E. catax*, particularly in understanding its habitat preferences and ecological needs for oviposition. These findings can directly contribute to the species conservation strategies and interventions.

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LACERTA AGILIS IN PIEMONTE: CONOSCENZE ATTUALI E LACUNE

La *Lacerta agilis* è uno dei rettili eurasiatici con l'areale più vasto. Il limite meridionale europeo è rappresentato dall'Italia, con la presenza di popolazioni marginali in Piemonte ed in Friuli-Venezia Giulia. Gli studi sull'ecologia e la biogeografia della specie sono numerosi ma le popolazioni italiane non sono mai state prese in considerazione. Lo scopo di questa ricerca è stato quello di ampliare queste conoscenze, tramite un censimento e caratterizzazione genetica della popolazione presente in Alta Valle Stura (Piemonte). Durante l'estate 2023, sono stati raccolti dati biometrici ed ecologici relativi a 26 individui (dei 54 osservati) con conseguente raccolta di campioni di tessuto per le analisi molecolari (autorizzazione ISPRA N.0028702/2023 del 26/05/23). Da questi sono stati amplificati e sequenziati tre marcatori mitocondriali (citocromo c ossidasi I, control r*egion, citocromo b ossidasi*). Dall'analisi genetica, risulta come la popolazione indagata presenti aplotipi unici e significativamente distanti rispetto a quelli ottenuti da altri studi già effettuati nel resto d'Europa. Inoltre, il presente lavoro conferma che la popolazione delle Alpi Marittime occupa un areale ristretto (*area of occupancy*: 12 km^2) e presenta basse densità. I dati ottenuti con l'analisi dei marcatori mitocondriali forniscono una prima caratterizzazione genetica, suggerendo una bassa variabilità ed uno scarso flusso genico nella popolazione campionata. Studi futuri dovranno essere mirati ad analizzare popolazioni limitrofe tramite l'utilizzo di altre tecniche molecolare quali microsatelliti o analisi su SNPs per verificare se la popolazione soffre di fenomeni di inbreeding e per misurare la presenza di flusso genico fra le località. Al fine di comprendere meglio la filogeografia della popolazione nord-occidentale italiana, sarebbe utile inoltre effettuare un confronto con le popolazioni del Sud della Francia di cui non sono presenti dati genetici in letteratura. Questi studi potrebbero essere utilizzati per valutare se il Colle della Maddalena (confine di Stato con la Francia) rappresenta per la specie un corridoio o una barriera ecologica.

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ANTHROPIC IMPACTS ON MACROZOOBENTHOS POPULATIONS IN THE GULF OF POZZUOLI

Located in the northwest of the Bay of Naples, the Gulf of Pozzuoli (GoP) is a semi-enclosed basin (Tyrrhenian Sea, Italy). It is notable for the high degree of anthropogenic activity due to population growth. The aim of this study is to examine how human pressures affect the macrozoobenthic communities in the Gulf of Pozzuoli. The study demonstrates the effects of human activity on these significant benthic communities by comparing the biodiversity and abundance of macrozoobenthos in three different areas: a unique hydrothermal vent site with extreme environmental conditions, a Marine Protected Area (MPA) with restricted human activities, and an area with high anthropogenic activity.Sediment samples were collected using a Van Veen Grab Sampler, and macrozoobenthos were extracted, identified, and counted. Grain size analysis and Total Organic Carbon (TOC) analysis were conducted to characterize the sediment at each location. The main results of data analysis showed a decline in species biodiversity (27 species in the MPA vs. 8 species in the anthropogenic area) and a reduction in the biomass of macrozoobenthos in the anthropogenic area compared to the MPA (Dominance_D = 0.03049 in MPA vs. 0.05455 in the anthropogenic area). The MPA also exhibited the highest biodiversity (Shannon index H = 3.437) and an elevated number of species (Fisher alpha = 34.42), indicating a healthier and more diverse ecosystem.These results highlight the detrimental effects of human activities, such as pollution and habitat disruption, on benthic communities. The significant difference in biodiversity and abundance between the MPA and the anthropogenic area suggests that protective measures, like those in place in the MPA, are effective in preserving marine biodiversity and promoting ecological health.

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MONITORING THE POPOLATIONS OF SARDINIAN TROUT (*SALMO GHIGII*, POMINI 1941) IN THE GUTTURU MANNU PARK (SARDINIA) THROUGH A CITIZEN SCIENCE APPROACH

The Gutturu Mannu Regional Natural Park, located in southwestern Sardinia, is renowned for its numerous endemic species, including the trout. Molecular Studies have confirmed teh exclusive presence of AD individuals (mtDNA) 100/100 (LDH-C1*) in this area (Berrebi et al., 2019; Delling et al., 2020; Splendiani et al., 2023), making the species native and worthy of conservation measures. To implement effective conservation plans, it is essential to have accurate data on the presence and distribution of the trout, obtained through continuous monitoring using non-invasive and low-cost methods. For this purpose, the Visual Census sampling method with underwater camera was chosen, already tested in other watercourses in Sardinia for monitoring Sardinian trout populations (Palmas et al., 2023). In this context, a monitoring project based on Citizen Science was developed, involving park visitors. Sampling is carried out using underwater cameras provided by the Park Authority, along with a simple operating protocol and a field sheet. Visitors can place the camera in a stretch of the river during their visit and retrieve it after an hour. The camera is then returned along with the field sheet indicting the exact coordinates of the sampling site.
This work presents the preliminary results obtained from the video analysis. In these first six months of investigation, a total of 40 videos were analyzed, of which 21 showed no presence of trout. Overall, 48 individuals were counted in the 19 videos with trot presence, with a maximum of 7 per pool and an average of 2,5 ± 1,8. Despite the limited number of videos collected, a map of the areas with the highest trout presence was created, and the river sections not occupied by the population were identified. This simple sampling method, which does not require specific training, has prove effective in providing the necessary data for conservation plan while raising awareness about the species and its vulnerability to extinction.

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NATURALISTS DO IT BETTER? HOW PISA UNIVERSITY STUDENTS ENGAGED IN BIOBLITZ VIA iNaturalist PLATFORM TO COLLECT ORGANISMS OCCURRENCE DATA

Citizen Science projects are among the innovative tools used to monitor environments and species, whether non-native or of conservation interest. These projects involve numerous activities aimed at communicating the importance of these practices, explaining their purposes, and how to implement them. Among the various online platforms and databases, iNaturalist stands out as one of the most user-friendly and freely accessible. However, despite its global use, not everyone is familiar with it, particularly those who do not work in naturalistic fields.

To promote acquaintance with the platform and gain occurrence data, two "BioVersity" photographic competitions have been organized for university students. To explore the feedback of the participants, metadata collected via questionnaires have been analysed in parallel with the results of the bioblitzes conducted in 2022 and 2024, connected with "BioVersity" and promoted by the University of Pisa. These observations provide insights into the interest and engagement of Pisa University students in this kind of activity and the utilization of the platform, with a focus on different groups of students, including those with "naturalistic" curricula and others.

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NEGLECTED BIODIVERSITY HOTSPOTS: FIRST ASSESSMENT OF HETEROBRANCH MOLLUSCS ASSOCIATED TO FOUR SICILIAN PORTS

Heterobranchs are a highly diversified group of specialized gastropods with more than 500 reported species in the Mediterranean Sea (BETTI et al., 2017). Due to its central geographical location and warm shallow waters, Sicily represents a key crossroad for investigating the Mediterranean Sea slug biodiversity and an early detection point of Non-Indigenous Species (NIS), both in natural and anthropized environments, like ports (KATSANEVAKIS et al., 2013). Recently, several studies dealt with the diversity of Heterobranchia in Sicily, mostly focusing on subtidal and coastal areas (LOMBARDO and MARLETTA, 2020; FURFARO *et al.*, 2023). The present study aims to screen the Heterobranchia diversity across four major Sicilian ports: Palermo (PA), Trapani (TP), Porto Empedocle (AG), and Augusta (SR) over a year cycle (late March, early July, late October and early January), through a standardized collection - using a surface-operated scraping tool - of benthic organisms living on port walls, floating piers and mooring lines. Collected specimens were *in vivo* photographed, identified, and then stored in 96% EtOH for future molecular analysis. The preliminary results obtained from the first two sampling campaigns (March and July 2024) revealed the presence of NIS and of species never reported before for the studied area, confirming the importance and the great potential of Sicilian ports in the study of heterobranchs diversity. Moreover, the trophic behaviours known for the recorded slug species allow to infer on the structure of sessile invertebrates communities characteristic of each investigated port.

In conclusion, the current findings confirm that the heterobranch fauna of Sicily may be greatly underestimated and encourage to increase investigations on the port-associated invertebrate fauna. This knowledge may also be important to detect future shifts in port-characteristic species composition, as early signals of marine community responses to global warming.

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IL VALORE DEL MONITORAGGIO SU LUNGO PERIODO PER LA GESTIONE DELLE SPECIE ALLOCTONE; IL CASO DELL’IBIS SACRO (*THRESKIORNIS AETHIOPICUS*) IN TOSCANA

I monitoraggi eseguiti su lungo periodo rappresentano uno strumento essenziale per segnalare tempestivamente l’insediamento di specie alloctone e controllarne la diffusione.

In Toscana il Centro Ornitologico Toscano (COT) esegue dal 1984 monitoraggi riguardanti vari aspetti dell’avifauna che hanno permesso negli anni di ottenere un quadro aggiornato delle specie presenti nella regione e della loro distribuzione e consistenza. Il presente lavoro si propone di delineare il quadro della colonizzazione della regione Toscana da parte di una specie alloctona visto come strumento utile all’indirizzamento di interventi mirati di gestione. L’ibis sacro (*Threskiornis aethiopicus*) è una specie esotica invasiva di rilevanza unionale (Regolamento UE 1143/14) ed è stato recentemente oggetto della stesura di un piano di gestione nazionale redatto da ISPRA nel quale il monitoraggio delle popolazioni svernanti e nidificanti riveste un ruolo fondamentale. In questo contesto, la regione Toscana ha avviato nel 2024 un progetto conoscitivo sulla consistenza e distribuzione dell’ibis sacro a livello regionale con la collaborazione dell’Università di Firenze e del COT con finalità ultime di controllo ed eventuale eradicazione.

Le prime segnalazioni di ibis sacro in Toscana risalgono al 1997; negli anni seguenti la specie è risultata di comparsa occasionale, con individui singoli osservati soprattutto nelle principali zone umide. La presenza nella regione è risultata sempre più regolare a partire dall’inverno 2013-2014 quando si è registrato l’insediamento del primo nucleo svernante stabile. Da allora gli individui svernanti sono aumentati in maniera esponenziale sino a superare i 3,000 individui nell’inverno 2023-2024. Alla presenza di singoli individui estivanti a partire dal 2013, hanno fatto seguito le prime nidificazioni, velocemente passate dalle poche coppie insediatesi nella garzaia del Lago di Sibolla (LU) nel 2017 alle oltre 150 nel 2023. Con la stagione riproduttiva 2024 sono state avviate specifiche attività volte a descrivere per la prima volta in Toscana la biologia riproduttiva e le caratteristiche delle aree di foraggiamento.

I dati raccolti fino ad oggi consentono di delineare un quadro chiaro e dettagliato della presenza della specie in Toscana che consentirà auspicabilmente alle amministrazioni competenti di compiere interventi laddove questi possano essere più incisivi e funzionali al raggiungimento degli obiettivi fissati dal Piano di Gestione nazionale.

Simposio 4
Modelli animali utilizzati a fini scientifici

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BEYOND THE CLASSICS: THE RISE OF NON-CANONICAL MODEL ORGANISMS IN ENVIRONMENTAL RESEARCH

The concept of model organism has broadened significantly with the development of diverse non-canonical models suitable for specific research areas (ELLEGREN, 2014; LAND *et al.*, 2015). Aquatic vertebrates, particularly teleost fishes - the most species-rich group of vertebrates - are increasingly recognised as valuable non-canonical models (RUSSELL *et al.*, 2017; MUTALIPASSI *et al.*, 2019). The killifish genus *Aphanius* (Aphaniidae) belongs to the order Cyprinodontiformes, which includes established model species such as *Fundulus heteroclitus* (Linnaeus, 1766) and *Nothobranchius furzeri* (Jubb, 1971). Within this genus, the Nono (*Aphanius fasciatus* (Valenciennes, 1821)) is promising for environmental research due to its unique characteristics (SCHARTL, 2014). This Mediterranean species thrives in fragile coastal environments such as lagoons and estuaries, showing remarkable resilience to harsh conditions (high salinity, low oxygen, pollutants). In addition, Nono has a short life cycle, high fecundity and sedentary behaviour, making it ideal for both laboratory and field studies (MOSESSO *et al.*, 2012). We propose Nono as a valuable model to study responses to various environmental stressors. Firstly, we characterise the anatomical and neuroanatomical features of Nono in natural, pristine conditions: subsequent comparisons with specimens from polluted sites will make it possible to assess the degree of impact of specific forms of pollution and environmental stress. Overall, their anatomical characterisation, coupled with DNA barcoding of isolated populations and future NGS RNAseq studies, may reveal environmental adaptations and responses to stressors.

Nono's potential as a sentinel species allows us to assess the impact of anthropogenic activities (pollution), ocean acidification and warming. The study of populations with potential resistance to pollutants can lead to the identification of the underlying mechanisms, ultimately helping to inform environmental protection strategies.

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AMPHIPRION SPP. AS A MODEL FOR AQUATIC VERTEBRATE NUTRITION

Over the past decade, research has increasingly focused on phytochemicals as dietary additives in aquaculture. This interest stems from their potential to improve farm productivity and fish welfare (ABDEL-LATIF, 2023). The present study focuses on caulerpin, a bisindolic alkaloid abundant in the green alga *Caulerpa cylindracea* Sonder, 1845, an exotic species that has invaded the Mediterranean basin, posing critical threats to local biodiversity. Caulerpin exhibits diverse biological activities with potential applications in food science and pharmacology. These include its role as a peroxisome proliferator-activated receptors (PPARs) agonist (VITALE ET AL., 2018), and its ability to influence metabolism, food intake, and reproduction in the freshwater model fish *Danio rerio* Hamilton, 1822 (SCHIANO ET AL., 2022). To expand our understanding of caulerpin effects across fish species, we investigated its impact on *Amphiprion* spp., commonly known as anemonefish or clownfish, a prominent marine model organism for behavioral, ecological, and genetic studies (LAUDET & RAVASI, 2022). Due to their ease of captive breeding and wide availability in the wild, anemonefish have emerged as a valuable model organism for diverse biological disciplines (HERRERA ET AL., 2023). In particular, the effects of dietary caulerpin on fish feeding behaviour, metabolism, and intestinal microbiota composition were evaluated on *Amphiprion clarkii* (Bennett, 1830) and *Amphiprion ocellaris*, Cuvier, 1830 using metabolomics and metagenomic NGS approaches. Obtained results are discussed in light of a possible valorization of the biomass from *C. cylindracea* as a source of a valuable animal feed additive of interest in the aquaculture sector.

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OPERANT LEARNING TASKS (VISUAL AND TACTILE EXPLORATION BEHAVIOR) IN *OCTOPUS VULGARIS*

Among cephalopods, *Octopus vulgaris* belongs to the behaviorally complex coleoids. The loss of the shell pushed them to adopt a benthic lifestyle and develop a diverse hunting strategy than squid and cuttlefish. To cope with the benthic lifestyle, octopuses underwent a radical modification. They, in fact, diverged from their relatives in terms of body plan, anatomy, behaviors, and intelligence. Octopuses have independently evolved the largest and most complex nervous system and sophisticated behaviors. They are equipped with arms capable of a wide range of movements with no skeletal support, allowing them to face and find solutions to different environmental challenges. These abilities are essential in exploring new environment, problem-solving, and play-like (DI COSMO *et al.,* 2018). This study explores the behavioral responses of *Octopus vulgaris* during controlled experimental conditions. The experimental protocol involves systematic, multi-stage training to familiarize the octopuses with the setup and ensure proper device interaction. The device used for the behavioral experiments is composed of three transparent boxes with lids that have a central hole, allowing the octopuses to insert its arm inside.

Our previous studies (MASELLI *et al.,* 2020) demonstrated that the *O. vulgaris* preferred prey is anchovies. During the experiment, two fake anchovies and one real anchovy are placed in the boxes. Training begins with acclimation to the environment, followed by increasing training to reinforce the correct interactions. Successful training will be confirmed when the octopuses consistently perform the required interactions in exchange for rewards. Behavioral performances have been evaluated by video recording of the trials allow for detailed analysis of arm movement and suckers employment using the software BORIS program, focusing on visual and chemoreception responses. This study assessed the repeatability of arms and suckers used during specific behaviors, providing insight into whether *O. vulgaris* employs consistent strategies under different food choice experimental tasks. The results highlight patterns of behavior that suggest specific strategies in the use of arms and suckers. We were able to determine the preferential strategy used by the octopus in food selection.

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KNOCK-OUT OF *SETD5* MATERNAL mRNA LEADS TO ABNORMAL BRAIN DEVELOPMENT AND BEHAVIOURAL DEFICITS IN A ZEBRAFISH (*DANIO RERIO*) MODEL

The zebrafish (*Danio rerio*) has been long recognized as an emerging model to study developmental processes and associated pathologies. Among the variety of advantages, zebrafish and mammals display strong similarities in neural cell types as well as conserved signaling pathways.

Despite zebrafish-human evolutionary differences, the main brain structures and fundamental molecular mechanisms remain highly conserved. Zebrafish neurodevelopment, in particular during embryonal stages ranging from the end of primary neurogenesis and the beginning of secondary neurogenesis, provides a useful comparative model for amniotes early brain development.

Finally, zebrafish is a highly social animal and the availability of a plethora of specific tests make it useful to assess different aspects of complex behaviors such as social interactions.

We had previously generated a loss-of-function zebrafish model by CRISPR/Cas9 gene editing for the gene *setd5*, an epigenetic regulator which has been strongly associated with intellectual disabilities and autistic spectrum disorders (ASD) in humans. Since we found that *setd5* is expressed in both maternal and zygotic transcripts in zebrafish, we also generated heterozygous fish carrying either wild-type or mutated *setd5* maternal mRNAs, investigating the relevance of these transcripts in neurodevelopmental processes.

Herein we focused on examining the role of *setd5* in the regulation of the cell cycle and in the distribution, differentiation and survival of neural progenitor. We found that absence of *setd5* mRNA during the very early stages of development determines prominent depletion in neural progenitors' proliferation, differentiation and survival. These alterations are coupled with the impaired expression of genes encoding for synaptic and neurotransmission proteins associated with GABAergic, serotoninergic and dopaminergic systems, thus affecting neuronal transmission. In particular, the entirety of our results emphasizes the significance of maternal mRNA contribution in nervous system formation, especially for late-stage events, such as the secondary neurogenesis wave.

Interestingly, the absence of a functional *setd5* maternal background is manifested also in adult individuals which display aberrant social behaviour and learning/memory defects, while a large portion of adults also exhibit repetitive swimming behaviors. Overall our results give detailed insights on the crucial role of *SETD5* in neurodevelopment and our mutant model emerges as a promising disease model for ASD.

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BRAIN AGING IN THE LONGEST-LIVED VERTEBRATE (*SOMNIOSUS MICROCEPHALUS*)

We are interested in the evolution of aging and the mechanisms of negligible senescence in vertebrates. In this regard, we have chosen chondrichthyans as ideal basal vertebrate models due to some neuro-developmental and molecular aspects they share with mammals, and because the longest-living vertebrate known is a shark: the Greenland shark, *Somniosus microcephalus*. This shark inhabits the abyssal part of the North Atlantic Ocean and is found near the surface along the Arctic coasts. It has a life expectancy of more than 270 years, based on radiocarbon dating of the cornea.

We are focusing on describing the neuroanatomy and analyzing specific markers of brain aging in *S. microcephalus*, comparing them with a sister species, the Little sleeper shark, *Somniosus rostratus*, a Mediterranean deep-sea shark that lives around 80 years. Specifically, we are investigating and comparing the presence of the aging marker lipofuscin, the distribution and spatial extent of adult neural stem cells, the morphology of the lysosomal component, and the presence of oxidative stress markers. Our preliminary immunohistochemistry data indicate the coexistence of young and old traits in the brain tissue of six specimens analyzed, such as neurogenic activity and accumulation of the aging marker lipofuscin, respectively. Additionally, lipofuscin, which usually accumulates in old tissues, is present in higher quantities in the brains of *S. microcephalus* than in *S. rostratus*, confirming the longer lifespan of this species.

Moreover, several pelagic species can be considered valid experimental outgroups in comparative analyses (e.g., *Etmopterus spinax*, *Galeus melastomus*), as they have a relatively short life expectancy compared to the Greenland shark. Finally, comparing the Greenland shark with the turquoise killifish, *Nothobranchius furzeri*, the shortest-lived vertebrate extensively used in laboratory aging studies today, could provide invaluable information on organism longevity.

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COMPARATIVE GENOMICS TO INVESTIGATE THE MECHANISMS UNDERLYING AGING AND LONGEVITY IN ANIMALS

The mechanisms underlying aging and longevity are complex and far from being understood. New methods and additional model species are necessary for elucidating such processes. In this context, studying animals with particularly long lifespans may provide new insights in this topic. Some species evolved indeed the capability to live longer: the features that allow such longevity are encoded in their genomes and the analysis of such features may help to identify key regulators that contribute to extension of lifespan.

In our research line, we are taking advantage of the remarkable lifespan disparity present in different taxonomic groups to investigate genes associated with lifespan extension, focusing our attention on genes showing convergent evolution in long-lived species. One of the most interesting taxa, in this context, is represented by bivalve molluscs, which show the highest lifespan disparity within a metazoan class. We analysed 30 bivalve species with a lifespan disparity ranging from 1 to 507 years, including the longest-lived non-colonial animal known so far, *Arctica islandica*. We found that a high number of genes showing convergent evolution in long-lived bivalves constitute a network of protein-protein interactions, which is enriched for factors with experimental support for a role in longevity in other animals. A different, promising, taxonomic group for aging studies is represented by birds. Despite their high metabolic rates and high production of harmful oxidative molecules, birds live up to three times longer than mammals of similar size. For this reason, we analyzed genomes from 141 bird species, with lifespans ranging from 3 to 84 years. We found that opposite selective forces act on the same genes in species with long and short lifespans. We found that such genes are mainly involved in cell-cycle regulation, DNA repair, and tumor progression.

Our results show that comparative genomics is a powerful method to investigate genes underlaying aging and longevity. Besides providing new interesting candidate genes underlying lifespan extension, these works highlight the presence of common genetic factors involved in aging and longevity in very distantly related species. This suggests that mechanisms of lifespan extension may be, at least partially, shared across animals, and highlights the contribution of species with extreme longevity in identifying new genes and pathways with a role in regulating lifespan of other species, including humans.

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USE OF PLANAR OPTODE TECHNOLOGY TO ASSESS OXYGEN AND PH DYNAMICS IN SOILS BIOTURBATED BY DUNG BEETLES

Dung beetles through the manipulation of livestock faeces provide a diversified range of ecosystem services. They contribute to dung redistribution in the soil, and organic matter mineralization and nutrient recycling. This, in turn, facilitate nutrient uptake from plants, and reduction of greenhouse gas emissions. Dung beetles are key organisms in maintaining the functionality of pasture ecosystems, secondary seed dispersal, and parasite control and suppression.

Here we use as a model *Onthophagus nuchicornis* (Linnaeus, 1758), a dung beetle which digs tunnels beneath the dung pat where it builds brood balls for larvae nutrition. We evaluated soil bioturbation and dung removal activity by investigating oxygen and pH dynamics with planar optode technology (MERL and KOREN, 2020; MERL *et al.*, 2023). The optodes were integrated into a thin (7 mm) soil sandwich (*i.e.*, microcosm) and by using a single-lens reflex camera we acquired two dimensional (2D) images (25x15 cm) over a > 24h period. A 405 nm UV LED was used to excite the optodes.

The results show that dung beetles strongly affect the amount of oxygen in the soil by tunnelling and transporting the dung, which is highly anoxic, deep into the soil. In addition, dung beetles, through bioturbation, increase the oxygenation of the dung still on the soil surface by allowing aeration and thus favouring aerobic decomposition processes. Soil pH also changes due to bioturbation and dung removal. The results show that dung beetles action produces an alkalinising effect on the soil (approx. from pH 6 to pH 8), especially in areas where dung has been buried. In addition, even the dung left on the surface shows an increase in pH due to bioturbation. With this technology, we were able to assess both the differences in the soil before and after the action of the dung beetles, as well as the actual way in which the dung beetles move underground by mixing the dung with the soil and building their nests.

In conclusion, this is an entirely new approach to understanding and quantifying some of the ecosystem services provided by these beetles, as planar optodes have, to our knowledge, never been used on dung beetles. Our pilot study well demonstrates the high potential of planar optode technology for application in this field.

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CLAVELINA LEPADIFORMIS (TUNICATA) AS AN EXPERIMENTAL MODEL TO STUDY THE EFFECTS OF NOISE EXPOSURE IN LABORATORY CONDITIONS

In the last century, the level of noise in the sea worldwide has risen considerably due to increased human activities, thus generating a growing international concern regarding its impact on marine organisms (CAMERLENGHI, 2021). Noise is now considered an emerging pollutant; however, we are still far from understanding how anthropogenic noise affects animal life. To date, there has been an almost exclusive focus on vertebrates while very few studies analyzed the effects of noise on invertebrates and especially on the sessile ones that can not avoid noise by escaping strategies. We took advantages of the amenability of the ascidian *Clavelina lepadiformis* as an experimental model to set up a protocol to study the effects of noise in laboratory conditions. *C. lepadiformis* is a compound Aplousobranchia ascidian common in the Mediterranean Sea that forms colonies of several zooids joint by short stolons. Differently from many solitary ascidians that spawn gametes in the seawater, adults of this species incubate the embryos in the atrial cavity where they complete their development.

In our experimental set-up, adults carrying developing embryos were exposed to a pink noise (higher intensities at 63-125 Hz) at different levels in tanks (120-180dB) using an underwater speaker, for six days. During this period, embryos completed their development, larvae were released in the seawater, settled on the bottom of the tanks and started the metamorphosis into sessile juveniles. To evaluate if swimming larvae can be influenced by the noise in the selection of the site where to adhere, we carefully annotated the position of each settled juvenile. Statistical analysis revealed that the distribution of juveniles in noise tanks was significantly different from that of juveniles in control silent tanks and that exposed juvenile were more abundant in sites far from the noise source then control ones.

Moreover, exposed juveniles displayed an impairment of the ciliated cells of the syphons, suggesting that noise can affect the sensory cell morphology of these animals. These data showed that the designed experimental set up is a valuable tool to unravel different effects of noise exposure in these sessile invertebrates. Anthropogenic noise recorded in different marine environments will be used to confirm the observed results in a realistic scenario.

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BIOACTIVE MOLECULES FROM CEPHALOPODS (*OCTOPUS VULGARIS* AND *SEPIA OFFICINALIS*): *GALLERIA MELLONELLA* LARVAE AS MODEL FOR TESTING ANTI-INFLAMMATORY ACTIVITY

Marine organisms are known to produce bioactive molecules (PRIN 2022, "Mediterranean marine organisms as sources of new anti-inflammatory and pro-resolving compounds"). Female octopuses care for their eggs by repeatedly touching them with suckers, and microbial biofilms on eggs occur when the mother is removed, leading to hypothesizing the antibacterial activity of their suckers. Indeed, the peptide octopartenopin has been isolated from *Octopus vulgaris* suckers, showing antibacterial and antimicrobic activities (MASELLI *et al*., 2018). In addition, cephalopod ink has demonstrated several bioactive activities (HOSSAIN *et al*., 2019). In this perspective, the present study aims to specifically evaluate the anti-inflammatory activity of peptides functionalized from octopartenopin and crude extracts of *Sepia officinalis* ink, using the larval life stage of *G. mellonella* lepidopteran which is already extensively used for testing antibacterial and antifungal activities since their immune system resembles that of mammals' innate immune system (PEREIRA *et al*., 2018).

Firstly, the colourimetric 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays are used as a preliminary test to evaluate the antioxidant activity of the bioactive molecules of interest. Subsequently, *G. mellonella* larvae are treated with lipopolysaccharides to induce inflammation and molecules that have shown the most promising antioxidant activity in preliminary assays. The survival rate of *G. mellonella* larvae following exposure to the selected molecules is monitored to assess their potential toxicity. Gene expression of inflammation-related markers (Cecropin-D, TNF-like, and NO synthase) is analyzed by Real-time PCR to determine the efficacy of these molecules in modulating the inflammatory response.

By employing *G. mellonella* larvae in this innovative approach, we identify novel anti-inflammatory compounds derived from cephalopods through a non-conventional model organism. This research not only expands the application of the larval model but also promises to advance therapeutic strategies for inflammatory conditions in human health.

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EXPLORING AND SAFEGUARDING INSECTS: THE BALANCE AMONG EXPERIMENTAL USE, FOOD CONSUMPTION AND WELFARE PROTECTION OF *TENEBRIO MOLITOR*

Life science research has traditionally exploited vertebrate species. Recently, insect models have gained interest mainly due to technological advancements, lower maintenance costs, and simpler controlled rearing. The study of their neurobiology and cognition has provided insights into the evolution of animal minds (Simons and Tibbetts, 2019; Rivi *et al.*, 2023) and clinical translational relevance (Adamski *et al.*, 2019; Tonk-Rügen *et al.*, 2022). While comparative analysis has shown certain shared higher-level aspects, policymakers are now promoting some insect species as a high-protein food source (FAO, 2021), urging regulation of their husbandry and welfare since they are not currently covered by European Welfare Legislation.

This poster presents a literature review on neurobiology, cognition, and behaviour of the beetle *Tenebrio molitor* (Linnaeus, 1758), whose larvae have recently been added among edible insects (EFSA, 2021), with the goal of highlighting how this species can be used as an emerging model and deserves to be protected under welfare frameworks originally developed for vertebrate species. We scanned the main bibliographic databases and found a limited number of studies on this beetle, even less especially focused on its larval stage. However, adult individuals possess rudimentary numerical abilities (Carazo et al., 2012), the pupae are already capable of habituation (Bernal-Gamboa *et al.*, 2021), and the larvae show spatial learning (Borsellino et al., 1970), and memory retention (Walrath, 1970) that are maintained across the metamorphosis from larval to adult stage (Alloway and Routtenberg, 1967; Borsellino *et al.*, 1970; Alloway, 1972). Moreover, we have recently demonstrated that *T. molitor* larvae represent the goal of their actions (Dissegna *et al.*, 2024a) and modulate their defensive behavior based on associative memories of previous negative experiences in a specific context (Dissegna *et al.*, 2024b).

The review revealed a gap in studies focusing on the cognitive abilities of *T. molitor* compared to other insects. Nonetheless, based on the neural, cognitive, and behavioral features identified insofar, this species can be considered an emerging animal model, as already proposed in the UZI database, and deserving of protection under the Five Freedoms framework. As it becomes increasingly significant both as an experimental model and a food source, continued research will ensure bidirectional benefits: enhanced knowledge and welfare improvements.

Simposio 5
Le innovazioni in Zoologia: dal microscopio all'intelligenza artificiale

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NUOVE METODOLOGIE PER L'ANALISI DEL PLANCTON (ZOOSCAN E A.I.): IL CASO DI STUDIO DEL SITO LTER PROMONTORIO DI PORTOFINO (LTER_EU-IT_015)

Lo studio del plancton coinvolge una gamma diversificata di metodi di ricerca finalizzati a comprendere la sua composizione, distribuzione e funzione al fine di rapportarlo ad uno stato di salute ambientale. I tradizionali approcci microscopici consentono l'identificazione e la quantificazione delle diverse specie di zooplancton e fitoplancton. L'avanzamento tecnologico ha permesso lo sviluppo di diversi strumenti che permettono di ottenere immagini digitalizzate utilizzabili da intelligenze artificiali dotate di algoritmi sempre più avanzati che consentono di automatizzare il processo di riconoscimento tassonomico e quindi semplificare e velocizzare l'analisi. L'obbiettivo principale di questo lavoro è stato quello di integrare alla metodologia classica quella più moderna di digitalizzazione delle immagini analizzate tramite l'utilizzo dello ZooScan e classificate tramite l'utilizzo di un'intelligenza artificiale (A.I.), mediante la piattaforma web EcoTaxa, al fine di migliorare ed implementare la metodica standardizzata di riconoscimento. Durante lo studio sono stati analizzati diversi campioni prelevati all'interno dell'area marina protetta di Portofino, in particolare nella stazione di Cala dell'Oro (sito LTER_EU_IT_015), in diversi periodi dell'anno, così da testare, ma al contempo addestrare, gli algoritmi dediti al riconoscimento dei vari organismi. Si è inoltre cercato di realizzare un nodo dell'A.I. sito-specifico per il riconoscimento dei Copepodi, gruppo che presenta ancora criticità nella classificazione dettagliata con metodologie automatiche. I risultati ottenuti evidenziano per i Copepodi, componente maggioritaria all'interno del plancton, degli ottimi risultati sia nel riconoscimento sia nell'apprendimento da parte dell'A.I.. Inoltre si è potuto notare che questa nuova tecnica risulta particolarmente efficace per la classificazione di organismi appartenenti ad alcuni taxa come Appendicularia e Diplostraca. Mentre per organismi gelatinosi molto fragili, come Cnidari e Thaliacea, si evidenziano ancora alcune problematiche. In conclusione questa metodologia basata sull'utilizzo del *machine learning*, con un continuo miglioramento dei dataset, può diventare un ottimo supporto ad altre tecniche di indagine del plancton (stereomicroscopio e *barcoding*) che richiedono maggior tempo di analisi e costi più elevati.

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UNVEILING HIDDEN LIFE OF MARINE ANIMALS: TECHNOLOGICAL APPROACHES USED TO INVESTIGATE LOGGERHEAD SEA TURTLE BEHAVIOUR

Investigating the behaviour of marine animals is a most challenging task due to the difficulty of obtaining continuous observations in animals dwelling in their natural environments. In recent years, substantial advancements in telemetry and biologging techniques have significantly increased our understanding of the behavioural patterns of air-breathing marine vertebrates. Our research group has investigated several options to study unexplored aspects of the behaviour of Mediterranean loggerhead sea turtles (Caretta caretta) actively moving in the open sea. In experiments on loggerheads nesting in Turkey, we have deployed next-generation GPS tags connected to the Iridium satellite system along with multi-sensor data loggers featuring depth sensors, triaxial magnetometers, accelerometers, and gyroscopes. GPS-linked satellite telemetry has enabled the precise reconstruction of individual routes, providing valuable information about the migration routes and the localization of foraging areas of tracked turtles. Data from multi-sensor loggers have revealed previously unknown fine-scale behavioural patterns displayed by loggerhead turtles travelling towards a specific destination, such as the alternation between subsurface and deep dives, rapid changes in body orientation, and low-activity deep dives suggestive of pelagic resting. Furthermore, loggerheads foraging in the coastal waters of Lampedusa Is. have been equipped with auto-detachable tags with multi-sensor data loggers and high-resolution video cameras, which allowed us to document interactions between individuals and searching or crawling events on the seafloor. These findings underscore the crucial role of integrating diverse techniques to study unexplored behaviours of marine animals, which have largely been neglected so far due to the lack of suitable technological and analytical approaches developed to this aim.

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DIGITIZATION OF THE BUTTERFLY COLLECTION OF ROGER VERITY

The butterfly collection of Roger Verity of the Natural History Museum of Florence, La Specola, includes about 100,000 specimens from Italy and other countries. Most of the butterflies represented by diurnal species that were mainly collected in the first half of the 20th century. Within the frame of the outreach activities funded within the National Biodiversity Future Center (NBFC) we carried out the digitization of this collection following the open science FAIR (Findability, Accessibility, Interoperability and Reusability) principles, in order to share its relevance and uniqueness with the scientific community.

High resolution photos of each box, bearing a unique code, a colour checker and a ruler were taken. Each single butterfly was then numbered, in post-production, using a self-made GIMP plugin to automatically set a unique code for each specimen (MIDS1, or Minimum Information on a Digital Specimen level 1; see https://www.tdwg.org/community/cd/mids/). The printed codes were placed in each box, ready to be permanently pinned together with the specimens once needed (after taxonomic revision, or annotation, or internal collection re-arrangement). All the photos are provisionally stored in a drive folder, while waiting to get ready for their final publishing on the Global Biodiversity Information Facility (GBIF).

The next step consisted in retrieving further metadata (MIDS2/MIDS3) for the original specimens (nomenclatural types) used by Roger Verity to describe new taxa. To this purpose, past-published catalogues (KUDRNA, 1982; KUDRNA and BALLETTO, 1984, 1985) were used as frameworks. After recognising the text of the documents via OCR, a Python script was written in order to extract regular expressions (regex) in a csv format. A review of the csv file was then performed, and completed with the missing/wrong information. Although Roger Verity was a very prolific collector and published many new taxonomic novelties at different ranks, he almost never associated types to such names. Several years later, Othakar Kudrna and other experts provided useful information to trace such types, but they still lacked the designation of lectotypes among (often) rich series of syntypes. Following each protologue, we started filling such gap with the proposed designation of lectotypes in the Nymphalidae within the scope of future taxonomic revisions.

After the digitization, 987 entomological boxes were labelled. Within these boxes, we have counted 99,926 specimens, among which ~ 9,000 “name-bearing types” as defined by the International code of zoological nomenclature (ICZN; https://www.iczn.org/the-code/the-code-online). Nevertheless, ~ 500 names of Nymphalidae taxa described by Roger Verity still needs a typification.

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AI IN ZOOLOGIA: RIVOLUZIONE NELLA RICERCA E CONSERVAZIONE ANIMALE

L'Intelligenza Artificiale (AI) sta rivoluzionando la zoologia, migliorando la ricerca e la conservazione animale. Le tecnologie AI offrono strumenti avanzati per l'analisi dei dati, la modellazione e la previsione dei comportamenti animali, trasformando la nostra capacità di comprendere e proteggere la fauna selvatica. Questo simposio esplora le numerose applicazioni dell'AI in zoologia, evidenziando come queste tecnologie migliorino la ricerca scientifica e contribuiscano alla conservazione della biodiversità.

L'AI permette di analizzare grandi quantità di dati biologici con precisione e velocità senza precedenti, facilitando scoperte scientifiche e migliorando la nostra comprensione del regno animale. Le tecnologie di riconoscimento delle immagini possono identificare e classificare specie animali con un'accuratezza del 96.6%, paragonabile a quella umana, ma in tempi molto più rapidi. Questo è utile per gestire immense raccolte di immagini catturate da trappole fotografiche e altre tecnologie di monitoraggio.

Applicazioni dell'AI in Zoologia

- Analisi delle Immagini: Algoritmi di riconoscimento delle immagini identificano e classificano automaticamente le specie animali in milioni di fotografie catturate da trappole fotografiche, riducendo drasticamente il tempo necessario per l'analisi dei dati.
- Monitoraggio dei Comportamenti: L'AI studia i movimenti e i comportamenti degli animali in natura. Tecniche di apprendimento automatico analizzano i dati raccolti da dispositivi di tracciamento, come collari GPS e sensori acustici, per determinare le abitudini di movimento, i territori di caccia e i modelli migratori.
- Modellazione Predittiva: Gli algoritmi di machine learning migliorano i modelli predittivi per studiare le dinamiche delle popolazioni animali e prevedere l'impatto dei cambiamenti ambientali, aiutando a sviluppare strategie di conservazione più efficaci.

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BLACK SPOTS IN THE BLUE: IS THERE ANY ELYTRAL PATTERN IN THE ITALIAN POPULATIONS OF THE PROTECTED BEETLE *ROSALIA ALPINA*?

Insect colouration has significant biological functions, such as escaping predation through mimicry and communicate with conspecifics (BADEJO et al., 2020) and may present large variation even within and among populations of the same species. Over time scientists have tried to investigate these patterns, providing physiological, ethological and evolutionary explanations to them (CLUSELLA-TRULLAS, et al., 2020; MILLER et al., 2023). Despite the great interest, many cases remain poorly understood, even in iconic and well-studied species with remarkable colouration, such as the Alpine longhorn beetle, *Rosalia alpina* (Linnaeus, 1758). *R. alpina* is a saproxylic beetle that shows a distinctive body colouration consisting of black spots on a light blue background on the pronotum and elytra (BENSE et al., 2003). This charismatic beetle is protected under the Habitats Directive (92/43/EEC), and anecdotal evidence suggests that the shape, location and size of the elytral spots vary greatly between individuals and/or populations. So far, little attention has been paid to this characteristic, mainly with regards to monitoring protocols (PAGOLA CARTE, 2011; ROSSI DE GASPERIS et al., 2016; CAMPANARO et al., 2017).

This given, the investigation of the elytral spots variation in *R. alpina* appears to be of great interest, searching for ethological, physiological or biogeographical reasons to it.

In this context, we developed a list of functions written in R capable of converting the elytral picture into a black and white grid corresponding to the colouration pattern. The homology of the position of each cell in the grid, in all the individuals, is ensured by the application of landmark-based methods (i.e., geometric morphometrics) that remove differences in position, rotation and dimension. Then, we acquired the closed outline of the elytra of the individuals to deform the grid based on the morphology of the elytra itself. As a proof of concept, we applied the technique to a dataset of *R. alpina* specimens (wild and museological samples), to investigate the reliability of the method and to preliminary assess the influence of two possible sources of variation, i.e. sex and individual size on coloration pattern. Our analytical framework proved to be user friendly and easily customizable to analyse and process large amounts of data, resulting in a reliable tool to effectively quantify and describe animal coloration variation and helping to explain its origin and evolution.

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HARNESSING LARGE LANGUAGE MODELS FOR THE EXTRACTION OF TRAITS FROM SCIENTIFIC LITERATURE

Gathering traits across the tree of life is one of the key aims of taxonomy and ecology. Accurate morphological, behavioural, ecological and life history trait measurements allow scientists to describe and catalogue the diversity of life and are often a reliable proxy for the functional role played in an ecosystem by a given species or taxonomic entity. Furthermore, traits have been linked to community dynamics and extinction threats. Although efforts to gather traits into accessible databases have been made, especially for vertebrates and plants, data on traits are unconnected and heterogeneous, resulting in confusing and time-consuming trait assignment. Extracting trait data from papers or scientific texts can aid in the fast implementation of analyses and foster a trait-based approach to the ecology of under-studied groups of organisms, such as invertebrates. Here, we present a Python pipeline to extract traits from the PDFs of scientific papers and books.

Large Language Models (LMM) are powerful artificial intelligence tools that aim to process texts, excelling in complex tasks related to natural language processing. We present a framework to extract traits, based on fine-funing of pre-existing LMMs (GPT2, BERT) in order to significantly increase their performance at the required task. The first step of the workflow consists in the annotation of a corpus of scientific papers, of various structures and referred to different taxonomic groups (with a main focus on invertebrates), in order to develop reliable and robust models. The annotation involves the species, the trait(s), the numeric value(s) of the trait(s) and the units of measure. The second step consists of splitting the annotated corpus in training and test datasets in order to evaluate the performance and avoid overfitting of the model. The third step is tuning and evaluation, in which the model is tuned and evaluated for its accuracy. The last step consists of the application of the tuned model to a set of PDFs to show its possible application to an un-annotated and novel corpus.

Our study is novel as no one has developed a user-friendly pipeline to extract species traits from PDFs so far. The extracted traits might then be useful for a wide array of purposes, given the growing relevance of trait-based ecology and zoology.

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TIGER MOSQUITO, *AEDES ALBOPICTUS*, LARVAL SALIVARY GLANDS CHARACTERIZATION

Mosquito salivary glands (SGs) play a major role in the pathogen transmission. Despite knowledge about vector SGs is constantly expanding, larval SGs, remain largely unexplored (OVIEDO et al., 2009), especially in *Aedes* mosquitoes. This study aims to better understand the structure, secretions, and gene expression of *Aedes albopictus* larval SGs.

We focused on the proteome of larval SGs using MS approach. Approximately one hundred fourth-instar larvae were dissected to collect SGs, and the extracted proteins were analysed with a mass spectrometer. To further investigate previous mentioned results, the tissue specificity of the genes encoding the aforesaid proteins was determined. RT-PCR was performed on total RNA extracted from larval different tissues (SGs, guts, heads, and carcasses). Subsequent PCR on the cDNA using specific primers confirmed the presence of a diverse array of proteins.

Additionally, to overcome the gap in morphologic data on the *Aedes* mosquitoes larval SGs we provided in this work images, obtained via light microscopy, and 3D models basing on tomography obtained with X-ray microscopy.

Four-instars larval heads were photographed with a light microscope after fixation, dehydration, resin embedding and ultramicrotome sectioning. In the immediate future, the tissue ultrastructure will be analysed via transmission electron microscopy (TEM). 3D models were produced using Dragonfly software.

As regard as morphological characterization, the different imaging techniques exploited in this work lead to new insight into the current knowledge of the larval salivary glands of *Aedes albopictus*. Furthermore, information about the biological function of the larval salivary secretions could be inferred from identified proteins most abundantly present in the larval SGs secretome. Mass spectrometry- based characterization of larval salivary gland proteome led to the identification of 31 proteins found over a set abundance threshold. According to their function, in mosquito larval salivary glands immune related proteins, enzymes involved in digestion or detoxification, transporters and others can be found.

Enhanced knowledge of larval SGs can provide insights into mosquito biology and identify potential targets for disrupting SG function, potentially leading to innovative vector control strategies and new approaches to interrupt mosquito-borne pathogen transmission.

Simposio 6
Oltre i confini della zoologia: multi/inter/trans-disciplinarietà nella zoologia

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A TEARFUL GLANCE: A VISUAL IDENTIFICATION KEY TO GENUS *LACRYMARIA* (CILIOPHORA, LITOSTOMATEA, HAPTORIA)

The ciliate genera *Lacrymaria* Bory de St. Vincent, 1824 and *Phialina* Bory de St. Vincent, 1824 have a long and complex taxonomic history, which includes genus *Phialina* being merged into *Lacrymaria* by Alfred Kahl (1930) and separated again by Wilhelm Foissner (1983) on the basis of various morphological features (the discriminant currently being the presence or absence of a contractile neck). Now it might be the time to merge them again as molecular studies show them to be non-monophyletic separately but monophyletic collectively (RAJTER et al., 2019; ALLIEVI et al., in preparation). These genera include a great number of morphologically diverse species many of which have been observed and/or described rarely or just once. We have therefore run an extensive bibliographic research meant to collect and summarize all information provided in literature about the species of these genera, in order to evaluate their taxonomic status and formally re-assign to *Lacrymaria* the species currently classified as *Phialina*.

As part of this effort, we provide a morphological identification key to about 78 species and likely new species of genus *Lacrymaria*. The key is based mainly on number and shape of macronuclear nodules, somatic ciliature, number and position of contractile vacuoles, trunk shape, and habitat. It is presented in form of a polytomous tree, which does not represent a phylogeny; each branch, labeled with a certain set of features, represents a group of species that is a subset of the parent branch, and which share the features indicated by the label. All branches end with individual species, and are accompanied with an illustration if any is available, generally from the most recent description. The species of the closely related genera *Phialinides* and *Pelagolacrymaria* are also included in the key, as they could be easily mistaken for *Lacrymaria*, and might even be brought into the genus by future studies.

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BEHAVIORAL PLASTICITY IN A SOCIALLY ISOLATED BONOBO (*PAN PANISCUS*): PLAY AS A PROMOTER FOR SOCIAL INTEGRATION

Prolonged social isolation can have lasting effects on the ability to engage correctly in social behaviors. However, the plasticity of play may offer a pathway for social integration, particularly in neotenic species like the bonobo (*Pan paniscus*). This case report focuses on Congo, an adult bonobo who was deprived of social stimuli, including interactions with conspecifics, for approximately thirty years. Our study examines Congo's social behaviors following his integration into the bonobo colony at Wilhelma Zoo (Stuttgart, Germany), with a particular emphasis on his playful interactions with two younger members of the group. Comparative analyses were conducted on dyadic play interactions between Congo and each of the juveniles versus other adult-juvenile play dyads within the colony. Despite his extensive period of isolation, Congo accepted the invitations to play by two juveniles of the colony. He exhibited playful facial expressions, signaling positive intent during interactions. However, he was never observed performing rapid facial mimicry, likely due to his difficulty in processing the rapid dynamics of playful actions. Additionally, play sessions involving Congo had a higher risk of aggressive escalation and were frequently punctuated by his screaming vocalizations, which often led to brief grooming pauses that helped de-escalate the situation. Furthermore, these sessions rarely involved object play. The findings highlight that, despite significant social deprivation, Congo managed to engage in social play, suggesting a degree of behavioral plasticity. This supports the notion that play, a behavior typically trained in youth, can serve a versatile role in an individual's life and can potentially be acquired in adulthood, albeit with some challenges. Although this is just a case report, the promising results indicate that play can be adopted as a key behavior for social integration. Its inherent flexibility allows it to serve as an icebreaker, facilitating the initial steps toward social engagement in a new environment. These insights underscore the potential of play behavior as a valuable tool in the social rehabilitation of isolated individuals.

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BRIDGING SPECIES DIVIDES: AFFILIATIVE INTERACTIONS BETWEEN *THEROPITHECUS GELADA* AND *PAPIO ANUBIS* IN AN UNPROTECTED AREA IN ETHIOPIA

Understanding interspecies interactions is crucial for elucidating the evolution of tolerance in complex societies and the flexibility of animal communication. We followed 11 gelada (*Theropithecus gelada*) one male units (OMUs) living in the Ethiopian highland of Debre Libanos area (North-West Shewa zone, Oromia regional state, Ethiopia), an anthropized unprotected area, from February to April 2024 (391 hours) covering the end of the dry and the beginning of the wet season. Here, we report positive interactions between geladas and olive baboons (*Papio anubis*), two sympatric monkey species characterized by complex but different social systems and ecological habits. Although geladas were observed co-feeding with olive baboons for four hours (1.02% of total observation time), such co-presence went beyond the mere spatial tolerance. We also recorded long sessions of inter-specific grooming (N=2) and a play invitation accompanied by reciprocal signal exchanges (e.g., lip smacking, mutual gaze, rear presenting) thus suggesting a communicative common ground due to a shared evolutionary history. Both species initiated social interactions by inviting and gazing each other with the involvement of males for geladas (N_{males}=5; $N_{immatures}$=3) and males and females for olive baboons (N_{males}=1; $N_{females}$=5; $N_{immatures}$=2). Agonistic interactions were never observed. Our pilot observations are in line with the socially tolerant nature of geladas, which seems to expand not only to intergroup but also to interspecies dynamics. Such tolerant propensity together with the high human pressure in some Ethiopian unprotected areas, altering the space occupancy of several primate species, can increase the risk of hybridization.

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GLI SQUALI ANGELO DEL MEDITERRANEO: ANALISI STORICA E CONSERVATIVA DELLE SPECIE A RISCHIO

Il Mar Mediterraneo ospita tre squali angelo *Squatina aculeata* (Cuvier 1829), *S. oculata* (Bonaparte 1840), *S. squatina* (L. 1758), tutte in pericolo critico di estinzione. Le loro caratteristiche biologiche, come taglia elevata alla nascita, crescita lenta, maturità tardiva e bassi tassi di riproduzione, li rendono particolarmente vulnerabili e ostacolano la ripresa demografica delle popolazioni. In quanto predatori bentonici, gli squali angelo svolgono un ruolo fondamentale nel mantenimento dell'equilibrio dell'ecosistema marino. La loro presenza è indicativa di un ambiente marino sano e stabile, poiché sono sensibili alla qualità dell'habitat e alla disponibilità di prede. Il recente declino di queste specie, situato all'intersezione dinamica tra la civiltà umana e gli ecosistemi marini, sottolinea l'urgente necessità di comprendere i fattori alla base della loro diminuzione e le implicazioni per gli sforzi di conservazione.
In questo studio, abbiamo utilizzato reperti museali e un approccio multidisciplinare per ricostruire la diversità specifica ed ecologica storica degli squali angelo nel Mediterraneo attingendo ad una ricchezza di campioni storici osteologici e umidi di oltre 30 musei e collezioni private datati dal XIX al XXI secolo. I campioni museali sono stati analizzati mediante tassonomia integrata morfometrica-molecolare e mediante analisi degli isotopi stabili. I dati ottenuti hanno permesso, in via preliminare, di: distinguere 53 esemplari di Squatina sp., conservati in formalina, tassidermizzati o osteologici, mediante analisi morfometriche. Quest'analisi ha permesso la corretta distinzione tra individui di Squatina aculeata, Squatina squatina e Squatina oculata. Inoltre, grazie a nuove tecniche di estrazione, è stato possibile ottenere DNA dai campioni in formalina, consentendo l'identificazione a livello di specie per individui le cui condizioni non erano adeguate per l'analisi morfometrica o per feti nelle quali le caratteristiche discriminanti erano assenti.
La ricerca sugli squali angelo storici ha inoltre permesso di redigere un catalogo degli Elasmobranchi del Museo di Zoologia "Doderlein" dell'Università di Palermo utile a rendere fruibile la collezione ai ricercatori e a valorizzare il contributo significativo delle collezioni museali naturalistiche alla conoscenza e alla conservazione degli Elasmobranchi, rafforzando l'impegno verso la tutela della biodiversità marina e l'educazione ambientale.

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HITCHIKING AND FREE HOUSING: PRELIMINARY DATA OF TWO EPIBIOTIC CILIATES, *LAGENOPHRYS VAGINICOLA* AND *CYCLODONTA BIPARTITA* (CILIOPHORA, SESSILIDA), LIVING ON A COPEPOD (ARTHROPODA, CANTHOCAMPTIDAE), FROM AN ITALIAN ALPINE LAKE

Members of the Peritricha Stein, 1859 consists of two distinct orders, the motile Mobilida Kahl, 1933 and the larger order Sessilida, Stein 1933 that develops a migratory telotroch stage. Sessilids inhabit a wide range of environments (freshwater, brackish and marine) on both inanimate and animate (as epibionts and in two instances endobiont) substrates and is generally assumed that the vast majority are filter-feeding bacterivores. Ciliates of the family Lagenophryidae Bütschli, 1889 are characterised by a flat body shape, an opereulariform peristome that is completely enclosed by a stalkless lorica with an elaborate aperture operated by a peristomial sphincter (CLAMP, 1999). Lagenophryiids are associated with crustaceans, where they attach to the basobiont integument via a cemented lorica to feed on particles that are caught in the microcurrents generated by the host's movements. Another family associated with crustacean basobionts, is the monotypic *Cyclodonta bipartita* (Stokes, 1885) Matthes, 1958 (Vaginicolidae de Fromentel, 1874), but these can extend their peristome beyond the aperture of their pseudochitonous lorica (MATTHES, 1958). Both *Lagenophrys vaginicola* Stein, 1852 and *C. bipartita* have previously been described from the harpacticoid copepod genus, *Canthocamptus* (also being the type-host genus for *L. vaginicola*) (FERNANDEZ-LEBORANS and TATO-PORTO, 2000). During a recent survey, 20 harpacticoid copepods (preliminarily identified as *Canthocamptus*) were investigated from the alpine lake Lago Nero (Pistoia, Italy). Both the afore mentioned ciliate species were observed on 18 copepod individuals and, interestingly, it was noted that each ciliate group preferred different locations on the host carapace; *L. vaginicola* was always only present on the caudal region while *C. bipartita* inhabited the buccal appendages of the copepod. Using an integrative taxonomy approach (Next Generation Taxonomy) (SERRA *et al.*, 2020), experimental procedures included classical morphology, whole genome amplification of both the host and ciliate epibionts, and phylogenetic analysis using the 18S rDNA marker. The study also includes preliminary data on the infestation rate and prevalence of the epibionts. This study aims to provide novel and much needed information on the taxonomy and the symbiotic relationship of this neglected group of ciliates.

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THE LARVAL GUT MICROBIOTA OF *HERMETIA ILLUCENS* CAN CONTRIBUTE TO THE DEVELOPMENT OF PROMISING STRATEGIES TO SOLVE PLASTIC PANDEMICS

Plastic accumulation in the environment represents a global concern, dramatically impacting ecosystems, as the physicochemical characteristics of plastic polymers make them exceptionally persistent and inaccessible to most natural enzymes. However, bacterial and fungal species isolated from disparate sources, such as marine ecosystems, soil, plastic landfills, and even invertebrate hosts, have been reported as plastic degraders. The interaction between microbial species and invertebrate hosts often results in a profitable functional interplay, as for the biodegradation of plastics by insect-microbiota synergistic action. Among promising insects, *Hermetia illucens* larvae have been shown to effectively degrade two plastic polymers (i.e., polyethylene and polystyrene) thanks to bacterial communities inhabiting their midgut.

Our research aimed to unveil the potential of *H. illucens* larvae and their gut microbiota in the biodegradation of polyethylene terephthalate (PET), commonly used in the manufacturing of products such as water bottles, food packaging, and textiles. Efforts were devoted to i) monitoring the growth and development of *H. illucens* on PET, ii) studying larval physiological responses to oxidative stress and damage triggered by PET exposure, and iii) characterizing shifts in microbial community composition in different larval gut regions caused by PET ingestion.

Our results demonstrate that *H. illucens* larvae can survive up to 60 days on PET-based diets, although with substantial growth and development impairment. We detected a remodelling of the larval fat body and the activation of an effective response to cope with oxidative stress induced by PET ingestion. Moreover, we observed shifts in microbial communities in the larval gut regions after the ingestion of PET. Results herein presented demonstrate that *H. illucens* larvae are able to resist to consistent PET exposure and give first evidence of the possibility of using this insect as a bioincubator for the isolation of PET-degrading microorganisms or enzymes.

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ONTOGENY AND PHYLOGENY ON NOVEL HYPOTRICH CILIATES (PROTISTA, CILIOPHORA) FROM FRESHWATER WETLAND IN NORTH-CHINA

Hypotrichs, one of the most confused ciliate groups, are highly differentiated and morphologically diverse. During a faunistic study of ciliates from freshwater wetlands in China, details of ontogenesis and molecular data of seven novel hypotrich species have been acquired, five of which have been published, i.e., *Chaetospira sinica* Song *et al.*, 2022, *Heterobakuella bergeri* Song *et al.*, 2021, *Heterodeviata sinica* Song *et al.*, 2023, *Heterouroleptus weishanensis* Song *et al.*, 2024, and *Metaurostylopsis alrasheidi* Song *et al.,* 2020. The other two unpublished represent two new genera. Phylogenetic analyses, together with morphological and morphogenetic data from *Chaetospira sinica*, supported the validity of family Chaetospiridae, of which the diagnosis was improved. The new genus *Heterobakuella* Song *et al.*, 2021 was established mainly for the single buccal and parabuccal cirrus, and the absence of caudal and frontoterminal cirri. Both morphological and molecular information indicated that the new genus *Heterodeviata* Song *et al.*, 2023 should be assigned to the monophyletic family Deviatidae. Improved diagnoses for *Metaurostylopsis* Song *et al.*, 2001 and new combination, *Monourostylopsis antarctica* (Jung *et al.*, 2011) Song *et al.*, 2020 was proposed.

Simposio 7
Premio UTET:
Didattica, Divulgazione e Social Media in Zoologia

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LA REALTÀ AUMENTATA NELLA DIDATTICA MUSEALE: UN CASO STUDIO

L'OCSE e il Consiglio Internazionale dei Musei (ICOM) hanno elaborato una guida dedicata al ruolo e alle potenzialità dei Musei, riconoscendoli come istituti di istruzione pubblica e sottolineando la loro importanza nell'integrare l'istruzione scientifica formale. Numerose ricerche hanno dimostrato che i contesti educativi extrascolastici, inclusi i musei, svolgono un ruolo fondamentale nell'istruzione, fornendo agli studenti un'esperienza che si estende oltre le mura della classe (MORTENSEN and SMART, 2007) svolgendo inoltre un ruolo fondamentale nel sensibilizzare il pubblico su complessi problemi socio-scientifici come il cambiamento climatico e la sostenibilità ambientale (NOVAK *et al.*, 2023).

La realtà aumentata (AR) si è dimostrata efficace come strumento per migliorare l'apprendimento in molti campi, compreso l'apprendimento delle lingue straniere (CHEN, 2020). L'idea del nostro progetto di ricerca è nata da una mostra permanente allestita presso il Museo dell'Ambiente (Rimuseum) dell'Università della Calabria. L'allestimento riproduce un vero e proprio supermercato, il "SiMU Market", progettato con le nuove tecnologie di AR. I visitatori possono scegliere tra numerosi prodotti con diverse caratteristiche (provenienza, imballaggio, tipo di produzione) e al termine della spesa la cassa virtuale restituisce un valore espresso in termini di C02 prodotta. Per la sperimentazione il campione è stato rappresentato dagli studenti del III anno del corso di studi in Scienze della Formazione Primaria (n=180) ai quali è stato somministrato un questionario costituito da 13 quesiti a risposta multipla sul tema impronta di Carbonio. Il questionario didattico è stato somministrato in tempi diversi: prima della sperimentazione, dopo una lezione frontale della durata di circa 45 min, dopo l'utilizzo del Sistema di AR (tempo medio 10 min) definendo così tre gruppi sperimentali (G1, G2, G3). L'analisi statistica del numero di risposte corrette ha evidenziato che sia la lezione frontale che l'esperienza di AR hanno prodotto un incremento significativo nel numero di risposte corrette dimostrandosi funzionali all'apprendimento del tema proposto. I nostri risultati inoltre hanno evidenziato che non vi sono differenze significative tra la didattica tradizionale e l'esperienza di AR dimostrando che le due modalità, sebbene di durata notevolmente differente, consentono entrambe il raggiungimento dello stesso obiettivo di apprendimento.

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THE CITIZEN SCIENCE PROJECT “TARDIGRADES GO TO SCHOOL!” ENRICHES TARDIGRADE BIODIVERSITY THROUGH SCHOOLS AND RESEARCHERS’ COLLABORATION

Background: To spread knowledge on biodiversity, to develop the environmental awareness among the younger generations, and to map Italian tardigrade diversity, a Citizen Science project was developed in a school context. Tardigrades are microscopic animals present all over the world, from desert to deep seas, colonizing habitats such as lichens, mosses, and water sediments. Even though tardigrades are little known to most people, they are gaining popularity, even among non-researchers, for their cryptobiotic abilities, appearance, making them a model for science education.

Methods: During a hands-on activity led by experts, the Italian Primary and Secondary school pupils collect mosses in the school garden to look for tardigrades. They extract tardigrades from substrates with everyday materials following a video tutorial (https://youtu.be/5q6-jR_M5h4), observe them at microscope, and draw a tardigrade and the laboratory procedures. After this experience, children collect other mosses during a school-trip, compile a datasheet provided by researchers, look for tardigrades, and send the samples to tardigradologists for diversity assessment. The researchers analyse tardigrades through an integrative taxonomy approach involving morphological (light microscopies) and molecular (*cox1* mitochondrial gene for DNA barcoding) studies. Finally, feedback to the school pupils about the biodiversity highlighted in mosses is given through written reports or meetings, and via the *EvoZooLab* webpage representing the schools–experts interface.

Results: The pilot classes of Comprehensive School Institution “A. Manzoni” (RE) attending the project collected five moss samples during a school-trip at the “Marola” natural park in Apennines of Reggio Emilia. Abundant biodiversity was recorded, including the rare tardigrade species *Notahypsibius pallidoides* and two candidate new species from the genera *Diphascon* and *Isohypsibius*. Experts provided feedback on tardigrades biodiversity and an attendance certificate to the classes during a meeting.

The project is proceeding with engaging other schools from Emilia-Romagna and Tuscany. Indeed, three samples from Reggio Emilia (agritourism “La Collina”) showed interesting biodiversity.

Conclusions: The pupils' contribution enables new scientific knowledge about Italian tardigrade biodiversity. Moreover, this allows involved people to understand the value of biodiversity, making its knowledge and its conservation a commitment of all citizens.

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LA RUBRICA "I MUSEI DELLE MERAVIGLIE" COME STRUMENTO DI VALORIZZAZIONE PER I MUSEI ITALIANI DI STORIA NATURALE E PER PROMUOVERE LE COLLEZIONI ZOOLOGICHE

I musei di storia naturale soffrono (in particolare in Italia) di una carenza diffusa di fondi, di personale, e, soprattutto, di attenzione da parte della politica e di un pubblico potenziale che potrebbe contribuire a mantenere un buon livello di fruizione. Spesso hanno anche difficoltà a interagire tra di loro e a essere identificati come organismi di ricerca. Congiuntamente alle funzioni di studio della geo e della biodiversità, (in questa sede con evidente riguardo alla componente zoologica) alle necessarie indagini tassonomiche e alle finalità di curatela e di raccolta di collezioni scientifiche di riferimento, la divulgazione scientifica è, oggi, una delle funzioni più rilevanti. Nel 2024, la rivista online Piemonte Parchi della Regione Piemonte ha inaugurato la rubrica "I Musei delle Meraviglie", con l'obiettivo di fornire una voce e una vetrina ai musei italiani, da Nord a Sud, dai più grandi ai più piccoli, con l'idea di diminuire la frammentazione che tradizionalmente caratterizza le realtà museologiche in Italia. Gli articoli finora prodotti hanno coinvolto direttamente il personale dei musei, i quali hanno, di volta in volta, messo in luce i reperti carismatici o le attività peculiari della sede. Il numero di musei finora conta soltanto una minima parte; tuttavia, è emersa una eccezionale ricchezza di collezioni e di competenze che ha superato ogni aspettativa, tanto da ideare un progetto editoriale di un numero speciale monografico di Piemonte Parchi, che raccolga tutti i contenuti online della Rubrica, in versione cartacea e distribuito in modalità gratuita. I risultati sono incoraggianti e contribuiscono anche a presentare istituzioni e musei poco noti e a creare le basi per una rete diffusa di musei. L'impatto di questa iniziativa è risultato molto positivo con una media di circa 900 visitatori unici mensili. Alla luce di questa esperienza, si ritiene che l'Unione Zoologica Italiana, al pari di altre società scientifiche, debba prendere atto delle potenzialità della grande risorsa scientifica e culturale rappresentata dai musei naturalistici italiani e contribuire a integrarli come partner indispensabili nella scoperta, divulgazione e salvaguardia del mondo naturale.

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STRENGTHENING SOIL MEIOFAUNA EXPERTISE: THE SoilMATs (Soil Meiofauna Advanced Taxonomy school) PROJECT

Biological taxonomy suffers from a drain of knowledge and manpower, so we tried to face this problem training a new generation of taxonomists on three phyla that can be considered among the "dark taxa" as Nematoda, Rotifera, and Tardigrada. The knowledge of these animals taxonomy is only partly robust (thanks to the new integrative approach), however a lot of work has still to be done, since for these three taxa, that represent the main aquatic fauna component of soil, there is scarce information regarding their diversity, distribution, ecological functions, and temporal dynamics in this habitat. This is due to the paucity of extensive sampling, the lack of a standardized workflow, and above all the scarcity of expert taxonomists.

Under the European Horizon project TETTRIs (Transforming European Taxonomy through Training, Research and Innovations), we were granted for the 3PP project "Soil Meiofauna Advanced Taxonomy school" (SoilMATs**)** to train a new generation of taxonomists expert on Nematoda, Tardigrada, and Rotifera.

The SoilMATs involves 3 teams from Italy (UNIMORE), Germany (Senckenberg Museum), and Czechia (Biologické Centrum) with experts on tardigrades, nematodes, and rotifers, respectively. The school aims to increase the experts in soil meiofauna with two consequent actions: i) training by TETTRIs trainers and SoilMATs experts to "future local trainers"; ii) training by the new "future local trainers" to other researchers (master and PhD students; post-doc) on TETTRIs topics implemented with focus on soil meiofauna in local protected areas. The researchers training is also aimed to strengthen the "future local trainers" training through teaching. Besides, an international network, able to address local requests about soil fauna conservation and foster the trainees' career as taxonomists and their involvement in conservation projects, will be established.

The project spans through all the 2025 and will involve 6 "future local trainers" and 20 European researchers that will be trained online for the theoretical classes and will visit the partners laboratories for the field activities, the workshops, and the hands-on part of the learning program.

The multilocal character of the project foresees an impact on raising the awareness and the understanding of the biodiversity conservation importance among European citizens, thanks to conferences, social media activities, and involvement of the stakeholders as the local institution managing the protected areas.

This project has received funding from the European Union's Horizon Europe Research and Innovation program within the framework of the TETTRIs Project funded under Grant Agreement Nr 101081903.

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CITIZEN SCIENCE AT SCHOOL: AN OPPORTUNITY FOR BIODIVERSITY CONSERVATION AND ACTIVE LEARNING

The topic of biodiversity is very important in a context of education for sustainable development. However, it is a complex concept, often associated with solely animal species diversity or distant and exotic ecosystems. These biases may represent barriers to the correct understanding of biodiversity and its value to humans for teenage students.

This project aimed to explore biodiversity and its conservation using the citizen science platform Inaturalist (www.inaturalist.org/projects/agnelli-bioblitz). Five classes of a secondary high school were involved with the goal of transforming smartphones from pervasive and distracting devices to tools for exploring and monitoring local biodiversity and strengthening attention and observation skills.

Naturalistic data collection, carried out from mid-November 2023 to the end of the school year, led to significant results from an active and innovative learning perspective. A total of 2943 observations and 891 species were recorded. The data, collected by 106 students and some teachers, referred to the entire Italian territory, although they were mainly focused in the Piedmont region.

The high diversity of taxa recorded, belonging to different life kingdoms, enabled students to understand the meaning and the value of local biodiversity. Observations of species with high conservation interest such as the Italian wolf (*Canis lupus italicus*) helped the students perceive the relevance of conserving these species. Furthermore, the investigation allowed us to monitor and study the distribution of some invasive species, especially in urban ecosystems. The data obtained, in line with the purposes of citizen science, have been useful in the context of international ecological research of invasive species.

Considering the promising results in terms of data collected and student participation, this project opened up new perspectives for active learning by also giving a significant contribution to biological conservation.

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L'EVENTO "ACQUARI NATURALI" E IL MUSEO DI STORIA NATURALE DELL'UNIVERSITÀ DI PISA: UN PONTE TRA APPASSIONATI E GRANDE PUBBLICO

Il Museo di Storia Naturale dell'Università di Pisa ospita il più grande acquario di acqua dolce d'Italia, con oltre 100 specie di pesci provenienti da tutto il mondo e una sala dedicata alle specie autoctone, inaugurata nel 2021. La presenza di animali vivi risulta stimolante e coinvolgente per il pubblico e permette di svolgere attività di divulgazione scientifica su diverse tematiche, in particolare legate alla perdita di biodiversità dulciacquicola. Questi ecosistemi sono infatti particolarmente minacciati dal forte impatto dell'uomo sulla natura; secondo il recente aggiornamento della Lista Rossa IUCN, che contiene la prima valutazione completa delle specie di pesci d'acqua dolce del mondo (quasi 15.000 specie valutate), il 25% è a rischio di estinzione (IUCN Red List, 2024).

In questi ultimi anni l'acquario ha investito in nuove esposizioni e attività volte a sensibilizzare i visitatori sulle principali minacce per la biodiversità dulciacquicola, sottolineando il forte legame tra le specie e i loro ambienti e la necessità di un utilizzo sostenibile delle risorse. Oltre alle usuali visite guidate ed attività didattiche, dal 2023 il Museo, in collaborazione con varie associazioni italiane di acquariofilia, organizza un evento specifico di divulgazione chiamato "Acquari Naturali"; questa iniziativa, della durata di due giorni, mira ad avvicinare questo mondo di appassionati al pubblico meno specializzato in visita al Museo, e viceversa. Il coinvolgimento di associazioni non universitarie, radicate sull'intero territorio nazionale, permette di capillarizzare la divulgazione inerente temi quali: l'approccio etico e sostenibile all'acquariofilia, il ruolo degli acquariofili nella conservazione e le problematiche che possono derivare da attività irresponsabili o non consapevoli.

L'evento, a cadenza annuale, comprende varie attività divulgative: l'esposizione di vasche tematiche con pesci provenienti da tutto il mondo, incluse specie pressoché sconosciute al grande pubblico; conferenze, laboratori didattici per bambini sui pesci e i loro adattamenti; visite guidate a tema ed il congresso annuale dell'Associazione Ciclidofili Italiana (AIC). Questo evento di diffusione e divulgazione scientifica, organizzato in collaborazione diretta con gli *stakeholders* delle associazioni di settore, permette di accrescere in un vasto pubblico, specializzato e non, la consapevolezza dell'importanza di tutelare ambienti e specie poco conosciuti e di fare scelte responsabili anche in ambito hobbistico.

Poster a tema libero

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ALL THAT FREEZES IS NOT ICE: TARDIGRADE FAUNA OF VICTORIA LAND, ANTARCTICA

Tardigrades are found worldwide, including the harsh Antarctica, where they are one of the main terrestrial faunal components. These animals possess adaptations to colonize the extreme and stochastic polar environments such as cryobiosis, allowing them to freeze along with their substrate. The Victoria Land area is a hotspot of biodiversity in Continental Antarctica, including two different biogeographic regions. Present study is primarily aimed at investigating the biogeography and species richness of tardigrades in Victoria Land, through the analysis of specimens collected in mosses, lichens, and temporary ponds during the XXVIth, XXIXth, and XXXIVth Italian Antarctic expeditions. Moreover, genera that either show a high specific biodiversity or a wide distribution in the study area are analyzed with an integrative approach, combining morphological and molecular analyses to explore their distribution and speciation. The taxonomical analysis pointed out the presence of 17 tardigrade species (10 genera, 5 families). We focused on the genera *Diphascon* (Hypsibiidae) and *Milnesium* (Milnesiidae). *Diphascon* includes four morphospecies in the area, one of which (found in one sample) is new to science. *Diphascon polare* (found in 7 samples), already known for Victoria Land, is redescribed from type locality adding molecular data (18S, 28S, COI, and ITS2 markers), while the other two species are still under investigation. *Milnesium* specimens identified as *Milnesium* cf. *validum* are found in 19 samples throughout Victoria Land. Two populations, collected about 167 km apart in linear distance, were more in depth investigated with the ITS2 molecular marker, revealing a p-distance of 3.7–4% suggesting the two populations being different species. This is also supported by the finding of males in only one population, while the other is composed by females only, implying a different reproductive mode. Individuals of the two populations were also observed through light, epifluorescence, and scanning electron microscopies, resulting extremely morphologically similar. Our integrative analysis suggests the presence of cryptic biodiversity within the genus *Milnesium* in Victoria Land. Overall, our results point out that the current knowledge on tardigrades from Victoria Land is far from being complete, and that an integrative approach is crucial to avoid the neglection of a hidden biodiversity, which is probably greater than expected.

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DO CALCAREOUS AND QUARTZITIC SUBSTRATES AFFECT BENTHIC COMMUNITY DEVELOPMENT?

Studies comparing carbonate- and quartz-rich marine habitats have highlighted the significant ability of benthic organisms to select specific rocky substrates, especially during settlement. The transition from pelagic to benthic habitats, occurring through the settlement of larvae and propagules, is crucial in the life history of marine organisms. The lithological properties of the substrate significantly influence the diversity and dynamics of hard-bottom benthic communities. Research on this topic remains relatively sparse, although there is evidence that quartz-rich bottoms host diverse benthic communities compared to carbonate ones, even affecting the surrounding fish community.

Our study aimed to investigate the influence of the mineralogical composition of the substrate at multiple levels, from microscopic analysis of bacterial diversity and biomass, to meiobenthic and macrobenthic communities. Moreover, we tested the responses of different stages of the life cycle of the two model organisms *Aurelia coerulea* von Lendenfeld, 1884 (Cnidaria, Scyphozoa) and *Eleutheria dichotoma* Quatrefages, 1842 (Cnidaria, Hydrozoa).

Quartz and carbonate sand, with different grain sizes, was used to set up artificial substrates to test the responses of organisms.

Contrary to previous studies, our results did not show an inverse relationship between meiozoobenthos abundance and the presence of quartz sand. Microscopic analysis of quartz sand revealed surface alterations caused by diatoms, confirming their ability to actively dissolve quartz. The results of experiments with *A. coerulea* indicated significant differences in substrate selection during planulae settlement, which prefer glass over quartz and carbonate. The health status of *A. coerulea* polyps and *E. dichotoma* medusae, on different substrates, was also assessed, showing significant differences only between carbonate and quartz substrates compared to glass.

Our findings highlight the need for further research to fully understand the dynamics of settlement and colonization in relation to substrate properties. Moreover, the two used model organisms were revealed to be suitable for studying the effects of the substrate mineralogical composition.

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SILENT KILLERS AMONG FLOWERS: ANALYSIS OF THE ECOLOGICAL NICHE OF THREE CRAB SPIDERS (ARANEAE, THOMISIDAE)

Spiders (Arachnida, Araneae) are a widespread group of generalist predators, principally feeding on insects. Among them, the crab spiders of the family Thomisidae Sundevall, 1833 are ambush predators, generally living on the ground or on plants, especially on their flowers. Previous studies have largely overlooked tri-trophic networks between spiders, their prey and the plants on which they hunt, especially at large spatial scale (e.g. national). The main aim of this study is to analyze, through Citizen-Science Data, the foraging ecology of three spider species of Thomisidae: *Misumena vatia* (Clerck, 1757), *Synema globosum* (Fabricius, 1775) and *Thomisus onustus* Walckenaer, 1805. Italian records of spiders with prey on plants were collected from the iNaturalist platform. Plants were identified at family level and prey at order level, except for Hymenoptera that were considered at family level. These observations were analyzed separately for three main climatic areas of Italy, defined through three Koppen classes: Mediterranean, Temperate and Alpine climates. The trophic niche of the three spiders was studied with bipartite networks analyses. A total of 627 observations of spiders with prey on plants were analyzed. Of these, 216 belong to *M. vatia* (34%), 222 to *S. globosum* (35%) and 189 to *T. onustus* (30%). *Misumena vatia* seems to be closely associated to the Alpine (55% of records) and secondarily to the Temperate (37%) climates, whereas *S. globosum* and *T. onustus* are primarily associated to the Mediterranean (47% and 36% respectively) and secondarily to the Temperate climate (41% and 34% respectively). Overall, the three species mainly hunt for Hymenoptera (especially bees in the family Apidae) on Asteraceae plants. However, depending on the climatic area, variations in the structure and specialization patterns of the networks were found. This study provides, for the first time, a large geographically-scaled overview on the ecological niche of three Thomisidae species, using simultaneously the two organisms with which they interact, i.e. plants and prey. This type of studies can be useful for assessing the impact of climate changes on trophic chains involving spiders.

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HUMAN AS A SOCIAL BUFFER FOR HORSES

This study examines the potential of human presence to mitigate stress ("social buffer") in horses, a species known for its close historical and working relationship with humans. The aim is to determine whether an unfamiliar human can serve as an effective social buffer for horses experiencing isolation. Horses underwent 15-minute isolation sessions alone (isolation) and with a passive human (social). Blood samples were collected to measure cortisol levels, and heart rate (HR) data were obtained using a Polar H10 sensor. Behaviors were observed and coded. Statistical analyses included Friedman's ANOVA, post hoc Wilcoxon tests, Spearman correlation, and Generalized Linear Models (GLM). Cortisol levels increased similarly in both conditions. The Friedman test showed significant within-group differences ($N=11$, $\chi^2=21.87$; $p<0.001$), but no differences between initial and final values. In the isolation condition, HR was negatively correlated with time ($\rho=-0.775$, $p<0.001$) and positively with stress behaviors ($\rho=0.395$, $p<0.001$). In the social condition, HR was also negatively correlated with time ($\rho=-0.898$, $p<0.001$) and positively with stress behaviors ($\rho=0.301$, $p=0.019$). The GLM analysis in the isolation condition suggested that stress-related behaviors may increase HR ($B=3.928$, $p=0.066$). The negative interaction between stress behaviors and time ($B=-0.004$, $p=0.032$) implies this effect diminishes over time. In the social condition, stranger-directed behaviors significantly decreased HR ($B=-2.886$, $p=0.017$). Conversely, stress-related ($B=5.419$, $p=0.010$) and door-directed behaviors ($B=5.219$, $p<0.001$) increased HR. The positive interaction between time and stranger-directed behaviors ($B=0.005$, $p=0.006$) shows the effect of the stranger on HR increased over time. The negative interaction between door-directed behaviors and time ($B=-0.009$, $p<0.001$) indicates the effect of discomfort on HR decreased over time. Lastly, the interaction between stress behaviors and time ($B=-0.006$, $p=0.092$) suggests their influence on HR may diminish over time. Additional analysis showed distinct HR trends: initially, HR was higher in the social condition but reversed later. In the first 5 minutes, HR was higher in the social condition (77.4±10.7) than in isolation (69.5±7.2, $p<0.001$). In the second 5 minutes, HR was higher in isolation (63.3±3.7) than in the social condition (58.1±2.0, $p<0.001$). This pattern persisted in the last 5 minutes. The presence of an unfamiliar human can provide a social buffer in horses following a short adaptation period.

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ON THE TRAIL OF FORGOTTEN ANNELID SPECIES: MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF *APONUPHIS RIGIDA* (CLAPARÈDE, 1868) AND *APONUPHIS GRUBII* (MARENZELLER, 1886) (EUNICIDA: ONUPHIDAE) IN THE MEDITERRANEAN SEA

Historically several polychaete taxa were put into synonymy with a few cosmopolitan species, factually denying their high diversity as a group, and leading to forgetting some species. One of these cases is represented by the genus *Aponuphis*, which has been surrounded by taxonomic uncertainties since the beginning of the XX Century. This genus includes several cryptic lineages in the Mediterranean Sea and eastern Atlantic Ocean only partially diagnosable based on live colour pattern. Moreover, two species considered close to *Aponuphis bilineata*, namely *A. rigida* and *A. grubii*, are considered valid, but they have never been redescribed. This study provides morphological and molecular characterization (based on 16S sequences) of individuals of *Aponuphis bilineata s.l.* sampled from several Mediterranean localities. We identified two mitochondrial lineages within *A. bilineata* occurring in the Mediterranean Sea, with a partially overlapping distribution: lineage A, including material from the north-eastern Atlantic and Corsica, and lineage B, including individuals from Elba, Salento, Cyprus and Morocco. Moreover, the genetic analysis confirmed that *Aponuphis grubii* sampled off Tuscany, which morphologically corresponds rather well to the original description, despite showing a distinct colour pattern is close to the *A. bilineata* species complex. The sequences obtained for *Aponuphis rigida*, sampled close to the type locality, showed correspondence to a Mediterranean sequence erroneously assigned to *A. bilineata*, which was rather distant from all *Aponuphis* clades currently known. Aside from confirming the validity of both *A. rigida* and *A. grubii*, this study highlighted that the colour pattern is possibly the most useful character allowing to discriminate between these species and the “true” *A.bilineata* , typically characterised by two longitudinal dark stripes along the body.

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MOLLUSCS ASSOCIATED WITH TWO MEDITERRANEAN SEAGRASSES: *POSIDONIA OCEANICA* (L.) DELILE *VS. CYMODOCEA NODOSA* (UCRIA) ASCH

Understanding the taxonomic composition of the biotic component of an ecosystem, referred to as "biocenosis", is crucial for assessing health and state of that ecosystem. In the Mediterranean Sea, the system developed by the French ecologists Pérès and Picard in 1964[1] identifies around 100 benthic biocenoses, classified based on bathymetry, sediment type, and plant and animal communities. The most common and widespread photophilic biocenosis in the Mediterranean Sea is represented by the seagrass *Posidonia oceanica* L. (Delile), indicated as HP, followed by *Cymodocea nodosa* (Ucria) Asch, indicated as Cym.

During the summer season of 2023, HP was sampled at Dino Island (Tyrrhenian Sea) and Cym was sampled at Amendolara (Ionian Sea) using a 40x40cm quadrat (6 replicates) and an air pump with a 0.5 mm mesh net. The aim was to investigate the possible similarities and/or differences in mollusc population structure occurring in the seagrasses canopies and their soft bottoms.

A total of 1579 individuals from 54 *taxa* were identified. Of these *taxa,* only 12 were shared between the two seagrasses, primarily represented by the genera *Bittium* and *Rissoa,* which accounted for 50% of all sampled molluscs. HP was primarily characterized by species closely associated with the canopy and rhizomes, including the macrograzers *Tricolia speciosa* (Megerle von Mühlfeld, 1824) and *Alvania* spp., as well as the byssate bivalves *Glans trapezia* (Linnaeus, 1767) and *Musculus costulatus* (Risso, 1826). In contrast, Cym was characterized by species typical of well-calibrated fine sands (SFBC) biocenosis, such as "*exclusive characteristic species*" *Fabulina fabula* (Gmelin, 1791), and by "*accompanying species*", like *Tritia varicosa* (W. Turton, 1825). Additionally, Cym was characterized by species associated with unstable mobile soft bottom (MI) biocenosis, like *Moerella distorta* (Poli, 1791) and *Fustiaria rubescens* (Deshayes, 1826).

These results describe the pattern of mollusc distribution in two relevant Mediterranean seagrasses, highlighting that differences in population structure are clear when the individuals were analysed at the species level. This study underscores the importance of taxonomic analyses at the lowest possible level to identify the key species structuring different biocenoses.

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ANATOMICAL INSIGHTS AND BEHAVIORAL STRATEGIES OF PREDATION IN THE POLYCHAETE *HALLA PARTHENOPEIA*

Introduction: Polychaetes show a wide array of feeding mode, ranging from suspension feeding to microphagous carnivory. Predatory polychaetes have evolved various methods to capture and consume their prey, including ambush predation, active hunting, and burrowing techniques, and some of them, rely also on peculiar anatomical structures and toxin secretion to attack their prey. The tube-dwelling polychaete *Halla parthenopeia* is known as a voracious predator of bivalves. During predation, *H. parthenopeia* emerges from its tube, searching for prey and enveloping it in abundant mucus emitted from the mouth through alternating contractions and relaxations. This feeding mucus induces the opening of the clam valves, allowing the worm to enter and consume the predigested meat. However, detailed information on the anatomical structures responsible for feeding mucus production and their correlation with the worm predatory behavior remains lacking.

Methods: Histological analyses were conducted using paraffin and resin sections stained with different techniques, (Hematoxylin-Eosin, Tetrachromic, Coomassie Blue), to examine the feeding apparatus and identify the structures responsible for mucus production. To characterize the predation activity phases of *H. parthenopeia*, video monitoring of worms maintained in captivity was employed.

Results: Histological analyses revealed an eversible muscular pharynx equipped with paired maxillae and mandibles and a unique longitudinal mucus gland responsible for producing feeding mucus. Serous cells in the mouth, reactive to Coomassie Blue, suggest the secretion of proteinaceous materials such as enzymes or toxins active on the prey. Connected with the mouth there is a spiraliform oesophagus with thick cuticle and abundant mucocytes for the protection and bolus transport facilitation.

Conclusions: Observations combining predatory behavior and anatomical details allowed for the description of a mechanism of prey consumption and assimilation in *H. parthenopeia*. Pharyngeal contractions squeeze the mucus gland, producing mucus that, combined with serous cell secretions, is deposited along the bivalve valves to paralyze and predigest the clam. The digested meat moves into the esophagus and towards the intestine, facilitated by the copious secretion of mucus. This study fills a gap in understanding the relationship between mucus secretions, their role, and the anatomical structures responsible for their production in *H. parthenopeia*.

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CHARACTERISATION OF ANTENNAL SENSILLA IN THREE SPECIES OF SOLITARY BEES (HYMENOPTERA, APOIDEA)

Solitary bees perform a pivotal role in ecosystem function, facilitating the pollination of both cultivated and wild plant species. From an ecological perspective, reproductive females require habitats that provide suitable nesting sites and food resources, as well as materials for nest construction. Despite the crucial role that sensory systems play in perceiving biotic and abiotic stimuli during foraging and nesting activities, the sensory pattern of the antennae of solitary bees remains poorly studied. The aim of this study is to describe, using scanning electron microscopy, the morphology and distribution of the sensilla present on the antennae of females belonging to three species of distinct families. We analysed two species that utilise pre-existing cavities as nesting sites, *Ceratina cucurbitina* (Rossi, 1792) (Apidae) and *Osmia scutellaris* (Morawitz, 1868) (Megachilidae), and one that nests in the ground, *Lasioglossum leucozonium* (Schrank, 1781) (Halictidae). A total of seven distinct morphological sensillum types and 13 subtypes have been identified and classified as follows: trichoid (subtypes STI, II, III), chaetic (subtypes SchI, II), basiconic (subtypes SBI, II, III, IV), placoid, campaniform, coeloconic and ampullaceous sensilla. The distribution and abundance of the various types of sensilla have been compared between the three species, and their functional role has been discussed in detail. The sensory pattern of chemoreceptors, photoreceptors and mechanoreceptors that emerges from this study indicates that the diversity and distribution of antennal sensilla between the species studied may be correlated with differences in their behavioural ecology. The findings offer valuable insights that can be used to enhance our understanding of the ecological and physiological attributes of wild bees, which are subjected to a range of threats for the development of effective strategies for the conservation and protection of pollinators in the context of environmental change.

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THE EPIGEAL ARTHROPODOFAUNA OF THE PRESIDENTIAL ESTATE OF CASTELPORZIANO: REPLICATING A HISTORICAL EXPERIMENT

The presented one-year study compared the present epigeal arthropodofauna of the Castelporziano Presidential Estate (Central Italy, retrodunal Thyrrenian Mediterranean ecosystems) with data collected in a sampling campaign conducted about 37 years ago (Bonavita 1987). Epigeal macroarthropods were collected using pitfall traps filled with a saturated solution of vinegar and salt as killing preservative. Thirty-three traps were positioned in the six sites of the original experiment, along the sea-to-land topographical gradient. Within each site, six pitfall traps were arranged along a straight transect parallel to the coastline, 8 m apart (Bonavita 1987). Due to the increased presence of ungulate fauna, steel-wire cages were used to protect the traps. The sampling campaign so far conducted (September 2023 - September 2024) obtained the taxa Collembola, Brachycera, Acariformes, Orthoptera, Araneae, Coleoptera, Hymenoptera, Diplopoda, Nematocera, Oniscidea, Lepidoptera, Chilopoda, Opilionida, Pseudoscorpiones, Psocoptera, and Hemiptera. The quantitative composition of our collection is described. The original collection by Bonavita and Vigna-Taglianti (1986-1987), including 25 jars of different invertebrate taxa, has been recovered at the Museo Entomologico di Roma, and a first taxonomic description is here provided. The activation times of the pitfall traps and numbers of individuals were recorded, computing density-activity measurements to conduct comparisons. Replicating the experiment of Bonavita (1987) provided insights on the structural changes occurred in the focal taxa, evaluated with density-activity measurements, within the study area.

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PRELIMINARY STUDIES FOR THE IDENTIFICATION OF SUITABLE MARKING METHODS TO IMPLEMENT THE EFFICACY OF THE PREDATORY HOVERFLY *SPHAEROPHORIA RUEPPELLII* (DIPTERA SYRPHIDAE)

Hoverflies provide crucial ecosystem services in particular pollination and biological control. Some species, such as *Sphaerophoria rueppellii* (Wiedemann, 1820), are specially reared in biofactories for biocontrol programs against aphid crop pests. The use of specific markers on adults could be functional in mark–release–recapture trials to detect hoverfly dispersion from release points, in order to implement biocontrol programs. Using the predatory hoverfly *S. rueppellii* as a model species, this study aimed to identify the most suitable marker among those already used in other insects in terms of persistence, visibility and absence of negative effects on vital parameters. Three different marking techniques were assessed: the trace element rubidium (RbCl) and fluorescein, administered via the food supplies, and fluorescent dusts, applied topically.

Three laboratory trials were conducted to assess potential interference with fertility, mating behaviour, and persistence over time in marked females for each treatment. Based on the results, fluorescent powders were selected for a field marking trial, as they showed sufficient persistence and were easily recognizable. Fluorescent powders labelled *S. rueppellii* were released in watermelon tunnel farms in the province of Reggio Emilia. In each farm, hoverflies were released in one greenhouse while another greenhouse served as a control. Visual transects and chromotropic traps were used to assess the dispersal of the marked insects. Unfortunately, no *S. rueppellii*, either marked or unmarked, were recaptured, probably due to the high summer temperatures.

In conclusion, fluorescent powders proved to be suitable candidates for long-term dispersion studies and will be used in further trials to evaluate the effects of hoverfly release on aphid populations. Fluorescent powders are inexpensive and tagged insects are easily detected using a portable UV lamp. In contrast, the use of rubidium is expensive, very laborious to detect, requires specialised equipment and the sample must be killed for analysis. Fluorescein has a very short persistence, making it unsuitable for long-term dispersal studies.

This work was partially supported by the “FAR2023PD” funding, project “MARK-HOVER” and by NRRP, Mission 4 Component 2 Investment 1.4 funded by NextGenerationEU; Project code CN_00000033, Project title NBFC

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A MULTI-SPECIES APPROACH TO RESTORE MARINE BARREN GROUNDS

Protection and conservation measures are necessary to address coastal habitats' degradation caused by human activities, but in many cases active restoration is required to restore ecosystem services and halt biodiversity loss. The coastal area of the Salento Peninsula was affected by the illegal fishing of the marine date mussel *Lithophaga lithophaga* (Linnaeus, 1758), causing the damage of large portions of the shallow rocky substrate with the creation of extensive barren grounds lately maintained by the overgrazing of sea urchins. To date, the multi-species approach to restoration has been under-explored in the marine environment, especially in the Mediterranean Sea, but it is widely recognized that the co-transplantation of species that are usually associated in nature can facilitate and enhance their mutual chances of surviving and thriving. With the aim of restoring barren grounds in the fully protected zone of Porto Cesareo MPA we used an innovative multi-species restoration approach. The restoration was performed using two habitat formers, the marine phanerogams *Posidonia oceanica* and the madreporarian *Cladocora caespitosa*, respectively associated to two sponges, namely *Chondrilla nucula* and *Aplysina aerophoba*. We hypothesise that the species may facilitate each other, thereby improving the outcome of restoration when being transplanted together. The experiment involves the coral *C. caespitosa* and the sponge *A. aerophoba*: the two species were transplanted close to each other in different combinations in 3 sites: coral + sponge (Treatment, N=15), two sponge fragments (Control, N=15), and two coral fragments (Control, N=15). The same experimental setup was employed for *P. oceanica* and *C. nucula*: plant rhizome + sponge, two rhizomes, and two sponge fragments. Initial monitoring carried out to assess the success of the restoration activities showed that the sponges effectively attached to the substrate (89%), and at the same time 89% of the rhizomes of *P. oceanica* and 96% of the colonies of *C. caespitosa* were in good health status.

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SeaUrch-INK: A NOVEL 3D-PRINTABLE BIOINK BASED ON SEA URCHIN COLLAGEN FOR TISSUE ENGINEERING APPLICATIONS

Sea urchins exhibit unique anatomical and physiological adaptations, such as the Mutable Collagenous Tissues (MCT) *i.e* collagen-based tissues with distinctive and dynamic mechanical properties. These features hold significant potential for interdisciplinary applications in the biomedical field. Indeed, we previously demonstrated that MCT collagen can be easily extracted from sea urchin waste through eco-friendly and non-destructive methods, preserving its truly native fibrillar nature and thus its mechanical properties. This makes sea urchin collagen an optimal candidate for the production of highly biomimetic collagen-based biomaterials for tissue engineering. The aim of this work was to develop the first sea urchin-derived bio-ink (SeaUrch-ink) for use in 3D-bioprinting techniques to create customized 3D biomaterials (fiber-reinforced hydrogels) that emulate the role and functions of the extracellular matrix. Collagen fibrils (C) were mixed with alginate (A), an algae-derived polysaccharide, in various percentages/ratios to determine the optimal printability parameters (*e.g.* pressure and speed) to obtain final hydrogels; these latter were subsequently cross-linked with $CaCl_2$ to improve stability. The best A:C ratio and water percentages were selected to produce the final biomaterials, which remained perfectly intact and stable for up to one week. To verify the suitability of the produced sea urchin-derived hydrogels for tissue regeneration purposes, 3D-printed biomaterials were evaluated in terms of ultrastructure (SEM), degradation kinetics (under physiological and enzymatic conditions), and water uptake/swelling. SEM analysis showed that the collagen fibres were geometrically aligned in a parallel arrangement, a key feature that may facilitate cell orientation in tissue regeneration and enhance the mechanical properties of the biomaterial.

In conclusion, this preliminary study demonstrates the potential to effectively use sea urchin collagen combined with algal polysaccharide for the production of innovative eco-friendly bioinks for 3D-printing applications, perfectly matching the blue growth and bioeconomy directives. Future perspectives include further *in vitro* and *in vivo* characterization tests to confirm the suitability and efficacy of these biomaterials for biomedical applications. The long term challenge will be to incorporate also aspects of mechanical dynamicity, thus producing truly smart biomaterials and mimicking the unique MCT properties.

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16S rRNA TARGETED METAGENOMIC SEQUENCING TO ASSESS SEAFOOD TRACEABILITY AND FRESHNESS

In a context of growing world population, seafood consumption is increasing over the years, and is expected to continue rising. Is therefore unavoidable to adopt sustainable solutions in order to avoid depletion of natural resources and meet consumer's requests regarding seafood safety and geographical origin assurance. Microbial communities associated to seafood are gaining interest as a tool to assess seafood tracking and safety. In this work, we propose targeted metagenomic approach to improve information depth obtainable by fish microbiota. We assessed gill microbiota of the two important fish commercial species *Dicentrarchus labrax* and *Sparus aurata* (Linnaeus, 1758), caught at different sampling sites of the Tuscan coast, trough targeted metagenomic sequencing on bacterial biomarker rRNA 16S (V3-V4 regions), as well as specimens of *S. aurata* from different European retailers (Malta, Scotland, Portugal). In addition, we simulated the retailer-consumer chain storing conditions, sampling the same specimens every day for three days, to assess the presence of SSOs (Specific Spoilage Organisms) and the overall microbial community adaptation in a short-term span. Finally, to make the method simple and reproducible, we tried sampling both trough gill biopsy and swabbing. Microbial diversity analysis showed clear distinction according both to species and sampling sites, with site-specific variation in bacterial ASVs, suggesting the possibility to discriminate the geographic origin of fish trough gills microbiota both at a local and at wider geographical scale. Spoilage experiment results revealed a quick adaptation of microbiota, with a rapid increase in relative abundance of known SSOs such as *Photobacterium* ssp. Finally, the two different sampling methods provided overlapping results, suggesting good effectiveness of gills swabbing (an easy, quick, and non-appearance-altering method). In conclusion, this work shows the great potential of metagenomic sequencing in seafood safety and traceability, with simple sampling methods that could be performed directly by the first producer.

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REVEALING FEEDING BEHAVIOUR OF *DENDROPHYLLIA RAMEA* (LINNAEUS, 1758) AND *PARAMURICEA CLAVATA* (RISSO, 1827) THROUGH MULTIPLE DAYS TIME-LAPSE IMAGE ACQUISITION

The use of photography to study marine organisms started in the 19th century and has since gained increasing popularity. The deployment of a fixed device for image acquisition is particularly suitable for ethological studies and to assess the use of a specific environment by associated faunal species. This system allows to overcome the limits of depth and time of SCUBA diving, further removing the disturbance due to the presence of divers or moving vehicles. We used a time-lapse approach over multiple days to investigate the feeding behaviour of two coral species, *Dendrophyllia ramea* (Hexacorallia, Scleractinia) and *Paramuricea clavata* (Octocorallia, Malacalcyonacea). The time-lapse device “Frankie®” was equipped with a camera set to take pictures at 3-minute-intervals. *D. ramea* was recorded for 5 days (2472 photos) at 70 meters depth, while *P. clavata* record lasted 2 days (999 photos) at 45 meters depth, in the Southern and Northern Tyrrhenian Sea, respectively. For both species, a single colony was framed to focus on the behavioural patterns of polyps. Preliminary results indicated the absence of diel periodicity of *D. ramea*, that never showed the retraction of all the polyps of the colony. However, a slight difference was noticed when considering the colony fully open during nighttime (88.2 ± 3.9% SE) compared to daytime (56.9 ± 18.5% SE), although not significant (t-test, p=0.1586). *P. clavata* did not show diel preferences, being open for 76% of the recorded time regardless of night or day. A relationship with high turbidity (estimated with a scale of low–medium–high considering the suspended particles in the frame) was reported in 56% of the photos showing open polyp condition. An important observation was related to associated crustaceans, that used to walk over the colony during night, determining the retraction of polyps due to physical contact and possibly influencing their feeding activity. These observations allowed us to extrapolate several aspects of coral ecology, that are to date underestimated. The evaluation and assessment of polyps feeding behaviour provides specific insights on the trophism of benthic cnidarians, possibly related to hydrodynamic conditions at small (e.g., micro-currents and micro-upwelling) and large scales (e.g., whether and tides fluctuations). The interactions with associated organisms are poorly studied at mesophotic depths and reveal a fundamental relevance since they can further affect activity patterns of corals.

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SEA URCHIN WASTE VALORIZATION: COMPARISON OF ANTIOXIDANTS AND COLLAGEN EXTRACTION FROM *P. LIVIDUS* AND *S. GRANULARIS*

Sea urchins possess unique and well-known mutable connective tissue (MCT) and antioxidant polyhydroxynaphthoquinones (PHNQs). These anatomical/physiological adaptations make sea urchins promising candidates for use in biomedical applications. Adopting a circular economy approach, the food waste deriving from processing of the main edible Mediterranean sea urchin species (*Paracentrotus lividus*) can be fully converted into high value-added products: fibrillar collagen can be extracted from the MCT, while antioxidants can be extracted from the rest of the waste (mainly test and spines). Both PHNQs and collagen can be used to produce collagen-based biomaterials useful for biomedical applications. Considering the wide biodiversity of edible sea urchins, we aim to investigate whether different species that make up the global waste are potentially suitable as sources of antioxidants and collagen, as well as for biomaterial production. Therefore, a comparative study was carried out between *P. lividus* and another edible Mediterranean species: *Sphaerechinus granularis*.

We compared different extraction yields of PHNQs and their relative abundances (by UPLC-ESI-MS) in the two species. The antioxidant activities were then evaluated (by ABTS assay) and correlated with the presence of PHNQs. Collagen was extracted from both species, its yield was determined and scaffolds were prepared and compared in terms of ultrastructural features and porosity (by SEM and squeezing method), degradation kinetics (in physiological or collagenase solution) and mechanical resistance to compressive stress.

The results showed a significantly higher yield of PHNQs in *P. lividus*, whereas *S. granularis* extracts showed better antioxidant activity. The extracted PHNQs were partially species specific and their antioxidant activity apparently depended on the relative ratio of specific spinochromes (e.g. spinocrome E).

A higher yield of collagen extraction was obtained in *P. lividus*. Collagen-based biomaterials from *S. granularis* showed minor differences in ultrastructural organisation compared to those from *P. lividus*, and they were more stable when hydrated, more resistant to degradation and to compression. In conclusion, waste from different species can be successfully valorised for PHNQs and collagen extraction. The species-specific properties of PHNQs and collagen-based biomaterials could make them suitable for different biomedical applications, depending on the specific technical requirements.

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EFFECTS OF DIETARY CHITOSAN ON HEPATOPANCREAS ULTRASTRUCTURE IN *TALITRUS SALTATOR* (MONTAGU, 1808)

In recent years, bioplastic development and production increased remarkably to replace petroleum-based plastic. Among bioplastics, chitosan, used in combination with other polymers like starch, is gaining particular interest. Due to natural and anthropic factors, bioplastics, like conventional plastics, can accumulate in marine littoral environments and ingested by supralittoral arthropods, e.g. *Talitrus saltator*. To assess effects of ingestion of chitosan-starch mixtures on this species, hepatopancreatic (HP) caeca have been investigated, which represent the main digesting and processing organs in amphipods. Accordingly, we carried out experiments under controlled laboratory conditions (photoperiod, temperature), feeding adult individuals exclusively with chitosan-starch mixtures (chitosan-50%, chitosan-25%, starch-100%) or paper and dry-fish food as control. As additional check, specimens in a further group were fasted for three days. After this timelapse, HP caeca were removed from specimens of all groups to be processed for morphological analyses using light and transmission electron microscopies. Additional groups of specimens were kept separately to assess survival rates in two weeks related to the different feedings. According to structural and ultrastructural evidence, caecal epithelia of control individuals exhibited the usual cytological variety of HP tubules, with obvious B-cells (involved in the storage phase and characterized by the presence of a secondary lysosome of gigantic size). A similar pattern was found in starch-100% specimens. Chitosan-50% group showed structureless bulks in the caecal lumen (possibly undigested material) and lack of B-cell differentiation, along with remarkable patterns of autophagy. Chitosan-25% group showed features consistent with enhanced degradation processes. Fasting specimens exhibited patterns of slight maturation of B-cells along with autophagy. Notably, chitosan-50% group hosted bacterial cells with some characteristic debris in caecal lumen, undetected in treated and control specimens. These results align with mortality data. After 14 days, survival rate of specimens fed with chitosan starch mixtures drastically dropped to zero, while it was around 90% for control and starch-100% groups. Overall, present findings suggest that chitosan-starch mixtures may be particularly dangerous for supralittoral amphipods, urging the need to avoid releasing both bioplastic and conventional plastic in the environment.

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OSSERVAZIONE IN SITU DI PREDAZIONE SU *FUNICULINA QUADRANGULARIS* DA PARTE DI *PSEUDOSIMNIA ADRIATICA*

La famiglia Ovulidae Fleming, 1822, molluschi gasteropodi ampiamente distribuiti in tutto il mondo, è caratterizzata da specie che si nutrono di cnidari, principalmente antozoi, tra cui ottocoralli appartenenti agli ordini Malacalcyonacea e Scleralcyonacea, ed esacoralli degli ordini Antipatharia e Scleractinia, ma anche di idrozoi dell'ordine Anthoathecata (Nocella *et al.*, 2023). In questo contesto, riportiamo l'osservazione in situ della predazione da parte del gasteropode *Pseudosimnia adriatica* (G. B. Sowerby I, 1828) su esemplari del pennatulaceo *Funiculina quadrangularis* (Pallas, 1766). Questo comportamento è stato osservato nell'ambito di uno studio di caratterizzazione di un fondale a pennatulacei al largo delle Isole Tremiti, su un substrato caratterizzato da un sedimento fangoso tra i 120 e i 140 m di profondità. Mediante l'uso di un Remotely Operated Vehicle (ROV) sono stati osservati due esemplari di *P. adriatica* nell'atto di predare i polipi di *F. quadrangularis*. L'identificazione specifica è avvenuta osservando i principali caratteri tassonomici osservabili attraverso l'analisi dei video, sia della conchiglia sia delle parti molli esposte. Le colonie di *F. quadrangularis* risultavano evidentemente danneggiate dalla predazione, con assenza di polipi e cenosarco nella zona in cui era presente il gasteropode e conseguente esposizione dell'asse del pennatulaceo. Questa osservazione conferma il ruolo di *P. adriatica* come predatore dei polipi di *F. quadrangularis*, come rinvenuto di recente (Nappo, 2024) nell'area adriatica di Jabuka, Croazia.

I dati di associazione dei gasteropodi corallivori con le specie di cnidari oggetto della loro alimentazione sono estremamente lacunosi per le specie che vivono a profondità non facilmente accessibili all'osservazione diretta in immersione subacquea. Tali dati sono tuttavia cruciali per la comprensione sia dell'evoluzione della corallivoria sia dei meccanismi che regolano tali interazioni negli ecosistemi.

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INFLUENCE OF BASIBIONTS, AND THEIR COMPLEXITY, ON ASSOCIATED ALIEN AMPHIPODS IN FOULING COMMUNITIES OF MEDITERRANEAN PORT ENVIRONMENTS

Factors that promote the introduction and establishment of non-indigenous species (NIS) within port environments are not completely understood. We investigated the structure of fouling community in three Northern Tyrrhenian port systems (W Mediterranean), focusing on the relationships between basibionts and the associated macrofauna. For each port system, fouling samples from two harbour types (large port and recreational marina) were collected and, within each large port, we considered two use destinations (commercial and touristic). Highly positive relationships were detected between macrofaunal basibionts and associated organisms, especially considering the alien amphipods *Caprella scaura* Templeton, 1836, *Jassa marmorata* Holmes, 1905 and *Jassa slatteryi* Conlan, 1990. Possibly due to their complex three-dimensional morphology, erected bryozoans showed marked association with the majority of vagile taxa, especially alien amphipods. This association could be explained by the arborescent structure of erected bryozoans, that provides a substrate suitable for caprellid and ischyrocerid amphipods. Caprellids colonize these basibionts using their pereiopods, which have a complementary shape to the branches of bryozoan colonies. On the other hand, ischyrocerids use bryozoan branches to anchor their semi-permanent tube made of silk and organic debris. These results suggest that high levels of spatial complexity of fouling basibionts promote the invasion success within port environments. Then, the influence of substrate morphology on early stages of fouling development was assessed submerging experimental substrates with different morphological complexity. The experiment was carried out within commercial and touristic harbours of the port of Livorno (Italy), analysing the communities at three steps of colonization. We assessed the effect of substrate complexity on recruitment of NIS, combined with the influence of port use destinations. Substrate morphological complexity significantly affected fouling colonization and particularly NIS assemblages. We found that high-complexity substrates are particularly suitable for alien amphipods establishment in comparison with less complex ones. The touristic harbour exhibited a potential for fouling colonization higher than the commercial harbour. These results contributed to the understanding of factors involved in NIS establishment and spread, as well as in their spatial-temporal dynamics within port environments.

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PREVALENCE OF GASTROINTESTINAL PARASITES IN BROWN HARE (*LEPUS EUROPAEUS*) AND ITALIAN HARE (*LEPUS CORSICANUS*)

Understanding the impact of parasitic infections on the Brown hare (*Lepus europaeus*) and Italian hare (*Lepus corsicanus*) is crucial for wildlife management and conservation of these species. Previous studies conducted in 2018 and 2022 laid the groundwork by identifying the primary endoparasites affecting hare populations in Tuscany and Sicily. Building on these findings, this follow-up study aims to further characterize the parasitic load and evaluate potential changes in the composition of the helminthes fauna. We analyzed gastrointestinal tracts of 25 Italian hares and 20 brown hares using the sedimentation and counting technique (SCT) to identify and quantify adult parasites. In brown hares, *Trichostrongylus retortaeformis* had a prevalence of 75%, and in one hare, the cestode *Andrya* spp. was found. In Italian hares *Trichostrongylus retortaeformis* was the only species found with a prevalence of 66%, but unlike the previous study, *Andrya* spp. was not detected. Our findings contribute to a research field that is more effective when conducted over a long period of time. Given the influence of increasing factors such as climate change on the distribution of parasites and their interactions with host species, ongoing research is crucial to understand the role of parasitic infections on the hare populations dynamics. Additionally, anthropic activities further complicate the dynamics of these infections, necessitating comprehensive studies to develop strategies that mitigate their impact on hare health and populations. Thus, continued research is essential for understanding the evolving dynamics of parasitic infections in hares and for devising proactive management and conservation measures.