

Sessione
Tema libero

Poster

How microhabitat characteristics influence the females' nest site selection of the invasive alien species *Trachemys scripta* in the Angitola Lake (Calabria, Southern Italy)

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Invasive alien species (IAS) represent a major threat to global biodiversity, ecosystem functionality, and native species, with particularly pronounced impacts in protected areas. The red-eared slider turtle (*Trachemys scripta elegans*) is recognized as one of the 100 most damaging IAS worldwide due to its adaptability and widespread distribution. This research focused on the nesting ecology of *T. scripta* within Angitola Lake, a significant Ramsar site and WWF Oasis located in Calabria, Southern Italy. The primary objectives were to gain a deeper understanding of its reproductive strategies in this environment and to provide crucial information to guide the development of effective management interventions.

The study was carried out during the active nesting season of 2007, spanning from April to October. Through field surveys, three primary reproductive zones and three secondary sites along the lake's shoreline were identified. A total of 309 natural predated nests were documented across these locations. At each recorded nest, four measurements of the nest microhabitat were taken: the distance to the shoreline, nest depth, vegetative cover, and soil characteristics. Univariate analysis and a Generalized Linear mixed model (GLMM) were employed to investigate the nest site preferences of the females.

The findings revealed a clear preference for mixed habitats characterized by soil combinations such as dirt/sand and dirt/clay, suggesting these conditions offer optimal characteristics for oviposition. Nesting activity was predominantly concentrated within the first 30 meters from the shoreline. Notably, habitat and soil selection appeared to shift throughout the nesting season, potentially in response to changing environmental factors, including water availability. However, the results of the generalized linear mixed model (GLMM) indicated that only the distance from the shoreline was significantly influenced by vegetation type (with greater distances observed in reforested sites). The ecological plasticity exhibited by *T. scripta*, enabling it to exploit a broad range of nesting microhabitats, represents a significant ecological threat to local biodiversity. This study provides detailed insights into the reproductive ecology of this invasive species, underscoring the need for the implementation of integrated management strategies aimed at mitigating its ecological impacts.

Insights into the solitary ascidian *Ciona robusta*: a zoological model to study innate immunity

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Ascidians (Tunicata) are important models for studying the innate immune system due to their evolutionary proximity to vertebrates. As sessile filter feeders, they are exposed to environmental stressors, including pathogens, requiring a strong immune response. *Ciona robusta*, a solitary ascidian found in temperate marine environments, is a widely used model in zoological research, known for its well-characterized immune system, transparent body, and simple anatomy. This species has distinctive morphological features, such as a thick outer tunic for water filtration and a prominent pharyngeal basket, where immune cells play a key role in pathogen defense.

This study investigates the immune responses of *C. robusta* under bacterial challenge, specifically focusing on the effects of lipopolysaccharide (LPS) exposure. Using immunohistochemistry and enzyme assays, we observed activation of key immune pathways, including Toll-like receptor (TLR) and nuclear factor kappa B (NF- κ B) signaling, central to the inflammatory response in both invertebrates and vertebrates. These immune molecules were significantly upregulated in the pharyngeal tissues 4 hours post-LPS injection. Inflammatory nodules were found in the lumen of pharyngeal vessels, with endothelial cells actively participating in the inflammatory process. Histological analysis of untreated and buffer-injected ascidians confirmed the positive immune reactions. Enzymatic assays revealed a marked increase in the activity of immune-related enzymes, such as phenoloxidase, glutathione peroxidase, lysozyme, alkaline phosphatase, and esterase, following LPS exposure. These enzymes, involved in pathogen recognition, oxidative stress response, and antimicrobial activity, play critical roles in mediating the inflammatory response.

Our findings underscore a complex, multi-faceted innate immune activation in *C. robusta*, resembling vertebrate immune processes, and provide valuable insights into the molecular and cellular dynamics of pathogen defense. These results suggest that ascidians may serve as an excellent model organisms, offering opportunities to explore universal principles of immune regulation from tunicates to vertebrates. Future research should focus on bridging knowledge gaps and further elucidating the evolutionary conservation of immune mechanisms across species.

Loss of meiofaunal biodiversity under thermal stress regime: the vulnerable seagrass-bivalves association

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Biological positive interactions are key drivers in shaping the biosphere, yet climate change can alter these relationships with consequences for biodiversity. In the marine realm, a notable example is the mutualism between lucinid bivalves and seagrasses, both acting as ecosystem engineers. Despite their ecological relevance, the response of such interaction and associated benthic biodiversity to extreme climatic events remains poorly understood. In this study we investigated how meiofaunal biodiversity and trophic status change in the presence or absence of the association between the seagrass *Cymodocea nodosa* and the lucinid *Loripes orbiculatus* under simulated marine heatwaves (MHWs). A 17-day mesocosm experiment was conducted in laboratory, including four conditions: bare sediment, sediment with *C. nodosa*, with *L. orbiculatus*, and with the association. A MHW was simulated by gradually increasing water temperature to +5°C above in-situ one, then maintaining it for 10 days, while control mesocosms remained at 25°C. The highest meiofaunal richness (8 taxa) was found in the *C. nodosa* condition, followed by the seagrass-lucinids association (7 taxa). After the MHW, all conditions showed a decline in taxa richness, with the greatest loss (43%) found in the plant-bivalve condition where we report the extinction of Copepoda, Polychaeta and Oligochaeta. At the end of the MHW, taxonomic composition significantly varied with a decrease in the abundance of heat-sensitive taxa (e.g., copepods and foraminifera) and an increase of more tolerant ones (e.g., nematodes). In parallel, the quantity and nutritional quality of sedimentary biopolymeric carbon declined across all impacted conditions, especially in the seagrass-lucinids one. Our results suggest that the ecosystem characterized by the association between *C. nodosa* and *L. orbiculatus* seemed to be the most sensitive one among those tested to extreme ocean warming events, showing the greatest loss of meiofaunal biodiversity and available resources after MHWs. This finding contributes to our understanding of how disturbances may impact biodiversity and reduce the resilience of ecosystems especially those with complex interactions.

Multimodal communication in ants: the interaction between chemical and vibro-acoustic signals in three Myrmicinae species

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Communication is a fundamental aspect of animal behaviour, with signals playing essential roles in courtship, foraging, and predator-prey interactions. In social insects like ants, chemical communication prevails, guiding a wide range of collective behaviours. However, some ant species use a stridulatory organ to produce vibro-acoustic signals that can trigger a behavioural response, either independently or in conjunction with chemical cues. Despite the widespread occurrence of stridulation in ants, its biological significance and interaction with other signalling modalities remain poorly understood. In this study, we explored the interplay between chemical and vibro-acoustic signals in the context of alarm communication in three Myrmicinae ant species: *Crematogaster scutellaris*, *Myrmica scabrinodis*, and *Manica rubida*. We used Laser Doppler Vibrometry and Stir Bar Sorptive Extraction (SBSE) to characterize vibrational signals and volatile pheromones produced by restrained ants. We designed an artificial arena to investigate workers' behavioural responses to pheromones and vibrations separately, as well as in combination. Our findings confirm chemicals and vibro-acoustic signals to be specie-specific. Furthermore, we found different response to multimodality in different species. In particular, chemical and vibro-acoustic signals are both necessary to trigger an attractive response in *C. scutellaris* and *M. rubida*, while only chemicals are sufficient to attract nestmates in *M. scabrinodis*. Interestingly, in *M. scabrinodis*, the addition of a concomitant stridulatory signal disrupts this attraction. These findings suggest the presence of modulatory effects that are potentially subject to environmental constraints. In particular, the differences in the active space of each signal component can result in varying responses depending on the environment or distance from the source. Furthermore, the three species occupy distinct microhabitats (*C. scutellaris* is primarily arboreal, *M. rubida* inhabits open high-altitude areas, and *M. scabrinodis* is a generalist) which may influence how chemical and vibrational signals propagate, ultimately affecting receiver perception. Overall, our results reveal species-specific response to multimodality in species that inhabit different micro-habitats, providing insights into potential selective pressures and constraints shaping the evolution of multimodality in complex social systems.

Peripheral sensory learning and "Taxonomic Behavior" in *Octopus vulgaris* arms

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Octopus vulgaris serves as an excellent model organism for the study of motor control and sensorimotor integration due to its highly specialized nervous system and advanced manipulative abilities. Each arm possesses a remarkable degree of functional autonomy, supported by a decentralized neural architecture in which over two-thirds of the neurons are located in peripheral ganglia. This organization enables the execution of complex movements without needing continuous central control. This decentralized neural organization, combined with a highly flexible morphology and refined manipulation skills, enables the octopus to explore and interact with its environment in a highly adaptable manner.

In this study, we present a novel experimental protocol developed for the controlled analysis of sensory systems at the level of individual arms. This protocol maintains the ability to monitor motor patterns accurately and assess taxonomic behavior in terms of species-specific behaviors. The protocol includes a progressive training sequence: the pre-training phase, in which the animal is trained to interact with a device containing a single real food stimulus, and the experimental phase, in which it is required to discriminate between two 3D-printed artificial anchovies and one real anchovy. The goal is to condition the animal to associate specific interactions with a food reward. For this protocol, a transparent plexiglass device was developed, consisting of three independent compartments, each equipped with an opening for arm insertion and a removable lid. A dedicated "ethological photo set" was also assembled for behavioral data collection, enabling detailed analysis using the BORIS software. *O. vulgaris* demonstrates the ability to distinguish between real and artificial stimuli using tactile/chemical and visual cues. Through the training phases, octopuses exhibit learning behaviors, such as associating specific tactile/chemical and visual stimuli with food rewards. This would be consistent with documented evidence of their ability to learn through conditioning and retain information over time and exhibit taxonomic behavior. Individual variability in response to stimuli could indicate behavioral plasticity and personality-like traits, suggesting that *O. vulgaris* can adapt its exploratory strategies based on past experiences and environmental changes.

Preliminary analysis of benthic fauna associated with vermetid reef along the Ionian coast of Apulia (Southern Italy)

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Vermetid reefs are considered very important ecosystems, due to their role in enhancing habitat complexity, modulating coastal morphological processes and providing shelter and food resources for many invertebrates. This study aims to investigate for the first time the benthic associated fauna with vermetid bioconstructions newly reported along the Apulian coast of the Ionian Sea, in the province of Taranto. The research area was located along 40 km of coastline, between the localities of San Vito and Campomarino (TA). Three study sites were selected and, at each site, two quantitative samplings, using squares 10 × 10 cm, were carried out along three transects by scraping off thin vermetid encrustation. Samples were sorted and preserved in 70% ethanol for species identification and counting.

The present study reports a rich and diverse fauna associated with vermetid bioconstruction, comprising 3266 individuals across 63 different taxa and 6 Phyla (Porifera, Nemertea, Mollusca, Annelida, Arthropoda, Chordata). Amphipods were found to be the most abundant, followed by Bivalves and Polychaetes. Some species (e.g., *Perinereis cultrifera*, *Syllis amica*, *Mytilaster minimus*, *Caprella grandimana*) were common across all three sites, representing typically species found in other Mediterranean vermetid reefs. Among the identified taxa, alien species such as *Pseudonereis anomala* and *Mesanthura romulea* were also detected. Notably, the high number of new crustacean species has never been reported in association with vermetids, partially addressing the knowledge gap for this taxonomic group.

The comparison with the benthic fauna associated with the vermetid bioconstruction newly described along the Adriatic coast of Apulia, revealed that only 27% of the species were shared, highlighting the unique species composition of each vermetid formation depending on the geographic location. This finding has important conservation implications, as it suggests that each vermetid bioconstruction supports site-specific biodiversity, underscoring the ecological value of vermetid bioconstructions in the coastal areas. Therefore, it is crucial to integrate these habitats into regional and national conservation policies to ensure the protection of their unique associated biodiversity.

New methodology for monitoring fishery-target species in Marine Protected Areas

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Marine Protected Areas (MPAs) represent ideal contexts for the application of experimental approaches and co-management models, facilitating the reduction of conflicts between the Managing Authority and local stakeholders, and promoting more effective and participatory governance. Among the most relevant activities in terms of biological impact and management interest is small-scale fishing, which represents an extensive pressure on target species and coastal habitats. This study focuses on the MPA “Penisola del Sinis–Isola di Mal di Ventre” (central-western Sardinia). Over the years, numerous monitoring campaigns of fishing activities have been conducted, but the reliability of the self-reported information by fishermen through logbooks has raised concerns about data accuracy. In 2023, to improve the spatial resolution and traceability of fishing activities, GPS geolocation systems were installed on a voluntary sample of nine vessels. Between 2023 and 2024, a total of 317 fishing trips were recorded over 130 days (an average of 1.5 ± 0.6 trips per day). Of the GPS tracks collected, 61% were complete and usable for analysis. Data collected from 2018 to 2025—via logbooks, onboard observations, and visual inspection of landings—enabled in-depth analysis of fishing dynamics within the MPA. These efforts contributed to a more detailed understanding of fishing patterns and behaviors. The comparison between the two data sources showed a match in 60% of cases (118 trips), indicating a fair level of reliability in the self-reported data, while also highlighting areas for improvement in field data collection and validation.

The integrated analysis of GPS tracks and catch records allows for the correlation of fishing trajectories with benthic habitats and zones of varying biological vulnerability, contributing to the understanding of the spatial ecology of fishing effort and its implications for fish communities. The technological advancement of these tools, combined with the expansion of their functionalities, makes them particularly effective in promoting constructive dialogue between MPAs and fishermen, fostering stronger, more transparent collaboration based on objective data.

First record and biological analysis of *Pyroteuthis margaritifera* in Sardinian waters

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The jewel enope squid, *Pyroteuthis margaritifera* (Rüppell, 1844), is the only known representative of its genus in the Mediterranean Sea. Specimens are rarely encountered, and to date, existing records have merely confirmed its presence in the region. Throughout its entire distribution range, only few biological data are available mainly focused on the comparative morphology of its distinctive photophores located on the tentacles, eyes, anus, gills, and abdomen. In June 2016, for the first time in Sardinian waters, a specimen identified through molecular analysis as *Pyroteuthis margaritifera* was accidentally captured by a trawl net at a depth of 250 meters. The individual, which measured 42 mm in dorsal mantle length and weighed 11.3 g, exhibited all the photophores previously described for the species except those on the tentacles. This absence contrasts with observations from six other Mediterranean specimens (from the Strait of Messina) recovered from the stomach contents of *Thunnus thynnus*, in which tentacular photophores were clearly present. Biological analysis of the Sardinian specimen revealed that it was a mature male, containing 372 spermatophores inside the Needham's sac, with a mean total length of 8.62 mm. Meristic data were recorded on the hooks present on the tentacle club and on the fourth arms, and the main measures of the beak were also recorded. Overall, the morphometric and meristic data reported herein represent the first comprehensive biological information and anatomical documentation available in Mediterranean for this elusive species. The absence of tentacular photophores in this specimen, in contrast with what reported in the literature, highlights the need for further studies on additional Mediterranean specimens using an integrative approach that combines morphological and genetic analyses.

The use of the otolith reading technique to determine the age of Silver eels in Sardinia, Italy

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The European eel *Anguilla anguilla* (Linnaeus, 1758) inhabits rivers and lagoons during its continental growth phase, where the silvering process, essential for reproductive migration, occurs. The timing of silvering is influenced by factors like latitude, environmental conditions, and food availability. This study investigates the metamorphosis to the reproductive stage within the Sardinian lagoon environment where eels are traditionally fished, focusing on the age by otolith analysis, size, and sex at the onset of silvering.

In 2019, 229 eel specimens were collected from two Sardinian lagoons: 109 from Porto Pino (495 ha, southern Sardinia), and 120 from Sa Praia (97 ha, eastern Sardinia). For each specimen parameters as livery, sex, total length, pectoral fin length, eye diameter, ocular index (OI), total weight, and gonad weight were recorded. For age determination, otoliths were processed using a grinding and polishing technique and stained with toluidine blue to enhance the visibility of annual growth rings. The age of each specimen was subsequently correlated with its biometric values and indices.

Out of 229 examined eels, 150 were yellow eels. Among these, 109 were females, with length range 26.0-72.8 cm and weight range 24.5-326.3 g. The remaining 41 were males, with length range 30.5-43.1 cm and weight range 33.8-170.0 g. The other 79/229 were silver eels. Of these, 41 were females, with length range 50.2-72.7 cm and weight range 191.6-778.5 g. The remaining 38 were silver males, with length range 34.0-45.6 cm and weight range 65.0-142.7 g. Female yellow eels age ranged between 2 and 10 years (mean: 4.47 ± 1.36), while male yellow eels age ranged between 2 and 7 years (mean: 4.37 ± 1.22). Silvering females age ranged between 6 and 12 years (mean: 8.49 ± 1.50), while silvering males age ranged between 3 and 7 years (mean: 5.21 ± 1.02). The OI was correlated with the transition from the yellow to the silver eel stage. For females, OI values exceeding 7.40 were consistently associated with the migrant stage. For males, this threshold was at 5.33.

These values are relatively low compared to those observed in continental Europe, which aligns with the evidence that maturation age increases with increasing latitude. Given that *A. anguilla* is both a commercially important species and critically endangered according to the IUCN, this information can be valuable for guiding conservation efforts related to both fisheries management and environmental protection.

A polychaete under pressure: *Sabella spallanzanii* as a zoological model for marine invertebrate immunity and environmental stress response

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The Mediterranean polychaete *Sabella spallanzanii* is emerging as a zoologically relevant model for studying innate immune mechanisms and stress responses in marine invertebrates. Its ecological niche, phylogenetic placement within Lophotrochozoa, and the presence of a mucus-based immune system make it a valuable organism for investigating interactions between environmental pollutants and invertebrate immunity.

Recent studies have focused on the response of *S. spallanzanii* to various stressors, including inorganic arsenic, copper sulphate, methylmercury, and bacterial infection (*Escherichia coli*). Mucus secreted by this species contains a calcium-dependent galactose-binding lectin (SsGBL), with specific agglutinating activity toward Gram-negative bacteria. This component plays a key role in non-self recognition and first-line immune defense.

Following arsenic exposure, which is naturally bioaccumulated in tissues possibly as an antipredatory strategy, the agglutination capacity of mucus lectins was significantly reduced, suggesting a trade-off between chemical defense and immune functionality. Seasonal variations in biological activity were observed, but arsenic tolerance seemed consistent across conditions, pointing toward possible evolutionary adaptation.

Additional experiments investigating the effects of copper sulphate and methylmercury, both alone and in combination with *E. coli* infection, revealed modulation of several immune markers, including esterase, alkaline phosphatase, glutathione peroxidase, lysozyme, and haemagglutination activity. Notably, co-exposure to bacterial and chemical stressors led to suppressed immune responses and altered protein expression profiles.

These findings support the use of *S. spallanzanii* as both a bioindicator in marine biomonitoring and a model system for understanding invertebrate immunity. Its complex immunophysiological responses to natural and anthropogenic pressures provide valuable insights into the evolution of immune strategies and resilience mechanisms in benthic marine organisms.

Terrestrial arthropods as indicators of restoration success in an active limestone quarry

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Limestone quarries are industrial sites that impact natural areas, thus their restoration is crucial for mitigating these impacts and reducing biodiversity loss. Restoration can be achieved through active management projects, such as hydroseeding and soil amelioration. Our study aims to evaluate the results of an active restoration project performed at a limestone quarry in Piedmont (NW Italy) using terrestrial arthropods as ecological indicators. We sampled four areas of the quarry restored in different periods between 1999 and 2016, following the quarry development, and showing different stages of vegetation succession, from sparse grassy vegetation to woodland. Additionally, we included a semi-natural xerothermic grassland outside the quarry as a control area. Arthropods were sampled at 20 points (four for each successional stage, and four at the control site), each consisting of three pitfall traps, filled with 100 ml of 20% propylene glycol as preservative liquid and spaced about 3 metres apart. All traps had been activated for seven days each month, from May to October 2024, for a total of five sampling sessions. Overall, we collected 13164 individuals belonging to 107 families. RDAs and GLMMs were applied to test for differences in community composition and biodiversity indexes according to the restoration stage, and between the quarry and the control area. RDAs showed that the community composition was mostly similar at the different restoration stages and in the control area. Some coprophagous beetle families, such as Scarabeidae and Geotrupidae, were associated with the semi-natural xerothermic grassland, while predators such as Carabidae and several spider families were associated to the earlier successional stages created by the quarrying activity. GLMMs revealed a higher arthropod diversity in the oldest successional stage, now colonized by broadleaved woodlands. On the other hand, species richness reached a higher value in the control area, outside the quarry. These results suggest that predator arthropods can thrive in restored quarries, leveraging on sparse grassy vegetation in the newly restored areas of the quarry. On the contrary, coprophagous and necrophagous beetles are more abundant outside the quarry. Notably, high arthropod diversity in the oldest part of the quarry suggests that active restoration management is a profitable tool for restoring these post-industrial sites and achieving biodiversity conservation targets.

First observations on the electrosensory system of the common smooth-hound shark, *Mustelus mustelus* (Linnaeus, 1758), from the Mediterranean Sea

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The common smooth-hound *Mustelus mustelus* (Linnaeus, 1758) is a demersal shark widely distributed in temperate and subtropical coastal waters, including the Mediterranean Sea. Like other cartilaginous fish, it possesses a specialized electrosensory system: the ampullae of Lorenzini (AoLs). These structures, functionally adapted to detect minute electric fields, consist of subdermal sensory organs connected to the environment via canals ending in pores. The morphology, histology and spatial distribution of AoLs vary among species.

This study provides the first description of the gross anatomy and histomorphology of AoLs in *M. mustelus*, aiming to clarify their structure and infer possible ecological adaptation.

Specimens were collected as by-catch from artisanal trammel net fishery in the Egadi Archipelago. Following deep anaesthesia with MS-222 Sandoz, AoLs samples were fixed in paraformaldehyde and processed for macroscopic and histological analysis. Cephalic distribution and gross anatomy were examined macroscopically, while histological sections were stained with Alcian blue and Milligan's trichrome to reveal cellular features.

AoLs are organized into distinct clusters (supraorbital, buccal, hyoid, and mandibular clusters), each composed of a canal and a dilated ampullary portion (AP), both filled with gel and surrounded by connective tissue. Each cluster comprises at least five ampullae, connected to an afferent nerve. Nerve fibre bundles branch at the base of each AoL, enabling separate innervation.

Histological analysis confirmed the general organization of the AoLs, which are primarily located in the rostral cephalic region. The canal is lined with simple squamous epithelium, while the AP is composed of simple columnar epithelium containing supporting and sensory cells. Each AP includes eight alveolar sensory chambers, which are proximally connected to afferent nerves. Nerve bundles reach the base of each AP beneath the central cup, with terminals extending to the base of the sensory cells only.

Our findings seem to highlight a high density of cephalic AoLs and pronounced alveolar subdivision in *M. mustelus*, suggesting enhanced electrosensory reception and signal processing. These characteristics may be linked to specific ecological traits, encouraging future studies on the evolutionary drivers and ecological implications of AoL system in this species.

Monitoring of eel ladder on the Casteldoria dam in the Coghinas River (northern Sardinia, Italy)

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The European eel *Anguilla anguilla* (Linnaeus, 1758), a catadromous species classified as “Critically Endangered” on the IUCN Red List, faces several anthropogenic pressures that have led to stock progressive decline since the late 1980s. One of the most severe ecological problems, beyond the direct effects of adult fishing and the capture of juveniles for aquaculture, is habitat loss due to the fragmentation of watercourses by weirs and dams.

This study describes the monitoring of the eel ladder installed at the Casteldoria dam (Sardinia, Italy), located along the Coghinas River, 15 km upstream from its mouth. The dam is used for power generation by Enel Green Power and the water is also used for drinking and irrigation purposes by ENAS and Consorzio bonifica of North Sardinia. The eel ladder is a total of 37 meters long with an average slope of 63%. It operates by means of water flowing down a covered canal with plastic bristle substrate, which allows eels to ascend upstream. Data for this study were collected through weekly sampling from fall 2022 to fall 2024. For each monitoring day, the total weight of eels was recorded, and biometric analyses of length and weight were performed on a subsample of 30 individuals.

During the reference period, a total of 8,754 eels were counted, with a total weight of 18,612 g. The highest number of individuals counted was 4,814 in winter 2022–2023, while the lowest number was 5 individuals in autumn 2024. The biometric values recorded from the eel subsample across the different seasons were: mean length ranged from 10.24 ± 0.32 cm in autumn 2023 to 17.50 ± 0.71 cm in autumn 2024, while mean weight varied from 1.03 ± 0.58 g in autumn 2022 to 5.47 ± 6.42 g in winter 2023–2024.

The eel ladder is of vital importance to ensure the natural and complete colonization of eels along the river’s main channel. The installed ladder has proven effective in allowing the transit of elvers and yellow eels. No glass eels were detected, likely due to the ladder’s 63% slope, which is probably too steep for this stage. Restoring river continuity, even with structures like eel ladder, is fundamentally important to protect the species and enable its presence even in habitats impacted by human activity. Adopting measures of this kind is definitely a strategy to complement the regulation of the direct exploitation of the species.

Integrative taxonomy unravels the origin and introduction pathway of non-indigenous *Ceratonereis* in the Mediterranean Sea

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The Mediterranean Sea is one of the regions most affected by biological invasions, especially after the opening of the Suez Canal determining the arrival of Indo-Pacific species from the Red Sea. Ports and marinas are important gateways for alien species, fuelling the spread of lessepsian migrants that raise concerns due to their impact on local ecosystems. Although polychaetes are among the most abundant taxa in marine benthic communities, genetic data and critical taxonomic revisions are still lacking for many groups compared to other invertebrate taxa, which hinders proper identification of non-indigenous species (NIS). With the aim of tracing the distribution and invasion routes of polychaete NIS in the Mediterranean, this study focused on *Ceratonereis mirabilis*, a species originally described for the tropical western Atlantic and reported as a NIS in the eastern Mediterranean. Specimens of *C. mirabilis* from Lebanon and Cyprus were compared with material from the Arabian Gulf, Oman and the Caribbean using an integrative approach combining molecular, morphological and ecological data. Genetic and morphological data highlight the presence of two distinct *Ceratonereis* species in the Mediterranean, one of which occurs on vegetated hard bottoms, while the other in soft sediments only. Even though these lineages were historically referred to as *C. mirabilis*, molecular data show a clear distinction from specimens collected in the western Atlantic. Conversely, the Mediterranean lineages also occur in the Red Sea, which suggests a lessepsian origin of both species and that *C. mirabilis* as currently identified represents a species complex, emphasising the need for a critical revision of this genus.

The arthropod communities of the *Posidonia oceanica* banquettes of Sant’Agostino (Latium, Central Italy)

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Banquettes are an important coastal ecosystem formed from the accumulation on the coasts of plant biomass, represented by the dead leaves of the marine phanerogamous plant *Posidonia oceanica* (L.) Delile, and many species of invertebrates and small vertebrates are present within them. These environments are still poorly studied from an ecological and faunistic point of view and are increasingly at risk due to human activities. In Latium, at the locality of Sant’Agostino, near Civitavecchia (Rome), there is one of the most well-preserved banquettes in the Region, which is part of the protected area “La Frasca” Natural Monument. Eight sampling points were selected in a portion of beach approximately 610m long. For each point, two pitfall traps were placed and remained active for 7 days, the first one placed at the surface level of the banquette and the second one at a depth of 50cm. In addition, two samples were taken for analysis by visual census and the other by Berlese-Tullgren funnel extraction. The sampling sessions were carried out approximately every 40 days from January 2024 to November 2024. Sampled individuals were identified at Order level. For spiders, a more detailed identification was conducted at Species level. GLMM models were developed to analyse abundances, the number of taxonomic groups and Simpson’s indices, in function of seasons and sampling method, and a CCA analysis to highlight the relationships between taxonomic groups, seasons and sampling methods. The most significant seasonal variations are in the abundances of individuals captured, with the subterranean pitfall and Berlese-Tullgren funnel recording the largest numbers of individuals. For taxonomic groups and Simpson’s indices, the differences are less marked or non-significant. Fifteen species of spiders were identified, including three new for Italy (*Dysdera cf. gamarrae* Ferrández, 1984; *Gnaphosa jucunda* Thorell, 1875; *Erigone dentosa* O. Pickard-Cambridge, 1894) and one new species for Latium (*Chaerea maritimus* Simon, 1884). The results obtained extend a still limited literature, helping to highlight the biological importance of *Posidonia oceanica* banquettes, which often remain underestimated and poorly studied.

Circadian and sleep-like rhythmicity in the scalloped hammerhead shark (*Sphyrna lewini*)

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Understanding biological rhythms in continuously swimming marine animals is crucial to elucidate their rest-activity balance and internal timekeeping. Scalloped hammerhead sharks (*Sphyrna lewini*) are ram ventilators with high energetic demands, and have recently been shown to display a rhythmic rolling swimming behaviour (RSB). We investigated the presence and function of biological rhythmicity in wild *S. lewini* using high-resolution accelerometry and time-series analysis. Our results reveal robust circadian rhythmicity in activity levels, with a consistent nocturnal acrophase and significant anticipation of light transitions, indicating entrainment by an internal circadian clock. Concurrently, we identified a structured ultradian rhythmicity in rolling behaviour, which differed markedly between day and night. Daytime rolling cycles were shorter, less frequent, and associated with low metabolic effort, suggesting a restorative function. Nighttime rolling was longer and more active, potentially supporting navigation and environmental sensing. We observed a homeostatic relationship between night and day rolling intensity, consistent with compensatory rest regulation. Additionally, we detected a modulatory effect of moonlight on nighttime activity and subsequent daytime rest, suggesting masking effects. Altogether, our findings support the presence of circadian and sleep-like processes in *S. lewini*, and raise new questions about how these rhythms support energy conservation, navigation, and sensory integration in pelagic predators.

Wild bees (Hymenoptera, Apoidea, Anthophila) and flowers: evaluation of visit frequencies in urban nature reserves

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Urbanisation is a pervasive global process that contributes to habitat fragmentation and biodiversity loss. Nevertheless, large urban green spaces can serve as important refuges for wildlife, offering critical habitats that support biodiversity conservation even within metropolitan contexts. Also in the urban ecosystem, wild bees represent key pollinators among urban fauna, playing a crucial role in ecosystem functioning. Their diversity and activity are closely linked to the availability and characteristics of flowering plants, which provide essential food resources. This study investigates the interactions between wild bees and spontaneous flowering plants within selected large urban nature reserves in Rome, Italy. Specifically, we analysed bee visitation frequencies at the genus and family level and examined the role of functional traits of flowers and bees in shaping these interactions. For bees, we considered traits such as the ligula's relative length and position of the scopa on the body. We focused on inflorescence type, corolla structure, and flower colour combinations for plants. Our goal was to characterise the structure of pollination networks in large urban green areas and to understand how morphological and functional traits of both bees and flowers influence interaction patterns. Preliminary results suggest that specific trait combinations are associated with higher visitation rates, indicating a degree of trait matching that may enhance pollination efficiency and network stability. Emerged that a few plant families are highly represented and visited by many bee species, while many other botanical groups are visited by small groups of wild bees. Regarding morpho-functional traits, long-ligula bees visit a wider range of flowers and preferentially visit deep and tubular flowers, while short-ligula bees visit a smaller number of plant taxa. From the analysis of scopa position to corolla structure, significant results didn't emerge. The findings highlight the importance of functional traits in mediating ecological interactions in urban ecosystems. This approach provides a deeper understanding of how urban green spaces can sustain complex pollination networks, offering practical insights for urban biodiversity management. By identifying the traits that facilitate effective plant-pollinator interactions, this research can inform targeted conservation strategies aimed at promoting pollinator diversity and abundance in cities.

Accumulation of trace elements and assessment of oxidative stress in three European eel (*Anguilla anguilla*) populations in Calabria

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The European eel (*Anguilla anguilla*), a widely distributed catadromous species currently classified as *Critically Endangered* by the IUCN, is experiencing a marked decline on a continental scale. This decline is attributable to multiple factors, including chemical contamination of aquatic ecosystems.

This study investigated the concentrations of 16 trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sr, Zn, Ba, Bi) in the muscle tissue of 20 specimens collected from three sites in Calabria (Crati River, Raganello Stream, La Vota Lakes), using inductively coupled plasma mass spectrometry (ICP-MS).

Statistical analysis revealed significant differences in the bioaccumulation of 10 out of the 16 elements among the populations, raising particular concern over the significant results for cadmium and arsenic.

In parallel, ecotoxicological assays were conducted to assess oxidative stress (SOD, TBARS, ketonic and aldehydic OMPs) in four target tissues (muscle, heart, liver, gills). In muscle tissue, populations showed statistically significant differences for all biomarkers: OMP values were highest in La Vota Lakes, while SOD was highest in the Crati River.

Correlation analysis between bioaccumulation and stress revealed site-specific responses: no significant relationships were observed in Raganello; in the Crati, Mo and Cr were positively correlated with TBARS, while Zn was negatively correlated with SOD; in La Vota Lakes, Al was negatively correlated with TBARS, Bi was positively correlated with aldehydic OMPs, Se and Co were positively correlated with SOD, and Cr was negatively correlated with SOD.

The results highlight complex interactions between chemical contamination and physiological responses, underscoring the vulnerability of *A. anguilla* in ecotoxicologically critical environments.

“That’s a trap!”: Identifying extracellular traps formation in invertebrates and vertebrates

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All living animals possess multiple conserved mechanisms for regulated cell death (RCD) like apoptosis, but among these, the formation of extracellular traps (ETs) or ETosis is an innate immune mechanism to cope with the invasion of pathogens, recent studies have also shown how ETosis takes part into various auto-immune diseases. Although described in many vertebrates, few are the descriptions in crustacean, and almost none for echinoderms and sharks. Thus, this research characterized ETosis in four species: two invertebrates (*Cherax quadricarinatus*, Crustacea and *Arbacia lixula*, Echinodermata), as well as two vertebrates (*Cephaloscyllium umbratile* and *Triakis scyllium*, Chondrichthyes). Haemolymph, coelom or blood was extracted from *C. quadricarinatus*, *A. lixula*, *C. umbratile* and *T. scyllium*, then the respective immune cells were fractionated. Consecutively, to induce the release of ETs the cells were exposed in-vitro to Gram+ and Gram- bacteria: *Escherichia coli* or its LPS alone, *S. aureus* or *L. garvieae* (DNase or cytochalasin-D as positive controls). Lastly, fluorescent microscopy analyses and further quantification using ImageJ were performed. The results show species-specific differences in the morphology of the ETs and the time of release after exposure to each pathogen. Specifically, *C. umbratile* and *A. lixula* exhibited more diffuse ETs. Whilst in *T. scyllium* and *C. quadricarinatus*, produced conspicuous ET filaments when induced by Gram- and Gram+ bacteria respectively. However, the amount of exDNA vary among species depending on the concentration and activator specifically, sharks undergo ETosis releasing a lower amount compared with invertebrates. Moreover, after analysing all the results obtained, mainly two ETs morphologies are the most common: filamentous and diffuse. Those results, according with previous studies, suggest an effective recognition and a extraneous molecules and consecutive clearance of the bacteria. Thus, this is the first comparative report among different taxa, assessing the highly specificity of ETosis in each organism, and how it is strictly influenced by the concentration of the stimulant. Overall, ETosis in both invertebrate and vertebrate's immune cells was identified, suggesting it is a conserved RCD mechanism with insights for further studies on the specific activation and the implications on how this process can be regulated.

A spatio-temporally explicit distribution model to support conservation strategies for isolated small-ranged species: a case study from Galápagos

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Species Distribution Models (SDMs) represent the gold standard to investigate how environmental factors affect the occurrence of animal species and estimate their actual and potential distribution. However, SDMs often lack a formal hypothesis-driven selection of environmental predictors and require independence among observations. In contrast, actual occurrence data are often spatially and temporally autocorrelated, particularly when most observations originate from a limited number of surveys, as frequently happens in studies focusing on isolated, small-ranged species of high-conservation priority. Here, we explored the application of a hierarchical Bayesian spatio-temporal SDM to model the probability of occurrence of small-ranged species living in isolated and difficult-to-access areas, combining Integrated Nested Laplace Approximation (INLA) with Stochastic Partial Differential Equation (SPDE). To do so we used the Galápagos Pink Land Iguana (*Conolophus marthae*) as a model organism. This species is well suited for our scope in being critically endangered and endemic to an extremely small and remote area on Wolf Volcano on Isabela Island, Galápagos. Our approach enabled us to obtain reliable estimates of the effects of environmental variables on species distribution using opportunistic occurrence data, which exhibit a pronounced spatio-temporal structure. A spatially explicit cross-validation of the model demonstrated that the INLA-SPDE approach allows elucidating the relationship between small-ranged species and their areas of distribution, resulting in accurate predictions (Cohen's K = 0.86; True skill statistics = 0.84). We also used the INLA-SPDE model to map the actual and potential distribution of the species across the Galápagos archipelago. Our approach provided a first formal measure of the range of *C. marthae* (ca. 42 km²) and indicated that abundance of trophic resources, availability of open areas, and terrain roughness limit species' distribution. We also identified ca. 400 km² of suitable areas clustered into three main sites outside the species' actual range. These results constitute an essential starting point to evaluate a species translocation, a key conservation strategy outlined in the Conservation and Management Plan for this species.

Phoresy by ants: dispersion of Tardigrades enhanced by anhydrobiotic capabilities

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Phoresy is a temporary symbiotic relationship where an organism, generally with limited active dispersal capabilities, uses a more mobile host for dispersion to reach new habitats or resources. Tardigrades are known to use as phoretic hosts various vertebrates and invertebrates with limited mobility. This study investigated the potential role of ants as dispersal hosts for tardigrades, a possibility not yet explored, as similar behaviours have already been documented in ants with other invertebrates.

To verify the tardigrade-ant associations, a field survey was conducted on arboreal ants. Oak galls of the cynipid wasp *Andricus quercustoxae* colonized by four ant species were collected, the presence of tardigrade in these ants' gall nests and on ant bodies were assessed. The transport of tardigrades by ants was also evaluated by isolating galls for one year and then exposing them to ant colonization, as well as by laboratory experiments on tardigrade phoresy focusing on the behaviour of the ant *Colobopsis truncata*.

Ants carried tardigrades on their bodies, providing the first evidence of ant-mediated tardigrade phoresy, especially with *C. truncata*. Moreover, ants transport moss and lichen fragments inside their nests, thus tardigrades and other meiofaunal organisms were retrieved in galls. The high humidity conditions within galls spongy material allow tardigrades to be alive and active. The intense foraging activity and long distance walked by ants together with the high number of potential “transporters” within colonies explain the diversity of the tardigrade species retrieved within the galls. Finally, desiccated tardigrades may withstand potential long-distance transport by ants. Indeed, animals able of undergoing anhydrobiosis could properly desiccate on the body of an ant or within fragments of substrates (e.g. mosses, lichens) and being transported for long distances withstanding extreme conditions.

Thanks to the ant widespread abundance, myrmecophoretic dispersal may contribute to the spread of meiofaunal organisms in terrestrial habitats. These findings represent just the “tip of the iceberg” of an unexplored passive dispersal modality over significant distances for terrestrial hydrophilic micrometazoans, broadening our understanding of ecological interactions and phoresy.

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Diversity and distribution of Apoidea across conventional and organic crops in the MAB-SILA UNESCO biosphere reserve (Calabria, Italy)

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Pollination sustains plants and is therefore pivotal for the environment. Wild bees (Hymenoptera, Apoidea, Anthophila) represent the most significant group of plant pollinators, with a total of over 20,000 species identified worldwide. Nonetheless, these species are in decline due to a range of stressors, including habitat fragmentation, pesticides and pollution. These factors primarily affect their diversity and abundance. In order to design effective conservation programmes, it is necessary to collect information about bee presence and distribution, however more than 50% of wild bee species in the IUCN Red List for Europe are data deficient. This underscores the need for more ecological studies on species distribution and diversity. Mediterranean countries have the most diverse bees, including more than 1,000 species in Italy. Nevertheless, data on wild bees in certain areas, such as Calabria, are either limited or lacking.

The present study was performed in a MAB area in Calabria (Italy), as part of the BIONETPARKS Project – Protecting Pollinators and Plant Species in the MAB-SILA UNESCO Biosphere Reserve. A total of four sampling sites were chosen in cultivated areas, of which two were under conventional management and two under organic management. The sampling method employed, in accordance with the EU Pollinator Monitoring Scheme (PoMS), was permanent transect walks. The monitoring period (2022-23) resulted in the identification of 38 species, which belonged to 14 genera and 4 families. The most prevalent genera were *Andrena*, with a total of 14 species documented, and *Lasioglossum*, which accounted for 7 species.

A diversity analysis was conducted, with the alpha and beta diversity indices employed as the primary analytical tools. Subsequently, these indices were correlated with agroforestry activity, temperature and altitude. Furthermore, the relative abundances of the various genera at different sites were taken into account, showing a greater abundance of social managed species (*Apis*, *Bombus*) in the conventional areas, while organic farms exhibited a greater abundance of wild bees (*Andrena*, *Lasioglossum*). In addition, the interaction between the identified wild bees and the host plants was analysed and an overview of the knowledge on the ecological characteristics and conservation status of the identified species was considered. This study presents a preliminary investigation into the current status and diversity of Apoidea in Calabria.

A national reference for wild bee identification: Italian adaptation of Michez et al. (2024) key to bee genera

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In recent years the interest and concern for nature and its safeguard has grown. The European Commission has adopted the Law for the Restoration of Nature, which establishes and guides member states in implementing measures to promote the conservation of biodiversity. Pollinators, and in particular wild bees, play a significant role in this plan because of their key role in the ecosystem functioning and the critical decline they are facing. Several European projects for monitoring and conservation of wild bees have been launched. These have encountered problems due to a lack of resources for taxonomic identification. It is therefore evident that there is a need for both information and tools for taxonomic identification, as well as for training a new generation of experts. Projects such ORBIT and SPRING were launched for this purpose. ORBIT was created to provide a tool to support the taxonomic identification of European wild bees, while SPRING project aimed to test the implementation of the EU Pollinator Monitoring Scheme (EU PoMS) for wild bees and other pollinators. In this framework an international team of experts is working on adapting at national scale the “Key to the genera of European bees (Hymenoptera: Anthophila)” (Michez et al., 2024), a reference identification tool, at genus level, for the Apoidea fauna of each European country. The purpose of this work is to introduce the Italian version of the key.

The Italian key, to be published as a volume, is an adaptation of the European key by Michez et al. (2024). It has been rearranged to focus only on the Italian fauna of Apoidea Anthophila, removing the genera not present in the peninsula. In Italy, we have 61 genera of the 77 European genera listed on the original key.

The volume encompasses a general overview of the life cycle, ecology and morphology of bees. It also summarises the families, subfamilies, tribes, and genera present in Italy, including the number of species and the main bibliographic references available for species-level identification. Finally, the work provides two illustrated identification keys, one for females and one for males, and also includes species factsheets covering some of the most common and representative Italian species. This work, intended for students but also enthusiasts or amateurs, aims to help facilitate the dissemination of taxonomic knowledge on Apoidea, and therefore, approach the monitoring and conservation goals set by the European Union.

Trophic shift in *Mustelus mustelus* and *Mustelus punctulatus*: a vertebral stable isotope approach

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The increasing vulnerability of demersal elasmobranchs to overfishing, and the consequent global decline of their populations and their ecological role as apex predators in epi-benthic ecosystems, has led to growing scientific interest in understanding their trophic ecology. This aspect is considered essential for developing effective conservation and management strategies, especially in areas subject to high anthropogenic pressure.

Stable isotope analysis has proven to be a powerful tool in this kind of investigation. Soft tissues such as muscle reflect dietary input over relatively short periods, weeks to a few months, due to their higher metabolic turnover. In contrast, hard tissues, like vertebrae, are known to incorporate isotopic signals more slowly, reflecting feeding patterns over months or even years. This slower turnover rate allows vertebral isotopes to serve as a biological archive, providing long-term insights into individual trophic histories and ecological changes across life stages.

In this study, we analysed the stable isotopes of nitrogen (¹⁵N) and carbon (¹³C) along the vertebrae of *Mustelus mustelus* and *Mustelus punctulatus*, two demersal shark species that are increasingly threatened in various Mediterranean regions. A total of thirty vertebrae from *M. mustelus* and thirty-five from *M. punctulatus* were examined using isotope ratio mass spectrometry, enabling the reconstruction of individual trophic trajectories across ontogeny. The results revealed distinct species-specific patterns. *M. punctulatus* exhibited a general increase in ¹⁵N values with increasing body size, suggesting a trophic level shift as individuals grow, reflecting a dietary transition toward larger or higher-level prey. In contrast, *M. mustelus* showed stable isotopic signatures throughout ontogeny, indicating a more consistent diet over time and potentially a narrower ecological niche.

These findings highlight the value of vertebral stable isotope analysis in detecting ontogenetic trophic changes and contribute to a better understanding of the feeding ecology of demersal sharks. Such insights are fundamental to inform conservation policies and improve the management of species like *M. mustelus* and *M. punctulatus*, which are especially vulnerable in heavily exploited marine environments.

An habitat fight: the interaction between the polychaete *Sabellaria spinulosa* and the phanerogam *Posidonia oceanica*

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Posidonia oceanica (L.) Delile (1813) is the most important Mediterranean seagrass, and its meadows perform a pivotal role within the coastal marine environment. The polychaete *Sabellaria spinulosa* (Leuckart, 1849) is a biogenic reef builder in sandy environments and is included in the Red List of Marine Habitats and it is a priority habitat in the OSPAR convention. In the framework of the projects MOSSHA and NBFC, we analyze the interaction between the two habitat formers in an area adjacent to the Nature Reserve “Le Cesine”, in the Southern Adriatic Sea. The morpho-structural characteristics of *P. oceanica* and both the qualitative and the morphometric characteristics of *S. spinulosa* bioconstructions were analyzed in eight subareas where the two habitats co-occur and where they are isolated from each other. A visual and photographic census was conducted in each sub-area using a standard 40 x 40 cm reference square. *P. oceanica* was analyzed following ISPRA’s reference methodologies, while the analysis of the bioconstructions of *S. spinulosa* was performed by both non-destructive (video and photographic records) and destructive (collection of 18 blocks of 20 x 20 cm) sampling. The morpho-structural characteristics and the macrofauna associated with each block were determined. Density, coverage, and leaf production of *P. oceanica* exhibited higher values in absence of *Sabellaria*, showing instead a decline in the areas where interaction took place. The analysis of phenology and lepidochronology demonstrated a marked decline in the health status of the meadow in the presence of *Sabellaria*. Conversely, the qualitative analysis of *S. spinulosa* revealed that the presence of *P. oceanica* caused erosion, reduced compaction, more fractures, and reduced cover of epibionts. No effect of the interaction on the size of *S. spinulosa* individual was detected while their density was higher in the interaction areas. Finally, the macrofauna associated with the blocks was more abundant and diversified in the interaction areas. Thus, *S. spinulosa* tended to impair the growth of *Posidonia*, reducing the production and development of new rhizomes and causing a considerable decline in the productivity of the plant, while the interaction caused both positive and negative effects on the polychaete bioconstruction.

Bridging a two-decade gap: new insights into wild bee diversity of Circeo National Park (Latium, Italy)

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Circeo National Park (CNP) is one of the most important natural areas in Central Italy, encompassing a remarkable variety of habitats — including coastal dunes, wetlands, forests, and agricultural landscapes — and preserving numerous endemisms. Among its faunal communities, wild bees represent key pollinators, providing essential ecosystem services that sustain both plant populations and agricultural production. Despite their ecological importance, their diversity within the CNP has been scarcely documented over time. In fact, the last systematic surveys date back to two distinct periods: the first in 1963, and a later campaign between 1993 and 1998. Since then, no comprehensive monitoring has been conducted. The present study is part of the national framework of actions titled “*Pollinating Insects: Biodiversity and Ecosystem Services*“, promoted and funded by the Italian Ministry of the Environment (MASE) through the 2019 Directive addressed to Italy’s National Parks and Marine Protected Areas, and aims to investigate current wild bee diversity across different environments of the CNP. Field sampling was carried out from March to September over three consecutive years (2021–2023). Bees were collected using entomological nets along fixed transects (200x4m), evenly distributed across three main habitat types: agricultural areas with varying management practices, coastal sand dunes, and the Circeo Promontory. The data obtained are compared with results from previous surveys to assess changes in species composition. Moreover, differences in wild bee communities among the sampled habitats are discussed. This study contributes to updating the knowledge on the current status of wild bees within CNP, providing essential data to support conservation strategies.

Peracarida fouling communities along a Mediterranean North-South axis

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Marine ecosystems, including marinas and harbours, are hotspots for Non-Indigenous Species (NIS), which pose significant threats to marine biodiversity. Boating activities are a major vector for the introduction of NIS, particularly Peracarida, whose natural dispersal capabilities are limited. This work aimed to examine the variability and dynamism of Peracarida fouling fauna in three Italian key sites: Palermo and Trapani marinas, both highly impacted by recreational boating and located near the Ustica and Egadi marine protected areas (MPA), and the marina of Licata. Two additional sites were sampled for the first time: the Bergeggi MPA, Italy, and the Kerkennah archipelago in Tunisia, enabling a comparative analysis of communities along a north-south latitudinal and anthropogenic impact gradient. In addition to simply assessing species diversity, the study also considered the species' biological traits. A total of 25 species were identified, including eight NIS or cryptogenic species. The NIS *Caprella scaura* Templeton, 1836 was abundant in all marinas and surpassing in both abundance and frequency the other present predators, *Phtisica marina* Slabber, 1769 and NIS *Paranthura japonica* Richardson, 1909, likely due to its plasticity of feeding behaviour, being able to rely on filter-feeding when fully matured instead of being limited to carnivorous predation. The cryptogenic grazer *Jassa slatteryi* Conlan, 1990, efficient in detritus generation, was found only in Trapani. Wherever *J. slatteryi* was absent, we found the detritivorous *Eritchtonius brasiliensis* (Dana, 1853) and NIS *Stenothoe georgiana* Bynum & Fox, 1977, in Palermo, Licata and a sampled pier in Trapani. The highly invasive detritivorous NIS *Laticorophium baconi* (Shoemaker, 1934) was found at all sites except one in Licata, where the NIS *Monocorophium acherusicum* (A. Costa, 1853) occurred. NIS *Paracerceis sculpta* (Holmes, 1904) was absent from all Trapani piers. In Kerkennah, NIS *Ampithoe bizseli* Özaydinli & Coleman, 2012 was recorded; in Bergeggi *S. georgiana* was present. Fouling community structures and NIS assemblage varied not only among marinas but also between individual piers in Trapani and Licata. These differences were not linked to seasonality. Across all sites, species showed a broad range of trophic strategies, with suspension-feeders dominating the community structure.

Experimental setup for the study of behavioral responses of *Octopus vulgaris* to acoustic stimuli

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The sea is a complex acoustic environment, encompassing a wide range of sounds - from natural to anthropogenic sources - that influence how marine organisms communicate, navigate, and detect predators or prey. Although marine vertebrates have been extensively studied in this context, the role of sound in the sensory perception of cephalopods, and particularly in *Octopus vulgaris*, still remains poorly understood. Physiological evidence indicates a potential sensitivity to low-frequency sounds (>400 Hz), but systematic behavioral studies are lacking.

The aim of this work is to develop an experimental protocol to evaluate the behavioral responses of *O. vulgaris* to artificial and natural sound stimuli in an acoustically controlled environment. We designed a dedicated experimental tank to perform a comprehensive assessment of animal behavior under different sound stimuli across a wide range of frequencies and sound pressure levels, monitoring background noise levels. The behavioral responses of individual *O. vulgaris* to each sound combination were categorized as follows: no response, posture, startle, body pattern change, arm movements, grooming, jetting, and inking. These responses are plotted against stimulus conditions for the construction of a behavioral audiogram.

This experimental approach allows us to explore the octopus's auditory capabilities and contribute to a better understanding of its sensory strategies, with potential implications in field ethology, zoology and conservation.

Tracking chromosomal damage across species: the micronucleus assay as a versatile biomarker

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The micronucleus (MN) assay is a cytogenetic technique that allows to detect small, extra-nuclear bodies ('micronuclei'), arising from an incorrect integration of chromosomes or chromosome fragments into daughter nuclei during cell division. The technique is widely applied to evaluate chromosomal damage resulting from exposure to environmental pollutants or physical stress. Our primary aim is to review how the assay has been applied across diverse aquatic and terrestrial animals. Moreover, here we develop two new protocols to further broaden the applicability of the test, on the freshwater snail *Lymnaea stagnalis* and on the invasive blue crab *Callinectes sapidus*.

Methodologically, the MN assay involves collecting specimens—whether from controlled laboratory cultures or from the field—from which cells are gathered, fixed, and stained on lab slides. Typically examined tissues are buccal mucosa and circulatory tissues like blood (for vertebrates) and hemolymph (for invertebrates). Microscopic examination of the lab slides then allows to score the frequency of micronuclei or other nuclear aberrations. Focusing on size, shape, and staining intensity, these structures can be discerned from the main nucleus or from staining artifacts.

This method's simplicity and cost-effectiveness have allowed it to be successfully employed on many vertebrates and invertebrates, revealing a wide spectrum of genotoxic responses, such as those caused by some industrial waste, pesticides, and heavy metals. Fish and amphibians generally represent sensitive models due to their permeable tissues and are tested through straightforward blood sampling, while terrestrial mammals have often been sampled using mucosal cells. Even invertebrates have provided valuable data, despite some sampling challenges. *L. stagnalis*' behaviour of extruding hemolymph when prodding the animal's foot with a micropipette makes it particularly suitable for this test, while *C. sapidus*' invasive nature in the Mediterranean makes it a widespread and easily accessible resource as well.

Overall, the assay is a valuable tool for environmental monitoring and risk assessment through the use of various taxa, and findings from *L. stagnalis* and *C. sapidus* further confirm its versatility. By implementing robust methodologies and exploring its application to new organisms, it is possible to exploit the assay to detect the effects of genotoxic pollutants in various environments.

Macrozoobenthic non-indigenous species colonization in a Mediterranean Coastal Lagoon: comparison between natural and anthropogenic substrates

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Macrozoobenthic coastal communities are among the most productive in marine ecosystems, often threatened by biological invasions. Proliferation of non-indigenous species (NIS) outside their natural boundaries depends on several factors, including life-cycle, physiological plasticity, introduction pathways and substrates availability. The aim of this study is to compare the variability of hard bottom macrozoobenthic diversity and NIS abundance related to substrate type. Sampling was carried out in the Natural Oriented Reserve of Capo Peloro Lagoon (Messina, Italy) in 2023, collecting 115 marine litter objects (Anthropogenic Substrate-AS) and 115 *Pinctada radiata* (Natural Substrate-NS). NSs and ASs were transported in laboratory and sorted to collect the associated macrozoobenthic fauna. A total of 201 associated species were identified in both the substrate types, belonging to Polychaeta (85 species), Crustacea (49), Mollusca (41), Echinodermata (10) and 16 species belonging to minor represented groups. Nineteen NIS were identified during the analysis, representing almost the 10% of the total number of detected species. Concerning abundances, NIS represented the 4.5% of the total macrozoobenthos abundance, with the 95% of NIS abundance detected from NS. SIMPER analysis based on substrate typology showed an average similarity of 16.07% for AS, due to high abundance of *Pileolaria pseudomilitaris*, *Aiptasia mutabilis* and *Janua heterostropha*, and 33.91% for NS, given by Serpulidae and *A. mutabilis* as well; dissimilarity between substrates was of 90.98%, with NIS contributing for the 11.73%. *Hydroides dianthus*, *Paracerceis sculpta* and *Dorvillea similis* were the most responsible for the detected dissimilarity. Diversity was explored through a species abundance Bray-Curtis similarity matrix followed by Hierarchical Cluster analysis and Multidimensional Scaling. Results showed the 40% of similarity among natural and anthropogenic substrates, and the 60% among the same substrates from different sampling sites. Findings from the present research confirmed the influence of substrates type and sampling site on macrozoobenthic assemblage composition and abundance. Considering the sensitivity of coastal lagoons to anthropogenic pressure, monitor the NIS spreading inside these ecosystems, and improve the knowledge base on their distribution and abundance, is essential to plan effective management actions to face and prevent potential negative impacts of biological invasion.

Otolith variability, age composition, and ontogenetic diet shift in *Trachurus trachurus*, Linnaeus, 1758, from the Southern Tyrrhenian Sea

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Overexploitation of marine resources poses a global threat to biodiversity and food security, with the Mediterranean Sea particularly affected due to intense fishing activity and multiple anthropogenic pressures. This study provides new insights into ontogenetic changes in diet and otolith morphology of *Trachurus trachurus*, a commercially important Mediterranean species. A total of 282 specimens were collected from the commercial fishery in the Gulf of Palermo (Southern Tyrrhenian Sea) for analyses of otolith morphometry, age structure, and diet composition. Otoliths were analysed using wavelet transformation methods, and age was determined by counting annual growth zones, each consisting of one translucent and one opaque ring. The diet composition was assessed through stomach content analysis. Wavelet analysis revealed clear differences in rostrum length, notch depth, and posterior dorsal margin, with six distinct morphotypes identified in the sample. Age composition was dominated by juveniles and intermediate-aged fish: 38.7% belonged to age group 1, 46.8% to age 2, 13% to age 3, and only 1.5% to ages 4–6. Dietary analysis revealed a total of 1,418 prey items from 33 taxa, with 254 stomachs containing food remains and 28 being empty. It was also examined the diet variability in relation to specimens age, revealing an ontogenetic dietary shift: juveniles (ages 1–1+) primarily consumed small pelagic crustaceans, individuals aged 2–2+ included more fish and decapods, and older fish (ages 3–6) specialised in larger crustaceans and fishes. These results demonstrate dietary specialization and trophic flexibility across age classes in response to resources availability. These findings highlight the importance of integrating morphological, age, and dietary data to better understand life history strategies and ecological dynamics in commercially exploited fish species.

Early-stage Medaka Embrioyoys (*Oryzias latipes*) as an alternative model for evaluating the toxicity of of bromuconazole in vivo

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Bromuconazole (BRO) is a triazole class of chemical fungicides widely used worldwide since the 1970s. BRO is currently authorized as a Plant Protection Product (PPP) under Regulation EC 1107/2009, with approval renewed until 30/04/2027 in 14 EU Member States. Despite its ongoing utilisation, the substance has been designated as an EU candidate for substitution, as it fulfils the criteria of “Persistent-Bioaccumulative-Toxic” according to the EU Pesticide Database and EFSA evaluations, namely with respect to its persistence in the environment and its toxicity to aquatic organisms. Due to concerns about its ecotoxicity, BRO has been included in the 5th Watch List established under the EU Water Framework Directive (2000/60/EC), which identifies emerging pollutants that require further environmental monitoring. BRO’s Predicted No-Effect Concentration (PNEC) for aquatic environments is 0.015 µg/L. Recent studies have shown that exposure to BRO may cause cardiotoxicity, oxidative stress, and lipid metabolism disorders in zebrafish (*Danio rerio*) larvae and adults. This finding raises further concerns about potential consequences of its use on aquatic organisms (Huang et al., 2024; Qin et al., 2022). Therefore, the current study aims to assess the effects of BRO on the development of medaka (*Oryzias latipes*) from early stage to 5 days post-fertilization (dpf). Medaka is particularly suitable for toxicity study due to the optical transparency of the eggs, rapid embryonic development, and the high sensitivity to environmental contaminants at early stages of its development. After determining the Lethal Concentration 50 (LC50) through acute toxicity tests, the embryos were exposed to various concentrations of BRO to assess potential effects on early development. The dose selection was based on environmentally relevant levels and available literature data. Morphological and functional endpoints such as survival, somite development, heartbeat, and overall embryonic morphology were evaluated. This approach represents a valuable tool for the early toxicological assessment of persistent fungicides such as Bromuconazole, providing the basis for future studies on their environmental impact and safety profiles. Furthermore, the use of medaka embryos up to 5 dpf allows for in vivo testing in compliance with the 3Rs principles and EU Directive 2010/63/EU.

Effects of environmental microplastics on soil microarthropods survival, behaviour biodiversity

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Microplastics (MP, min di 1 mm) are ubiquitous in terrestrial ecosystems. Their presence is associated with potential alterations in the physico-chemical characteristics of soil and with impacts on the behavior and survival of soil organisms. Nevertheless, to date, studies on the effects of MP have mostly been limited to model organisms, such as the collembolan *Folsomia candida*.

In this study, the effects of MP derived from the degradation of plastics present in urban environments were assessed on different species of soil microarthropods. MP were tested on three wild collembolan species (*Parisotoma notabilis*, *Onychiurus* sp., and *Ceratophysella denticulata*), on the model species *F. candida*, and on the oribatid mite *Scheloribates* sp. The wild species used were acclimated in the laboratory for 2 years before testing, achieving stable populations. The MP tested originated from HDPE paving blocks commonly used in urban parking areas, which were fragmented in the laboratory to obtain the sub-millimetric fraction (10–832 µm) used in the tests. The effects of MP (at 1000 mg/kg) on microarthropod behavior were evaluated with ISO avoidance tests, while the effects on survival were assessed in *P. notabilis* and *F. candida* using OECD chronic exposure tests, exposing organisms to increasing concentrations of MP (from 10 mg/kg up to 1000 mg/kg). The standardized protocol for *F. candida* (28 days and 20 °C) was modified for the wild species *P. notabilis* based on its life cycle characteristics, extending the duration of the test to 35 days and setting the temperature at 17 °C.

The results show a clear trend of avoidance of MP-contaminated soil by all tested microarthropod species, with significant differences compared to the control groups. *F. candida* proved to be the least sensitive species to the presence of MP (avoidance of $34 \pm 13\%$), while the highest values were recorded for the collembolan *Onychiurus* sp. and the mite *Scheloribates* sp., with avoidance rates of $53 \pm 13\%$ and $53 \pm 19\%$, respectively.

In chronic exposure experiments (28–35 days), a significant decrease in collembolan survival was observed in response to MP exposure, with mortality rates at the highest concentration tested reaching $57.8 \pm 13\%$ in *F. candida* and $92.2 \pm 4.4\%$ in *P. notabilis*.

This study contributes to understanding and quantifying the effects that MP present in terrestrial environments have on both model microarthropod species and wild species endemic to the Italian territory.

Conservation insights from morphometric and demographic monitoring of *Patella ferruginea* in Northern Sardinian MAPS

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Patella ferruginea Gmelin, 1791 is an endemic limpet of the Mediterranean Sea and is currently considered the most endangered marine species. Its distribution has been drastically reduced by human collection for ornamental, food and bait purposes and is now largely restricted to protected or inaccessible coastal areas. In this context, long-term monitoring is essential to support effective conservation strategies. This study, to be conducted between 2024 and 2026, aims to assess the population density and structure of *P. ferruginea* in La Maddalena Archipelago National Park through a complete census of the smaller islands and representative transects on the main islands. All individuals were and measured using biometric parameters (length, width, height). Morphotypes (*rouxi* vs. *lamarcki*) were identified based on the ratio of shell height to shell length, and sex was determined based on the length of the shell.

During the first survey phase (summer–autumn 2024), a coastal section of 3,709 meters was surveyed, in which 205 individuals were recorded, corresponding to a linear density of approximately 0.055 individuals per meter. The *rouxi* morphotype was dominant with 175 individuals (85%). Statistical analyzes revealed significant differences in size metrics and sex ratios between sites. The population in La Maddalena had the highest proportion of females (78%) with 160 individuals, 35 males and 10 immature individuals. The data were compared with other data from previous surveys (2018) in two other MPAs in northern Sardinia: Asinara NP and Tavolara MPA. All sites showed a similar dominance of morphotypes. However, there were significant differences in biometric variables and sex composition. Specifically, the mean shell length in Tavolara was 49.6 ± 17.1 mm, lower than in Asinara (53 ± 17.8 mm) and La Maddalena (50.7 ± 14.7 mm). Tavolara also had the highest

proportion of males (27.4%), which probably affected the size differences, as males are generally smaller than females. The distribution of immature individuals was the same at all sites and did not appear to affect morphometric variability.

These results emphasise the importance of including morphometric and demographic data in conservation efforts. Continuous monitoring of *P. ferruginea* populations is crucial to identify local vulnerabilities and initiate management measures to conserve one of the most endangered endemic marine invertebrates of the Mediterranean.

From degradation to recovery: 30 years of illegal date mussel fishing impact on Apulian rocky shores

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This research evaluated the environmental consequences of the illegal harvesting of date mussels (*Lithophaga lithophaga*) along the Apulian coastline. The study primarily investigated the spatial distribution of habitat degradation, the formation of barren grounds, and the relationship with the population densities of sea urchins, specifically *Paracentrotus lividus* and *Arbacia lixula*. Between summer 2023 and winter 2024, a total of 42 locations were monitored, organized into six distinct sectors, covering an area of 59,196 m². The findings indicated a generally low impact of date mussel extraction, with a mean Damage Index (Dw) of 0.07, suggesting that recent disturbances are limited and localized. Nonetheless, sporadic instances of more recent damage were detected in both the Ionian and Adriatic Seas. The Rock Desertification Index (RDI) revealed the presence of barren grounds, particularly pronounced in the Ionian sector, where elevated sea urchin densities were recorded in the most severely affected areas. Statistical analysis demonstrated a strong association between sea urchin abundance and barren grounds, with larger individuals appearing to stabilize these degraded zones, while smaller specimens were linked to the freshly impacted areas from *L. lithophaga* exploitation. This study underlines the necessity of continuous monitoring and proactive conservation to safeguard biodiversity and the ecological balance of Mediterranean coastal environments. Additionally, it provides an updated cartographic representation of the current state of rocky coastal zones, forming a foundation for forthcoming ecological restoration initiatives.

Exploring neglected marine invertebrate fauna in Tunisian ports and marinas: NIS, rare taxa and first records.

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The introduction and spread of Non-Indigenous Species (NIS) are widely recognized as a primary global threat to native biodiversity, with variable consequences by regions and ecosystems. Maritime transport and global warming are key drivers of this process, primarily through vectors associated with international shipping. The Mediterranean Sea, hosting over 6% of all known marine species, is both a biodiversity hotspot and a major NIS introduction zone, particularly in ports and marinas. Owing to its central position in the Mediterranean basin and relatively warm waters, Tunisia represents a strategic region for NIS monitoring and dispersal dynamics, notably related either to Lessepsian species advancing westward from the eastern Levant Sea, and to taxa introduced through the Gibraltar Strait. However, comprehensive studies of NIS in Tunisian waters remain scarce, particularly for small invertebrate taxa, whose status is still poorly documented. To address this gap, eight Tunisian ports and marinas (Marina Bizerte, Sidi Bou Said, Port Yasmine Hammamet, Port el Kantaoui, Sousse port, Marina Cap Monastir, Sidi Mansour, Sfax port) were surveyed in April 2025. At each site, a standardized sample of four liters of fouling organisms was collected from artificial substrates, including concrete walls, floating pontoons, mooring lines, and buoys, using surface-operated scraping tools. Samples were sorted, organisms photographed alive, preserved in 80% ethanol, and identified via integrative taxonomic method. This survey yielded new records of NIS and rare native species within Heterobranchia (Mollusca: Gastropoda), Peracarida (Arthropoda: Crustacea), and Polychaeta (Annelida), increasing our knowledge on the marine invertebrate fauna of Tunisia, particularly on the marine invertebrate diversity and distribution patterns in port environments, paving the way for future biogeographic and comparative studies. Furthermore, filling gaps on NIS distribution across the Mediterranean is critical for achieving effective biodiversity management and conservation planning.

***Antennal sensillar* equipment in the Apoidea: morphology, diversity and proposition of a consistent terminology**

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Insect sensilla are the structural units responsible for the perception of chemical, hygro-thermal and mechanical stimuli, and they are especially abundant in the antennae. A number of studies have reported that the *Antennal sensilla* play a critical role in kin recognition, detection of natural enemies, foraging activity and mating activity. Given the wide range of ecological diversity of insects, it is not surprising that differences in antennal sensory system were reported between species with different life-history traits and within species between sexes. In the Apoidea (Hymenoptera: Aculeata), a large group including bees and stinging wasps, the morphology and distribution of *A. sensilla* have been investigated in several species, though the level of detail is very variable and quantitative analyses are rare. Moreover, entire lineages have not yet been investigated. We present here new data from a Scanning Electron Microscopy (SEM) analysis of *A. sensilla* in 14 species of bees, spanning six of the seven known bee families (Andrenidae, Halictidae, Colletidae, Apidae, Megachilidae and Melittidae), including lineages that have been particularly underrepresented in previous studies. A total of six types of *Sensilla* are present in the studied bee species: *Sensilla trichoidea* (ST, with three subtypes), *S. basiconica* (SB), *S. placodea* (SP), *S. coeloconica* (CO), *S. ampullacea* (AM) and *S. campaniformia* (CA). All these sensillar types occurred in the studied species. However, there was considerable variation in their density, which can be partly explained by body size variation. Indeed, head width (a proxy for body size) positively affected both antennal length and density of several sensillar types. Some differences among species, however, seem to be unrelated with variation in body size and may be linked to differences in life-history traits such as pollen specialization. These findings provide a foundation for more detailed investigations into the ecological pressures that may have influenced the evolution of antennal sensillar equipment in the Apoidea. Furthermore, this work has also given us the opportunity to review the currently available information on the antennal sensilla of Apoidea with the aim to address issues, such as the lack of a standardised terminology for sensillar types, which is essential for future comparative studies.

The Italian wall lizard *Podarcis siculus* (Rafinesque-Schmaltz, 1810) as a bioindicator of environmental quality in three Calabrian sites with different degrees of anthropic disturbance.

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Podarcis siculus (Rafinesque-Schmaltz, 1810) is a synanthropic species widely distributed and often used as an environmental bioindicator. This study evaluated its role as a bioindicator of the ecotoxicological quality of environments placed under different degrees of anthropization, verifying both the effectiveness of indirect methods such as leukocyte count, and of minimally invasive methods, such as the use of the tail, to analyze the different environmental conditions and quantify the levels of trace elements. Two sites were identified in the municipality of Rende (CS, Calabria): the Industrial Zone (IZ) and the Botanical Garden of the University of Calabria (BO); a third control site was located in the municipality of Fagnano Castello (CS, Calabria). From 2022 to 2024, five samplings were carried out per site on a seasonal basis, allowing a total of 250 lizards to be captured. Sex, age, morphometric measurements (SVL, TL, weight), ectoparasite load were recorded for each individual, leukocytes (basophilic, heterophilic, eosinophilic) and haemoparasites were counted. At the same time, pectoral muscles and unregenerated tails were analyzed for 30 individuals (10 per population) to evaluate their concentration of trace elements by ICP-MS spectrometry. In the ZI, individuals are larger and have a higher leukocyte load than those in BO; in both sites, females show higher leukocyte values than males. Lizards in BO have greater ectoparasite diversity and haemoparasite abundance than in the ZI; no significant difference between parasite load and leukocyte and emerged response. Regarding bioaccumulation, there were no statistically significant differences between males and females, except for copper. For the three populations, however, it emerged that for As, Mn, Fe, Cu, Mo and Ba the differences were statistically significant; elements such as As and Mo increased with increasing anthropization. Thus, the tail can actually be used as a substitute for muscle, but only for some elements of all those evaluated and cited previously, such as As, Se, Sr, Mo and Ba. *Podarcis siculus* confirms itself as an effective bioindicator for all three types of analyses carried out, capable of highlighting differences in the state of health of environments placed under different pressures. Caudal sampling represents a valid alternative to more invasive analyses.

Out of sight, out of mind? A Sicilian case study on the distribution of less charismatic species

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Addressing the biodiversity crisis requires an accurate knowledge of species distribution and their conservation status. However, our current knowledge remains largely fragmented, hampered by several well-known “biodiversity shortfalls”, including the Linnean and Wallacean ones. These knowledge gaps particularly affect the so-called neglected species, i.e., those species that are less charismatic and often excluded from research and conservation priorities, despite their diversity and potential ecological roles.

Sicily is a recognised biodiversity hotspot in the Mediterranean area with a high number of endemic species. In this context, we investigated the inland water copepod fauna in a small area of northwestern Sicily, i.e., the San Vito Peninsula, with the explicit aim of assessing species richness in a restricted sampling area, estimating expected species occurrence, and evaluating the sampling effort required for an exhaustive faunal survey.

Through extensive sampling over two years, we identified 18 taxa, i.e., approximately 42% of the freshwater copepod species known for the entire island, also finding one species previously unrecorded in Sicily. These results, obtained in such a geographically restricted area (about 165 km²), highlight the outstanding biological diversity currently overlooked, and often hidden within seemingly “minor” areas and habitats. Moreover, species accumulation curves, analysed using different asymptotic species richness estimators based on occurrence data, pointed out that the real copepod species richness of the area is expected to be considerably higher than what currently observed, suggesting that many species may have gone undetected despite the intensive sampling effort we realised.

These findings highlight the need of extending scientific surveys dealing with neglected taxa and environments. In fact, small water bodies, often overlooked in conservation agendas, actually represent biodiversity hotspots that harbour rich, unique and often vulnerable faunal communities. Therefore, it is essential to allocate sufficient resources to the systematic study of these habitats in order to close existing knowledge gaps and strengthen conservation strategies from an inclusive perspective on biological diversity.

Effects of heavy metal contamination on *Hermetia illucens* (Diptera: Stratiomyidae) physiology and development

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The larvae of the black soldier fly (BSFL), *Hermetia illucens* (Diptera: Stratiomyidae), are widely used to reduce organic waste and produce a protein-rich insect biomass which has potential applications in various industrial fields. However, contaminated substrates may expose BSFL to numerous pollutants, including persistent xenobiotics as heavy metals (HMs), which can not only negatively affect larval development and health, but also accumulate in insect tissues and organs, raising concerns about their potential transfer into BSF-derived products.

To explore the effects of HMs on insect growth, physiology, and immunity, BSFL were reared on the organic fraction of municipal solid waste supplemented with two PbCl concentrations (15 and 1000 mg/kg of diet), and their development and survival rates were monitored over the time. Additionally, markers of the cellular (hemocyte count and phagocytosis) and humoral (antimicrobial and lysozyme activities) branches of the immune system were analyzed. Finally, lead accumulation in whole larvae, midgut epithelium, and rearing residue (the so-called frass) was measured at the end of the bioconversion process.

The results revealed a concentration-dependent accumulation of lead in the larval tissue. The exposure to the highest PbCl concentration caused reduced weight and higher mortality rates, indicating the strong toxic effects of lead on BSFL. Moreover, the activation of both cellular and humoral immune responses was observed, highlighting negative impacts of heavy metal exposure on larval health.

This research offers valuable insights into how BSFL react to HMs contamination, reinforcing the evidence of lead's toxicity on this insect, and emphasizing the importance of thoroughly assessing the safety of using BSFL for the bioconversion of contaminated-substrate bioconversion for industrial applications.