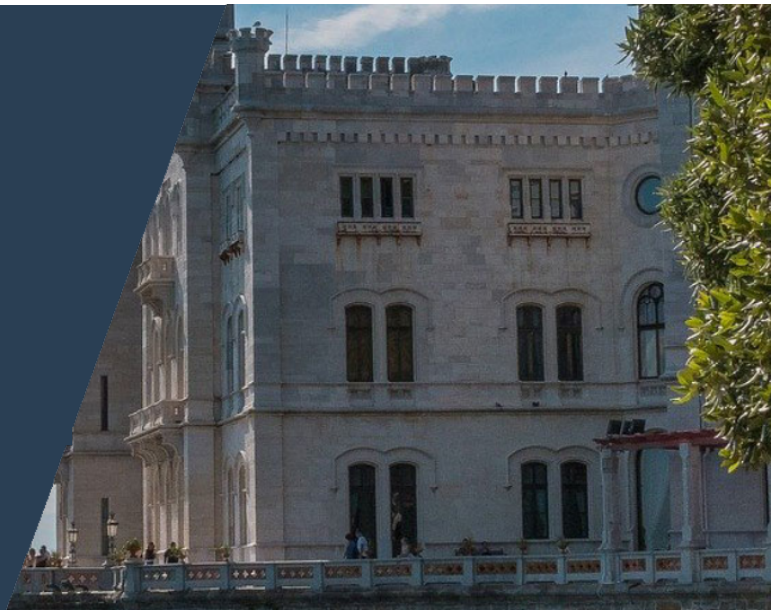
81° Congresso
Unione Zoologica Italiana

81° CONGRESSO UZI 2022



BOOK OF ABSTRACTS

20 - 23 Settembre 2022
Trieste

UNIVERSITÀ DEGLI STUDI DI TRIESTE

Dipartimento di Scienze della Vita

COMITATO SCIENTIFICO PER LA FAUNA D'ITALIA

comune di trieste

UDINE MUSEI | MUSEO FRIULANO DI STORIA NATURALE

UTET UNIVERSITÀ

ETPI

Stazione Zoologica Anton Dohrn Napoli

Book of Abstracts

81° Congresso
Unione Zoologica Italiana 2022

L'81° Congresso UZI si svolgerà a Trieste dal 20 al 23 settembre 2022 e prevede lo svolgimento di tre Simposi: “Risposte ai cambiamenti globali: omeostasi, difese interne e plasticità fenotipica”, “La ricerca *in vivo*: modelli animali emergenti, nuove competenze professionali e nuovi approcci metodologici e di comunicazione”, “Monitoraggio e gestione della biodiversità”.

Durante il Congresso, è in programma una Tavola Rotonda, dedicata ai "Ruoli tradizionali e emergenti degli zoologi: in ambito forense, museale, in stabulario, nella comunicazione e nella divulgazione".

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PROGRAMMA

20 SETTEMBRE

13.30
Apertura della Segreteria organizzativa dell'81° Congresso dell'Unione Zoologica Italiana

14.00 - 14.30
Cerimonia apertura: Rettore dell'Università di Trieste, Direttore del Dipartimento di Scienze della Vita
Presidente UZIETS, Presidente Comitato organizzatore

SIMPOSIO: Risposte ai cambiamenti globali: omeostasi, difese interne e plasticità fenotipica

14.30 - 15.15
Inaugural lecture: Bella Galil
The Suez Canal and the transformation of the Mediterranean biota

15.15 - 15.35
L'urbanizzazione determina variazioni contrastanti in tratti funzionali legati al volo e allo stress in due specie di insetti impollinatori: Bombus terrestris e B. pascuorum
NICOLA TOMMASI, EMILIANO PIOLTELLI, PAOLO BIELLA, MAURIZIO CASIRAGHI, ANDREA GALIMBERTI

15.35 - 15.55
Stress granules in ascidians: an overview
LAURA DRAGO, ALESSANDRO PENNATI, UTE ROTHBÄCHER, GIANFRANCO SANTOVITO, AND LORIANO BALLARIN

15.55 - 16.15
Living on the edge of a warming environment: the case of the olm Proteus anguinus (caudata, proteidae) and other stygobionts exploiting spring habitats
RAOUL MANENTI, BENEDETTA BARZAGHI, VERONICA ZAMPIERI, FILIPPOMARIA CASSARINO, GIULIA PACINOTTI, EDGARDO MAURI, MARCO RESTAINO, ROBERTA PENNATI, MATTIA FALASCHI, & GENTILE FRANCESCO FICETOLA

16.15 - 16.35
Pausa caffè

16.35 - 16.55
Entomofauna fluviale e cambiamento globale: temi, problemi e possibili scenari
STEFANO FENOGLIO

16.55 - 17.15
Le lucertole insulari, un modello di plasticità adattativa come risposta ai cambiamenti globali
DOMENICO FULGIONE

17.15 - 17.35
Pomacea canaliculata immunity and environmental stress response rely on common metabolic features
AGNESE FERRARI, FRANCESCO DEMETRIO LOFARO, ALICE MONTANARI, GIULIA BERGAMINI, FEDERICA BORALDI, DAVIDE MALAGOLI

17.35 - 17.55
Consequences of increasing temperature on life cicle and gene expression in an antarctic species of tardigrades
ILARIA GIOVANNINI, CHIARA MANFRIN, TIZIANA ALTIERO, SAMUELE GRECO, JOEL VINCENZI, ROBERTO GUIDETTI, PIERO GIULIANINI, LORENA REBECCHI

17.55 - 18.15
Studio preliminare sulle differenze fenotipiche che manifestano le popolazioni di proteo (Proteus anguinus) in grotta e quelle trovate in ambiente superficiale
BENEDETTA BARZAGHI, GENTILE FRANCESCO FICETOLA, EDGARDO MAURI, MARCO RESTAINO, RAOUL MANENTI

21 SETTEMBRE

SIMPOSIO: La ricerca in vivo: modelli animali emergenti, nuove competenze professionali e nuovi approcci metodologici e di comunicazione

09.00 - 09.30
Dall'attivitá di singolo neurone al comportamento: zebrafish come modello in vivo per studiare la funzione del sistema nervoso
Giada Cellot

09.30 - 09.50
Dendritic spine morphology and density of granule cells in the olfactory bulb of Anguilla anguilla (l. 1758)
CINZIA PODDA, RICCARDO PORCEDDU, GIOVANNA MULAS, FRANCESCO PALMAS, LUCA PICCI, CLAUDIA SCANO, SATURNINO SPIGA, ANDREA SABATINI

09.50 - 10.10
Sensory system, behaviour and differential gene expression: new insights from common octopus (Octopus vulgaris)
VALERIA MASELLI, KRISHANTHA SAMEERA DE ZOYSA HEETHAKA, BRUNO PINTO, GIANLUCA POLESE, MICHAEL J. KUBA, TAMAR GUTNICK, MARIANGELA NORCIA, ANNA DI COSMO

10.10 - 10.30
Pausa caffè

10.30 - 11.00
Are invertebrates proper model organisms? What comparative research on protostomia can teach us about evolution
Michael J. Kuba

11.00 - 11.20
Alien metabolites vs. Synthetic compounds: a comparative study on Mytilus galloprovincialis
TANIA RUSSO, FRANCESCA COPPOLA, CARMELA CELENTANO, ERNESTO MOLLO, MARIANNA CARBONE, ANNA DI COSMO, ROSA FREITAS, GIANLUCA POLESE

11.20 - 11.40
Tardigradi: un modello animale emergente?
ROBERTO GUIDETTI, ILARIA GIOVANNINI, MICHELE CESARI, ALTIERO TIZIANA, LORENA REBECCHI

11.40 - 12.00
The Crinoid antedon mediterranea as an emerging model organism in developmental and evolutionary biology

SILVIA MERCURIO, GIACOMO GATTONI, BENEDETTA BARZAGHI, GIORGIO SCARÌ, MICHELA SUGNI, ELIA BENITO-GUTIÉRREZ, ROBERTA PENNATI

12.00 - 12.20
Il baco da seta: un modello alternativo per lo screening di molecole ad attivitá antimicrobica
AURORA MONTALI, FRANCESCA BERINI, ALESSIO SAVIANE, SILVIA CAPPELLOZZA, FLAVIA MARINELLI, GIANLUCA TETTAMANTI

12.20 - 14.00
Pausa pranzo

14.00 - 14.20
Formazione e aggiornamento nell'ambito dei modelli animali emergenti
Giuliano Grignaschi

14.20 - 14.40
Pulcini geometri, gamberi che generalizzano, polpi che imitano, api che astraggono, vermi che contestualizzano: infiniti (insospettabili) modelli di studio per la cognizione
CINZIA CHIANDETTI, PAOLA ZARATTINI

14.40 - 15.10
The Erasmus+ project "International zoo conservation & management" – outputs, challenges and opportunities
Stefano Vaglio

15.10 - 16.00
Presentazioni finaliste premio UTET

16.00 - 16.30
Pausa caffè

16.30 - 18.00
Assemblea ordinaria UZI

22 SETTEMBRE

08.50 - 09.50
Presentazioni finalisti premio UZI

09.50 - 10.05
Characterization of two neurodegeneration pathways in the colonial tunicate Botryllus schlosseri
CHIARA ANSELMI, LUCIA MANNI

10.05 - 10.20
Assessing anhydrobiotic performance - the diversity and evolution of desiccation resistance in tardigrada (macrobiotidae)
MATTEO VECCHI, LORENA REBECCHI, ŁUKASZ MICHALCZYK, DANIEL STEC, SARA CALHIM

10.20 - 10.40
Pausa caffè

SIMPOSIO: Monitoraggio e gestione della biodiversità

10.40 - 11.00
Aliens in the dark: a synthesis of knowledge on non-nativ species in subterranean ecosystems
GIUSEPPE NICOLOSI, STEFANO MAMMOLA, LAURA VERBRUGGE, MARCO ISAIA

11.00 - 11.20
Benthic foraminifera for the monitoring of tourist marinas
ANNALISA FRANZO, MAURO CAFFAU, ELENA MARROCCHINO, MARIA GRAZIA PALETTA, MATTEO BAZZARO, TAMARA CIBIC

11.20 - 11.40
A whole-genome sequencing approach to study and protect italian endangered endemic species: the case of the Ponza grayling Hipparchia sbordonii
MARCO GERDOL, MBARSID RACAKU, MARCO SOLLITTO, ALBERTO PALLAVICINI, ANDREA BENAZZO, LORENA ANCONA, SEBASTIANO FAVA, ALESSIO IANNUCCI, CLAUDIO CIOFI, VALERIO SBORDONI, EMILIANO TRUCCHI, GIORGIO BERTORELLE

11.40 - 12.00
An "Oasis" of biodiversity in deep waters: the use of remoted operated vehicles (rovs) as a tool to assess the ecological role of Atrina fragilis (pennant, 1777)
ALESSANDRA DAVANZO, SIMONA IANNUCCI, ROCCO AURIEMMA

12.00 - 12.20
Avian biodiversity within the mediterranean basin: first insights from two passerine taxa
MARTINA NASUELLI, LUCA ILAHIANE, GIOVANNI BOANO, MARCO CUCCO, ANDREA GALIMBERTI, MARCO PAVIA, EMILIANO PIOLTELLI, GARY VOELKER, IRENE PELLEGRINO

12.20 - 12.40
Detenction of endangered mediterranean trout Salmo trutta L.,1758 complex (osteichthyes: salmonidae) using non-harmful visual methods
FRANCESCO PALMAS, PAOLO CASULA, CINZIA PODDA, FRANCESCO CURRELI, ANDREA SABATINI

12.40 - 13.00
First insight into meiofauna inhabiting the shallow CO_2-seeps around castello aragonese (Ischia, Italy)
ELISA BALDRIGHI, MATTHIAS LÓPEZ CORREA, LUIGIA DONNARUMMA, MARIA CRISTINA GAMBI, FEDERICA FERRIGNO, NICOLA SIMONCINI, LUCA APPOLLONI, ROBERTO SANDULLI, ANNALISA FRANZO, ELEONORA GRASSI, ADELE COCOZZA DI MONTANARA, LINDA CATANI, FEDERICA SEMPRUCCI

13.00 - 14.20
Pausa pranzo

14.20 - 14.40
From ecological niche models to species distribution models: a framework to capitalize on data and support biodiversity management
MATTIA IANNELLA, MAURIZIO BIONDI

14.40 - 15.00
Ghost nets as substrate for marine invertebrates
SARA IGNOTO, EMANUELE MANCINI, BIANCA MARIA LOMBARDO, GAETANO DI MARIA, SABRINA LO BRUTTO, FRANCESCO TIRALONGO

15.00 - 15.20
How can different taxonomic structure and functional traits of nematodes reflect different human pressures in “heavily modified water bodies”?
ANNALISA FRANZO, ELISA BALDRIGHI, ELEONORA GRASSI, MATEJA GREGO, MARIA BALSAMO, MARCO BASILI, LINDA CATANI, ADELE COCOZZA DI MONTANARA, ROBERTO SANDULLI, FEDERICA SEMPRUCCI

15.20 - 15.40
Il progetto “Life Imagine Umbria”: coleotteri e lepidotteri della direttiva habitat
MATTEO PALLOTTINI, GIANANDREA LA PORTA, ANTONIA CONCETTA ELIA, FABIO MANELI, ROBERTO VENANZONI, ENZO GORETTI

15.40 - 16.00
Impact of Hermodice carunculata (Pallas, 1766) (polychaeta: amphinomidae) on artisanal fishery: a case study from the mediterranean sea
FRANCESCO TIRALONGO, SARA IGNOTO, SEBASTIANO MARINO, RICCARDO MARTELLUCCI, BIANCA MARIA LOMBARDO, EMANUELE MANCINI

16.00 - 16.20
Local extinction? Not yet. Conservation plan for a protected buttefly species
IRENE PICCINI AND SIMONA BONELLI

16.20 - 16.40
Pausa caffè

16.40 - 17.00
Monitoring the fauna through the eDNA analysis
CHIARA MANFRIN, MASSIMO ZANETTI, MARIJAN GOVEDIC, PIERO G. GIULIANINI, ALBERTO PALLAVICINI

17.00 - 17.20
Never stop exploring: novelties and perspectives on italian odonates
ANDREA GALIMBERTI, FAUSTO RAMAZZOTTI, GIACOMO ASSANDRI, DAVIDE MAGGIONI, MAURIZIO CASIRAGHI

17.20 - 17.40
Utilizzo di un approccio integrato per l'identificazione degli apoidei urbani: strutturazioni inattese e casi criptici nella citta' di Milano
FAUSTO RAMAZZOTTI, MARCO CATANIA, ANDREA GALIMBERTI, PAOLO BIELLA

17.40 - 18.00
"Plastic eater" sea slugs (aplysiidae, mollusca) in the mar piccolo of Taranto
GIULIA FURFARO, MARCELLA D'ELIA, MICHELE SOLCA, EGIDIO TRAINITO, STEFANO PIRAINO, GENUARIO BELMONTE

20.30
Cena sociale e consegna premi UTET
Antica Trattoria Suban Via Emilio Comici, 2 - 34128 Trieste - www.suban.it

23 SETTEMBRE

09.00 - 10.30
Tavola rotonda: Ruoli tradizionali e emergenti degli zoologi
Cristina Giacoma (Presidente UZI): Introduzione su parchi zoologici, acquari e iniziative UZI per i giovani
Cristiano Piani (Responsabile dell'unità di staff Comunicazione e Relazioni esterne dell'Università di Trieste): Startup e aziende emergenti
Ambiente forense: Luciano Garofano, generale in congedo dell'Arma dei Carabinieri, comandante del R.I.S. di Parma dal 1995 fino al 2009
Ambiente museale: Fausto Barbagli, Presidente ANMS - Associazione Nazionale Musei Scientifici
In stabulario: Paola Zarattini, responsabile dello stabulario dell'Università di Trieste
Nella comunicazione e nella divulgazione: Telmo Pievani, filosofo della biologia ed esperto di teoria dell'evoluzione

10.30 - 10.50
Pausa caffè

10.50 - 11.30
Tavola rotonda: Ruoli tradizionali e emergenti degli zoologi

11.30 - 12.00
Consegna premi UZI

UTET UNIVERSITÀ

LA DIETA DELLA LEPRE EUROPEA (LEPUS EUROPAEUS) IN UN AMBIENTE ESTREMO
MARIA BUGLIONE

EFFETTO DELLA DENSITÀ E DELLA DISPOSIZIONE SPAZIALE DEI NIDI SUL TASSO DI COLONIZZAZIONE, PARASSITISMO E SEX RATIO NELL'APE SOLITARIA OSMIA CORNUTA
ANDREA FERRARI, CARLO POLIDORI

SMELL BY TOUCH IN COMMON OCTOPUS (OCTOPUS VULGARIS)
KRISHANTHA SAMEERA DE ZOYSA HEETHAKA, VALERIA MASELLI1, BRUNO PINTO, GIANLUCA POLESE, MICHAEL J. KUBA, TAMAR GUTNICK, ANNA DI COSMO

BEE RESPONSIVE TO PM: GENETIC AND BEHAVIORAL HONEYBEE RESPONSE TO PARTICULAR MATTER
IRENE PICCINI

PIANO DI GESTIONE DELLA PESCA DEL POLPO (OCTOPUS VULGARIS) NELLE AREE MARINE PROTETTE DELLA REGIONE CAMPANIA
BRUNO PINTO, VALERIA MASELLI, PIERPAOLO CONSOLI, ELEONORA RIVECCIO, RICCARDO MINICHINO, KRISHANTHA SAMEERA DE ZOYSA HEETHAKA, GIANLUCA POLESE, ANNA DI COSMO

SVILUPPO DI UN METODO DI TRACCIAMENTO DEI PRODOTTI ITTICI MEDIANTE TECNICHE DI METAGENOMICA NGS
ALESSANDRO RUSSO

A SCUOLA CON I TARDIGRADI: UN MODELLO MICROSCOPICO PER L'EDUCAZIONE SCIENTIFICA
JOEL VINCENZI

Inaugural lecture

BELLA S. GALIL

Steinhardt Museum of Natural History, Tel Aviv University, Israel

THE SUEZ CANAL AND THE TRANSFORMATION OF THE MEDITERRANEAN BIOTA

Invasive alien species have become a concern in marine coastal ecosystems worldwide, but no where more so than in the Mediterranean Sea, where over 1000 multicellular introduced species have been recorded. This is an invasion of calamitous proportions, resulting in the main from ever expanding invasion pathways and a warming sea.

A pathway unique to the Mediterranean is the Suez Canal. There is no other vector of marine bioinvasions that delivers as high a propagule supply for so long to the same locale. For over a century Erythraean aliens were confined to the Levantine upper shelf, and largely ignored by scientists, conservationists, policy makers and managers. However, in the 1980s -following major enlargement of the canal, increasing seawater temperature and marine heat wave duration, severety, frequency - the rapid spread and injurious impacts of some Erythraean aliens helped raise awareness of the invasion. Recent surveys documented Erythraean aliens at the westernmost Mediterranean and beyond the shelf edge to depth of 250 m, suggesting that thermal niche estimations based on the species' native range likely underestimated their thermal tolerance, and thus their potentail bathymetric and geographic expansion.

Bearing in mind that climatic models predict that the Mediterranean Sea will be markedly affected by warming and heat waves, native stenothermal biota unable to shift their range to deeper and/or colder water will likely endure increasing stress and demographic attrition, presaging degradation of native communities on the Mediterranean shelf, perhaps even the upper slope. The synergic and additive effects of warming and the greater volume of trans-isthmian propagules may increase the plausibility of displacement by Erythraean aliens.

The invasion has long posed a challenge to the environmental ethics and policies of the Mediterranean countries.

SIMPOSIO

Risposte ai cambiamenti globali: omeostasi, difese interne e plasticità fenotipica

NICOLA TOMMASI1,2, EMILIANO PIOLTELLI1, PAOLO BIELLA1, MAURIZIO CASIRAGHI1, ANDREA GALIMBERTI1,2

1 ZooplantLab, Dipartimento di Biotecnologie e Bioscienze, Università Milano Bicocca, Milano, Italia
2 INFN Sezione Di Milano Bicocca, Milano, Italia

L'URBANIZZAZIONE DETERMINA VARIAZIONI CONTRASTANTI IN TRATTI FUNZIONALI LEGATI AL VOLO E ALLO STRESS IN DUE SPECIE DI INSETTI IMPOLLINATORI: *BOMBUS TERRESTRIS* E *B. PASCUORUM*

Gli effetti indotti dall'espansione delle aree urbane sulle comunità di insetti impollinatori sono ancora poco compresi. Le peculiari condizioni dell'ambiente urbano possono indurre variazioni in importanti tratti funzionali legati alle prestazioni di volo e indicatori di stress subito durante lo sviluppo larvale di questi insetti come la dimensione corporea e l'asimmetria di forma e dimensione delle ali. Le conseguenze di queste variazioni possono essere rilevanti in termini di salute degli impollinatori ed efficienza del servizio ecosistemico di impollinazione. In questo studio abbiamo valutato l'influenza di fattori di stress legati all'urbanizzazione sulla dimensione corporea e asimmetria di forma e dimensione delle ali in due specie di bombi: *B. terrestris* (Linnaeus, 1758) e *B. pascuorum* (Scopoli, 1763). Per farlo abbiamo campionato 348 individui (169 *B. terrestris* 179 *B. pascuorum*) in 37 siti distribuiti lungo un gradiente di urbanizzazione comprensivo della città metropolitana di Milano e città limitrofe. In particolare, abbiamo valutato gli effetti della composizione e configurazione dell'uso del suolo, della temperatura dell'aria, degli inquinanti atmosferici e della disponibilità di risorse fiorali sulla variazione dei tratti funzionali indagati. Le due specie investigate hanno mostrato variazioni idiosincratiche dei tratti funzionali investigati in riposta all'urbanizzazione. Le temperature più elevate dell'ambiente urbano sono risultate correlate a dimensioni corporee più piccole in *B. pascuorum* e ad una maggiore asimmetria nella dimensione delle ali in *B.terrestris.* La disponibilità di risorse fiorali, inoltre, è risultata correlata ad una maggiore dimensione corporea in *B. terrestris* ed a una minore asimmetria dimensionale delle ali in *B. pascuorum.* Le variazioni specie specifiche che abbiamo evidenziato in questo studio hanno dimostrato che i fattori di stress ambientale associati all'urbanizzazione hanno un impatto negativo sui tratti funzionali associati alle prestazioni di volo e alla stabilità dello sviluppo di questi due impollinatori ampiamente rappresentati in ambiente urbano. I risultati ottenuti supportano l'adozione di interventi di gestione del territorio utili a mitigare l'impatto negativo dell'urbanizzazione su questi importanti insetti impollinatori.

LAURA DRAGO[1], ALESSANDRO PENNATI[2], UTE ROTHBÄCHER[2], GIANFRANCO SANTOVITO[1], AND LORIANO BALLARIN[1]

1 University of Padova, Department of Biology, Via Ugo Bassi 58/B, 35131 Padova, Italy
2 University of Innsbruck, Department of Zoology, Innrain 52, 6020 Innsbruck, Austria

STRESS GRANULES IN ASCIDIANS: AN OVERVIEW

Anthropic pressure is causing changes, sometimes irreversible, to ecosystems. A clear example of damage to ecosystems relate to human activities is pollution, especially by metals which accumulate in coastal waters and sediments. Metals, in turn, can induce oxidative stress on aquatic invertebrates leading to the weakening of their defenses, including immune responses.
Stress granules (SGs) are stalled translational initiation complexes preserving mRNAs for anti-stress proteins and so regulating stress responses. This is possible thanks to the presence of mRNA-binding proteins such as TIA-1 related nucleolysin (TIAR), considered an important core component of SGs. They disassemble in the presence of an acute stress so to unlock the translation of mRNAs into anti-stress proteins.
Until now, very few works have been devoted to study SGs in invertebrates, especially in marine species.
By using TIAR as SG marker we explored the possible roles of these foci in the solitary ascidian *Ciona robusta* and in the colonial ascidian *Botryllus schlosseri*, both collected in the Lagoon of Venice.
We started with an evaluation of their involvement in the responses to oxidative stress induced by metals, such as Cu, Zn, Fe and Cd, the impact of which on marine ecosystems is well documented. We carried out gene expression studies by qRT-PCR and in-situ hybridisation. To validate the hypothesis of SG posttranscriptional control, we used specific anti-TIAR antibody in immunocytochemistry and immunohistochemistry and visualized their subcellular localization in immunocytes through transmission electron microscopy. In addition, the importance of SGs in the regulation of stress responses during embryonic development was investigated in *C. robusta*, using a construct for reporter gene (LacZ) expression, containing the promoter region for TIAR.
B. schlosseri, due to its peculiar capability to reproduce sexually and asexually, was chosen to investigate the SG role during non-embryonic development, with microinjection experiments of the anti-TIAR antibody. The latter experiments suggest that SGs are involved not only in the control of stress, for example the one related to the high extent of efferocytosis occurring during the weekly generation change, but also in modulating the cell proliferation required for the full development of new adult individuals.

RAOUL MANENTI[1,2], BENEDETTA BARZAGHI1,2, VERONICA ZAMPIERI[1], FILIPPOMARIA CASSARINO[1], GIULIA PACINOTTI[1], EDGARDO MAURI[3], MARCO RESTAINO[4], ROBERTA PENNATI1, MATTIA FALASCHI[1], & GENTILE FRANCESCO FICETOLA1

1 Dipartimento di Scienze e Politiche ambientali, Università degli Studi di Milano, Via Celoria, 10, 20133, Milan, Italy
2 Laboratorio di Biologia Sotterranea "Enrico Pezzoli", Parco Regionale del Monte Barro, 23851, Galbiate, Italy
3 Speleovivarium Erwin Pichl, Via Guido Reni, 2/C, 34123, Trieste, Italy
4 *Società Adriatica di Speleologia, Via Domenico Rossetti, 59/a, 34141, Trieste, Italy*

LIVING ON THE EDGE OF A WARMING ENVIRONMENT: THE CASE OF THE OLM *PROTEUS ANGUINUS* (CAUDATA, PROTEIDAE) AND OTHER STYGOBIONTS EXPLOITING SPRING HABITATS

Groundwater-dwelling animals are considered as strongly vulnerable to climatic changes and environmental perturbations being adapted to the stable conditions of aquifer. For the same reason they are also often regarded as dead-end points when it comes to their evolution. However, in the past different observations of *Proteus anguinus* typical troglomorphic populations have been reported for spring habitats along with a community rich in stygobionts. Spring habitats provide a potential source of selective pressures for groundwater-dwelling animals together with potential energy to adapt, but are more exposed to global warming consequences than subterranean environments. The aim of this work is to point out the non-random active use of surface habitats by stygobionts, understanding if plastic and adaptive responses to spring ecotones may occur. Since 2020 we started multiple day and night surveys (ranging from 8 to 20 surveys for each habitat) of P. anguinus and other five taxa of stygobionts in 60 springs and 11 caves. Each spring and cave habitat monitored has been characterised by respect to multiple abiotic and biotic features. Additionally, cave and spring populations of Troglocaris sp. were tested to determine whether they showed any behavioural response to different light stimuli as potential adaptive/plastic response to the constraints posed by surface sunny environments. Five stygobiont taxa out of six (including the olm) have been regularly recorded in spring habitats. Through RDA analysis we assessed that spring features significantly explain occurrence and distribution of the whole stygobiont community. Most common taxa in springs were Troglocaris sp. and P. anguinus; for both detection probability in springs and caves was similar. Olms seem to prefer temporary springs without predator fish, while Troglocaris sp. exploit deeper springs. Preliminary results of the behavioral experiments show no susceptibility to light neither for spring nor cave populations of Troglocaris sp. During 2022 due to extended drought period 60% of the springs dried up including the 48% of springs that were stable across 2021 and 2020. We suggest that epigean habitats and borders with surface may have an underestimated importance for animals adapted to subterranean environments including the olm. Further studies focusing on the hypogean community which actively exploits spring habitats are underway, with the aim of determining the diel occupation of these complex ecotones.

STEFANO FENOGLIO

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ENTOMOFAUNA FLUVIALE E CAMBIAMENTO GLOBALE: TEMI, PROBLEMI E POSSIBILI SCENARI

Nell'ambito delle conseguenze del cambiamento globale, il crollo della biodiversità faunistica nei sistemi fluviali è un fenomeno drammatico, che supera per dimensione quanto sta accadendo negli ambienti marini o terrestri. In particolare, gli insetti che popolano le acque correnti rappresentano una porzione tanto importante quanto spesso poco considerata della biodiversità locale: gli insetti dominano le acque interne, dove costituiscono oltre il 60 % delle specie animali conosciute e occupano praticamente ogni nicchia disponibile. Circa la metà degli Ordini di cui è composta la classe Insecta comprende specie acquatiche o semiacquatiche e addirittura in cinque ordini tutti i taxa sono strettamente acquatici. Nei fiumi gli insetti rappresentano la componente principale della biomassa e biodiversità animale, costituendo un campo di ricerca di estremo e attuale interesse dal punto di vista evolutivo (tra gli insetti fluviali annoveriamo i più antichi Pterygota), biogeografico, biomolecolare, applicativo e conservazionistico.
Purtroppo, per la fragilità dei loro ambienti di fronte al cambiamento globale, gli insetti fluviali versano in una situazione drammatica. In questa comunicazione sono sintetizzati i risultati di numerose ricerche scientifiche, realizzate in gran parte nei fiumi alpini ed appenninici del nostro Paese. Tra le componenti del cambiamento che esercitano l'impatto maggiore ricordiamo:
- Alterazioni idrologiche. L'incremento e la diffusione delle secche provocano un crollo della biodiversità faunistica in un numero crescente di fiumi. Vengono qui presentati risultati in cui si nota come la recente intermittenza idrologica stia portando alla scomparsa di taxa semivoltini, come Plecoptera Systellognatha ed alla contrazione di taxa specializzati come Ephemeroptera Heptageniidae.
- Innalzamento della temperatura. Il riscaldamento delle acque ha importanti ripercussioni, sia dirette sia indirette, su numerosi gruppi di insetti acquatici, legate ad esempio a: crollo dell'ossigeno disciolto, riduzione degli habitat disponibili, alterazione della fenologia e dei cicli vitali di alcune specie.
Un'altra componente del cambiamento globale, legata alla diffusione di specie invasive, è sicuramente meno evidente nell'ambito dell'entomologia acquatica: anche in questo caso vengono presentati e discussi alcuni lavori. In conclusione, è estremamente probabile che l'attuale scenario di rapido cambiamento ambientale porterà in breve tempo ad una profonda alterazione delle entomocenosi fluviali, con la banalizzazione delle comunità, la scomparsa o contrazione delle specie longeve e stenoterme fredde e l'espansione di quelle euriterme, tolleranti e a rapida crescita.

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LE LUCERTOLE INSULARI, UN MODELLO DI PLASTICITÀ ADATTATIVA COME RISPOSTA AI CAMBIAMENTI GLOBALI

Le variazioni globali sono una minaccia alla biodiversità, influenzando la pressione di selezione sulle popolazioni naturali che spesso non esprimono risposte adeguate. Le popolazioni naturali hanno tre vie per rispondere alle nuove sfide della selezione naturale: spostarsi, adattarsi o estinguersi. Spostarsi significa seguire modelli di habitat tracking che non sempre possono essere attivati per limitazioni fenotipiche o spaziali. La capacità di adattare il fenotipo, per essere efficace, deve verificarsi in poche generazioni e se non fa riferimento a variazioni ereditabili, è identificata come plasticità. Le capacità plastiche possono salvare dall'estinzione, laddove non è possibile spostarsi. Indagare questo tema prevede modelli adeguati che possano evidenziare, in brevi tempi e spazi limitati, le possibili variazioni adattative. I lacertidi insulari sono modelli ideali per indagare la variazione espressa e la limitazione spaziale imposta dalle piccole isole. In questo contributo evidenzio come la specie *Podarcis siculus* (Rafinesque 1810) sia capace di affrontare le sfide imposte dai cambiamenti ambientali, tipiche delle piccole isole, ma spesso esacerbate dai cambiamenti globali, attraverso una plasticità che coinvolge la morfologia, la riproduzione, il comportamento e la composizione microbiotica. Oltre alle osservazioni e misurazioni su diverse popolazioni, ho comparato i dati con la sequenze del trascrittoma annotato, evidenziando come le variazioni fenotipiche possono essere imputate a espressioni geniche differenziali piuttosto che differenze genetiche ereditabili. Queste agiscono in tempi relativamente brevi, come ho misurato per le popolazioni del faraglione di Capri e per l'isolotto di Licosa. Particolarmente, interessanti risultano le variazioni trovate a livello del microbiota e le capacità di variazione di questo in ragione di cambiamenti ambientali inattesi. Questi studi, oltre a una importante indicazione sulle modalità di evoluzione delle popolazioni insulari, rappresentano la base per meglio collocare la diversità di questa specie in una adeguata strategia di conservazione.

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POMACEA CANALICULATA IMMUNITY AND ENVIRONMENTAL STRESS RESPONSE RELY ON COMMON METABOLIC FEATURES

The South American *Pomacea canaliculata* (*Pc*) is a polyphagous and highly invasive freshwater snail, that is affecting agriculture and public health, especially in Asia. European Food Safety Authority indicated Italy and other EU countries as areas of establishment for the genus *Pomacea*, prompting for sustainable prevention and/or control of its distribution. Aiming at the immune system as the target for *Pc* biocontrol, we tested the effects of Nemaslug®, a commercial product based on the nematode *Phasmarhabditis hermafrodita.* We exposed the snails to 3 concentrations (0.17, 1.7 [recommended dose] and 17 g/L), each tested at 3 temperatures (18, 25 and 30 °C), representing the optimum and the limits for the growth of *Pc*. The biopesticide had a dose-dependent effect on mortality and food consumption at 18 and 25 °C, killing respectively 30 and 14% of the animals, and strongly diminishing snail feeding rate. At 30 °C, the molluscicide was ineffective. Histological analysis showed the presence of nematodes after incubation at 18 and 25 °C in the anterior kidney (AK) and gills (*i.e.*, organs also involved in the immune response of *Pc*), respectively. Thus, we investigated these two organs for the expression of *Pc*-BPI (bactericidal/permeability increasing protein), a conserved immune-related soluble mediator. Quantitative PCR showed a decrease in *Pc-bpi* expression in AK (at 18°C) and in gills (at 25 °C), suggesting that temperatures affect the multi-organ-based immune response of the snails. Further analysis on *Pc* immune and stress response was performed at proteomic level on the ampulla, a saccular organ located immediately downstream the heart, that store nitrogen-based compounds and contribute to the oxidative stress control in specific physiological situations, like the arousal from aestivation or hibernation. The exposure to nematodes (1,7 g/L at 25 °C) is associated with the up-regulation of the antioxidant molecules, crucial for detoxification, and of heat shock proteins. In addition, we observed a significant increase of several enzymes involved in metabolic pathways suggesting a rise up of energy demand.
Our data suggested a suitable candidate for the sustainable control of *Pc* in non-original countries. Furthermore, our findings shed light on the molecular bases of the adaptability of *Pc*, that is able to exploit physiological mechanisms useful for facing environmental challenges, like absence of water or low temperature, also to contrast immune challenges.

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CONSEQUENCES OF INCREASING TEMPERATURE ON LIFE CICLE AND GENE EXPRESSION IN AN ANTARCTIC SPECIES OF TARDIGRADES

The temperature in Antarctica is predicted to increase by 0.34°C per decade (BRACEGIRDLE et al., 2008) as a consequence of global climate changes. This could affect Antarctic organisms, especially animals with limited dispersal capability such as tardigrades. Since tardigrades are sensitive to prolonged periods of high temperature exposure (REBECCHI et al., 2009; GIOVANNINI et al., 2018; NEVES et al., 2020), they may be vulnerable to the potential effects of global warming. This study aims to investigate the molecular mechanisms involved in the adaptation of the tardigrade *Acutuncus antarcticus* to increasing temperature, to identify the genes responsible to heat stress resistance, and to analyse its life cycle at 5°C evidencing the consequences on life history traits due to the increasing temperature. *A. antarcticus* specimens collected at Victoria Land (Antarctica) from a temporary freshwater pond were individually reared at 5°C from birth to death (12h/12h, L/D) with algae *Chlorococcum* sp. as food source, according to ALTIERO et al. (2015). Data on life history traits of two successive generations were collected: lifespan, age at first oviposition, total eggs per life span, eggs per clutch, time interval among oviposition, egg hatching time, and egg hatching percentage. Moreover, groups of adult animals were exposed to gradually increasing temperatures (from 5°C to 10°C, 15°C, and 20°C) and kept at each temperature for 1 day (short-term exposure) or 15 days (long-term exposure). A reference transcriptome for *A. antarcticus* was *de novo* assembled and compared with other transcriptomes at different temperatures and conditions to find differentially expressed genes. Specimens of the first generation reared at 5°C lived 541.3±60.7 days, laying 78.8±12.7 eggs, which hatched in 21.9±4.8 days. First oviposition occurred at 33.9±8.0 days. Females laid eggs once a fortnight, and the oviposition number per life span was 29.8±3.5. Similar results were obtained for reared specimens belonging to the second generation. Comparing the data of *A. antarcticus* reared at 5°C with those at 15°C obtained previously (ALTIERO et al., 2015), a significant effect due to the temperature increase was evidenced on the life history traits of this species. Indeed, animals at 15°C have a reduced lifespan, lay less eggs, but reach sexual maturity earlier, and their eggs hatch faster. The reference transcriptome is made up by 95511 contigs with a mean GC content of 45.82%. Sixty-seven genes resulted differentially expressed in the short-term exposure to heat stress. In particular, the expression of transcripts for Me31b (RNA-binding protein), protein-argonaute1, protein-Gawky, Ubiquinol-Cytochrome C Reductase and Pupal cuticle protein C1 was down-regulated, while that for ND3, ND4, ND5, SAHS1 and CAHS1 was up-regulated. Moreover, the Cox-2 was down-regulated until 10°C, but up-regulated after 15°C. These data indicate an increase in expression of genes related to oxidative metabolism. As regards the long-term exposure, 14 genes resulted differentially expressed, of which two annotated: F-box/WD repeat-containing protein was up-regulated, while Von Willebrand factor A domain-containing protein was down-regulated. The first transcriptome from the genus *Acutuncus* and the transcriptomic response of *A. antarcticus* to increasing temperature provide new insights into molecular mechanisms of tardigrades.

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STUDIO PRELIMINARE SULLE DIFFERENZE FENOTIPICHE CHE MANIFESTANO LE POPOLAZIONI DI PROTEO (*Proteus anguinus*) IN GROTTA E QUELLE TROVATE IN AMBIENTE SUPERFICIALE

Il proteo (*Proteus anguinus*) è da sempre considerato come uno degli animali più rappresentativi della fauna troglobia. Tuttavia, è anche segnalato in alcuni habitat epigei del Friuli Venezia Giulia (Nord-Est Italia). *P. anguinus* presenta alcuni adattamenti all'ambiente sotterraneo, compresa la depigmentazione. Studi passati hanno tuttavia dimostrato che la pelle di *P. anguinus* contiene un'elevata quantità di riboflavina che può dare un aspetto giallastro ad alcuni individui e che l'esposizione alla luce può determinare la melanizzazione della pelle. L'obiettivo di questo studio è verificare se ci sono differenze nella colorazione del proteo e nella quantità di melanina presente nella loro pelle tra gli individui trovati nelle grotte rispetto a quelli campionati nell'ambiente esterno. Durante il 2021, 28 protei, 18 provenienti da grotte e 10 da siti epigei, sono stati fotografati in condizioni standard con una scala colorimetrica di riferimento e, successivamente, su carta millimetrata. Le immagini sono state elaborate utilizzando i software Photoshop e R. Per analizzare la melanina presente nella pelle, sono stati esportati 2 mm^2 dalla coda di ogni individuo. Ogni campione è stato analizzato utilizzando uno spettrofotometro.

I risultati mostrano una differenza significativa nella presenza di melanina tra i protei campionati in grotta e quelli ritrovati in habitat epigeo. I protei provenienti da siti esterni presentano livelli di melanina maggiori, inoltre mostrano una colorazione gialla più marcata. Questi risultati forniscono una prima prova delle differenze osservate tra le popolazioni di protei che vivono nelle grotte e quelli trovati in superficie.

SIMPOSIO

La ricerca *in vivo*: modelli animali emergenti, nuove competenze professionali e nuovi approcci metodologici e di comunicazione

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FROM SINGLE NEURON TO BEHAVIOR: ZEBRAFISH AS AN IN VIVO MODEL FOR STUDYING THE FUNCTION OF THE NERVOUS SYSTEM

During the last decade, zebrafish has been emerging as an outstanding model in the field of neuroscience. Due to its unique features, such as the quick, extra-uterine development of transparent embryos, early stage zebrafish is amenable to a broad range of *in vivo* methodologies, including patch clamp electrophysiology, confocal imaging and behavioral analysis. In addition, the accessibility of the zebrafish spinal cord coupled with a detailed knowledge of spinal neuron anatomy and locomotor function makes this an attractive system for exploring the correlation between synaptic function and behavior.

By combining a nanomaterials based approach for manipulating synaptic activity with a high-throughput analysis of early-stage zebrafish locomotor behavior, we demonstrated that graphene oxide nano-flakes with increasing degrees of thermal reduction could impact differently on the electrical activity of spinal cord, resulting in a modulation of zebrafish swimming performance, that was dependent on the chemo-physical properties of the nanomaterial. These findings validated zebrafish as an *in vivo* model to study neuronal function from synaptic activity to the correlated emerging behavior.

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DENDRITIC SPINE MORPHOLOGY AND DENSITY OF GRANULE CELLS IN THE OLFACTORY BULB OF *ANGUILLA ANGUILLA* (L. 1758)

Olfaction could be considered as pivotal process involved the orientation and migration behaviour of fish. Olfaction signals are managed by the olfactory bulb which shows properties of synaptic plasticity in granular cells (GCs) whose dendritic spines are capable to change their morphology and structural stability after environmental odours enrichment or removal.
The GCs' dendritic spines density and morphology were analysed post-mortem using a cytomorphological Golgi's staining approach along life stages of the catadromous European eel *Anguilla anguilla* (L. 1758). According to the morphology of the head and neck, dendritic spines have been classified as mushroom (M), long thin (LT), stubby (S), and filopodia (F). Dendritic spines density decreased progressively from juvenile migrants to no-migrant stages, to increase again in the adult migrant stage. The number of dendritic spines is comparable between glass eels and silver eels, showing quantitative and qualitative adaptations during migratory phases. Relationships were found between spines' density and the catadromous life cycle of this species rather than with aging. This trend could be explained by the decrease found in M and LT spines in non-migrating trophic phases. Spines' density increased again in silver eels due to the increment of M, LT, and S spines. These results demonstrated the presence of great activity of synaptic development during the migratory phases, which regresses in the trophic phases, but which must necessarily be recreated by tracing the migratory memory of the routes previously travelled in the juvenile phases. Because of its ancient phylogenetic attribution to the Elopomorph teleosts group, and its complex life cycle that requires high physiological plasticity under several conditions (adaptation to several aquatic environments, marine, freshwater, brackish; sexual maturation, growing or fasting phases), *A. anguilla* could be recommended as model species to study olfaction in catadromous fish species. The development of dendritic spines in GCs of the olfactory bulb could be considered an index of synaptic plasticity involved in the modulation of olfactory stimuli that play a role in the memory, orientation, and migration mechanisms of *A. anguilla*.

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SENSORY SYSTEM, BEHAVIOUR AND DIFFERENTIAL GENE EXPRESSION: NEW INSIGHTS FROM COMMON OCTOPUS (*Octopus vulgaris*)

The Common Octopus (*Octopus vulgaris*) is well known for its cognitive abilities, morphological innovations and amazing camouflage. The octopus's central nervous system (CNS) is built around the esophagus forming a set of lobes with differentiated functions that, beside their learning and memory capabilities, support complex visual and limb-based tactile systems.
Including the extended neuropils, an octopus CNS may contain as 200 million neurons or more, almost the same amount as several vertebrates and even some mammals. Their CNS is the most complex nervous system in invertebrates. The genome of octopus (like many coleoid cephalopods) is highly rearranged relatively to other mollusks, with cephalopod-specific innovations. In particular, hallmarks are extensive A-to-I mRNA editing (tissue-dependent) and some gene families expansions. However, most of our knowledge on octopus behavior, neurophysiology, neuroanatomy and, recently, on genomic and transcriptomic novelties, have come from single field non-integrated approaches. In our approach, to better understand the complexity of the behavior of the animal, we use a combination of methods: ethological, functional-morphology, and molecular, focusing our research on one of the most unique sensory systems of the Common Octopus, the suckers. Here we explored their chemoreceptive and photoreceptive abilities, deepening the understanding of both molecular structural identification and function.
Additionally, we analyzed the differential gene expression in specific brain areas in adult animals under different cognitive tasks, using one of the more critical expanded gene families in the brain, the protocadherin genes (PCDHs) and other genes considered adult neurogenesis markers. As we deepen the knowledge of octopus behavior we gain essential information to help improve protocols for captive care, in the light of the ethical principles of the 3Rs on animal welfare (replacement, reduction, refinement). Thus, we show that the common octopus is a great animal model for performing both basic and applied research.

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ARE INVERTEBRATES PROPER MODEL ORGANISMS?

WHAT COMPARATIVE RESEARCH ON PROTOSTOMIA CAN TEACH US ABOUT EVOLUTION

The study of behaviour, neurobiology, morphology and molecular biology seeks to reveal how underlying body and brain organization and function enable an animal to survive and adapt to its environment. In this regard, there are several areas where an invertebrate study can benefit a wider scientific community. Recently, researchers have begun to examine how phylogenetically diverse organisms solve similar tasks in similar or different ways to better understand general evolutionary trends how to adapt to the habitat and challenges. Invertebrates often process the sensory world in a way that is very different from that of the vertebrate with whom they share an environment. This makes them an important subject for comparative studies in psychophysiology, perception, eco-morphology and learning. Understanding the evolutionary relationship and general procedures in their biology we will be able to use invertebrates instead of vertebrates to study important biological topics.

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ALIEN METABOLITES VS. SYNTHETIC COMPOUNDS: A COMPARATIVE STUDY ON *MYTILUS GALLOPROVINCIALIS*

Human activities along coastlines have prepared a fertile ground for alien species invasions. Among them, the green alga *Caulerpa cylindracea*, one of the most perturbing invasive species in the Mediterranean Sea, contains bioactive metabolites that could affect native communities and biodiversity. Studies have revealed that some *Caulerpa* metabolites could be of interest for pharmacological applications. In particular, the bisindolic alkaloid caulerpin (CAU) has shown anti-tumour, anti-microbial and anti-inflammatory properties, possibly due to its activity as an agonist of peroxisome proliferator-activated receptors (PPARs), nuclear transcription factors that regulate development, metabolism and tumorigenesis.
Aquatic environments are also affected by the increase of pharmaceutical pollutants. Among them, fenofibrate (FFB), a synthetic drug used to treat hyperlipoproteinaemias in humans, was detected in trace concentrations in groundwater, surface water, and drinking water, but at concentrations ranging between 0.08 ng/L up to 0.2 µg/L in effluents from wastewater treatment plants. Studies have shown that FFB reduces the lipid contents, including eicosapentaenoic and docosahexaenoic acids in aquatic organisms, through the selective activation of PPARα, (a PPAR isoform with which CAU also interacts). To date, there are no comparative studies about the biological effects of CAU and FFB on marine organisms.
Here, we have compared the effects of CAU and FFB through biochemical and histopathological analysis on a relevant bioindicator species, the mussel *Mytilus galloprovincialis*. The mussels were fed three times per week, for 28 days, with food enriched with CAU or FFB. In the end, biomarkers and histological alterations were evaluated. The results revealed metabolic capacity impairments, higher cellular damage, and changes in acetylcholinesterase activity in mussels fed with FFB-enriched food. In contrast, higher antioxidant defences were recorded in mussels fed with CAU-enriched food. Histopathological indices showed higher morphological alterations in gills and digestive tubules of mussels fed with FFB. These findings indicate that CAU produces no significant harmful effects compared to FFB. In conclusion, the results obtained pave the way for a possible valorisation of the huge invasive biomass from *C. cylindracea* in the Mediterranean Sea as a source of a PPAR agonist to overcome some of the side effects that can occur with the use of fibrates.

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TARDIGRADI: UN MODELLO ANIMALE EMERGENTE?

Grazie allo sviluppo delle tecnologie e dei metodi di analisi dati, diversi gruppi animali che fino a qualche decennio fa erano trascurati stanno assumendo il ruolo di modelli animali per studi focalizzati su specifiche tematiche.
Uno di questi gruppi è rappresentato dai tardigradi. Grazie alla loro facile reperibilità (colonizzano svariati substrati di tutti i continenti), alla possibilità di essere allevabili in laboratorio e alle loro peculiarità biologiche, i tardigradi vengono utilizzati per diversi studi. I tardigradi hanno evoluto la possibilità di attuare la criptobiosi, cioè la capacità di seccarsi o congelarsi insieme al substrato, sospendendo completamente il metabolismo per poi riattivarlo quando le condizioni ambientali ritornano idonee, cioè quando l'acqua torna ad essere disponibile in forma liquida. In condizione di criptobiosi, gli animali sono in grado di resistere a condizioni chimico-fisiche estreme (es. temperatura, radiazioni, pressione, sostanze chimiche). Durante la criptobiosi i tardigradi devono resistere a diversi stress come la produzione di ROS, iperosmosi, e la rottura di macromolecole (es. RNA e DNA) e delle membrane cellulari per cui hanno evoluto strategie adattative per proteggere molecole, cellule e tessuti. I tardigradi vengono quindi utilizzati per lo studio dei meccanismi fisiologici, metabolici e molecolari coinvolti nella criptobiosi per potere applicare le conoscenze in svariati campi quali, ad esempio, preservazione di cellule, organi o organismi in biobanche, incrementare la resistenza a stress osmotici/siccità, ridurre gli effetti dell'invecchiamento controllando i fenomeni di ossidazione e rottura di macromolecole, curare/prevenire malattie preservando e/o riparando i danni al DNA, o ridurre i danni da radiazioni (es. radioterapia, lunghi viaggi spaziali). In questi contesti i tardigradi sono diventati modelli animali nello studio della biologia spaziale e nell'individuazione di molecole bioprotrettrici. I tardigradi potrebbero diventare un modello animale anche per altri studi in quanto sono a disposizione anche due genomi di specie diverse, e numerosi transcrittomi di specie mantenute a diverse condizioni fisiologiche. Nuove proteine coinvolte nella criptobiosi ed esclusive dei tardigradi sono state individuate e la loro trasfezione in cellule umane ha evidenziato un'azione protettiva nei confronti di stress osmotici e radioattivi. I tardigradi quindi si stanno evidenziando sempre più come modelli animali per la scoperta di molecole bioattive per approcci biotecnologici.

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THE CRINOID *ANTEDON MEDITERRANEA* AS AN EMERGING MODEL ORGANISM IN DEVELOPMENTAL AND EVOLUTIONARY BIOLOGY

Crinoids are basally-branching echinoderms, the only living representatives of the Pelmatozoa clade (WRIGHT et al., 2017). Their phylogenetic position and their many ancestral features make these animals particularly interesting in evo-devo studies of deuterostomes. However, the developmental biology of these animals has remained elusive for decades, due to difficulties in collecting wild specimens and culturing both adults and embryos in laboratory. Indeed, most crinoids live below 20 m depth and their embryos develop attached to female arms until doliolaria larvae hatch. These are lecithotrophic barrel-shaped larvae which swim only for few hours looking for the proper substrate on which to metamorphose into pentacrinoids (BARBAGLIO et al., 2012a; MERCURIO et al., 2019). *Antedon mediterranea* is a Mediterranean stalkless crinoid that has been traditionally exploited in regenerative studies (ALLIEVI et al., 2022). Moreover, extensive research has been performed on its reproductive biology and physiology (BARBAGLIO et al., 2008; BARBAGLIO et al., 2012b). Starting from these data and taking advantage of the accessibility of this species, we are developing a novel evo-devo model system that could help answering many unsolved questions of deuterostome evolution.

First, we set up *in vitro* embryo culture: embryos detached from female arms were successfully cultured in petri dishes, allowing the investigation of embryogenesis from zygote to hatching larva stage. Optimization of culture conditions has led to a hatching rate of up to 90%. Then, timing of the developmental stages was precisely determined and each phase was described in details by means of light and confocal microscopy. General morphological features of doliolaria larvae and pentacrinoids were described; particularly nervous system organization and distribution of neural populations in both stages has been characterized. We optimized standard molecular techniques, such as whole mount *in situ* hybridization and immunolocalization, and transcriptomic and genomic resources are being produced.

Overall, we provide a novel model organism with numerous applications in developmental and evolutionary studies. In particular, our research will contribute to shed light on deuterostome nervous system evolution: comparative analysis and molecular approaches will provide the basis for understanding nervous system in echinoderm and its ancestry in deuterostome lineages.

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IL BACO DA SETA: UN MODELLO ALTERNATIVO PER LO SCREENING DI MOLECOLE AD ATTIVITÁ ANTIMICROBICA

L'uso smodato ed inappropriato degli antibiotici ha contribuito negli ultimi decenni ad un incremento dei microrganismi resistenti, pertanto la ricerca e lo sviluppo di nuove molecole ad attività antimicrobica rappresentano una delle principali sfide per la comunità scientifica. Lo screening di nuovi antibiotici prevede test preclinici effettuati su mammiferi ma, a causa dei problemi etici e degli alti costi della sperimentazione condotta su questi animali, diventa sempre più urgente la necessità di individuare modelli di infezione alternativi tra gli invertebrati. I Lepidotteri e, in particolare il baco da seta, *Bombyx mori*, si sono rivelati dei validi modelli in grado di affiancare, ed in parte ridurre, l'utilizzo dei mammiferi in questo contesto.
Nel presente studio è stato valutato l'utilizzo di larve di *B. mori* come modello di infezione per testare l'efficacia di antibiotici glicopeptidici (vancomicina, teicoplanina e dalbavancina) contro patogeni Gram-positivi appartenenti al genere *Staphylococcus* (*Staphylococcus aureus* e *Staphylococcus epidermidis*). Le larve sono state allevate a 37 °C per riprodurre le condizioni fisiologiche umane e l'andamento dell'infezione è stato monitorato tramite l'analisi della sopravvivenza delle larve, la misurazione della carica batterica nell'emolinfa e la valutazione di marcatori immunitari. In particolare, l'attivazione della risposta cellulare e di quella umorale è stata valutata mediante l'analisi della vitalità degli emociti, l'attività del sistema della profenolossidasi e del lisozima, e l'espressione di peptidi antimicrobici. I dati ottenuti mostrano come, per entrambi i batteri, tutti e tre gli antibiotici siano efficaci nel contenere l'infezione, riducendo la mortalità degli insetti e bloccando l'attivazione del sistema immunitario.
Questi risultati dimostrano come il baco da seta possa essere introdotto nei test preclinici ed utilizzato come modello di infezione per uno screening rapido e economico di nuove molecole antimicrobiche contro infezioni da stafilococco.

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FORMAZIONE E AGGIORNAMENTO NELL'AMBITO DEI MODELLI ANIMALI EMERGENTI?

Con l'entrata in vigore, nell'agosto del 2021, del Decreto Formazione nell'ambito della normativa inerente la sperimentazione animale (D.LVo 26/2014), si pone in maniera sempre più pressante la problematica relativa alla formazione e all'aggiornamento di tutto il personale coinvolto (operatori, membri scientifici dell'OPBA etc) sulle 3R ed in particolare sul Replacement totale o parziale. Il Replacement infatti è la prima delle 3R (reduction e refinement sono le altre due) che deve essere presa in considerazione e applicata ogni qualvolta sia possibile, anche solo ad una parte del progetto sperimentale. Ma chi può formare e aggiornare il mondo della ricerca, composto non solo da biologi ma anche da farmacisti, biotecnologi, biotecnologi, medici etc. sulla possibilità di utilizzare specie che, ad oggi, si ritiene abbiano un minor livello di senzienza e quindi possono rappresentare un tipico caso di partial replacement? Il contributo degli zoologi in questo ambito è quindi, a mio avviso, essenziale non solo nella formazione dei ricercatori che approcciano i modelli animali ma anche nel loro continuo aggiornamento. Non va peraltro dimenticato il fatto che anche i membri dell'Organismo Preposto al Benessere Animale che la normativa italiana ha chiamato ad esprimere un parere motivato sulla validità tecnico/scientifica dei progetti di ricerca e sulla possibilità di applicazione delle 3R (membri scientifici, responsabili del benessere animale, veterinari) hanno bisogno di continuo aggiornamento su questo tema per poter garantire di svolgere al meglio il proprio ruolo. Troppe volte infatti, ancora oggi, nel modulo per la richiesta di attivazione di un progetto (All. VI° del D,Lvo 26/2014) nel paragrafo relativo alla sostituzione (Punto 20.1) si trova scritto "Questo studio prevede l'utilizzo del topo che rappresenta il modello animale a più basso sviluppo neurologico che va incontro a patologia ……. con caratteristiche simili a quella umana" senza una vera discussione del possibile utilizzo di altri modelli preclinici.

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PULCINI GEOMETRI, GAMBERI CHE GENERALIZZANO, POLPI CHE IMITANO, API CHE ASTRAGGONO, VERMI CHE CONTESTUALIZZANO: INFINITI (INSOSPETTABILI) MODELLI DI STUDIO PER LA COGNIZIONE

Al mondo, in questo istante, ci sono circa 22 miliardi di polli domestici (*Gallus gallus domesticus*) ma la loro presenza è invisibile, come invisibili sono, ai più, le loro spiccate capacità cognitive. Eppure, molti dati sono stati accumulati nei più disparati ambiti della cognizione, dalla percezione (ZANFORLIN, 1981) all'apprendimento e memoria (ROSE, 2000), dalla lateralizzazione cerebrale (ROGERS *et al*., 2013) alla risoluzione di problemi di natura geometrica (CHIANDETTI and VALLORTIGARA, 2008), fisica (CHIANDETTI and VALLORTIGARA, 2011), numerica (RUGANI, 2017), e logica (DAISLEY *et al*., 2021). Il pulcino offre svariati vantaggi per rispondere a quesiti da sempre centrali anche nel pensiero filosofico, come ad esempio se certe funzioni siano innate o dipendano dall'esperienza (CHIANDETTI *et al*., 2015), dimostrandosi quindi un eclettico modello d'indagine al crocevia tra saperi umanistici e scienze dure. Insospettabili sono anche le abilità mentali di ordine superiore evidenziate attraverso rigorosi esperimenti scientifici in polpi (*Octopus vulgaris*, ad es. l'apprendimento per osservazione, FIORITO and SCOTTO, 1992), api (*Apis mellifera*, ad es. l'astrazione di concetti, AVARGUES-WEBER *et al*., 2012), nematodi (*Caenorhabditis elegans*, per una rassegna si veda RANKIN, 2004) e moltissimi altri invertebrati che si rendono di volta in volta interessanti per lo studio di specifiche questioni, dalla semplificazione o differente implementazione del sistema nervoso per comprendere la basi neurali della cognizione, agli aspetti legati ai vantaggi adattivi. A questo proposito, i gamberi della Louisiana (*Procambarus clarkii*) che, a differenza dei già citati pulcini sono invece sempre più presenti sulla terra e anche ben visibili a causa della loro capacità invasiva, sembrano possedere una spiccata abilità di generalizzazione che potrebbe supportare il loro successo adattivo (DISSEGNA *et al*., 2020). *Proprio nell'indagine comparata* si apre la strada per la scoperta di specifici adattamenti cognitivi che, oltre a quelli biologici, dotano alcune specie di una estrema flessibilità rispetto ad altre. *E solo un'indagine comparata* può portare alla risposta alle 4 domande di Tinbergen (1963) supportando la completa comprensione dei fenomeni, a differenti livelli di analisi (BATESON and LALAND, 2013), che i ricercatori di svariati ambiti d'indagine investigano con disparati approcci e metodologie.

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THE ERASMUS+ PROJECT 'INTERNATIONAL ZOO CONSERVATION AND MANAGEMENT' – OUTPUTS, CHALLENGES AND OPPORTUNITIES

There is a wealth of experience and knowledge in the zoo sector based on large numbers of institutions of various sizes and individuals with diverse backgrounds. Zoo sector employees often enter on relatively low qualifications or are employed on the basis of degree-level programmes, yet whatever the background over time they accumulate extensive experience and knowledge that can be developed further through the application of the critical evaluation and wider subject contextualisation that both professional development and academic progression bring. Whilst competency-based training is commonplace in the sector in maintaining the currency of practical skills, there is a lack of similar opportunities at higher levels of study.

Both the higher education and the zoo sectors are inherently international with personal, institutional, sectorial and professional networks extending well beyond individual country boundaries. The overarching aim of the Erasmus+ 'International Zoo Conservation and Management' (InterZoo) project was therefore to bring these two sectors together to develop a structured training programme that blends the applied/experiential-based knowledge of the zoo sector with the rigour of higher education-based pedagogy and research. The outcome was a defined training curriculum incorporating three specific training modules that target: international zoo welfare and management; international management of zoo-based conservation; and ethical and legal issues with international zoo management.

Ultimately the aspiration is to develop the modules into a formal international post-graduate qualification, thus giving the project outcomes and impacts beyond the time limit of the project itself.

Presentazioni giovani zoologi all'estero

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CHARACTERIZATION OF TWO NEURODEGENERATION PATHWAYS IN THE COLONIAL TUNICATE *BOTRYLLUS SCHLOSSERI*

With elderly populations among the fastest-growing demographics of many nations, understanding mechanisms responsible for neurodegenerative diseases is an important challenge for our society. Studying the evolutionary origin of these traits in a model organisms like *Botryllus schlosseri*, a marine colonial tunicate of an evolutionary clade considered to be a sister group of vertebrates, can provide new insight into the cellular and molecular processes mediating pathways of neurodegenerations. During asexual reproduction, the colony exhibits the periodical regression of adult individuals that, in a phase called takeover, are substituted by their buds. Within a single colony, three generations coexist: the adult zooids, their (primary) buds and small (secondary) buds on the latter. When takeover occurs, the adult zooids degenerate, the primary buds become the new generation of adult zooids, and the secondary buds become primary buds and produce a new generation of small buds. Although wild colonies only live for 1-2 years, in laboratory conditions they can persist for >20 years. Morphological, behavioral, and transcriptomic analyses show that the nervous system degenerates in adult zooids during both the takeover (rapid neurodegeneration) and in aging (slow neurodegeneration) colonies. In both processes, adult zooids exhibit a progressive reduction in neuron number, accompanied by an increasing number of immunocytes (phagocytes and morula cells) close to and infiltrating the brain. The reduction in the number of neurons is associated with impaired behavioral performances, as zooids in takeover and older zooids (~20 year of age) are less sensitive to mechanical stimuli than active zooids and zooids belonging to younger colonies. Rapid neurodegeneration during takeover is characterized by changes in the expression of 73 mammalian homologous genes associated with neurodegenerative diseases, such as Alzheimer's, Parkinson's, Huntington's diseases and frontal dementia. Moreover, older colonies exhibit changes in the expression of 148 such genes, 35 which are differentially expressed across both the rapid and the slow neurodegeneration processes. In conclusion, *B. schlosseri*, with its two different yet apparently related neurodegenerative processes, represents a model organism capable of providing insight concerning the mechanisms balancing neural regeneration and degeneration that are crucial to the onset of neurodegenerative diseases.

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ASSESSING ANHYDROBIOTIC PERFORMANCE - THE DIVERSITY AND EVOLUTION OF DESICCATION RESISTANCE IN TARDIGRADA (MACROBIOTIDAE).

Introduction. Anhydrobiosis (the ability to survive environmentally induced loss of body water) is the most widespread and studied form of cryptobiosis. Unfortunately, not only do laboratory protocols vary considerably between research groups and across study organisms, but there are few phylogenetic comparative studies on how this trait evolves. Our aims are to develope new analysis methods for data from anhydrobiosis experiments and to address how this trait evolves using a model system for the study of desiccation tolerance: tardigrades. **Methods.** We developed a new approach based on the Bayesian fitting of a cumulative exponential distribution function on anhydrobiotic recovery curve of the proportion of motile individuals against monitoring time points. This method estimates both the overall survival (where the curve flattens) and recovery speed (the rate of increase of the proportion of motile individuals with time). These two measures can also be combined into a single anhydrobiotic index as the area under the fitted recovery curve. We analyzed both published data on the effect of different chemicals on anhydrobiotic performances and newly produced data. For the newly produced data, we estimated the anhydrobiotic index for over 20 Macrobiotidae populations under a standardized desiccation protocol. These values were then analyzed in a phylogenetic context. **Results.** Based on the reanalysis of published data, we first demonstrate that our method not only can estimate reliable anhydrobiotic performance measures, but also does so for any combination of anhydrobiotic protocol, study species, and experimental setup and provides an intuitive visualization of the estimated parameters. Secondly, the phylogenetic comparative study highlighted a considerable variability in anhydrobiotic performance, even between closely related species of Macrobiotidae. The three measures of anhydrobiotic performance (overall survival, recovery speed and anhydrobiotic index) show weak phylogenetic signal (the best fitting model is white noise model, which assumes data come from a single normal distribution with no covariance structure among species), suggesting a fast evolution of anhydrobiotic performances in response to environmental pressures. **Conclusions.** Our approach provides three distinct measures of anhydrobiotic performance. These measures are objective, reliable, and have both biologically meaningful interpretation and wide applicability. The fine-scale variability observed within this single tardigrade family (Macrobiotidae) is suggestive of different responses to desiccation in closely-related species. Therefore, the application of our method in a comparative approach can improve our understanding of variation in anhydrobiotic performance.

SIMPOSIO

Monitoraggio e gestione della biodiversità

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ALIENS IN THE DARK: A SYNTHESIS OF KNOWLEDGE ON NON-NATIVE SPECIES IN SUBTERRANEAN ECOSYSTEMS

Invasive alien species are a serious threat to native species and ecosystems. Despite global efforts to address the challenge, their number is rapidly increasing worldwide. The effect of biological invasions may be difficult to quantify and the magnitude of the impact may significantly vary across different ecosystem types. Furthermore, some habitats may be less prone to biological invasions than others due to their abiotic and biotic characteristics and only invaders possessing traits closely related to the native organism may be more successful than others. Subterranean ecosystems are quintessential examples of habitats with strong environmental filters, selecting for convergent adaptations in species that have successfully adapted to life in darkness. Despite caves being quitless considered among the most fragile ecosystems on the planet, the true effect of the presence of alien species is still unknown, and in-depth studies are intensely needed. The number of records of alien species in subterranean ecosystems has increased in the last decades, but literature on the subject remains scarce and largely fragmented. We here provide the first global systematic literature survey to synthesize the available evidence base on alien species in subterranean ecosystems. The review is supported by a detailed database summarising available literature on the topic, aiming at filling gaps in the potential distribution and spread of alien invertebrate species in subterranean habitats, laying the foundations for future management practices and interventions. Our results have yielded information on the presence of 250 subterranean alien species, pronominally invertebrates, belonging to 18 different classes, mostly insects and arachnids. Most species were reported in terrestrial subterranean habitats from all continents except Antarctica. Palearctic and Nearctic bioregions represented the main source of alien species. The main routes of dispersions are linked to commercial activities (26.8% of species). Furthermore, negative impacts have been documented for a small number of subterranean alien species (27.2%), mostly related to increased competition with native species. Only for a limited number of subterranean aliens (5.2%) management strategies are in place, but the effectiveness of these interventions has rarely been quantified. Approximately half of the species in our database can be considered established in subterranean habitats. According to our results, the presence of suitable traits grants access to the stringent environmental filter posed by the subterranean environments, facilitating the establishment of the new habitat. We recommend future studies towards a deeper understanding of invasiveness into subterranean habitats, raising public and the scientific community awareness of preserving these fragile ecosystems.

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BENTHIC FORAMINIFERA FOR THE MONITORING OF TOURIST MARINAS

Leisure boating is a growing tourist sector that provides noticeable incomes for coastal and insular economies. The other side of the coin is represented by the detrimental impacts exerted on marine environment by some of the activities associated to leisure boating (i.e. careening, cleaning, refueling, etc.), especially inside the several small marinas that dotted the coastline. Among these activities, the hull cleaning causes the dispersion in the marine environment of even noticeable amounts of antifouling residues. Since these compounds contain biocidal metals (Pb, Cr, Zn and Cu), the sediments nearby the boathouse areas can be severely polluted, especially inside the marinas with a sheltered shape and a low water renewal. In order to support the authorities for an effective monitoring of these maritime spaces, the feasibility of benthic foraminifera as biological indicators was investigated in three Mediterranean tourist ports. The small size, rapid turnover, high abundance and diversity of these organisms might minimize the sampling effort leading to a rapid and cost-effective surveillance. In each marina the stations were located from the boathouse areas towards the port entrance along a confinement gradient.
Besides foraminifera abundance, vertical distribution and species composition, we calculated and compared several foraminiferal indices that differ for the concepts on which they rely: indices based (1) on morphological alterations, (2) on diversity and (3) on the dichotomy 'sensitive-tolerant species (e.g. foram-AMBI).
High concentrations of biocidal metals were measured in the sediments nearby the boathouse areas, confirming the careening activity as one of the main source of metal pollution. Foraminifera clearly responded to this contamination with scarcely numerous and poorly diverse assemblages at these stations. The highest biodiversity was observed at intermediate metal levels and seemed to be influenced by the presence of submerged vegetation that likely provides additional ecological niches. Diversity indices and foram-AMBI gave apparently contradictory results since one of the least biodiverse assemblage was observed at the lowest metal concentrations. Notwithstanding, at this site the dominance of *A. parkinsoniana,* a well-known sensitive species, suggested a sort of complementarity between the two kinds of indices that therefore deserve to be synoptically considered for an effective monitoring of tourist marinas.

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A WHOLE-GENOME SEQUENCING APPROACH TO STUDY AND PROTECT ITALIAN ENDANGERED ENDEMIC SPECIES: THE CASE OF THE PONZA GRAYLING *HIPPARCHIA SBORDONII*

EndemixIT is a project funded within the frame of the PRIN program, which aims at obtaining high quality and annotated reference genomes for five Italian endangered endemics. These resources will be used as reference to map inter-individual sequence variation data, reconstructing demographic trajectories and estimate the accumulation of deleterious mutations that may increase the susceptibility to extinction. Ultimately, the data generated by EndemixIT may help in predicting the effects of possible genetic rescue plans, promoting the use of genomics in conservation biology. The Ponza grayling *Hipparchia sbordonii*, endemic to the Pontine archipelago, has an area of occupancy lower than 500 km^2, whose quality has been constantly declining due to an increased anthropic impact and the progressive abandoning of traditional agricultural practices. Due to the decline in the number of individuals observed over the past few decades, *H. sbordonii* is listed as an endangered species by IUCN. Using a combination between second- and third-generation sequencing approaches, we generated a high quality, near-chromosome scale reference genome assembly for this species. The combined use of four different de novo genome assembly algorithms, paired with several rounds of polishing and scaffolding, allowed to select the assembly produced by Canu as the reference for a further manual refinement, based on completeness and contiguity metrics. This step exploited the availability of chromosome-scale assemblies for other Satyrinae to create high-resolution synteny maps, allowing to resolve several major assembly gaps and to discard several small scaffolds likely deriving from alternate haplotypes. The final assembly included 389Mb sequence, with just 36 scaffolds (the expected number of chromosomes was 29), 98.7% completeness and very little evidence duplication (0.3%) and fragmentation (0.2%), based on BUSCO metrics. The annotation process, supported by RNA-seq data, carried out with the BRAKER2 pipeline, led to the annotation of 16,345 protein-coding genes, a value in line with other Satyrinae, further confirming the high quality of this resource.
The analysis of resequencing data from historical samples and from mainland congeneric species will help to better assess the risk of extinction of this species, also allowing an improved planning of genetic rescue strategies, which may be needed in the future.

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AN "OASIS" OF BIODIVERSITY IN DEEP WATERS: THE USE OF REMOTED OPERATED VEHICLES (ROVs) AS A TOOL TO ASSESS THE ECOLOGICAL ROLE OF *ATRINA FRAGILIS* (PENNANT, 1777).

Most marine environments still lack comprehensive habitat assessments, despite they are subjected to increasing threats. Therefore, the development of new technologies, like cost-effective Remotely operated vehicles (ROVs) have been providing an opportunity to carry out ecological surveys, solving some constraints related to the restricted accessibility to some environments (i.e dive time and depth). Moreover, this method permits to obtain a huge quantity of images and video, creates permanent records, facilitates resamplings (RIEGL, 2001; LAM, 2006) and hence is widely used for long-term monitoring activities, in particular for protected areas and threatened species, like the fan mussel *Atrina fragilis* (PENNANT, 1777) (SOLANDT, 2003). This Atlantic-Mediterranean species is the second largest bivalve living in the Adriatic Sea, where inhabits deep pelitic sandy bottoms (25-50 m depth), often severely affected by trawling, especially rapido trawling (HALL-SPENCER and FROGLIA, 1999). *A. fragilis* plays an important ecological role as ecosystem engineer, providing a hard substrate for sessile species and fishery targets, thus representing an "oasis" of biodiversity in a predominantly sedimentary biotope (HALL- SPENCER and FROGLIA, 1999). Currently, little is known about the ecology of *A. fragilis* and, as far as we know, there are no data for the Mediterranean Sea on the epibiontic community that lives on its shell. Therefore, this work is intended to be a preliminary study to describe the associated fauna inhabiting the shell of *A. fragilis*. Images of 40 live specimens of the fan mussel were randomly selected after the analysis of videos and HD images obtained during a four-year (2018-2021) monitoring plan with ROVs in an area of the northern Adriatic Sea. The study area, with a mean depth of 29 m, was characterised by pelitic-sandy sediments with scattered biogenic outcrops locally known as "trezze or tegnùe". The assessment of associated fauna was performed on a single valve, considering both sessile and vagile organisms, taxonomic identification was carried out at the lowest possible level. In addition, the number of species and percent coverage were estimated. A total of about 36 taxa were identified, most of which were sessile (encrusting or massive sponges, ascidians, cnidarians and bryozoans). As regard the vagile fauna, few species were observed, but in high abundances. From initial observations, it appeared that only a few specimens of *A. fragilis* were found living in proximity to the outcrops, while aggregations were observed far from them. Moreover, specimens that lived farther away and isolated from the tegnùe, were more colonized than the nearest ones. These results confirm the hypothesis of the importance of the ecological role of *A. fragilis* as an ecosystem engineer in sedimentary bottoms. In addition, Remotely Operated Vehicles (ROVs) have proved to be a useful non-destructive tool for this first attempt to describe the fauna associated with threatened species such as the fan mussel *A. fragilis*, that inhabit deep bottoms, that are normally difficult to reach and study.

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AVIAN BIODIVERSITY WITHIN THE MEDITERRANEAN BASIN: FIRST INSIGHTS FROM TWO PASSERINE TAXA

The Mediterranean Basin is known to be a hot spot of biodiversity for many vertebrate taxonomic groups, with birds numbering more than 370 breeding species. Although avian alpha diversity is well known, the genetic structure of many Mediterranean bird taxa still lacks a proper characterization across all, or some portion, of their distributional range. For this reason, research focused on clarifying taxonomic uncertainties, identifying polytypic species and geographic variation, and understanding the processes shaping biodiversity in time and space is urgently needed. We selected two species, *Lanius senator* and *Curruca melanocephala*, aiming to deepen our understanding of their biogeography and genetic diversity. The breeding range of both species is circum-Mediterranean, they are polytypic with three (*L. senator*) and four (*C. melanocephala)* accepted subspecies (GILL *et al.,* 2022). *Lanius senator* is a long-distance migrator whose breeding populations declining at a fast rate, whereas *C. melanocephala* is a sedentary or partially migrating species with expanding populations (KELLER *et al*., 2020). We analyzed a set of mitochondrial and nuclear markers from individuals sampled across each of the two species' breeding range. Our results highlighted a significant difference in patterns, when compared to existing information on the distribution of subspecies. For *L. senator,* we found two well supported clades: one clade including all individuals from Armenia, Iraq and Iran attributable to the subspecies *L. s. niloticus*, and another one including all other individuals. We did not identify a clade that supports *L. s. badius* a subspecies described for the Balearic Islands and Sardinia, and we could not confirm the exclusive presence of *L. s. niloticus* in Israel, where we found all the individuals included in the second clade, except one. Results for *C. melanocephala* highlighted three main clades, as well as several widely distributed haplotypes indicating generally low geographic variation across the species' range. However, individuals from Israel clustered in a single clade, supporting the differentiation of the subspecies *C. m. momus*. A second clade includes some individuals from Morocco and El Hierro (Canary Islands), which can be attributed to the subspecies *C. m. valverdei*. Lastly, in the Canary Island, where the subspecies *C. m. leucogastra* occurs, we found the coexistence of three clades which confirms a complex genetic differentiation pattern in this archipelago (DIETZEN *et al*., 2008). Phylogeographical analyses of each of these two species highlight gene flow among populations and a complex geographical structure. Further studies should be applying a finer approach by analysing genome wide SNPs variation and an integrative approach by adding morphological, bioacoustics and ethological data to genetic data to clarify the processes of differentiation, the directions and amount of gene flow, the relationships among populations and the morphological characteristics of the taxa. In conclusion, our results provide reliable evidence that there is still much to discover about the genetic pattern and processes of differentiation between avian species and subspecies distributed around the Mediterranean and on it's islands. Furthermore, investigation in this field is strongly needed to properly develop and implement effective policy and conservation measures.

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DETENCTION OF ENDANGERED MEDITERRANEAN TROUT *SALMO TRUTTA L.,1758 COMPLEX* (OSTEICHTHYES: SALMONIDAE) USING NON-HARMFUL VISUAL METHODS

Monitoring the status of endangered freshwater fishes using effective and, at the same time, non-harmful methods are critical issues for ecologists and conservationists. In small streams, one well established monitoring technique is electrofishing, even if it has been shown that it can cause injuries, physiological stress and post release mortality of organisms. These detrimental effects should be avoided in monitoring programs of rare or endangered species. Beyond the emerging use of environmental DNA analysis, visual observation techniques represent non-harmful and low-cost alternative methods. In this context, the aims of the present study are (1) to compare relative effectiveness of the two visual methods in detecting patterns of patch occupancy of Mediterranean native trout and (2) to investigate the effect of a set of environmental variables that could affect detection probability of fishes. The study was conducted in the Piras River, an intermittent stream located in the south east Sardinia (Italy). Field samplings were conducted in 47 pools and replicated during two sampling seasons: during highest flow regime (HFR) and during the lowest flow regime (LFR). Each pool was sampled with two survey methods: 1) Visual Survey from Streambanks (VSS) and (2) Underwater Visual Survey (UVS) using underwater cameras. Environmental variables that characterized the pool habitat, possibly affecting detection probability or occupancy of fishes, were also recorded: pool size, chemical-physical characteristics of water, substrate and vegetation cover. For each visual method, the relationships between habitat variables and presence/absence (1-0) of the species were modelled as a binary logistic regression, by means of Generalized Linear Models (GLMs). With UVS, trout were detected in 51% of pools during HFR and 64% during LFR, while using VSS, trout were detected in 0.08% and 59% of pools during HFR and LFR, respectively. The best model for UVS revealed that detection probability was positively correlated with pool volume, whereas mass of green algae negatively affected detection probability. On the contrary, detection from stream banks (VVS) was negatively affected by a high turbulence rate and high percentage cover of boulders in streambeds. In conclusion, our results suggest the utility of visual methods to describe patterns of patch occupancy of Mediterranean trout in intermittent streams. However, methods can be differently affected by environmental variables: while UVS method appears effective regardless of turbulence rates and in streams with low percentage of submerged vegetation, VSS performs well only with low turbulence and low percentages of high size of substrate cover (boulders) that can provide protection to fish. Therefore, monitoring programs based on these methods should consider or model such effects to provide a reliable description of pool occupancy rates.

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FIRST INSIGHT INTO MEIOFAUNA INHABITING THE SHALLOW CO_2-SEEPS AROUND CASTELLO ARAGONESE (ISCHIA, ITALY)

Increasing atmospheric CO_2 concentrations are causing an alarming rise in pCO_2 concentrations at the ocean surface, generating the well-known phenomenon of 'Ocean Acidification'(OA). Recent studies in naturally acidified environments pointed out that benthic community structure and diversity might be altered by high *p*CO2 levels.

For the present study, sediment samples were collected along a pH gradient (mean range 7.4-8.1) nearby the CO_2 vents located on the northern and southern sides of Castello Aragonese (Ischia island, Italy), to characterize the meiobenthic community inhabiting the sediments of these naturally acidified environments. Total organic carbon (TOC) content and the grain size have been determined at each sampling station: N1, N2, N3 on the northern side; S1, S2, S3 on the southern side. We considered as a null hypothesis that there would be no significant difference in meiobenthic community abundance and composition along the pH gradients investigated. Overall, major differences were observed between intermediate stations (N2 and S2) vs. acidified (N3 and S3) and control stations (N1 and S1). The meiobenthic diversity seemed not particularly affected by lower pH conditions, while the lowest abundance value (167±75 ind/10cm^2 at station N2) did not characterize the most acidified stations (S3 and N3). Among the total of 16 taxa identified, the most abundant were Nematoda, Copepoda and their *nauplii* at all sampling stations. The total contribution of rare taxa (i.e. taxa <1% of total meiofaunal abundance) ranged from 1% (N2) to 2.6% (S3). Significant changes in meiobenthic community composition were detected between stations located both on the northern and southern sides of Castello, particularly, when only rare taxa were considered (dissimilarity ranging from 51% to 96% at S3 vs. S1 and N3 vs. N2, respectively). The pH conditions appeared to be a secondary factor that governed meiofauna distribution and variability among stations at the Castello vents, with respect to grain size and food availiability, which seemed more important. Although in this study case the meiofauna was not strongly affected by acidified conditions, an overall continuous increase of marine CO_2 levels could result in a changed functional diversity. The study of the nematode taxonomic and functional composition (in progress) will help in exploring the issue. This study aims to advance our understanding of how marine benthic communities may react to OA.

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FROM ECOLOGICAL NICHE MODELS TO SPECIES DISTRIBUTION MODELS: A FRAMEWORK TO CAPITALIZE ON DATA AND SUPPORT BIODIVERSITY MANAGEMENT

Global change is currently posing a serious risk to many animal species, with unprecedented rates of ecosystem disequilibria and local extinctions. In recent years, forecasts of species' distributions based on their occurrence localities and sets of environmental predictors have permitted to assess possible future changes in range, with important implications for biodiversity management. The modeling techniques used for this aim depend on different algorithms, and the corresponding outcomes can be used for many applications, spanning from biogeography and conservation to invasion biology and landscape management. Nowadays, one of the challenges is to convert models representing a part of the ecological niche of a target species to a more realistic one, where many aspects of a species' niche are comprised. This can be achieved if considering the advances in the ecological modeling techniques together with geostatistical analyses, but the scientific debate is far from having defined the "best practices". In this contribution, we present a hierarchical framework that takes advantage of the state-of-art of ecological niche modeling techniques and geostatistical analyses, combining both in a "couple-and-weigh" approach. Using occurrence localities and environmental predictors as input data, this framework permits to narrow of the target species' potential distribution (estimated through the ENMs procedures) to the realized one, in the context of post-modeling GIS analyses. We apply this framework using an updated dataset of all the four subspecies of the meadow viper *Vipera ursinii*, in the five different European regions they occur within. First, we predict current and future "weighted" environmental suitability, to assess the threats possibly arising in the subspecies' ranges in the next years. Then, considering the continuous nature (i.e., not discrete) of the weighted approach, we use the predictions to infer landscape corridors. Specifically, we measure and map the connectivity both among existing populations and to future suitable areas, also assessing whether current populations could recolonize extinct ones; finally, we evaluate the role of protected areas in covering these connections. In conclusion, the purpose of this contribution is to provide an integrative workflow to be applied as a comprehensive assessment method, to properly inform the protected areas' managers and other stakeholders, supporting informed biodiversity conservation actions.

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GHOST NETS AS SUBSTRATE FOR MARINE INVERTEBRATES

Fishing gears can be lost from vessels for several reasons, such as gears being lost or abandoned during or after fishing operations or very damaged gears discarded while at the sea (RICHARDSON *et al*., 2018). When concerned with nets, these gears are called "ghost nets" and originate from fishing vessels operating both legally and illegally (KIESSLING, 2003). In the last few years, the political and social awareness of the presence and problems caused by "ghost nets" has grown considerably. In this regard, several actions such as removal of these gears from the sea bottom or removal of beached gears are promoted by governments, academics and local associations. However, during the period they lay on the marine bottom, "ghost nets" are colonized by filamentous algae in the first stage, and subsequently by encrusting, sessile and vagile organisms, such as macroalgae, bryozoans and crustaceans (RUITTON *et al*., 2019). Afterwards, when the nets are totally colonized, their removal should be evaluated carefully. Indeed, in some cases, the removal of a "ghost net" causes not only the death of all associated organisms but can physically damage some important habitats, such as the coralligenous one. As a preliminary assessment of such biodiversity, we evaluated, through a quali-quantitative analysis the invertebrate fauna associated with "ghost nets" recovered along the Ionian coast of Sicily in 2022. A total of four different sections of "ghost nets" was recovered between Riposto and Catania, at a depth range of 12–35 m. Immediately after removal, "ghost nets" were analyzed by taxonomic experts and all associated fauna was collected using tweezers and preserved in alcohol for identification in the laboratory, under a stereomicroscope. Results showed a quite high diversity. Associated vagile fauna was dominated by crustaceans (e.g. Amphipoda –*Abludomelita* cf. *gladiosa*, *Gammarella fucicola* (Leach, 1814), *Pseudoprotella phasma* (Montagu, 1804), *Elasmopus* sp. and *Lysianassa* sp.; Decapoda –*Alpheus macrocheles* (Hailstone, 1835), *Pisidia* sp.) and, in some cases, also of polychaetes (*Eunice torquata* Quatrefages, 1866, *Hesione* sp., *Lepidonotus clava* (Montagu, 1808)). Our study underlines how "ghost nets" can also be, after a relatively long period, an important substrate for marine life. Hence, they can be used as a useful substrate for the study and monitoring of marine diversity. Indeed, "ghost nets" could attract a species assemblage and abundance that can be different from that of nearby areas. For example, a high abundance of species commonly recorded on hard or mixed substrates, such as *E. torquata*, *Hesione* sp., Hippolytidae members and *P. phasma* were sampled in a "ghost net" set on soft bottom and vertically partially suspended. In conclusion, the collaboration between authorities, organizations and experts is of fundamental importance for the correct management of "ghost nets".

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How can different taxonomic structure and functional traits of nematodes reflect different human pressures in "Heavily Modified Water Bodies"?

Ports can be regarded as "Heavily Modified Water Bodies" since their waters and sediments cannot achieve the Good Environmental Status (GES) as required by the Marine Strategy Framework Directive. These areas are in fact prone to the accumulation of noticeable amounts of contaminants (metals, hydrocarbons, pesticides, etc.) due to the presence of several productive and industrial activities settled in the surroundings. At the same time this aspect makes the commercial ports as key-areas for studying the long-term effects of chronic contaminantion on marine biota and, in case, for detecting any kind of adaptation. In order to shed light on the issue, free-living nematodes were selected as a target assemblage to analyse in three commercial ports of the Adriatic Sea (Trieste, Ancona and Koper). This choice was made according to the high tolerance of some nematode genera to contamination, which ensures the presence of living specimens even in very polluted conditions. In each port, free-living nematodes were investigated in terms of abundance, genera composition, diversity and functional traits. Overall, the sediments of all sampling sites were inhabited mainly by colonizers. The port of Trieste was characterised by the highest level of biodiversity notwithstanding the overall higher contamination level, suggesting a possible adaptation of the nematodes to the long-standing contamination. Total Polycyclic Aromatic Hydrocarbons (ΣPAH) were the main environmental variable that influenced the nematode assemblages, followed by Total Organic Carbon (TOC) content and the sediment grain-size. A recalibration and implementation of the indicator genera lists were carried out specifically for such heavily modified environments by means of a co-occurrence analysis that allowed to identify which genera cohesively respond to site-specific conditions. Furthermore, we defined some simple guidelines for the use of the most common indices applied to nematodes (i.e. Maturity Index, Index of Trophic Diversity, Shannon index) in order to encourage the use of these organisms as biological indicators for the environmental quality assessment of large commercial ports.

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IL PROGETTO "LIFE IMAGINE UMBRIA": COLEOTTERI E LEPIDOTTERI DELLA DIRETTIVA HABITAT

Il progetto LIFE19 IPE/IT/000015 (LIFE IMAGINE UMBRIA) è un progetto LIFE integrato della durata di 7 anni (2020-2027) che ha la finalità di sostenere lo sviluppo di una strategia gestionale di conservazione della rete Natura 2000 nella regione Umbria. Partecipano al progetto dieci partner, tra cui quattro università.

Tra gli obiettivi principali del progetto c'è quello di mantenere e migliorare lo stato di conservazione degli habitat e delle specie, sia animali che vegetali, inserite negli allegati della Direttiva Habitat e della Direttiva Uccelli. In particolare, in alcune azioni del progetto, è previsto un approfondimento delle conoscenze su varie specie di insetti e sui loro habitat biologici in Umbria, insieme a interventi di conservazione che verranno effettuati tra il 2022 e il 2027 all'interno di siti Natura 2000. Le specie target sono i coleotteri *Rosalia alpina* (Linnaeus, 1758), *Osmoderma eremita* (Scopoli, 1763), *Cerambyx cerdo* Linnaeus, 1758 e *Lucanus cervus* (Linnaeus, 1758) e i lepidotteri *Eriogaster catax* (Linnaeus, 1758), *Euphydryas provincialis* (Boisduval, 1828) e *Melanargia arge* (Sulzer, 1776).

Nei primi due anni di progetto, per tutte le specie sono stati effettuati degli approfondimenti conoscitivi che hanno contributo ad aumentare la conoscenza e ad aggiornare le loro mappe di distribuzione nella regione.

Le attività di conservazione individuate per i coleotteri saproxilici consistono nella realizzazione di microhabitat sostitutivi, cioè aree artificiali o naturali (definite aree di senescenza) costituite da alberi morti in piedi o a terra e alberi "habitat", cioè alberi senescenti, cavitati, morti, deperiti o parzialmente deperiti, con un'estensione minima di 5 x 5 m per ettaro (totale 100 ettari). Per la specie *O. eremita*, inoltre, saranno realizzate ed installate 30 *wood mould boxes*, cioè delle cavità artificiali costituite da cassette piene di rosura legnosa e lettiera, utilizzate come microhabitat sostitutivo per le sue larve.

Le attività di conservazione individuate per i lepidotteri consistono, per *E. catax*, nella realizzazione di fasce ecotonali attraverso la piantumazione di arbusti, in particolare la sua pianta nutrice, il prugnolo, per un'estensione complessiva di 1 km, in 10 installazioni di circa 100 m l'una; per le specie *E. provincialis* e *M. arge*, è stata invece effettuata la selezione di una serie di tecniche di conservazione degli habitat prativi che prevedono la limitazione o l'eliminazione dell'insediamento arbustivo.

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IMPACT OF *HERMODICE CARUNCULATA* (PALLAS, 1766) (POLYCHAETA: AMPHINOMIDAE) ON ARTISANAL FISHERY: A CASE STUDY FROM THE MEDITERRANEAN SEA

The bearded fireworm, *Hermodice carunculata* (Pallas, 1766) (Polychaeta: Amphinomidae) is a large species whose length can exceed 50 cm. This amphinomid is considered a neonative invader of the Mediterranean Sea. Indeed, this species is expanding its range in the Mediterranean Sea (RIGHI *et al*., 2020). *Hermodice carunculata* prefers rocky bottom of the upper infralittoral (SIMONINI *et al*., 2021). The species is considered a voracious generalist and opportunistic predator and scavenger; preys are caught through a bulbous muscular pharynx which is ejected from the mouth (SCHULZE *et al*., 2017). This study aims to present first and preliminary data on the impact of *H. carunculata* on artisanal fishery (longlines) in the Mediterranean Sea. Indeed, the only available data on this aspect is the work of CELONA and COMPARETTO (2010), in which it was first observed the opportunistic predation of the species in fishes trapped in set net (trammel net and gill net). Our study was conducted in the southern Ionian coast of Sicily, from June 2017 to February 2018, and was carried out in collaboration with local fishermen. Data were collected on board and at the landing point. For each survey, the following data were collected: period (month), number of hooks used, bait used, length in m of the longline, depth in m, bottom nature, species caught, hours of fishing and the economic damage caused by *H. carunculata* on the catch. Results indicated a significant direct and indirect economic loss due to the presence of *H. carunculata*. Indeed, the bearded fireworm caused several types of damage to both valuable and not valuable species, and takes the hooks itself (3.3% on total hooks per fishing day), reducing the efficiency of the fishing gear. Furthermore, although the economic damage to catch was recorded during the whole period of study, this was higher during warmer months, indicating as this species effectively benefits from warm waters. Further studies on the geographical and bathymetric expansion of this species and constant monitoring of local populations are essential to mitigate ecosystemic alterations and prevent economic damage to artisanal fishery.

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LOCAL EXTINCTION? NOT YET. CONSERVATION PLAN FOR A PROTECTED BUTTEFLY SPECIES

Megaprojects radically change the landscape due to their large-scale and high investments. Forests and clearings are often one of the most affected habitats, as they are frequently included in megaproject construction sites. These habitats can support rich communities, including threatened and protected species. *Zerynthia polyxena* is an ecotonal butterfly species, listed in the Habitats Directive (92/43/CEE). Here, we present research developed to conserve the species in Susa Valley (Piedmont, Italy) where it was threatened by the expansion of an industrial site, part of the megaproject Turin–Lyon high-speed railway (TAV).

First of all, we estimated the i) effective loss, the exact amount of the population threatened, its mobility and the possibility to survive. Then, we have proved that ii) the species is locally monophagous on *Aristolochia pallida* (PICCINI et al. 2021). In order to understand how to restore the habitat and micro-habitat conditions of host plant and butterfly, we have studied iv) the optimal ecological conditions of both the host plant and the species at local scale (PICCINI et al. 2022a). Considering those results, we have developed iv) a specific conservation plan to preserve the species in the site and its surroundings by the construction of an ecological corridor.

In accordance with Piedmont Regione, ARPA Piemonte and the TAV company (TELT), we apply a the field-base conservation strategy that we have developed. This plan included habitat restoration for the species through the construction of an ecological corridors that might link two subpopulations. For the first time, compensation funding, derived from the forest and clearing habitat loss, was used to restore, improve habitats and to support local threatened species by developing an ecological corridor that would link two subpopulations and favour the species mobility (PICCINI et al. 2022b). Once the clearings of the ecological corridor have been made and considering that the two subpopulations were still genetically similar, we moved to the clearings host plants and 100 larvae that would have been dead in consequence of the expansion.

We have developed and applied a local conservation plan and its success would be evaluate in the next years through the collection of new genetic data and checking the presence of the species in the ecological corridor. However, in 2021, we have already found some eggs on host plants, thus the species has already used the clearings for oviposition. In conclusion, this approach represents a good example to reduce mega project impacts and it can be exported to other megaprojects to support local biodiversity.

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MONITORING THE FAUNA THROUGH THE eDNA ANALYSIS

Environmental DNA (eDNA) analysis is a rapid, non-invasive, cost-efficient biodiversity monitoring tool with enormous potential to inform aquatic and terrestrial conservation and management. The eDNA methodology takes advantage on the use of species-specific assays based on hydrolysis probe chemistry, which are designed to target mainly mitochondrial DNA (mtDNA) portions, since it has significant divergence across species and there are thousands of copies of mtDNA per cell. Friuli Venezia Giulia region alongside the neighbouring Slovenia have recently invested on environmental monitoring of both native and alien species through eDNA and here successful applications are presented. Projects related to the monitoring of invasive species have interested both FVG region and Slovenia in an attempt to rapidly achieve a map of distribution of species of interest as *Pseudorasbora parva* and *Procambarus clarkii*, both species listed amongst the worst 100 invasive species in Europe (DAISIE 2009). In FVG, a species-specific assay matching a short region of the mitochondrial Cytochrome c Oxidase I (COI) was developed to target topmouth gudgeon (*Pseudorasbora parva*) The eDNA detection was tested in water samples using both quantitative Real-Time PCR (qPCR) and quantitative digital PCR (qdPCR). Field results for both qPCR and qdPCR were largely in agreement thus, we judged the presence/absence by combining the results from the two methods and found that nine sites showed “strong positive” signal of *P. parva* eDNA (at least 2 positive replicates), 3 showed “suspected” (only 1 positive replicate), and 42 showed “absent” (MANFRIN et al., 2022a). Similarly, in 2018 and 2019, we monitored the presence of *Procambarus clarkii*, after a deliberate release near Celje (Slovenia) using SPY primers (TRÉGUIER et al., 2014) to invesitgate the presence of *P. clarkii* after a massive eradication campaign immediately following the release of the crayfish. During this campaign, sites other than the intentional release area were monitored using eDNA to potentially detect new sites with *P. clarkii*. Five sites showed a “strong positive” signal, one of them near the Italian border and far from the site of the release, resulting in a new detection of *P. clarkii* on Slovenian territory, never reported before. Monitoring by eDNA has also been used in the FVG for conservation purposes. A study based on the development of two assays able to distinguish *Austropotamobius torrentium* and *A. pallipes complex* eDNA. Fourteen sites within the Danubian Slizza basin were analysed to collect preliminary information on the distribution of *A. torrentium,* and one site known to contain *A. pallipes* (Tagliamento River) to test the performance of the *A. pallipes* probe. The presence of *A. torrentium* was confirmed at 6 out of 15 sites, four of which were new, and the Tagliamento River site confirmed the presence of *A. pallipes* (MANFRIN et al., 2022b). The latter study was conducted to fill the knowledge gap on the distribution of this species in Italy, after *A. torrentium* was reported as “data deficient” and the occurrence of the species was classified with Scientific Reserve and represents the most up-to-date information on the stone crayfish in Italy.

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NEVER STOP EXPLORING: NOVELTIES AND PERSPECTIVES ON ITALIAN ODONATES

Italy is considered one the richest European countries in terms of odonate diversity, with at least 89 reproducing species. Many aspects regarding the distribution and ecology of Italian dragonflies and damselflies are still incomplete with some taxa showing fragmented or relict populations that likely indicate complex biogeographic histories. Given the extreme importance of this taxonomic group as a bioindicator of water quality and climate-driven environmental changes, it is of fundamental importance to better characterize the genetic, morphological and ecological traits of Italian populations.

In the last four years, our "DNA barcoding Italian Odonates" project contributed to increasing the knowledge of these insects at the national level, with the assembling of a biological collection for integrative taxonomy analyses. It now counts more than 1000 specimens collected from 328 localities representing almost all the Italian administrative regions and ecoregions, > 500 deposited DNA barcoding sequences (on average 5 DNA barcodes per species) and several scientific collaborations aimed at comparing the diversity patterns shown by Italian odonate populations with those at the European, Palearctic, and Holarctic scale. A multi-approach species delimitation analysis involving two distance (OT and ABGD) and four tree-based (PTP, MPTP, GMYC and bGMYC) methods were used to explore these data. Of the 88 investigated morphospecies, 75 (85%) unequivocally corresponded to distinct molecular operational units, whereas the remaining ones were classified as 'warnings' (i.e., showing a mismatch between morphospecies assignment and DNA-based species delimitation). The species causing warnings were grouped into three categories depending on if they showed low, high or mixed genetic divergence patterns. The analysis of haplotype networks revealed unexpected intraspecific complexity at the Italian, Palearctic and Holarctic scales, possibly indicating the occurrence of cryptic species, one of which is endemic to Italy (i.e., *Coenagrion castellani*). Finally, studies conducted on bacterial endosymbionts from the genus *Ischnura*, revealed how their occurrence can shape spatial variation in their host evolutionary genetics, rising new insights into the maintenance of reproductive barriers among congenerics.

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UTILIZZO DI UN APPROCCIO INTEGRATO PER L'IDENTIFICAZIONE DEGLI APOIDEI URBANI: STRUTTURAZIONI INATTESE E CASI CRIPTICI NELLA CITTA' DI MILANO

Nonostante gli ambienti urbani siano considerati di minor importanza per la biodiversità, studi recenti hanno dimostrato che possono costituire aree di estrema ricchezza per molti gruppi di animali, tra cui gli insetti impollinatori. Questi trovano infatti le condizioni idonee per completare il loro ciclo biologico e instaurare popolazioni stabili grazie, ad esempio, all'elevata presenza di risorse trofiche in termini di piante ornamentali e alla presenza di siti di nidificazione idonei. Tuttavia, per valutare e comprendere meglio la biodiversità di queste comunità e per progettare idonee strategie di mitigazione e di supporto degli ecosistemi in contesti urbani, l'identificazione delle specie rappresenta un punto fondamentale. Considerando i soli imenotteri (Anthophila) esistono diverse difficoltà nel riconoscimento morfologico di molti taxa, inoltre, a fronte dell'elevata diversità presente nel nostro paese (*i.e.*, oltre 1.000 specie di apoidei), la continua perdita di esperti tassonomi e la velocità con cui le popolazioni di impollinatori stanno scomparendo, lo sviluppo di un approccio tassonomico integrato risulta cruciale. Grazie al supporto offerto dalle tecniche di identificazione molecolare (*e.g.,* DNA *barcoding*) è possibile accelerare i tempi di identificazione, ottimizzando allo stesso tempo gli investimenti in termini di operatori e risorse economiche. Lo scopo di questo studio è quello di utilizzare la città metropolitana di Milano come target "pilota" per creare un primo dataset di riferimento morfologico e molecolare per gli Apoidea urbani del Nord Italia. Nel corso del progetto, iniziato nel 2019, sono stati raccolti oltre 300 campioni, appartenenti a 5 famiglie. In seguito all'identificazione morfologica, gli esemplari sono stati processati anche a livello molecolare tramite un approccio standard DNA *barcoding*. I risultati ottenuti hanno permesso di identificare con un elevato tasso di successo casi morfologicamente criptici (gruppo *Andrena* e *Halictus)* confermando l'affidabilità di questo metodo a supporto della tassonomia per questo gruppo di animali. Per alcuni generi (*e.g., Andrena*, *Hylaeus*, *Anthidium*) è stato necessario l'utilizzo di primer alternativi, o interni alla regione target mtDNA COI, per superare limiti di specificità di *annealing* e/o co-amplificazione di simbionti batterici (*e.g.*, *Wolbachia*). Inoltre, attraverso il confronto con sequenze italiane ed europee presenti nelle banche dati di riferimento (NCBI e BOLD) l'approccio molecolare ha anche rivelato la presenza, in alcuni generi (*e.g.*, *Andrena*), di un'inattesa strutturazione delle popolazioni di alcune specie.

Questo studio conferma l'importanza dell'utilizzo di un approccio integrato per l'identificazione delle specie di apoidei urbani e pone le basi per l'avvio di progetti su scala nazionale volti alla tutela e alla stima della biodiversità di questo gruppo, nonché alla produzione di una banca dati molecolare di riferimento per automatizzare e rendere processiva l'identificazione (*e.g.*, DNA *metabarcoding*) e che al contempo permette di inferire aspetti ecologici rilevanti (*e.g.*, *network* di interazione piante-impollinatori).

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"PLASTIC EATER" SEA SLUGS (APLYSIIDAE, MOLLUSCA) IN THE MAR PICCOLO OF TARANTO

The Mediterranean Sea is widely considered as a hotspot of biodiversity, with high rates of cryptic diversity and endemism. Unfortunately, it is also particularly sensitive to microplastic pollution, a worldwide emergent threat: the number of new scientific papers on this topic is continuously on the rise and the impacts of plastics on marine organisms increasingly reported. The Mar Piccolo of Taranto (Apulia, Ionian Sea) is a natural semi-closed coastal basin subject to high impacts from shoreside industries and activities at sea, including fish and mussel farming, causing severe environmental pressures and diverse pollution phenomena. Due to its geomorphological peculiarities, it can be considered as a natural laboratory where to observe, understand, and eventually foresee the negative effects that may occur on a larger geographical scale. In this context, a recent publication (FURFARO et al., 2022) highlighted interactions between a heterobranch mollusc (*Bursatella leachii* Blainville, 1817) living in the Mar Piccolo and the microplastics spreading in its natural habitat. By SEM/EDX analyses, plastic fibres and debris were found in the stomach contents of all investigated specimens and the available information on the nature and source of plastics assembled into a reference database. Nowadays, information on key biological aspects of *B. leachii*, such as its trophic behavior, prey preference, and inter-population morphological variability, is still scant. To reduce this gap of knowledge, stomachal content analyses, anatomical dissections, and optical and SEM observations were carried out on slug specimens collected from two populations (Mar Piccolo, Ionian Sea; Porto San Paolo, Sardinia). Significant differences were unexpectedly recorded both in anatomy and stomachal content in the Mar Piccolo specimens, respect to what originally known in the literature and found in Sardinian individuals (Tyrrhenian Sea). Namely, the degree of divergence in the shape of the radula (the most important and diagnostic morphological character in Heterobranchia), together with differences in the sexual reproductive system and prey preferences, altogether suggest the possible occurrence of cryptic diversity within this taxon. Molecular investigations are planned to verify whether these discrepancies fall within an intraspecific or interspecific range of variability.

POSTER

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RELATIVE BILL SIZE CONFORMS TO ALLEN'S RULE VIA DEVELOPMENTAL PLASTICITY IN A MEDITERRANEAN RAPTOR

Endotherms need to respond properly to temperature changes in their environment to avoid loss of their physiological functions. Whereas evaporative cooling favours rapid heat dissipation, it requires big energy investments and can lead to dehydration if prolonged. Another mechanism endotherms rely on is heat dissipation through dry heat exchange, by means of little-insulated appendages. In the next decades, temperatures will increase, on average, +1.5 ^{o}C above pre-industrial levels. Endotherm populations recently exposed to climate change show increased size of their appendages, accordingly to Allen's rule (bigger appendage sizes in warmer latitudes). Long-term population monitoring and museum data do not respond the question if these changes are evolutionary responses or reflect developmental plasticity. We provide the first evidence that temperatures close to the upper thermal limit during development are associated with relatively longer bills in a wild bird population of lesser kestrels *Falco naumanni*. We experimentally manipulated nestboxes in order to reduce the maximum internal temperature (MIT) and measured the growth rates of different body traits in nestlings (body mass, forearm, tarsus and bill length). We found a negative correlation between MIT and both body mass and forearm, but not with thermoregulation-related appendages (such as the bill and tarsus), leading to relatively longer appendages. Our results demonstrated for the first time that increasing temperatures due to climate change significantly can influence bird body shape according to Allen's rule through developmental plasticity.

POSTER N. 1

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EFFECTS OF POLYVINYLCHLORIDE MICROPLASTICS ON THE BRITTLE STAR *OPHIACTIS VIRENS* (ECHINODERMATA: OPHIUROIDEA)

Plastic represents the dominant type of litter in the aquatic compartment. Polyvinylchloride (PVC) is among the most common synthetic polymer used, hence frequently detected in the marine environment. Regardless their density, over time plastic fragments invariably sink in the water column due to surface fouling, thus ending up in sediments. The impact of sunken debris and their degradation micro-products on benthic organisms is not entirely clear, nevertheless there is some evidence of negative effects.
This study aims to investigate the potential effects of PVC microparticles on the physiology of *Ophiactis virens*, a small fissiparous ophiuroid highly abundant in the Ligurian Sea. Irregularly shaped microplastics were obtained by cryo-milling fragments of a PVC tube commonly used in building. Different parameters at different levels of biological organization were considered, including mortality, behavioural response (righting time and speed), arm regeneration efficiency (differentiation index = number of regenerated segments/regenerate length), presence of anomalies in the external/internal anatomy. A semi-static assay was performed (complete medium renewal every 48 hours): juveniles of *O. virens* were subjected to arm amputation and exposed to clean artificial seawater (control) and three suspensions of PVC fragments (0.1, 1 and 10 µg/mL) for two weeks. Each experimental condition was tested in triplicates.
At the end of the exposure period, no effects induced by PVC were recorded on mortality, righting time, speed as well presence of anomalies in the external and/or internal anatomy. However, a significant reduction of the arm differentiation index was observed at the higher tested doses. PVC fragments were observed in the preoral cavity of some sample, but they were not detected into the stomach.
Overall, PVC exposure only partially affected *O. virens* physiology: while individual-level parameters were not influenced by the treatment, some tissue-level effects were observed at the highest concentrations. The significant reduction of the arm differentiation index prompts to an unequal energy allocation between lengthening and tissue differentiation, whereas the fragments observed in the preoral cavity highlight the risk for the ingestion of plastic materials, possibly resulting in a reduced feeding efficiency in the long-term period. Further multidisciplinary investigations are needed to understand the real environmental risk of plastic contaminants.

POSTER N. 2

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ASSESSMENT OF THE ENVIRONMENTAL ACIDIFICATION LETHAL EFFECT: TARDIGRADES EXPOSURE TO SYNTHETIC ACID RAIN AND ITS PRINCIPAL COMPONENTS

Acid rains are a phenomenon that has been increased by anthropization. Local emitted pollutants are fastly transported over the globe and spread also over uncontaminated areas by atmospheric currents. The chemical composition of acid rains can vary, but high concentration of sulfuric and nitric acids in raindrops is a clear sign of human activities. The main aim of this work is assessing the toxicity lethal effect, evaluated by $LD_{50,}$ and phenotypic reactions of exposure to Synthetic Acid Rain [SAR] and its compounds (i.e., H_2SO_4: HNO_3, 3:1), and of other naturally occurring acids on three species of tardigrades collected from three different latitudes and environments. Among animals, tardigrades are a suitable model containing calcium carbonate structures such as the stylets of the feeding apparatus that can be damaged by SAR. Specimens of *Acutuncus antarcticus*, *Hypsibius exemplaris*, and *Macrobiotus* cf. *hufelandi* were singularly exposed to different pH (4.0, 4.5, 5.0, 5.5) of SAR and to H_2SO_4, HNO_3, HCl, and CH_3COOH at pH 4.5. Inter- and intra-species differences in LD_{50} calculated for each treatment and control (distilled water, pH 6.2) were tested in a Bayesian frame in R. Experiments with SAR evidenced a statistically significant correlation between the increase of acidity and the mortality in all tested species. The freshwater species *A. antarcticus* and *H. exemplaris* show a better resistance than the moss-dwelling species *M*. cf. *hufelandi. Acutuncus antarcticus* has higher resistance to short exposure to SAR, but it accumulates negative effects during the exposure. *Hypsibius exemplaris* reacts similarly to *A. antarcticus* in withstanding SAR, but its probability of death remains stable over the time. *Macrobiotus* cf. *hufelandi* is the least resistant, but the animals slightly acclimate over the time. Nature of acid also influences mortality: SAR and its components (H_2SO_4 and HNO_3) are less endangering than acetic and hydrochloric acid for *H. exemplaris* and *M*. cf. *hufelandi,* on the contrary, *A. antarcticus* results less endangered by acetic and hydrochloric acids. The animals that survived to acid exposure maintained an unaltered morphology of the stylets showing tolerance to tested acidity and hence capabilities in buffering the proton charge incoming from the environment. The autecology of tardigrade species could have a major role in influencing animal mortality exposed to specific acids. *A. antarcticus* inhabits the Antarctic habitats rich in HCl and CH_3COOH; this peculiarity of Antarctica could influence the resilience of *A. antarcticus* to these two acids even if this species reacts similarly to the other tardigrade species when exposed to SAR for instance. Specific metabolism of tested species may have a role in tardigrades ability to detoxify specific counterions and therefore pollutants. Phenotypic variability in the population seems to be instead the key for the survival of a species over environmental stressors. In fact, animals carrying an “acidic buffer” phenotype are potentially able to help the population to restor after chronic or repeated exposures to acid rains.

POSTER N. 3

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BODY SIZE SHRINKING IN HEAVILY URBANIZED SITES? LESSONS FROM A BEE AND A WASP

Urbanization is one of the main causes of natural habitats alteration. Particularly, urbanization is associated with a reduction and fragmentation of green areas and an increase in temperatures, in a phenomenon called “Urban Heat Island Effect” (UHI). Wild bees and wasps (Hymenoptera) can be affected by – or adapt to - these changes by shifting some functional traits such as their body size. In turn, variation in body size may alter their fitness in these anthropized landscapes. To date, the effect of urbanization on body size of these insects have been either largely neglected (wasps) or have yielded contrasting results (bees). Here, we investigated how body size of the solitary bee *Osmia cornuta* (Latreille, 1805) and the social wasp *Polistes dominula* (Christ, 1791) responds to urbanization level within a large metropolitan city (Milan). Insects were sampled in 8 and 9 sites (for *O. cornuta* and *P. dominula* respectively) along a gradient of temperature, Normalized Difference Vegetation Index (NDVI) and green area fragmentation (i.e. Edge density); all obtained through a remote sensing approach. We found urbanization level to affect body size of both the considered species, though with a variable role of the urbanization traits. Edge density negatively affected body size in *O. cornuta*, while NDVI positively affected body size of *P. dominula.* Fragmentation of green areas, found in highly urbanized sites, may reduce the quantity and quality of floral resources, thus the reduction of *O. cornuta* body size may be due to an impoverishment of the larval diet. Smaller body sizes are associated with reduced dispersal capacities, possibly making smaller bees more prone to local extinctions. Greater NDVI values, found in low urbanized sites, correlates with ecosystem properties such as arthropod diversity. Thus, the positive association between NDVI and body size of *P. dominula* may be due to a greater availability of trophic resources (prey for the larvae). Altogether, our results highlight that heavily urbanized sites within cities tend to shrink body size of wild bees and wasps, with possible consequences on ecosystem services provided by these insects (pollination, pest removal). This calls for the adoption of better practices of green areas management. Further, comparative studies on wild bee and wasp species with different nesting substrates, diet specialization and social organization should broad our knowledge on the effects of urbanization on these insects.

POSTER N. 4

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BRAIN AND GILLS OF THE ANTARCTIC NOTOTHENIOID *TREMATOMUS BERNACCHII* ARE TRANSCRIPTOMICALLY SENSITIVE TO A SMALL TEMPERATURE INCREASE AND SPATIAL CONFINEMENT

Global warming is expected to reach a worldwide 1.5°C average temperature increase within this decade, with a possible impact on stenotherm animals such as antarctic fish. To model its responses to a warmer Antarctic Ocean, the nototheniod *Trematomus bernacchii* was exposed to a 1.5°C temperature increase for up to 20 days.
The RNA-seq technology was used to study the response to the applied heat in terms of differential gene expression, and thanks to the availability of naïve control sample it was possible to also infer the effect of spatial confinement. Gill and Brain tissues were sampled after catch (naïve), after acclimation to tank (T0), and after 6 hours, 7 days and 20 days of exposure, at -0.9°C and +0.6°C. mRNA was extracted and sequenced, then several bioinformatic tools were applied to perform differential expression, expression pattern clustering and gene set enrichment analyses.
The response of the gill tissue to the heat stress followed a time progression, and consisted in the activation of pathways related to cell replication and DNA repair. Gills also responded to confinement: we observed early compensatory activation of genes involved in muscular contractile activity and in cellular remodelling, followed by a later activation of vesicle trafficking and innate immune response.
The brain was much more sensitive than gills, as both stresses produced great shifts in the transcriptional landscape of this tissue. The heat stress started after 7 days of exposure, mainly consisting in activations of innate immune response by classical and alternative complement pathways. Such response changed at the 20 days time point in downregulation of genes involved in protein synthesis, mitochodrial activity and autophagy, and in upregulation of genes related to structure and function of several synapses, including typical stress related ones such as glutamatergic. The response of brain to confinement was the greatest of all, showing strong alteration of metabolic pathways and heavy effects on neutoransmitters and synapses, suggesting that the observed effects of heat in brain may be a consequence of the impossibility to behaviorally move to a cooler place.
Overall our results are an high resolution resource for modelling the effects of ocean warming on stenotherm fish, and indicate that future studies should take the stabling stress in serious consideration, specially when working on wild captive animals, with light stressors and with sensitive techniques.

POSTER N. 5

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COMPARING THERMAL PERFORMANCE BETWEEN TROPICAL AND TEMPERATE STRAINS OF THE ARBOVIRAL VECTOR *AEDES ALBOPICTUS*

Because mosquitoes are poikilotherms, temperature greatly influence mosquito physiology, life cycle, distribution and behavior, possibly accompanied with greater risk of disease outbreaks. A major public health concern is the arboviral vector *Aedes albopictus*, which recently expanded from its native home range in Southern East Asia to temperate regions of the world, resulting in the (re)-emergence of arboviral outbreaks in Europe and North America. *Aedes albopictus* is known for its ecological plasticity, including diapausing eggs, which allows the species to overwinter in temperate climates. However, current climatic changes include an increase in environmental temperature and extreme weather events that can be disjointed from the photoperiodism controlling diapause. To start investigating the mechanisms underlying *Ae. albopictus* invasive success besides diapause, we compared various fitness and thermal traits of a native strain from China (Foshan) and an invasive strain from temperate Italy (Crema). The two strains were reared at the optimal rearing temperature of 28°C and lower and higher temperatures of 18°C and 32°C, corresponding to average spring and summer temperatures in Italy, respectively.
Our results show significant differences between the two strains in thermal acclimation through survival and development. The strongest acclimation responses were found at 32°C in Foshan in terms of larval viability, developmental time and female percentage (sex ratio). At 28°C both strains showed the same performance, except for a higher larval viability in Foshan and a longer lifespan in Crema females. At 18°C Crema prevailed in terms of developmental time, females and males' longevity, resulting in potentially higher reproductive success. The fitness curve of Foshan showed a wider distribution in thermal preference, which was shifted towards higher temperatures. Foshan highest performance was estimated at 33°C. Crema showed preference for a narrower range of temperatures and a curve shifted towards colder temperatures, reaching an optimal temperature of 26°C.

Overall, these results highlight clear differences between the two strains, with the native tropical strain acclimated to warmer temperatures than the Crema temperate one. These results also suggest that the genetic background of the two strains, resulting from their different geographic origin, may play a role in the detected fitness differences because the two strains had been maintained in the laboratory for more than 10 generations before our experiments.

POSTER N. 6

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MARINE ANNELIDS AS MODELS FOR INNOVATIVE ROBOTS

Due to the evolution of a great variety of forms, marine organisms represent novel models for inspiring ground-breaking technologies. The Horizon Europe EIC Pathfinder Open project MAPWORMS (www.mapworms.eu) is aimed at using marine annelids as models for next-generation soft-bodied robots able to autonomously respond to environmental stimuli and adapt to the surrounding environment. The project follows a multidisciplinary approach and integrates biological, chemical, physical and mathematical data, focusing on annelid species occurring along the Salento Peninsula that is characterized by a variety of habitats that are expected to support high annelid diversity. Their distribution along the Salento Peninsula is being reconstructed using both literature, and unpublished data, to identify suitable sampling sites and target species to be used as models. Currently, 271 marine annelid species, 15 of which non-indigenous, have been reported for the Salento Peninsula according to literature data, which show sizable gaps of knowledge for most of the coastline, and a high amount of data available only for a few localities. A detailed distributional map was obtained for the sole *Hermodice carunculata* (Pallas, 1766). Present sampling activities carried out mostly in port environments, intertidal coralline algal habitat and coralligenous outcrops revealed the occurrence of 36 species hitherto unknown for the area. Among the up to now recorded taxa, some intertidal Sipuncula, such as *Phascolosoma stephensoni* (Stephen, 1942), and Opheliidae, such as *Ophelia barquii* Fauvel, 1927, appear as suitable model organisms for the study of the burrowing behaviour, as a first step towards the development of worm-inspired soft-bodied robots. Their simplified morphology, the ability to thrive in intertidal environments, and to withstand variable and sometimes extreme environmental conditions make these organisms relatively easy to rear in laboratory conditions and particularly suitable as models in robotics. The ability to adapt their body structure to the surrounding environment, and to elongate or contract their body without changes in the overall volume, make these organisms promising for the study of functional adaptations through an integrative approach considering behavioural and anatomical data, and ultimately to develop robot prototypes.

POSTER N. 7

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MYTILUS GALLOPROVINCALIS (LAMARCK, 1819) AS BIOINDICATOR OF MARINE POLLUTION INDUCED BY NANO- AND MICROPLASTICS

Mytilus galloprovincialis is a bivalve mollusc living as sessile suspension-feeder on marine hard bottoms. Being a bioaccumulator, it provides a strong and specific response to pollutants (CAPPELLO *et al*., 2021) and, since it is easy to identify, collect and maintain, it represents a good bioindicator of environmental conditions. Micro- and nanoplastics are among the main contemporary pollution sources in marine environments, reported on a global scale from the poles to the equator, impacting from the water surface down to the deep sea (LLORCA *et al*., 2020). Plastics accumulate in a large variety of organisms at different trophic levels and could cause harmful toxicological and/or physical effects (ANDRADY, 2011). The aim of this work is to evaluate the cytotoxic effects of nano- and microplastics in *M. galloprovincialis* at histological levels, finalized to a risk assessment of marine pollution.
Acclimated animals, held in aquaria at 18 °C, pH 8.0, 38‰ salinity, and 12:12h light:dark photoperiod, were exposed to 5□m (microplastic) or 0,1□m (nanoplastic) polystyrene beads for 1, 3, or 11 days. Gills, gonads, digestive glands, and byssus were fixed and processed. Preliminary results on gills show that both micro- and nanoplastics interfere with tissue morphology, at all experimental times. Alterations in the septum and lamellae organization were detected, together with the formation of cytoplasmic granules and melanin infiltrations. Mucus cells are differently distributed and increased in number, especially after nanoplastics exposure; cells proliferation was demonstrated by the positivity to the PCNA antibodies in immunocytochemical investigations.
In conclusion, the presence of polystyrene induces tissue damage in the mussels' gills, probably altering their function. Future investigation will clarify how the two pollutant sizes affect cellular stress response with consequences on fitness and reproductive success, representing a risk for their health, for the trophic chain, and for human consumers.

POSTER N. 8

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NUOVI OBBLIGHI FORMATIVI PER IL PERSONALE COINVOLTO NELLA SPERIMENTAZIONE *IN VIVO*: QUALI PROSPETTIVE SI SONO APERTE

I nuovi obblighi formativi ai sensi del DM 228/2021 e del DD del Ministero della Salute del 18 marzo 2022 riguardano in generale quanti sono impegnati a lavorare con gli animali a fini scientifici e educativi ed in particolare coloro che operano presso gli stabulari. Lo stabulario è il luogo in cui si ospitano animali vertebrati ed invertebrati impiegati nella ricerca biomedica. All'interno dello stabulario può accedere solo personale autorizzato e adeguatamente formato che svolge le seguenti funzioni: a – realizzazione di procedure su animali; b – concezione delle procedure e di progetti; c - accudimento degli animali; d - esecuzione di metodiche eutanasiche. Il personale addetto a tali funzioni deve possedere competenze specie-specifiche inerenti alla biologia, alla fisiologia, all'etologia e all'anatomia del modello animale utilizzato, oltre a conoscenze più strettamente collegate alle mansioni da svolgere.
La formazione di base, che prevede l'acquisizione di 22 crediti formativi professionalizzanti (CFP) per la funzione a, 24 CFP per la funzione b, 18 CFP per la funzione c e 20 CFP per la funzione d, dovrà essere completata dall'aggiornamento professionale continuo a partire dal 1° gennaio 2023.
Una corretta formazione ed il continuo aggiornamento sono alla base della piena applicazione del principio delle 3R (Russell & Burch, 1959) su cui si fonda tutta la normativa vigente sulla protezione degli animali impiegati a fini scientifici (D.lgs. 26/2014). Infatti, solo la diffusione della conoscenza sempre più approfondita delle esigenze e delle potenzialità delle specie con cui si lavora, assicura il contenimento della sofferenza animale attraverso la riduzione del numero di animali impiegati ed il perfezionamento delle tecniche di allevamento e di manipolazione. Inoltre, il principio delle 3R invita anche ad impiegare specie alternative come modelli di Replacement parziale. Questo dovrebbe essere di stimolo per tutti i biologi animali a comunicare e trasmettere le caratteristiche peculiari delle specie che utilizzano, che meglio conoscono e che potrebbero essere utili nella sperimentazione biomedica.

POSTER N. 9

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A STANDARDIZED PROTOCOL TO MAXIMISE ENVIRONMENTAL DNA METABARCODING STUDIES ON MARINE BENTHIC COMMUNITIES

Environmental DNA (eDNA) metabarcoding is considered as a powerful tool for biodiversity monitoring in terrestrial and aquatic environments. eDNA may allow a quick, cost-effective and sensitive surveillance of the environmental status, providing a rapid snapshot of the biodiversity that would be hardly possible by the traditional morphological methods. Nevertheless, the use of genomic methodologies for biodiversity inventories is still rather limited, also due to the lack of standardized sampling protocols and the inherent difficulties of setting appropriate experimental designs.
For eDNA investigations, a basic prerequisite is the optimization and definition of an optimal workflow for eDNA analysis. The main problems are: low replication (in time and space), contamination, expensive instrument and low repetitiveness.
We report here a user-friendly protocol for eDNA metabarcoding sampling, *ad hoc* developed to carry out a comparative analysis of eDNA collected at three bordering sites in the Southern Adriatic Sea, involving i) a marine protected area (Torre Guaceto); ii) the nearshore zone adjacent to the Brindisi harbour, and iii) a coastal site impacted by a thermal power plant (Cerano). Scope of the study is the assessment of the changes of marine benthic biodiversity across a gradient, based on the hypothesis of local community changes driven by anthropogenic impacts.
At each site three sampling stations were located along a linear transect at 100, 500 and 1000m distance from the shoreline. Both water and sediment samples were collected by SCUBA diving, in eight points at each station: setting the anchor point as a centre, a 20m-long rope allowed the SCUBA diver to sample at eight points equally distributed along the perimeter drawn by the rope as radium. Water was collected using a 1 l white sterilized bottle, previously filled with deionized water, at the sediment-water interface. A 40x50cm sterilized plastic bag was used to collect the first 3cm of sediment. Water samples were immediately filtered on board on 0.22□m cellulose filters. Each sediment bag was homogenized, and sub sampled thanks to three 10ml sterilized tube. Both filters and tubes were kept cold and in sterile condition on board and then stored at –80°C until eDNA extraction.
At the same stations, a morphological analysis of benthic invertebrates is carried out following the EU Water Framework Directive. By integrating the two methodologies, we aim to develop a novel integrative index for water quality assessment, to carry out cost-effective routine surveys on benthic communities.

POSTER N. 10

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EFFECTS OF GEOTEXTILES FOR COASTAL EROSION CONTROL ON MARINE FOULING SETTLEMENT

In recent years, the prevention of coastal erosion has arisen as a top priority for reducing the damage to structures and loss of lands. Climatic change is making the erosion rates worse by increasing in storm frequency and intensity. The high coastline recession rates cause significant concern in heavily populated locations. New tools are represented by nonwoven geotextiles. They are made of polypropylene (PP) and polyester (PET) fibres, which are useful in artificial structures for coastal protection. On the other hand, they represent an alternative to natural hard substrates and could interfere with the settlement of fouling species in respect of natural substrates. To better understand the effects of geotextiles on the colonisation capability of macrofouling organisms, a 10-months study was carried out in the Lagoon of Venice (Italy).

Three different needle-punched staple fibre geotextiles were chosen: 1) White-PP, 2) hot-calendered White PP&PET, and 3) multi-layered Coloured PP&PET. Fouling settlement was monitored and analysed on panels replaced monthly with the aim to investigate significant differences among the covering surfaces of each fouling species. The measures of the areas (cm^2) per month were compared using PERMANOVA considering two fixed factors, i.e., geotextiles and month.

All geotextiles revealed a negative effect on the settlement of green and red algae, bivalve molluscs and barnacles. Conversely, they showed a positive selective effect towards both solitary and colonial ascidians. As regards the 18 organisms observed, PERMANOVA showed that for four of the organisms (*Ulva rigida*, *Ceramium ciliatum*, *Janua heterostropha*, *Bugula neritina*) the settlement takes place according to a significant negative selection on basis of both fixed parameters considered in the analysis (month and geotextile). Conversely, for two tunicates (*Ciona robusta* and *Diplosoma listerianum*) the positive selection on settlement capacity appears to be significantly dependent only according to month and not to the type of geotextiles.

Therefore, it must be considered that a long-term and extensive use of geotextiles worldwide could negatively affect local biodiversity and community growth on coasts. The progressive loss of pivot species in favour of the selection of dominant and/or invasive species could trigger negative consequences in both trophic chains of coastal ecosystems and in economical relevant activities of fishing industry and aquaculture.

POSTER N. 11

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EFFICACIA DI PCR E QPCR NELLA DIAGNOSTICA DEI PATOGENI ASSOCIATI ALLA MORIA DI MASSA DI *PINNA NOBILIS*: DUE METODICHE A CONFRONTO.

A partire dal 2016 le popolazioni appartenenti al più grande bivalve endemico del Mediterraneo, *Pinna nobilis*, sono andate incontro ad un declino repentino che ha portato la specie sull'orlo dell'estinzione. I numerosi eventi di mortalità di massa sono attribuibili ad una malattia multifattoriale, che nel tempo ha interessato l'intero bacino del Mar Mediterraneo espandendosi in direzione ovest-est e le cui cause, ad oggi, non sono ancora del tutto chiare (SCARPA *et al*. 2020). Nell'ottica di un piano di recupero e ripopolamento della specie iniziato nel 2021 con il Progetto Europeo *LIFE Pinna* (di cui gli scriventi dell'Università di Sassari sono partner), nel presente studio sono stati analizzati mediante tecnica PCR e qPCR 49 individui di *Pinna nobilis*, 2 individui di *Pinna rudis* e 30 bivalvi "sentinella" provenienti dall'Isola dell'Asinara, Olbia e Oristano (Sardegna), al fine di valutare la presenza, nei tessuti degli individui campionati, dei patogeni attualmente associati alla moria di *Pinna nobilis*. L'obbiettivo finale del presente studio è stato quello di mettere a confronto l'efficacia dei due metodi di indagine diagnostica (PCR e qPCR) per valutare la presenza/assenza dei patogeni *Haplosporidium pinnae* e *Mycobacterium* spp. I risultati ottenuti attraverso PCR e qPCR sono stati messi a confronto mediante analisi di regressione lineare, utilizzando il modello GLM implementato nel pacchetto R lme4 (BATES *et al*. 2015). È stato evidenziato un incremento del potenziale diagnostico (+55%) nei risultati ottenuti dalla qPCR per l'individuazione di batteri appartenenti al genere *Mycobacterium*. Al contrario, la capacità diagnostica delle due metodiche (con un lieve aumento del 14% di diagnosi di positività per la qPCR) appare similare per il rilevamento del protozoo *Haplosporidium pinnae*. Considerando che per la diagnostica di *Mycobacterium* spp. vengono utilizzati primers universali, la discrepanza fra l'efficienza delle due metodiche potrebbe ridursi con l'utilizzo di primers specie specifici come nel caso di *Haplosporidium pinnae*.

POSTER N. 12

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FIRST APPLICATION OF NISECI INDEX IN TUSCAN WATERCOURSES

Tuscany Region applied the classification based on the NISECI (New Fish Community Ecological Status Index) method for updating the knowledge framework of the Water Protection Plan. The NISECI (MACCHIO *et al.*, 2017) has been adapted to the requirements of the EU Water Framework Directive (Directive 2000/60 / EC). It is identified in its original formulation ISECI by Italian legislation as the official method for river fish fauna (D.M. 260/2010).
In NISECI, the main among the criteria for evaluating the ecological status of a watercourse is the completeness and consistency of the composition in native fish species expected. They are quantified positively for the expected indigenous species and negatively for the aliens in relation to the zoogeographic and ecological framework, then the biological condition of populations present are considered in terms of abundance and population structure.
Comparing the populations sampled and the communities taken as reference to be consistent, *ad hoc* lists have been drawn, thanks to the DILETTA database that collects about 20 years of field experiences of the local fish communities in Tuscany watercourses and grey literature. Our lists of species are indicated in tables that partially differ from those indicated in the NISECI Manual. The sub-metrics relating to population density and size were also compared with previous monitoring experiences. Sampling was performed across seasons over one year (2020) and present different environmental characteristics.
In 5 out of 25 monitored stations, the method was found to be inapplicable: in 4 rivers the fish community was monospecific while in 1 the outflow appeared discontinuous and completely insufficient for the survival of the fish fauna. Ten waterways have a Moderate NISECI ecological status, none of them has an Elevate.
The logic of the NISECI of considering the increase of number of native species as wealth index of the fish community clashes with the reality of many Apennine waterways. In fact, the number of native species is rather low in Tuscany and therefore the NISECI risks underestimating situations of value of this area, where fish populations are often represented by a monospecific community of *Telestes muticellus* or *Cottus gobio*, but this does not mean that they do not represent a naturalistic reality of value.
In 2022 the implementation of NISECI method is in progress and additional 20 Tuscan watercourses are under consideration.

POSTER N. 13

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HIDDEN BIODIVERSITY WITHIN THE "FLOWERS OF THE SEA" (SABELLIDAE, ANNELIDA)

Sabellidae Latreille, 1825 account for 42 genera and 512 valid species (WoRMS). Despite the low number of specialists worldwide, a high number of new species has been described in the latest years leading to the necessity for a deep review of most genera. (CAPA *et al*. 2021). The availability of modern methodologies and technologies, allowed the detection of species complex, often cryptic, widely distributed within the family. However, a good morphological analysis remains the basis for taxa definition and identification. We present 2 cases of study, the first relative to *Amphiglena mediterranea* (Leydig, 1851), the latter to *Myxicola infundibulum* (Montagu, 1808). In both cases a deep morphological analysis was achieved, not only increasing the resolution for traditional characters, but also taking in account biometric relationships among and within different body elements. *Amphiglena mediterranea* is a tiny sabellid inhabiting photophilic algae in shallow waters, characterized by direct development and intratubular brooding (ROUSE and GAMBI, 1998). It is the type species of the genus, described for European water, and, until recently, considered a cosmopolitan taxon. Recent molecular analysis unveiled the presence of a cryptic species complex in the Mediterranean Sea, falling under the name *A. mediterranea* (CALOSI *et al*. 2013; TILIC *et al*. 2019). Our work led to give a morphological identity to the population used in the previous molecular analysis with the description of 9 new species (GIANGRANDE *et al.* 2021). *Myxicola infundibulum* is the type species of the second genus erected in Sabellidae family (CAPA *et al.* 2021). Originally reported on softbottom environments, this species was described for the first time in British waters. At the present it is still considered a cosmopolitan as well. This was due to the work of Fauvel and Hartmann, who synonymized most American species with *M. infundibulum*. Today, every large-sized sabellid with a funnel-shaped crown, peculiarity if this species, is reported as *M. infundibulum*, despite macroscopic differences. A recent molecular analysis confirmed the Mediterranean origin of the species in Australian water and the divergences of these from one American taxon (DANE, 2008). We studied the morphology of different populations inhabiting enclosed systems along the Italian coast, recognizing at least 4 different new taxa before identified as *M. infundibulum.* Moreover, available extra-Mediterranean material synonymized with *M. infundibulum* allowed us to assess important differences that can justify the re-establishment of original taxa. A molecular analysis will be performed to evaluate if the highlighted morphological differences have intraspecific or interspecific resolution, other than to clarify phylogenetic relationships between British, Mediterranean and American forms. These examples indicate as diversity within Sabellidae family is still greatly underestimated. Molecular analysis is a fundamental help in detecting cryptic complex of taxonomically demanding species. However, easily recognizable taxa can hide a completely overlooked diversity when not properly analyzed. The morphological approach, still essential, coupled with modern molecular methodologies, is the best bet for the future of biodiversity studies.

POSTER N. 14

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IL POPOLAMENTO A PORIFERI DELLE GROTTE MARINE PUGLIESI: STATO ATTUALE DELLE CONOSCENZE

Le "Grotte marine sommerse e semisommerse" costituiscono uno degli habitat europei di interesse comunitario elencati nell'Allegato I della Direttiva Habitat - codice 8330, di cui è necessario monitorare e conservare lo stato ecologico al fine di mitigare eventuali impatti umani e rimediare a possibili situazioni di degrado. L'estrema variabilità geomorfologica e topografica determina in ogni cavità condizioni diversificate che inducono la presenza di popolamenti caratteristici, la cui unicità ne evidenzia l'importanza della loro salvaguardia (SARÁ, 1974). Le grotte marine custodiscono uno dei più importanti patrimoni di biodiversità del Mediterraneo, svolgendo il prezioso ruolo di rifugio per specie endemiche e/o "relitte" di popolamenti profondi (CICOGNA *et al*., 1997; MANCONI *et al*., 2009). I popolamenti animali di grotta includono quasi tutti i *phyla* marini e comprendono sia forme vagili che sessili. Dei circa 2.000 taxa animali censiti nelle grotte marine mediterranee, tra gli organismi sessili il phylum più rappresentato è quello dei poriferi (GEROVASILEIOU and BIANCHI, 2021). Lungo le coste pugliesi, le grotte marine sono numerose alle Isole Tremiti, sul Gargano, lungo il tratto di costa compreso fra Bari e Brindisi e in Salento. Tra le grotte pugliesi quelle delle Isole Tremiti e quelle salentine sono fra le più studiate, mentre ampi tratti di costa regionale, pur essendo particolarmente ricchi di cavità marine, risultano ad oggi inesplorati. Gli studi effettuati sulle grotte marine delle Isole Tremiti hanno riguardato la caratterizzazione delle biocenosi di grotte semisommerse, con particolare riguardo al comparto bentonico (CARDONE *et al*., 2022; CORRIERO *et al*., 1998; 1999; 2000; DE ZIO GRIMALDI *et al*., 2001; GALLO D'ADDABBO *et al*., 2001; LONGO *et al*., 2003; SANDULLI *et al*., 1999; SARÀ, 1961). Le numerose ricerche condotte nelle grotte marine salentine (oltre 40 articoli) hanno censito più di 600 taxa animali (ONORATO and BELMONTE, 2017). L'analisi della letteratura disponibile (pubblicazioni scientifiche nazionali ed internazionali e letteratura grigia) ha evidenziato conoscenze sulla composizione del popolamento a poriferi per 28 grotte marine pugliesi così distribuite: 10 in Salento, 2 lungo la costa barese e 16 alle Tremiti. Sebbene lo sforzo di ricerca non sia confrontabile tra tutte le cavità, l'analisi critica dei dati di letteratura ha permesso di censire complessivamente 144 taxa di poriferi, 132 identificate a livello di specie. Il popolamento comprende: 10 Calcarea, 125 Demospongiae e 9 Homoscleromorpha. Le grotte salentine contano complessivamente 76 taxa di poriferi, quelle della costa adriatica barese 13, mentre le grotte tremitesi, che ospitano il maggior numero di taxa, ne contano 122. Escludendo i popolamenti delle due grotte della costa barese, il 39,44% dei taxa sono in comune tra le grotte del Salento e quelle delle Tremiti, mentre le specie esclusive nelle due aree sono il 12,67% ed il 46,48% per le grotte del Salento e delle Tremiti, rispettivamente.

POSTER N. 15

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IS THERE A UNIVERSAL DNA BARCODE GAP IN TARDIGRADES?

Widely regarded as a revolutionary taxonomic tool, DNA barcoding represents, even two decades after its introduction, a crucial framework for species delimitation across all organisms. However, the definition of a clear-cut method to determine species boundaries probably represents the main issue of this method. One of the methods to delimit species is the identification of the barcode gap (i.e. the limit between smaller intraspecific distances and larger interspecific distances), which is very fast and computationally undemanding. In tardigrades, the first application of DNA barcoding was on *Macrobiotus* species, where it was proposed that the barcode gap allowing to distinguish between eutardigrade species is around 3% p-distance. The integrative taxonomic approach, adopted by an increasing number of researchers, has allowed to expand the dataset of available *cox1* sequences that, once analysed, will allow understanding if there is a universal value of DNA barcoding gap in Eutardigrada. The *cox1* sequences belonging to both eutardigrade Apochela and Parachela classes were analysed using the Assemble Species by Automatic Partitioning method (ASAP). Basing on the barcode gap, ASAP sorts the sequences into groups corresponding to putative species. The program first detects the barcode gap as the first significant gap beyond a model-based one-sided confidence limit for intraspecific divergence, and then uses it to produce several partitions of the data. Then ASAP computes an *ad hoc* ASAP-score for each defining partition, with the lower score indicating the better partition, thus overcoming the challenge of a priori definition. Analyses performed with ASAP identified strongly differing threshold values of the barcoding gap, depending on the different considered taxa. For example, in the *Macrobiotus* genus the threshold value was 4.5%, while, when considering all the superfamily Macrobiotoidea taxa, the threshold value was highly different (13.9%). A clear-cut barcode gap for tardigrades is at moment complicated and difficult to achieve. Data point out that relying on a single gene and a single method for species delimitation will probably give a potentially distorted assessment of species boundaries, thus suggesting that using different tools based on different methods and frameworks would possibly achieve a most comprehensive biodiversity assessment.

POSTER N. 16

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LA DIVERSITA' ENTOMOLOGICA DELLE SERRE CALABRESI (APPENNINO CALABRO)

La Calabria ospita un'entomofauna molto diversificata, osservabile soprattutto in aree naturali protette come quelle dei parchi, dove purtroppo non mancano i disturbi antropici. La diversità dell'entomofauna in Calabria deriva dalla sua posizione nella regione mediterranea e dal suo passato zoogeografico. Come in altre aree geografiche, le alte montagne rappresentano importanti rifugi e "hot spot" per la diversificazione dei taxa. In Calabria, i parchi nazionali e le aree forestali sono aree in cui si osserva una elevata biodiversità (BONACCI *et al.,* 2020). L'area delle Serre Calabresi è una zona collinare e montuosa caratterizzata da habitat prevalentemente forestali, poco studiata dal punto di vista della diversità entomologica. Nonostante sia un'area fortemente naturale, presenta al suo interno numerosi centri abitati ed è interessata da attività antropiche legate all'industria del legname e all'agricoltura. Nel corso di vari progetti di ricerca è stato possibile indagare la biodiversità entomologica delle Serre Calabresi attraverso varie tipologie di campionamento. Tra i metodi utilizzati vi sono metodi diretti (raccolta a mano) e indiretti (*bait bottle traps*, Malaise, *pan traps*, *light traps* e *pitfall traps*). Le attività di campionamento sono state svolte dal 2020 al 2022 in 42 siti. Sono stati raccolti 51346 esemplari di insetti appartenenti a 135 famiglie. Molte specie raccolte sono endemiche, altre hanno un notevole interesse ecologico, medico-veterinario, forense ed economico. Alcune sono incluse nella Lista Rossa Europea, come alcuni coleotteri saproxilici. Tra le specie di interesse economico vi è ad esempio la specie alloctona, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), dannosa per le piante da frutto. Sono state inoltre raccolte specie di importanza medico-veterinaria appartenenti all'ordine Diptera, famiglie Calliphoridae, Fanniidae e Tabanidae. Sono presenti anche specie di interesse forense appartenenti all'ordine Diptera, famiglie Calliphoridae, Muscidae e Sarcophagidae, e all'ordine Coleoptera, famiglie Cleridae, Dermestidae e Silphidae. Dalle indagini sono emerse nuove ed interessanti segnalazioni che richiedono ulteriori studi entomologici in questa area della Calabria. Lo studio sulla biodiversità nelle Serre Calabresi oltre ad aver evidenziato l'eccezionale endemicità e ricchezza dell'entomofauna di questo territorio, fornisce le basi per ulteriori analisi che potranno servire per adottare misure appropriate, finalizzate alla conservazione e gestione dell'entomofauna e degli ecosistemi coinvolti.

POSTER N. 17

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MONITORAGGIO DEGLI IMPOLLINATORI DEL PARCO DEL POLLINO: CONSISTENZA E DISTRIBUZIONE DI APOIDEI E FARFALLE IN AREE NATURALI E COLTIVATE

Numerosi studi hanno recentemente documentato una riduzione della consistenza delle popolazioni degli impollinatori e in particolare degli apoidei, uno dei taxa di insetti terrestri che evidenziano tassi di declino tra i più accentuati (SÁNCHEZ-BAYO and WYCKHUYS, 2019). Il MITE ha avviato nel 2019 un programma di ricerca finalizzato ad incrementare la conoscenza di Apoidei e Lepidotteri Ropaloceri nei Parchi Nazionali. In questo studio presentiamo i primi risultati delle indagini realizzate nel PN del Pollino. I campionamenti sono stati effettuati lungo 66 transetti, , localizzati in maniera tale da garantire una adeguata conoscenza della maggior parte del territorio del Parco. Tra i transetti localizzati in aree naturali ve ne sono alcuni già percorsi in passato (SCALERCIO et al., 2014), così da registrare eventuali variazioni incorse negli anni. I transetti in aree agricole sono localizzati all'interno delle principali tipologie colturali presenti nel Parco e in aziende a differente modalità di conduzione (tradizionale o biologica), così da cogliere le eventuali differenze determinate dall'applicazione di pratiche agricole a diverso grado d'intensità. Le dimensioni dei transetti e le modalità di raccolta sono coerenti col metodo descritto nel Rapporto ISPRA 330/2020. Per i Lepidotteri diurni si utilizzano transetti di 500m. Gli Apoidei vengono campionati lungo i medesimi tracciati ma utilizzando transetti di 250m. Il PN del Pollino non è mai stato finora oggetto di studi organici relativamente alla presenza e allo stato di conservazione degli impollinatori. Questa ricerca ha consentito di costruire un quadro conoscitivo approfondito, che fornisce elementi utili alla definizione di una strategia organica di tutela degli impollinatori, ad esempio identificando le aree di maggiore valenza per le specie di interesse conservazionistico. Inoltre, ha potuto confermare la tendenza già osservata in passato delle modificazioni in atto nelle comunità delle farfalle in risposta al cambiamento climatico.

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POSTER N. 18

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MONITORAGGIO E GESTIONE DELLA LEPRE ITALICA (*LEPUS CORSICANUS*)

La lepre italica (*Lepus corsicanus,* de Winton 1898) è una specie endemica dell'Italia centro-meridionale e della Sicilia. E' classificata come *vulnerabile* dalla IUCN, soprattutto per la perdita di habitat idonei, la frammentazione dell'areale, l'isolamento delle popolazioni e la competizione con la lepre europea (*Lepus europaeus*, Pallas 1778).
Le strategie di conservazione si basano principalmente sull'acquisizione di informazioni sulle sue esigenze ecologiche. Solo così è possibile agire correttamente, con la gestione dell'ambiente potenzialmente idoneo, la creazione di corridoi ecologici, riproduzioni ex-*situ* o riducendo le pressioni di altre specie che competono per le stesse risorse.
Per acquisire tali informazioni è necessario un montaggio teso alla comprensione dei tratti ecologici, minimizzando il disturbo alla specie. Noi, abbiamo approfondito le conoscenze sulle esigenze spaziali e trofiche, includendo quasi il 70% dell'areale di distribuzione peninsulare. I dati sono stati raccolti con metodiche differenziate, come il monitoraggio attraverso spot light e il campionamento di pellet fecali lungo transetti a piedi e/o con segugi addestrati alla ricerca. La georeferenziazione delle fonti (geneticamente assegnate tramite HRM) è stata il primo passo per un'analisi della distribuzione reale e potenziale (sviluppata mediate modelli di massima entropia). Inoltre, un con un approccio di DNA metabarcoding e HTS su materiale genetico estratto dai pellet, sono state definite la dieta e le sue variazioni in accordo con le stagioni e in differenti contesti ambientali.
Le analisi mostrano che la lepre italica sembra essere una specie adattabile, capace di esplorare un'ampia varietà di risorse ecologiche, spesso in simpatria con la lepre europea. Ad oggi, gli Appennini mostrano molte tipologie di habitat idonee alla presenza della lepre italica, Infatti, la reale minaccia sembra risiedere piuttosto nella frammentazione del paesaggio indotta dall'uomo, potenzialmente connessa a riduzione di flusso genico e fenomeni di inbreeding. Sulla base di queste evidenze, abbiamo avviato un programma di zoo-conservazione per incrementare la connessione tra le popolazioni isolate, liberando in natura individui allevati in cattività, partendo proprio dagli habitat ecologicamente idonei. Ulteriori sforzi sono necessari per approfondire altri aspetti, come la competizione ecologica con la lepre europea, in modo da definire sempre migliori e fattive strategie di conservazione.

POSTER N. 19

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MONITORING AMPHIBIANS AND CRUSTACEANS OF THE SERCHIO RIVER WITH AN INTEGRATED APPROACH: TRADITIONAL MONITORING AND SETTING UP THE eDNA APPROACH

Freshwater environments are the most endangered worldwide, due to climate change and to anthropogenic factors, resulting in a strong decrease of their biodiversity. In the last decade, molecular ecology has been strongly focused on developing new methods to evaluate and monitor the biodiversity of various ecosystems. Amid these methods, environmental DNA (eDNA) metabarcoding reached great acclaim, mainly due to its sensitivity, non-invasiveness, and ability to simultaneously identify species. Notwithstanding its advantages, eDNA metabarcoding requires calibration at a local scale, in particular as regards the selection of primer pairs able to depict the resident biodiversity. Moreover, it is crucial to correctly integrate molecular and traditional methods for a proper comparison with previous datasets. In this study we selected the Serchio river basin to develop and shape a synergic interaction between traditional monitoring and metabarcoding. Monitoring through standardized transects was carried out for several species of amphibians (the most endangered vertebrates) and two species of crustaceans. Trough QGIS and R, physical and geographical parameters of waypoints have been elaborated to understand local habitat selection for various species. To set-up metabarcoding methods at a local scale, and to account for primer and sequencing biases, a mock community was established by pooling equimolar concentrations of DNA extracted from tissue samples of 39 known local species of ecological interest, including frogs, salamanders, and freshwater crayfish and crabs. The mock community obtained was amplified trough PCR with three published molecular markers, and PCR products were sequenced with Novaseq 6000. Sequences have been processed through QIIME2 bioinformatic tools and R. Positional data collected, representing, for many of the species studied, the newest and largest distribution database for the region, were consequentially used to estimate habitat selection models, which are helpful to better understand the autecology of those species, predict new areas of distribution, correctly select eDNA sampling sites and soundly interpret future metabarcoding data. The designed metabarcoding assay portraited the complete biodiversity of the mock community with an excellent taxonomic resolution. Through traditional methods, this study updated the distribution of several endangered species in the Serchio River basin, and at the same time allowed to set up a molecular and bioinformatic pipeline for biomonitoring, usable by research groups and stakeholders.

POSTER N. 20

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PIANO DI GESTIONE DELLA PESCA DEL POLPO (*Octopus vulgaris*) NELLE AREE MARINE PROTETTE DELLA REGIONE CAMPANIA

Il polpo è considerato una prelibatezza alimentare tipica del Mediterraneo e una risorsa fondamentale per la pesca in quest'area geografica. Annualmente milioni di polpi di diverse specie vengono catturati con metodi di pesca industriale ed artigianale, non adeguati alla vigente legge europea relativa protezione dei cefalopodi. La mancanza di una tutela legislativa adeguata ha indotto la Regione Campania, sensibile alla problematica, ad interpellare il mondo della ricerca ad individuare un piano di gestione per la pesca del polpo comune (*Octopus vulgaris*) più sostenibile ed etico. L'elaborazione di questo piano è frutto dell'analisi dei dati di cattura e sforzo relativa ad un sub-campione di imbarcazioni operanti nell'area marina protetta del Regno di Nettuno (Golfo di Napoli) e che utilizzano il sistema a trappola (nassa). Sono state individuate due barche appartenenti al settore della piccola pesca, operanti rispettivamente nelle marinerie di Ischia e Pozzuoli, ed è stata condotta una rilevazione periodica dello sbarcato. Sono state raccolte informazioni inerenti al numero di polpi pescati giornalmente e alle tecniche di pesca. Si è poi proceduto con l'acquisto con un numero congruo di esemplari di *O. vulgaris*, di peso variabile tra i 400 e i 1500 g, sottoposti a rilevamenti biometrici riguardanti la maturazione sessuale (indice gonadosomatico), la sex ratio, l'osservazione dello stress da pesca mediante l'analisi di indicatori macroscopici (vitalità, escoriazioni del mantello e mutilazioni delle braccia) e ad indagini molecolari finalizzate all'identificazione della specie, mediante *DNA Barcoding*. Inoltre, durante il periodo di monitoraggio sono stati campionati diversi esemplari di polpo al fine di rilevarne i dati morfometrici e comparare gli indicatori macroscopici dello stress di quelli pescati con le nasse con quelli degli individui catturati nelle reti a tramaglio. Queste analisi hanno dimostrato che tra i polpi pescati con le nasse, il 66% presentavano escoriazioni anche molto estese e il 18% mutilazioni dovute ai tentativi di fuga, a lotta intraspecifica o alla presenza nella gabbia di predatori, come le murene. Tra gli esemplari catturati nelle reti a tramaglio, invece, il 48% presentavano piccole escoriazioni ed uno solo era mutilato. Inoltre, a valle della somministrazione ai pescatori di un questionario elaborato dal gruppo di ricerca, è emerso che questi ultimi praticano la pesca del polpo circa 300 giorni l'anno. Le nasse, contenenti granchi o sarde come esche, vengono posizionate fino a profondità di 70 m, in quantità comprese tra le 300 e le 700 unità, e sono interamente costruite in reti di plastica, spesso fragili e quindi potenzialmente inquinanti. Infine, in base alle le stime di entrambe le imbarcazioni, si evidenzia un andamento spaventosamente decrescente nei kg di pescato, negli ultimi 20 anni. L'importanza strategica dell'elaborazione di un piano di gestione della pesca del polpo risulta impellente, in quanto i nostri i risultati dimostrano che lo sforzo di pesca stia diventando sempre meno sostenibile per la resilienza delle popolazioni di *O. vulgaris* nel golfo di Napoli e che i metodi di pesca non risultano adeguati alle caratteristiche etologiche ed al ruolo ecologico che questi animali svolgono nell'ambiente marino.

POSTER N. 21

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RECRUITMENT VARIABILITY OF *SABELLA SPALLANZANII* (ANNELIDA, SABELLIDAE) IN THE MAR GRANDE OF TARANTO

During the past 30 years the Zoological Research group of the Salento University has been studying the biology of *Sabella spallanzanii*, in the area around the Mar Grande of Taranto (Ionian Sea) also for possible application purposes. During this period a high variability in the settlement rate was observed each year, this variability was, however, only the object of simple observation and until now no data were available to evidentiate a true pattern. Considering its high abundance in the area, and its easiness of collection, this species is at present exploited, within the life project 'RemediaLife', as a bioremediator of the water column in mariculture with its biomass used for different purposes in different fields, from baits to row material for fodder production. Therefore, during the time span of this study, data on density and settlement of the species in the area has been available. To obtain a large quantitative of worm biomass, in the RemediaLife Project each year several collectors (coconut ropes) were placed around the fish cages and the settlement of larvae was observed. Each year *S. spallanzanii* reproduces massively in winter and placing the collectors just before the spawning period of this target species a community dominated by this worm can be obtained. So, we have had a large amount of material to analyse recruitment rate during 4 years of productive cycle. We observed a bimonthly pattern of abundance with densities going from 1300 to 400 individuals for square meter. Recruitment was higher in the years 2019 and 2021 than in the years 2020 and 2022. The highest recruitment rate was observed in 2021 and the lowest in 2020. Previous studies suggested as the final fouling community is mostly determined by biological factors, such as types and abundance of larvae and propagules, and as settlement variation patterns were implicated as drivers of fouling succession. A better knowledge of such processes could improve the management and the employment of fouling biomass towards useful purposes and so reducing its negative impact on the facility structures.

POSTER N. 22

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SEASONAL VARIATION OF MOLLUSC DIVERSITY CHARACTERIZING THE SOFT-BOTTOM SUBLITTORAL ZONE OF CAPRI ISLAND (GULF OF NAPLES, ITALY)

Molluscs are widely known to be efficient biological indicators of environmental conditions, both in pristine and human-altered sites (e.g., with organic enrichment, heavy metals). In this study, an investigation of the indicator species among the mollusc assemblages characterizing the circalittoral soft-bottom of the north side of Capri Island (Gulf of Naples, Mediterranean Sea) was carried out. In winter (C1), summer (C2) and autumn (C3) 2020, sediment samples were collected with a Van Veen grab (25L), at 20 stations within the depth range of 40-110 m. Here, the soft-bottom is mainly characterized by biogenic and coarse sand deposits, and bivalves are the main representatives of this substrate, classified by RAC\SPA as "Coastal Detritic Biocenosis (DC)". Consistent with this, bivalves were dominant in species richness (54 sp.) and abundance (565 ind.), followed by gastropods (49 sp.; 147 ind.), and scaphopods (4 sp.; 107 ind.). *Abra prismatica* (Montagu, 1808), *Cardiomya costellata* (Deshayes, 1835) and *Modiolula phaseolina* (Philippi, 1844) were the "exclusive characteristic species" of the DC, occurring in all sampling periods, even though the gastropod *Cylichna cylindracea* (Pennant, 1777), the bivalve *Bathyarca pectunculoides* (Scacchi, 1835), and the scaphopod *Antalis inaequicostata* (Dautzenberg, 1891) were the most representative species, accounting for 50% of the similarity among the three assemblages (SIMPER analysis: C1=44.22%; C2=40.16%; C3=49.69%). Overall, molluscs displayed a seasonal trend in species richness and abundance, with the highest values observed during winter and the lowest ones recorded during summer. Statistical differences were detected among seasons (PERMANOVA analysis; F=2.383 – p=0.0002) and stations (F=1.619 – p=0.0002), while variations of mollusc assemblages among stations for each sampling period were not significant (F=1.051 – p=0.245). Despite differences among seasons, probably due to the reproductive life cycle of structuring species, a mollusc assemblage structure consistent with the DC biocenosis is evident. Moreover, our findings suggest a good environmental status of the study area with the occurrence of biological indicators, such as the dominant species. They are sensitive to organic enrichment and occur in "unpolluted or normal environmental conditions" (I and III order in the marine Biotic Index).

POSTER N. 23

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VALUTAZIONE DELLE COMUNITA' DI MACROINVERTEBRATI IN SISTEMI LOTICI ASSOCIATI A CARTIERE NELLA PROVINCIA DI LUCCA

Gli ambienti dulciacquicoli costituiscono habitat unici per la biodiversità ed una risorsa fondamentale, fortemente minacciata da cambiamenti climatici, sfruttamento eccessivo, ed altri fattori che possono portare al degrado se non alla perdita di tali ambienti. La direttiva 2000/60/CE, recepita nell'ordinamento nazionale tramite il D.Lgs 152/2006, istituisce un quadro di riferimento per l'azione comunitaria in materia di acque ai fini della tutela e gestione delle risorse idriche, inserendo il monitoraggio come strumento di salvaguardia e prevenzione. Oltre a parametri chimico fisici, viene indicato il monitoraggio delle comunità biologiche. In Italia, fino all'abrogazione del D.Lgs 152/1999, il metodo di riferimento è stato l'Indice Biotico Esteso (I.B.E.), oggi integrato nell' indice STAR_ICMi, ma ancora valido per l'assegnazione di classi di qualità alle acque superficiali. La Provincia di Lucca è sede di uno dei più importanti poli cartari italiani. Tra i principali impatti derivanti dalle cartiere figurano il consumo d'acqua, un grave problema nella piana lucchese, e lo scarico delle acque reflue che, in seguito a depurazione, vengono spesso immesse in acque superficiali. Dalla seconda metà di Novembre 2021, sono stati effettuati campionamenti delle comunità di macroinvertebrati presenti in quattro torrenti tributari del fiume Serchio, associati a quattro cartiere nella provincia di Lucca, individuando zone di prelievo a valle e a monte dello scarico degli impianti. I campioni raccolti sono stati identificati morfologicamente per procedere ad analisi di comunità e calcolo dell'Indice Biotico Esteso. Inoltre, da alcuni organismi, prelevati come voucher per analisi molecolari, è stato estratto il DNA e su tale materiale sono state effettuate analisi molecolari per la caratterizzazione di geni barcode e la realizzazione di un relativo database. I risultati ottenuti dall'analisi delle unità sistematiche recuperate hanno restituito valori differenti per le diverse stagioni di campionamento. Il confronto degli indici di Bray-Curtis per i dati della stagione di campionamento del 2021 ha generato una rappresentazione che suggerisce una effettiva differenza tra le componenti a monte e a valle dei singoli stabilimenti. I dati raccolti possono fornire un valido punto zero per futuri studi su macroinvertebrati in contesto locale, per il monitoraggio dello stato di qualità dei sistemi lotici e delle componenti faunistiche dipendenti da questi, ponendo l'attenzione sui cambiamenti climatici in atto e l'utilizzo delle risorse idriche.

POSTER N. 24

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DIVERSITY AND BIOGEOGRAPHY OF DEEP-SEA ONUPHIDAE (ANNELIDA: EUNICIDA) FROM THE MEDITERRANEAN SEA

The deep Mediterranean Sea is characterised by homeothermic conditions, a low nutrient load, and a low abundance of most organisms, which hinders their study. For these reasons, deep-sea Mediterranean fauna was historically regarded as an impoverished Atlantic fauna, and it is largely unknown from the taxonomic and biogeographical point of view. In this context, the project DeePoly focuses on Mediterranean deep-sea polychaetes, which represent the dominant component of bathyal benthic assemblages in terms of both abundance and diversity, to give a deeper insight into their diversity and biogeography. Representatives of the family Onuphidae are particularly widespread in bathyal Mediterranean environments, where they attain relatively large sizes, and might contribute to sediment stability through their permanent tubes. In this study we analysed Mediterranean Onuphidae specimens from depths ranging from 100 to 800 m from the morphological and molecular point of view. Molecular characterisation was carried out through two mitochondrial markers: 16S rRNA and Cytochrome c Oxidase subunit I genes. We identified six species belonging to four genera, namely *Aponuphis* Kucheruk, 1978, *Hyalinoecia* Malmgren, 1867, *Nothria* Malmgren, 1867, and *Paradiopatra* Ehlers, 1887. The genera *Aponuphis* and *Hyalinoecia* include one species each, with their distribution encompassing the whole north-eastern Atlantic and the Mediterranean Sea. The genus *Paradiopatra* included two species, *Paradiopatra calliopae* Arvanitidis & Koukouras, 1997, occurring off West Africa and in the Mediterranean between 100 and 400 m depth, and *Paradiopatra fjordica* (Fauchald, 1974), known from off Norway and here firstly recorded in the deep Mediterranean below 600 m depth. Lastly, the genus *Nothria* includes two species, a larger one occurring between 200 and 300 m depth, close to the Atlantic *Nothria conchylega* (Sars, 1835) and tentatively identified with the overlooked taxon *Onuphis jourdei* Marion, 1883, and a smaller one occurring below 600 m, probably corresponding to *Nothria maremontana* André & Pleijel, 1989. These results suggest the co-occurrence in deep Mediterranean environments of two biogeographical components: a) Senegalese with warm affinity, occurring in low circalittoral and intermediate environments; and b) Lusitanic with cold affinity, occurring in deeper environments.

POSTER N. 25

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ACTIVITY OF TRANSPOSABLE ELEMENTS RELATED TO SALINITY CONDITIONS IN TELEOSTS

Teleosts include species adapted to a wide range of environments. Some of them have to face consistent salinity changes during their life cycle due to reproductive purposes. We considered the giant marbled eel *Anguilla marmorata* and the chum salmon *Oncorhynchus keta*, both diadromous species, and the marine medaka *Oryzias melastigma*, a euryhaline organism *sensu stricto*. We investigated the transcriptional contribution of transposable elements (TEs), one of the most intriguing components of the genome, in the gill transcriptomes of these species treated with different salinity conditions. Our results highlighted changes in TE activity in the case of juvenile eels, commonly adapted to salty water, when exposed to brackish and freshwater conditions. In addition to the activity of *Ago* and *Piwi* genes and of genes involved in heterochromatin formation, we also reported the activity of NuRD complex in fish species. Intriguingly, in sarcopterygians, this mechanism needs a krüppel-associated box (KRAB) proteins and KAP1/TRIM28 to recruit the NuRD complex. Both proteins are absent in actinopterygians. However, we identified a KRAB-like protein specific of fish and tested its interaction with TRIM33 through molecular docking analyses.

POSTER N. 26

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ARRICCHIMENTO DELLA CUTICOLA CON METALLI DI TRANSIZIONE E ALCALINO-TERROSI NELLE VESPE PARASSITOIDI DELLA FAMIGLIA MUTILLIDAE

Uno dei tratti più variabili associati alla struttura della cuticola degli insetti riguarda la presenza sia di metalli di transizione che di metalli alcalino-terrosi, che si ritiene, e in pochi casi è stato dimostrato, aumentino la durezza e la resistenza all'usura. Le vespe parassitoidi della famiglia Mutillidae (Hymenoptera: Aculeata) sono comuni nemici naturali di api e vespe e sono noti per possedere una cuticola molto forte. In questo studio, abbiamo analizzato con microscopia elettronica a scansione (SEM) associata a spettroscopia EDS (Energy Dispersive X-ray Spectroscopy) la presenza e l'abbondanza di metalli in sei specie di mutillidi, e in cinque specie di altri imenotteri aculeati appartenenti a famiglie sfruttate da questi parassitoidi come come ospiti. Abbiamo trovato evidenze di un diverso profilo di metalli nella cuticola dei mutillidi rispetto agli altri aculeati. Complessivamente, i mutillidi presentano alluminio (Al), zinco (Zn), calcio (Ca), manganese (Mg) e ferro (Fe) nella loro cuticola, mentre le altre specie mancano di Fe e, ad eccezione delle formiche, mancano anche di Zn. Al, Zn e Ca si trovano generalmente in maggiore abbondanza nei mutillidi rispetto alle altre specie. Il cloro (Cl) co-varia con lo Zn, suggerendo che quest'ultimo è presente in una forma molecolare in congiunzione con questo alogeno. Ancora più interessante è il fatto che il profilo dell'arricchimento in metalli nella cuticola varia considerevolmente con la parte del corpo considerata. Infatti, i mutillidi hanno soprattutto la cuticola degli occhi e della porzione della testa tra gli occhi – ma non la cuticola del mesosoma e del metasoma – più ricche di Al e Ca rispetto alle altre specie, mentre Zn e Fe sono stati trovati esclusivamente nelle mandibole. Al contrario, si è evidenziata una debole tendenza ad una maggiore % di Mg nei non-mutillidi rispetto ai mutillidi. Suggeriamo che i mutillidi possano trarre vantaggio da una cuticola rinforzata in metalli nella parte anteriore del corpo (capo e strutture sul capo) durante l'invasione del nido dell'ospite, che spesso implica violenti incontri con gli occupanti. Tali parti del corpo rinforzate potrebbero essersi originariamente evolute nei mutillidi per proteggersi dall'attacco di vertebrati predatori, in linea con molti altri loro adattamenti (es. lungo aculeo, colorazione aposematica), ma risulterebbero efficienti anche per loro attività parassita.

POSTER N. 27

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ASSESSING ANHYDROBIOTIC PERFORMANCE - THE DIVERSITY AND EVOLUTION OF DESICCATION RESISTANCE IN TARDIGRADA (MACROBIOTIDAE).

Introduction. Anhydrobiosis (the ability to survive environmentally induced loss of body water) is the most widespread and studied form of cryptobiosis. Unfortunately, not only do laboratory protocols vary considerably between research groups and across study organisms, but there are few phylogenetic comparative studies on how this trait evolves. Our aims are to develope new analysis methods for data from anhydrobiosis experiments and to address how this trait evolves using a model system for the study of desiccation tolerance: tardigrades. **Methods.** We developed a new approach based on the Bayesian fitting of a cumulative exponential distribution function on anhydrobiotic recovery curve of the proportion of motile individuals against monitoring time points. This method estimates both the overall survival (where the curve flattens) and recovery speed (the rate of increase of the proportion of motile individuals with time). These two measures can also be combined into a single anhydrobiotic index as the area under the fitted recovery curve. We analyzed both published data on the effect of different chemicals on anhydrobiotic performances and newly produced data. For the newly produced data, we estimated the anhydrobiotic index for over 20 Macrobiotidae populations under a standardized desiccation protocol. These values were then analyzed in a phylogenetic context. **Results.** Based on the reanalysis of published data, we first demonstrate that our method not only can estimate reliable anhydrobiotic performance measures, but also does so for any combination of anhydrobiotic protocol, study species, and experimental setup and provides an intuitive visualization of the estimated parameters. Secondly, the phylogenetic comparative study highlighted a considerable variability in anhydrobiotic performance, even between closely related species of Macrobiotidae. The three measures of anhydrobiotic performance (overall survival, recovery speed and anhydrobiotic index) show weak phylogenetic signal (the best fitting model is white noise model, which assumes data come from a single normal distribution with no covariance structure among species), suggesting a fast evolution of anhydrobiotic performances in response to environmental pressures. **Conclusions.** Our approach provides three distinct measures of anhydrobiotic performance. These measures are objective, reliable, and have both biologically meaningful interpretation and wide applicability. The fine-scale variability observed within this single tardigrade family (Macrobiotidae) is suggestive of different responses to desiccation in closely-related species. Therefore, the application of our method in a comparative approach can improve our understanding of variation in anhydrobiotic performance.

POSTER N. 28

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BIOPLASTIC INGESTION BY SUPRALITTORAL AMPHIPODS

Supralittoral amphipods, detritivores and scavengers, are important in the recycling of beached organic material and can ingest microplastics. The various types of bioplastics are spreading more and more, even in the environment, therefore we set out to evaluate the possible role of *Talitrus saltator* (Montagu, 1808) as a fragmenter and degrader of bioplastics. A "starch-based" bioplastic widely used for the production of disposable bags, was characterized by FTIR-ATR. 10cm side squares of bioplastic were placed in wet sand trays together with 20 adults of *T. saltator*. After 30 days we measured the survival rate, bioplastic consumption, lipid content of the amphipods. The controls consisted of wild-caught individuals and groups of individuals kept under the same conditions as the experimental ones but fed with paper and dry fish food. The results showed higher survival rate and higher consumption in the batches supplied with bioplastic than in the controls. The passage of the bioplastic into the intestine of the sandhoppers determines the lowering of a peak of the NMR spectrum associated with the lipid component, suggesting a degradation of the material. Through GC-MS, the concentrations of ten different phthalates were detected in the grasshoppers, noting a decrease in total concentrations in animals that ate bioplastic compared to individuals collected in the wild, suggesting a possible scavenging effect by the bioplastic (SCOPETANI *et al*, 2018). Finally, the sandhopper gut microbiota composition has been investigated through targeted metagenomic sequencing on the Illumina Miseq platform; respectively, the amplification of the rRNA 16S gene (V3-V4 regions) and the ITS1 region were performed to characterized both the bacterial and fungal communities, after 7 and 21 days with bioplastic as unique food source. A number of amplicon sequence variants (ASV) were described as differentially enriched in the different conditions. It is noteworthy that after 21 days, an enrichment of the genus *Rhodococcus* was detected, which is important as some strains of *R. ruber* have been shown to be of interest in the degradation of bioplastics. In conclusion, the results show a possible role of supralittoral amphipods and of microbial consortia in their gut microbiomes in the fragmentation and degradation of starch-based bioplastics.

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POSTER N. 29

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EFFECTS OF SHORT-TERM EXPOSURE TO TWO ENVIRONMENTALLY RELEVANT CONCENTRATIONS OF LEAD (PB) ON *DANIO RERIO* LIVER: AN HISTOPATHOLOGICAL AND FUNCTIONAL INVESTIGATION

Due to their non-degradability, high bioaccumulation tendency, and long persistence, heavy metals raise a great concern worldwide and are recognized as priority pollutants in the environment (KORTEI et al., 2020). Lead (Pb) is one of the most toxic and persistent heavy metals, and it is listed in the Priority List of Hazardous Substances (SHI et al., 2020). Although Pb is a natural constituent of the earth's crust, anthropogenic activities have altered its geochemical cycles and environmental distribution. Once dispersed in the environment, Pb can move in waterways as soluble complexes and ions, contaminating water bodies (ALI et al., 2016; LI et al., 2019). In fish, Pb quickly accumulates in several organs, such as the liver and gills. Available studies on the Pb-induced effects on the liver are scarce, and literature data refer to chronic exposure or high concentrations (KIM and KANG, 2017; PAUL et al., 2019). However, when evaluating the toxic potential of chemicals, it is crucial to test realistic concentrations, which can be found in a natural ecosystem. To provide a more comprehensive overview of Pb toxicity, here we investigated for the first time the effects induced by two environmentally relevant concentrations (2.5 - 5 µg/L) in *Danio rerio* liver. Given its small body size, short reproductive cycle, high fecundity, and easy husbandry, zebrafish has become a popular model for ecotoxicological studies. After 48, 96, and 192 hours, the pathological effects were evaluated through an in-depth histological examination. Moreover, by applying a standardized method, we determined the organ index that allows us to quantify the frequency and the severity of histological changes. We demonstrated that the histological abnormalities arose after 48 hours of exposure, and the most frequent alterations detected were cytoplasmic vacuolization, lysed areas, bile duct degeneration, and vessel congestion. Both the incidence and intensity of lesions were dose and time-dependent. Moreover, the organ index significantly increased ranking in Class V (index > 40, severe histological alterations) at the end of the experiment. Pb exposure also resulted in the upregulation of two markers of oxidative stress induction (superoxide dismutase1-*sod1* and catalase-*cat*). Our study emphasizes the crucial role of histopathological examination in disclosing the harmful potential of heavy metals, highlighting the use of *sod1* and *cat* a valuable biomarkers of lead-induced oxidative stress.

POSTER N. 30

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FIRST CHROMOSOME ANALYSIS ON *ZAMENIS LINEATUS* AND *ELAPHE QUATUORLINEATA* (SERPENTES: COLUBRIDAE)

Squamates are exceptional models to study karyotype and sex chromosome evolution in vertebrates. They display a remarkable variability in chromosome number and morphology, number and location of different chromosome markers, the occurrence of environmental or genetic sex determination and simple and multiple sex chromosome systems with either male or female heterogamety (ALAM et al., 2018; MEZZASALMA et al., 2021). However, despite the growing interest in their evolutionary cytogenetics, only a small fraction of squamate species has a known karyotype. We performed a comparative cytogenetic analysis on *Elaphe quatuorlineata* (Bonnaterre, 1790) and *Zamenis lineatus* (Camerano, 1891), using a combination of staining and banding techniques. We present the first karyotype description of these two species and an assessment of their chromosomal characters. Chromosome analysis was performed with standard karyotyping, chromomycin A3-methyl green staining (SAHAR and LATT, 1980), Ag-NOR staining (HOWELL and BLACK, 1980), C-banding (SUMNER, 1972), sequential C-banding + CMA_3 + DAPI and NOR-FISH (SIDHOM et al., 2020). The karyotypes of *E. quatuorlineata* and *Z. lineatus* are composed of 2n = 36, with 8 macro- and 10 microchromosome pairs. In *E. quatuorlineata*, the pairs 1, 3-5 are metacentric, the pairs 2, 7 and 8 are submetecentric and the pair 6 is telocentric. In *Z. lineatus*, the pairs 1, 3-5 and 8 are metacentric, the pairs 2 and 7 are submetacentric and the 6^{th} pair is telocentric. In both species, the pair 4 represents a sex chromosome pair with female heterogamety (ZZ/ZW) and loci of NORs were localized on a microchromosome pair. By adding our data to those available from the literature on phylogenetically closely related species (ITOH et al., 1970; SINGH, 1972; DE SMET, 1978; AUGSTENOVÁ et al., 2017; ROVATSOS et al., 2018), we analyse the occurrence of plesio- and apomorphic chromosomal states in the genera *Elaphe* and *Zamenis*. We show that autosomal rearrangements occurred in both *Elaphe* and *Zamenis* mostly by means of chromosome inversions and translocations of NORs. Noteworthy, the progressive evolutionary stages of the W chromosome reported in different species of *Elaphe* and *Zamenis* are consistent with the heterochromatinization model of sex chromosome diversification (see ALAM et al., 2018; MEZZASALMA et al., 2021). Finally, we discuss citotaxonomic evidences supporting the species status of *Z. lineatus* and *Z. longissimus*.

POSTER N. 31

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IL SIMBIONTE *WOLBACHIA* ALTERA IL PROFILO CHIMICO CUTICOLARE DELLE FEMMINE, MA NON DEI MASCHI, DI UNA VESPA PARASSITOIDE

È noto che i batteri intracellulari del genere *Wolbachia*, che sono trasmessi per via materna, infettano diverse specie di insetti. Tuttavia, i loro effetti sulla fisiologia dell'ospite rimangono oscuri per molti insetti. Qui, riportiamo un'associazione tra l'infezione di *Wolbachia* del supergruppo A ed il profilo chimico cuticolare (profilo CHC) nella vespa parassitoide *Myrmilla capitata* (Lucas, 1849) (Hymenoptera: Mutilidae), fornendo la seconda evidenza di tale fenomeno negli insetti (precedentemente noto solo in *Drosophila*). Confrontando individui infetti e non infetti di *M. capitata* campionati in un'aggregazione di nidi del suo ospite, l'ape eusociale *Lasioglossum malachurum* (Kirby, 1802), abbiamo trovato che il profilo CHC delle femmine infette da *Wolbachia*, ma non dei maschi, possiede un numero inferiore di idrocarburi e una maggiore abbondanza di idrocarburi a catena lunga, dunque meno volatili, rispetto alle femmine non infette. Le femmine di *M. capitata* infette hanno anche mostrato una minore variazione interindividuale nel profilo CHC, suggerendo che *Wolbachia* produca un'alterazione fisiologica uniforme. Il profilo CHC sia delle vespe infette che di quelle non infette differiscono notevolmente dal profilo CHC dell'ape ospite, che nel complesso presenta un profilo chimico meno complesso, costituito da circa la metà dei composti presenti nel parassitoide e privo di un'intera classe di idrocarburi che si trova nelle vespe. Sulla base dei nostri risultati, ipotizziamo in via preliminare che *Wolbachia* possa alterare la comunicazione sessuale in queste vespe, forse in modo da aumentare l'attrazione dei maschi infetti verso le femmine infette (che emettono i feromoni), riducendo di conseguenza la letalità zigotica per incompatibilità citoplasmatica. Ciò è in linea con quanto recentemente osservato in *Drosophila*, dove i maschi (che emettono i feromoni), ma non le femmine, sintetizzano un profilo CHC modificato da *Wolbachia*. Inoltre, possiamo escludere che il simbionte aumenti il successo riproduttivo di *M. capitata* – e dunque la sua frequenza di trasmissione – conferendogli mimetismo o insignificanza chimica verso l'ospite, strategie note per essere utilizzate da altre specie di imenotteri parassiti per ridurre l'aggressività dell'ospite durante l'invasione del nido.

POSTER N. 32

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INGESTED BIOPLASTICS AFFECT FUNCTIONAL CYCLES OF B-CELLS IN THE HEPATOPANCREAS OF TWO SUPRALITTORAL AMPHIPODS

This study reports the effects exerted by ingestion of bio-based plastics used for customary shop bags on the hepatopancreas (HP) of supralittoral amphipods. For two weeks we fed adult *Talitrus saltator* (Montagu, 1808) and *Orchestia montagui* (Audouin, 1826) with two starch-based bioplastics, green and white, classified as biodegradable and compostable according to UNI EN 13432, and characterized by FTIR analysis in ATR mode with a Cary 630 FTIR (Agilent Technologies) and GC-MS analysis (GC: Agilent 7890A and MS Agilent 5975 C) after extraction with methanol mixed with methyl heptadecanoate as internal standard. Control specimens were fed with blotting paper and dry fish food. HP is a multi-role organ despite its relatively plain histology, and is involved in nutrient internalization, endo-cellular digestion, and waste removal. The two species express different dietary habits: *T. saltator* is more generalist (detritivore scavenger, and grazer) than O. *montagui*, which suggests possible roles of model organisms. LM semi-thin sections from proximal regions of HP caeca in control specimens exhibited active B-cells in both species, spanning from the nutrient engulfing/processing stage to the “mono-vacuolar” step. The range of morpho-functional diversity was wider in *T. saltator*, where multistage processing of nutrients largely prevailed. In comparison, B-cells of *O. montagui* were mostly involved in storing tiny alimentary residues in single “vacuoles”, i.e. large secondary lysosomes. Dietary schedules affected such differential patterns dramatically, smoothing functional diversity to the monotonous occurrence of bleb-like B-cells with single, large lysosomes. Actually, this remarkably homogeneous pattern included occurrence of dense grains in specimens of both species fed with white bioplastic. TEM micrographs of *O. montagui* HP confirmed LM evidences, and highlighted the functional polarization of B-cells. The above data allow a preliminary differential analysis, of both control (a) and treated (b and c) samples. In our opinion: a) the larger morphological variety of *T. saltator* is a side feature of its dietary generalism, which modulates B-cell activity to various extents; b) the morphological flattening derives from functional synchronization due to the limitative-type feeding; c) the dark grains represent undigested residues/additives of white bioplastic.

POSTER N. 33

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SEA URCHIN WASTE VALORIZATION FOR THE DEVELOPMENT OF INNOVATIVE TOOLS IN REGENERATIVE MEDICINE

Marine organisms are highly exploited as a source of food and their consumption often produces large amount of waste and by-products. Their valorisation and recycling towards high added-value products is one of the current main societal challenges. In line with this "circular economy approach", we recently developed innovative sea urchin collagen-based biomaterials for skin regeneration. Native collagen was obtained from part of the wastes (up to 90% of the animal mass) of the sea urchin food industry. These biomaterial prototypes were partially characterized in term of structure, mechanical properties, *in vitro* biocompatibility, and preliminary *in vivo* tests.
The aim of this work was to: 1) further characterize these prototypes in terms of degradation kinetics and antioxidant activity and 2) pave the way for the development of a second-level bioactive composite biomaterial, combining collagen and antioxidants always extracted from sea urchin wastes, for a 100% recycling.
1) We evaluated the *in vitro* biomaterial degradation kinetics in both physiological and enzymatic (collagenase) conditions, trying to simulate the *in vivo* performance of the collagen-based biomaterials. Indeed, the *in vivo* physiological degradation of these biomaterials leads to the formation of collagenous peptides, which display certain antioxidant properties. The degradation test showed that in enzymatic conditions the biomaterial is degraded more than 50% after 48 hours, while a commercial bovine collagen membrane used as control (Integra®), by less than 10%. Subsequently, the antioxidant activity of collagen peptides was evaluated, in comparison to Integra, using ABTS assay. The analyses show that collagen peptides have greater antioxidant activity than both collagen-based biomaterial and the commercial membrane.
2) A preliminary protocol was developed for collagen-antioxidant composite preparation. Obtained biomaterials were characterized in terms of structure and antioxidant activity using ABTS assay. Results indicate that composite prototypes are structurally similar to the simple collagen-based counterpart and that antioxidants apparently remain adsorbed on the matrix of the biomaterial, without significant release over time, maintaining the pristine antioxidant power.
Overall, the results suggest that sea urchin wastes can be efficiently valorised obtaining high value molecules which can be used for the development of innovative biomaterial for tissue regeneration.

POSTER N. 34

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SPERM MODELS IN EUROPEAN PLECOPTERA

The sperm of Plecoptera is of a typical polyneopteran pattern, filiform and flagellate, with an elongated and compact nucleus topped by an acrosomal complex and a "9+9+2" axoneme flanked by two mitochondrial derivatives. The structure shows a certain heterogeneity, in particular as regard to morphology of acrosomal complex (mono-bi-three layered), shape of nucleus, presence or not of one or two accessory bodies flanking the axoneme and degree of crystallisation of mitochondrial derivatives. Many systematic issues regarding Plecoptera are still debated, and it seems that the use of molecular data is unable to definitively clarify the systematic relationships within the order. We believe that sperm characters can be useful also in the phylogenomic molecular era to help solving some of the unanswered systematic questions. We studied in the past the spermatozoa structure of ten Plecoptera species belonging to all the seven European stoneflies families (FAUSTO et al., 2001; 2002a; 2002b). Our comparative analysis of sperm characters allowed us to suggest the presence of a "sperm model" valid at family level; a certain degree of heterogeneity was, however, found in sperm structure within the seven European families analyzed. We describe in the present communication the sperm structure of four more species belonging to European stoneflies genera not analyzed in our previous studies, i.e. *Capnopsis schilleri* Rostock, *Amphinemura sulcicollis* Stephens, *Rhabdiopteryx neglecta* Albarda, *Tyrrhenoleuctra zavattarii* Consiglio. Based on the data obtained, it is possible to infer affinity between all the taxa examined so far and discuss regarding the systematic arrangement within the order.

POSTER N. 35

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TO SEE OR NOT TO SEE: THE COMPLEXITY OF SENSORY EQUIPMENT IN A VULNERABLE SAPROXYLIC BEETLE

Mycetozoa-feeding beetles are an important component of biodiversity in old-growth forest communities. However, their high degree of adaptation to biotic and abiotic conditions of this habitat results in high sensitivity to intensive forest management, which reduces deadwood abundance, altering ecosystem characteristics and changing resource availability, endangering the connected organisms. Despite their ecological importance, little information is available on the morphological and functional adaptation of these species. In the present study, the focus was on the sensory structures of a saproxylic beetle, *Clinidium canaliculatum*, Costa 1839 (Coleoptera, Rhysodidae), listed as vulnerable on the International Union for Conservation of Nature (IUCN) Red List. Observations were made by coupling traditional scanning electron microscopy (SEM) with synchrotron radiation X-ray phase contrast microtomography (SR-PhC micro-CT), as an alternative non-destructive technique. SEM analyses showed the presence of moniliform antennae and allowed the identification of seven different types of sensilla: two chaetic, two basiconic, one campaniform, one coeloconic, and one Böhm sensilla. This antennal equipment revealed the ability to detect chemical and physical environmental parameters for habitat, food, mate and oviposition site selection. However, SR-PhC micro-CT data provided, for the first time, evidence on the compound eye structure of this beetle, previously known to be anophthalmic. Virtual sections and 3D volumetric renderings of the head enabled us to study the internal dioptric and sensory parts of the eyes. Although the absence of facets, the eyes of *C. canaliculatum* show the typical sensorial structure of a superposition vision, such as in nocturnal insects, taking advantage of the low light present in deadwood and barks. SR-PhC micro-CT is an emerging method useful to avoid time-consuming destructive techniques such as dissections and classical histology, which is unsuitable for small, rare samples, difficult to collect or present in limited numbers. Our results enrich knowledge about the ecological and behavioural characteristics of this species, which has developed olfactory and chemotactile perceptions, but also a so far unknown visual and light-detecting ability. Moreover, virtual sections and 3D reconstructions could be a useful data set of information for further physiological and ecological studies and to promote the knowledge and conservation of rare and vulnerable species.

POSTER N. 36

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STRUCTURAL EVOLUTION OF *EUPLOTES* PHEROMONES AND PHEROMONE CODING-GENES

Species of the ciliate *Euplotes* thrive in the most disparate aquatic habitats, in which they represent an ordinary component of every microbial food web. A major factor of their extraordinary evolutionary and adaptive success resides in a common ability to synthesize cell type specific water-borne protein pheromones, each determined by one of species-specific series of multiple alleles that are inherited at the same *mat* (mating-type) locus of the germinal (micronuclear) genome and expressed as individual gene-sized DNA molecules in the somatic (macronuclear) genome. Being closely related by structural homology, *Euplotes* pheromones thus function by competitively binding target cells in autocrine (self) fashion to promote cell growth, and heterologous (non-self) fashion to induce sexual unions in mating pairs. Limited for long to only *E. raikovi* and *E. octocarinatus*, the knowledge of *Euplotes* pheromone and pheromone-gene structures has lately been widened to a number of species (*E. aediculatus*, *E. crassus*, *E. focardii*, *E. minuta*, *E. nobilii*, and *E. petzi*) that are variously diversified in relation to their phylogenetic relationships and habitat colonization, making possible to outline an initial picture of how these structures evolve in concert with *Euplotes* speciation. The pheromone and pheromone-gene sequences similarly undergo marked increases in length. In the former case, the increase is consequent to the extension of one or more sequence segments spanning between tightly conserved Cys residues, and the addition of new amino acid combinations clearly provides pheromones with the necessary structural substrate to adapt and optimize their activity to diverse environments. In the latter case, the increase is consequent to the inclusion of intron sequences in the regulatory 5'-leader region or, exceptionally in *E. octocarinatus* and *E. aediculatus*, in the coding region, and this inclusion directly contributes to amplify and diversify the mechanism of the pheromone-gene expression.

POSTER N. 37

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PROTOCOOPERATION IN ANTHOZOANS: THE COLLECTIVE PREDATION OF LARGE PREY BY SMALL-SIZED CNIDARIAN POLYPS

Heterotrophy in cnidarians is usually carried out through suspension-feeding, the tentacles collecting particulate organic matter in the water column. Although, a different feeding modality has been reported in anthozoans (MUSCO *et al.*, 2018; TER HORST and HOEKSEMA, 2021; GREGORIN *et al.*, 2022), referred to as protocooperation: it is a facultative mutualism in which small-sized polyps cooperate to catch large prey, usually gelatinous zooplankton pushed against the polyps by currents. Hence, jellyfish could represent an ephemeral but abundant food resource that can be exploited through the cooperation of spatially close polyps. Here we present field observations of five anthozoan species catching jellyfish. Observations were made by SCUBA diving in several locations (Tyrrhenian Sea, Adriatic Sea, Red Sea). The phenomenon was filmed and photographed with underwater cameras during 2021/22. When possible, a metric reference was left near the polyps to measure the size ratios of animals. The species observed are *Anemonia viridis* (Forsskål, 1775), *Astroides calycularis* (Pallas, 1766), *Parazoanthus axinellae* (Schmidt, 1862), *Cladocora caespitosa* (Linnaeus, 1767) and *Tubastraea* cf. *micranthus* (Ehrenberg, 1834). Videos and images were processed and analyzed (ImageJ, Photoshop), focusing on size ratios of prey and predators and the number of polyps involved. The prey is from 6 to more than 10 times larger than the single polyp; for colonial species, often more than one colony is involved into cooperative predation on a single jellyfish; the involved polyps from one colony vary from 3 to more than 12. The capturing phase is very fast, the jellyfish is blocked as it touches the polyps, but their ability to hold it depends on hydrodynamic conditions, influencing the success of the predation. The present descriptions improve the investigation of the protocooperative strategy in cnidarians, being more frequent than one might think, at different latitudes from temperate to tropical seas. In an evolutionary perspective, the need for polyps to be close neighbours to successfully protocooperate suggests that this strategy could represent an additional and still unexplored driver that encourages benthic cnidarians to form aggregations.

POSTER N. 38

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CHARACTERIZATION OF TWO NEURODEGENERATION PATHWAYS IN THE COLONIAL TUNICATE *BOTRYLLUS SCHLOSSERI*

With elderly populations among the fastest-growing demographics of many nations, understanding mechanisms responsible for neurodegenerative diseases is an important challenge for our society. Studying the evolutionary origin of these traits in a model organisms like *Botryllus schlosseri*, a marine colonial tunicate of an evolutionary clade considered to be a sister group of vertebrates, can provide new insight into the cellular and molecular processes mediating pathways of neurodegenerations. During asexual reproduction, the colony exhibits the periodical regression of adult individuals that, in a phase called takeover, are substituted by their buds. Within a single colony, three generations coexist: the adult zooids, their (primary) buds and small (secondary) buds on the latter. When takeover occurs, the adult zooids degenerate, the primary buds become the new generation of adult zooids, and the secondary buds become primary buds and produce a new generation of small buds. Although wild colonies only live for 1-2 years, in laboratory conditions they can persist for >20 years. Morphological, behavioral, and transcriptomic analyses show that the nervous system degenerates in adult zooids during both the takeover (rapid neurodegeneration) and in aging (slow neurodegeneration) colonies. In both processes, adult zooids exhibit a progressive reduction in neuron number, accompanied by an increasing number of immunocytes (phagocytes and morula cells) close to and infiltrating the brain. The reduction in the number of neurons is associated with impaired behavioral performances, as zooids in takeover and older zooids (~20 year of age) are less sensitive to mechanical stimuli than active zooids and zooids belonging to younger colonies. Rapid neurodegeneration during takeover is characterized by changes in the expression of 73 mammalian homologous genes associated with neurodegenerative diseases, such as Alzheimer's, Parkinson's, Huntington's diseases and frontal dementia. Moreover, older colonies exhibit changes in the expression of 148 such genes, 35 which are differentially expressed across both the rapid and the slow neurodegeneration processes. In conclusion, *B. schlosseri*, with its two different yet apparently related neurodegenerative processes, represents a model organism capable of providing insight concerning the mechanisms balancing neural regeneration and degeneration that are crucial to the onset of neurodegenerative diseases.

POSTER N. 39

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INVASORI POST-GLACIALI DELLA PENISOLA ITALIANA: IL CASO PARADIGMATICO DI *CANIS AUREUS* (MAMMALIA: CANIDAE)

Nei giacimenti di vertebrati fossili italiani lo sciacallo dorato non è rappresentato e ciò dimostra la sua penetrazione post-glaciale nel nostro paese. Dopo una breve disamina delle penetrazioni post-glaciali della teriofauna italiana (*Arvicola amphibius*, *Apodemus agrarius*, *Erinaceus roumanicus*, ecc.), l'Autore esamina nel dettaglio il caso di *Canis aureus*, la cui spontanea espansione nella Penisola Italiana è paradigmatica dell'influenza di varie attività antropiche sulle comunità di mammiferi. Il recente ingresso della specie in Italia (1984) è infatti diretta conseguenza di interventi di gestione delle comunità di carnivori nell'Europa sud-orientale. La forte pressione antropica esercitata verso la metà del XX secolo sulle popolazioni balcaniche di lupo (*Canis lupus*) ha ridotto notevolmente le popolazioni del grande canide, naturale antagonista di *Canis aureus*. L'abbondante presenza di lupi limitava la distribuzione dello sciacallo dorato balcanico, riducendola sostanzialmente alle garighe delle coste greche, dalmate e montenegrine.

L'espansione naturale dello sciacallo dorato in Italia costituisce uno dei più bei paradigmi delle conseguenze della pressione antropica sugli eco-sistemi europei.

POSTER N. 40

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IL MUSEO FRIULANO DI STORIA NATURALE E I MONITORAGGI FAUNISTICI A TUTELA DELLA BIODIVERSITÀ

La *mission* di un Museo scientifico si articola attorno ai punti cardine della conservazione dei reperti, della ricerca e della divulgazione. La ricerca è dunque un aspetto fondamentale che funge da volano per l'acquisizione di nuovi reperti e per la conoscenza ai fini della divulgazione. Il MFSN da decenni si è impegnato nella conoscenza del territorio, attraverso lo studio dei reperti e mediante specifici programmi di ricerca.
Negli ultimi anni il MFSN ha compiuto varie azioni di studio del territorio e in particolare delle aree protette, nonché alcuni monitoraggi finalizzati alla comprensione a livello regionale di singoli gruppi animali oggetto di tutela. La grossa mole di dati e di conoscenze provenienti dalle collezioni e raccolta nel corso dei sopra menzionati progetti e in varie altre ricerche, ha permesso al Museo di aggiornare continuamente le liste faunistiche delle aree protette regionali, in special modo dei siti Natura 2000, in accordo con la Regione Autonoma FVG. Tra gli anni 2011 e 2022 sono state continuamente aggiornate e verificate le banche dati georiferite con la situazione distributiva di alcune specie animali oggetto di tutela ai sensi del DPR 357/97 (allegati II, IV e V della Direttiva 92/43 "Habitat").
Il numero di taxa trattati complessivamente per ciascun gruppo curato è il seguente:
Gastropoda: 7; Annelida: 1; Arthropoda: 27; Amphibia: 15; Reptilia: 20; Mammalia: 52.
Su un totale di 112 celle 10x10 km (secondo il reticolo ERTS89) che coprono il territorio regionale (terrestre), sono state analizzate 2744 informazioni di presenza. Il 100% delle celle è coperto da dati, e più del 60% da almeno 20 dati di specie per cella. Il numero medio di specie protette per cella è di 24,5, mentre la mediana è 23. La densità media di specie protette per quadrante differisce nelle province, con il suo massimo nella provincia di Trieste. La densità media di specie è più elevata inoltre nelle zone che includono aree protette.

Il gruppo tassonomico più indagato (con più specie inserite negli Allegati) è quello dei Chirotteri (30 taxa). Dal 2011 al 2018 in questo gruppo di mammiferi vi è stato un notevole sviluppo nelle conoscenze, con un aumento di informazioni del 500% e un incremento della copertura media per specie di più del 100%. Questo si deve in particolare a uno specifico incarico di monitoraggio affidato al Museo dalla Regione nel 2013, a ulteriore conferma dell'importanza fondamentale dei monitoraggi biologici per la conservazione e la tutela.

POSTER N. 41

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SMELL BY TOUCH IN COMMON OCTOPUS (*Octopus vulgaris*)

Octopuses are intelligent animals, with highly evolved sensory organs, which exhibit complex behaviours, These abilities are coordinated and controlled by a cephalized central nervous system (CNS), which is linked and interacts with an extensive peripheral nervous system (PNS). Hundreds of suckers line the flexible octopus arms, and each sucker is a unique multimodal sensory organ. These suckers allow the animal to both collect multiple types of information, and to manipulate the environment. This system has been considered as a "sense of taste" which is able to detect molecules by contact, exhibiting a behaviour defined as "taste by touch". As the majority of volatile odorant molecules are insoluble or have a very low solubility in water, in octopuses, as in all other aquatic animals, detection of them is akin to the terrestrial sense of smell. Among these animals, octopuses stand out, possessing both a central chemical detection system in the olfactory organ, and the extremely large and diffuse sucker system.
So far, experiments with a capacity for tactile olfaction have been conducted to show the *Octopus vulgaris* olfaction ability.
We designed behavioural experiments that tests the octopuses' ability to detect water-insoluble chemical substances using the *O. vulgaris* in the facility of the Department of Biology, University of Naples Federico II.
Our results demonstrate that the suckers of the *O. vulgaris'* arm can detect odour stimuli. This shows, tactile forms of olfaction and CNS perception of olfactory information from the arms, a "smell by touch" behaviour. In future experiments, discrimination learning based on specific odour stimuli will help to elucidate, stimulus specific discrimination and olfactory memory in *O. vulgaris*. Those experiments increases knowledge about octopus behaviours and improve animal welfare in captivity.

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