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Abstracts

Summer School 2024
PhD in Biological and Natural Sciences

Perugia, 6 –7 June

- 8.30 Summer School Opening

Chairs: *Manuela Rebora, Silvana Piersanti*

- 9.00 Summer school work start (PhD Course Coordinator Manuela Rebora)

- Coevolutionary dynamics in biological interactions
    - 9:15 - **Adolfo Cordero-Rivera** - *'Sexual conflict in aquatic insects: an ecological perspective'* - Universidade de Vigo, ECOEVO Lab., E.E. Forestal, Campus Universitario, 36005 Pontevedra, Spagna

    - 10:15 - **Paolo Masini** - *'Host location in the parasitoids of the genus* Sclerodermus *'* - *Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università degli Studi di Perugia*

- 10:45 coffee break and poster session

    - 11:30 - **Régis Cereghino** - *'What drives ecosystem resistance to drought in the Neotropics? A test using tank-bromeliads'* - Biodiversity & Environment Research Center (CRBE), CNRS, University Toulouse Paul Sabatier, Toulouse, France

    - 12:30 - **Massimiliano Scalici** - *'Plastics in running waters: a fluent issue of concern'* - University Roma Tre, Department of Sciences, Viale Guglielmo Marconi 446, 00146 Roma, Italy

- 13.00 Lunch and poster session

Chairs: *Antonia Concetta Elia, Gianandrea La Porta*

- Bridging Theory and Practice in Environmental Research
    - 14:30 - **Paolo Pastorino** - *'Global change and environmental pollution in aquaculture: emerging issues in the 21st century'* - Istituto Zooprofilattico

Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino

- o 15:00 - **Régis Cereghino** - *'River macroinvertebrates as indicators of ecosystem health'* - Biodiversity & Environment Research Center (CRBE), CNRS, University Toulouse Paul Sabatier, Toulouse, France

- o 15:30 - **Corrado Marcenò** - "*Floristic and vegetation studies in the era of big data: challenges, trends, and applications*" - Department of Chemistry, Biology and Biotechnology, University of Perugia, Italy

- o 16:00 - **Michele Croce** - "*Management of Invasive Alien Species in Umbria*" - Sezione Tutela Patrimonio Ittico e Pesca Sportiva, Regione Umbria

- 17.00 Closure of works

7 June - Vital Processes and Human Well-being

- 8.30 Summer School Opening

Chairs: *Giovanna Traina, Antonio Michelucci, and Luigi Catacuzzeno*

- Muscle and neuron physiology
  - o 09:15 - **Robert T. Dirksen** *"Role of Orai1 in tubular aggregate myopathy"* (Dept. of Pharmacology and Physiology - University of Rochester Medical Center)

  - o 10:15 - **Simona Boncompagni** *"Tubular Aggregates and their possible role in skeletal muscle during ageing"* - Dept. of Neuroscience, Imaging and Clinical Sciences - University *G. d'Annunzio* of Chieti-Pescara

- 10:45 coffee break and poster session

- o 11.30 - **Antonio Michelucci** *"Store-operated Ca^{2+} entry as a key mechanism in Duchenne muscular dystrophy"* - Dept. of Chemistry, Biology and Biotechnology - University of Perugia

- o 12.00 - **Guglielmo Sorci** *"Use of the immunomodulatory Sertoli cells to treat Duchenne muscular dystrophy"* - Dept. of Medicine and Surgery, University of Perugia

- o 12.30 - **Rosario Amato** *"The retina as a tool to study the neurovascular physiology"* - Dept. of Biology, University of Pisa

- 13:00 Lunch and poster session

Chairs: *Ermanno Federici*

- ### Sciences transition from the benchtop to the desktop
    - o 14:30 - **Daniele Pietrucci** "*Bioinformatics for computational genomics: characterizing prokaryotes and eukaryotes'* - Department for Innovation in Biological, Agro-Food and Forest Systems University of Tuscia, Viterbo

    - o 15:00 - **Davide Chiasserini** "*Next-generation proteomics: an overview of techniques and computational approaches*" - Dipartimento di Medicina e Chirurgia, sezione di Fisiologia e Biochimica, Università di Perugia

    - o 15:30 - **Davide Petri** "*Systematic reviewlution: artificial intelligence for evidence synthesis*" - Dipartimento Ambiente e Salute, Istituto Superiore di Sanità

- 16:15 Closure of Summer School works

6 June

Sexual conflict in aquatic insects: an ecological perspective

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Keywords: behaviour; sexual selection; cryptic female choice; sperm competition

Sexual conflict occurs when the optimum value for a trait is different for females and males and is the consequence of pressures related to natural and sexual selection. Therefore, here I consider sexual conflict as a part of a continuum of sexual selection mechanisms. My hypothesis is that the dimensional structure of the habitat will affect the intensity of sexual conflict over mating rate, copulation duration and postcopulatory guarding. To test this idea, I review the literature on sexual conflict in three groups of aquatic insects, whose habitat is markedly different: water-striders (Gerridae), dragonflies (Odonata) and diving beetles (Dytiscidae). The bidimensionality of the water surface allows an easy monopolization of females by males, and water striders conform to this rule, with the commonest mating system characterized by strong conflicts and struggles before and after copulation. For fast flying animals, like odonates, the opportunities for males to force females to copulate are certainly limited, but conflicts over mating frequency and duration are intense in certain species. In the case of diving beetles, the situation seems more favourable for the females, as they could control male approaches by hiding easily in the vegetation, or even in the case of extreme male density, they could fly away and move to a different water body. I highlight that a comprehensive understanding of sexual conflicts in animals requires the study of both male and female anatomies, as well as their behaviours, avoiding assumptions or gender stereotypes, which have historically biased research to a male-view approach.

Host location in parasitoids of the genus *Sclerodermus*

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Keywords: Flat wasps; chemical ecology

The *Sclerodermus* genus encompasses more than 80 species of ectoparasitoids of woodboring beetles. Many species of this genus, still poorly investigated regarding their taxonomy and biology, resulted in a very promising biological control agent against insect pests and were reported as responsible for the insurgence of sting dermatitis in humans. In order to develop sustainable methods to control insect pests and to better understand the genesis of human sting dermatitis, two populations of bethylid wasps were collected in the field and then identified taxonomically as *S. cereicollis* and *S. domesticus*. The two species were redescribed and illustrated, with molecular support. Moreover, the morphology and ultrastructure of the antennal chemoreceptors on *S. cereicollis* were investigated, with the description of different chemoreceptors useful for interspecific and intraspecific relationships. The host location processes of the two species were evaluated through behavioral responses in a Y tube and in a still-air olfactometer. Stimuli coming directly or indirectly from their natural hosts (*Trichoferus holosericeus* and *Hylotrupes bajulus*) were tested: 1) chemical stimuli deriving from the host microhabitat like seasoned-wood sawdust from host plants; 2) chemical stimuli associated with the presence of the host, like frass from larvae; 3) cuticular hydrocarbons (CHC) from host larvae. Naïve micropterous females of *S. cereicollis* and *S. domesticus* both responded to volatile stimuli from seasoned wood sawdust from the host plant of their natural hosts and to CHC from host larvae. A further statistically significant response was recorded towards semivolatile stimuli derived from the host's frass. Furthermore, extracts from these stimuli and CO_2 will be tested on *Sclerodermus* antennae with electroantennographic investigations.

What drives ecosystem resistance to drought in the Neotropics?

A test using tank-bromeliads

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Keywords: Climate change; functional traits; experiments

Future climate scenarios vary in the direction and magnitude of precipitation changes, depending on geographic location. The discrete communities found within natural freshwater microcosms (e.g., tank bromeliads, tree holes) allow ecologists to take advantage of systems that are small and contained, to test ecological theory over geographic gradients. We conducted coordinated experiments to understand how aquatic invertebrate traits mediate the responses of multitrophic communities to changes in the quantity and evenness of rainfall in tank bromeliads across the Neotropics. We asked whether geographic variation in community response to climate change is driven by physiological matching to local conditions (local adaptation hypothesis) or by differences between species pools in functional redundancy (insurance hypothesis). Our results predict uneven impacts of precipitation change on communities and energy channels across Neotropical regions. Geographic differences in community responses to precipitation regimes were not consistent with the local adaptation hypothesis, as responses did not correlate with the current amplitude in precipitation. Differences were consistent with the insurance hypothesis: sites with the lowest functional redundancy had the strongest response to uneven precipitation. Hydrological variability induced a shift from communities using green and brown energy channels to brown energy communities. If such geographic variation is due to differences in the size and redundancy of species pools, then strategies for climate change adaptation should seek to identify and preserve functionally-unique species and their habitats.

Plastics in running waters: a fluent issue of concern

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Keywords: Plastics; River; Ecological threat

To date, plastics represent one of the main and increasing threats to aquatic habitats worldwide in the Anthropocene so much to receive attention from many scientists. Although the effects of plastics have mostly been studied in marine environments and organisms, rivers (proposed as the main source of plastics for seas and oceans) received less attention. Exposure of plastics to various environmental conditions degrades and fragments them into smaller particles, from macroplastics to nanoplastics, even if there is no standardized definition for the size fraction. Although in the last decade some improvements were conducted in the comprehension of plastic pollution in freshwaters, many topics are still under-investigated. My research lines aim at providing a contribution to the knowledge of *plastics' behaviour* in riverine ecosystems by a multi-level approach, from the ecosystem to organism level, analyzing for each level a different plastic size. The first main line aims to provide an assessment of floating macroplastic transport in riverine ecosystems using a harmonized approach and suggest some improvements to further investigate plastic categories. This study contributes to increase knowledge on the origin, composition and transport of riverine litter entering the Mediterranean Sea. The second line regards the acquisition of field data on the rule of riparian zone in the distribution of macro- and mesoplastics, allowing the collection of important information on how riparian vegetation can be exploited in management activities for removing plastic litter and restoring riverine ecosystems. The third mainly investigates plastics and additives accumulation in freshwater organisms.

Global change and environmental pollution in aquaculture:

emerging issues in the 21ˢᵗ century

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Keywords: climate change, fish diseases, emerging contaminants, water scarcity

Aquaculture plays a crucial role in global food production, but it faces increasing challenges due to the interconnected effects of climate change, water scarcity, (re)emerging diseases, and emerging contaminants. These issues are of great importance in the context of aquaculture research and development. Climate change, characterized by rising temperatures, poses substantial threats to aquaculture ecosystems by altering water quality, disrupting species behavior, and exacerbating disease dynamics. Concurrently, shifting precipitation patterns contribute to water scarcity, imposing constraints on aquaculture productions. The (re)emergence of diseases in aquaculture systems is another pressing issue exacerbated by environmental stressors. Understanding and managing disease dynamics are imperative for sustaining aquaculture productivity and ecosystem health. Furthermore, the rise of emerging contaminants such as micro(nano)plastics, pharmaceuticals, and persistent organic pollutants presents complex challenges to aquaculture sustainability. These contaminants accumulate in aquatic environments, posing risks to both aquatic organisms and human consumers of aquaculture products. Emphasis will be placed on innovative research approaches and technological interventions aimed at enhancing the resilience and sustainability of aquaculture systems in the face of global change and environmental pollution. The talk will also highlight the importance of interdisciplinary collaboration and knowledge exchange in addressing these multifaceted challenges, offering insights and strategies to guide future research directions.

River macroinvertebrates as indicators of ecosystem health

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Keywords: Bioassessment; freshwater; anthropogenic disturbance

In recent water policies in Europe, the causes and extent of various disturbances and the reference conditions of rivers are determined using physical–chemical parameters, whereas biological quality elements (e.g. diatoms, macroinvertebrates, fish, macrophytes) serve as indicators of the ecological consequences of river impairment. In natural rivers, changes in macroinvertebrate communities along the downstream gradient correspond to the continuous gradient of the physical characteristics of streams from headwaters to mouth. Some pollution-sensitive species tend to decline in abundance or to disappear under disturbed conditions. Therefore, deviations from predictable invertebrate-habitat relationships can quantify the severity of anthropogenic impacts on freshwater ecosystems. Here, I illustrate the main directions in water policies that rely on macroinvertebrates in ecological health assessment: indicator species, functional traits, and community-based indices. The presence or absence of an indicator species often matches certain environmental conditions, but doesn't provide a quantitative estimate of how much disturbance is present. Functional traits are efficient to measure impacts of various types of disturbance, because traits account for the niche space occupied by organisms in terms of the environment's effect on individuals, and of individuals' impact on their environment. By combining different categories of community-derived metrics and functional traits that respond to different aspects of environmental conditions (e.g. species richness, abundance, functional traits), multimetric indices are more reliable than single metrics to provide integrated assessments of ecosystem health.

Floristic and vegetation studies in the era of big data: challenges, trends, and applications

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Keywords: big data; plant science; citizen science platforms

In plant science, species data occurrences and vegetation plot databases are two of the most common and powerful tools to supplement existing research and provide new perspectives on more complex and geographically broader questions. During the seminar, we will examine the challenges, trends, and applications that have emerged in this context. Challenges include data quality management, standardization, and analysis methodologies. However, promising trends also emerge, such as the integration of citizen science platforms and the adoption of advanced analytical techniques like machine learning and remote sensing. Through case studies and real-world applications, this abstract highlights how big data is revolutionizing our understanding of plant species and communities. In conclusion, the importance of addressing methodological challenges to fully harness the potential of big data in future floristic and vegetation studies is emphasized.

The management of invasive alien species in Umbria

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Invasive alien species represent one of the main threats to biodiversity conservation. The Umbria Region is committed to counteracting the spread of alien species according to national regulations and EU directives. The main actions that are put in place are: prevention, monitoring and eradication.

In recent years, a major contribution to combating invasive alien species has been the LIFE IMAGINE Project (LIFE IPE/IT/000015) and L.N. 234, 30 December 2021, which established the Fund for Combating Invasive Alien Species with a total allocation of 5 million euros for each of the years 2022, 2023 and 2024.

In this paper, as an example, we analyze in detail the interventions implemented by the Umbria Region against: beaver (*Castor fiber*), nutria (*Myocasor coypus*), California crayfish (*Pacifastacus leniusculus*), and American marsh tortoise (*Trachemys scripta*), the last 3 classified as invasive alien species of EU interest according to EU Regulation 1143/2014 and Dlgs 230/2017.

It is important to consider that invasive alien species have a negative impact not only from a conservation point of view, but also from an economic and health one.

Therefore, it is necessary to continue to raise awareness among the public and interest groups about the importance of this phenomenon in order to promote responsible behavior.

7 June

Role of Orai1 in tubular aggregate myopathy

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Tubular aggregate myopathy (TAM) is a heritable myopathy primarily characterized by progressive muscle weakness, elevated levels of creatine kinase (CK), exercise intolerance, and the presence of tubular aggregates. Here, we generated a knock-in mouse model of TAM harboring a glycine-to-serine point mutation in the Orai1 pore ($Orai1^{G100S/+}$ or GS mice) that results in a severe, early-onset form of TAM in humans. By 8 months of age, $Orai^{G100S/+}$ mice exhibit significant muscle weakness, exercise intolerance, elevated CK levels, and robust presence of tubular aggregates. Unexpectedly, constitutive Ca^{2+} entry due to the Orai1 pore mutation is only observed in muscle during early development and is abolished in adult skeletal muscle, in part to a reduction in Orai1 expression. Proteomic analyses of muscle of 2-week and 8-month-old WT and GS mice revealed significant alterations in mitochondrial-related pathways. GS mice represent a powerful model to investigate the pathophysiological mechanisms that underlie the muscle weakness, exercise intolerance and formation of tubular aggregates, as well as compensatory responses to limit the damaging effects of uncontrolled Orai1-mediated Ca^{2+} influx.

Tubular Aggregates and their possible role in skeletal muscle during ageing

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Tubular aggregates (TAs), ordered arrays of elongated sarcoplasmic reticulum (SR) membranes, are present in skeletal muscle fibers from patients with various myopathies. In 2011, we showed that TAs also form in muscle of male mice during ageing starting from the remodeling of SR at the I-band. TAs have been recently linked to mutations in STIM1 and Orai1, the two main molecular players in store-operated Ca^{2+} entry (SOCE), a ubiquitous mechanism that allows recovery of extracellular Ca^{2+} when internal stores are depleted. In 2017, we demonstrated that exercise triggers the formation of unique intracellular junctions between SR and T-tubules, named *Ca^{2+} Entry Units,* or CEUs, that promotes co-localization of Orai1 with STIM1 and improves muscle function in presence of external Ca^{2+}. Using a combination of structural, molecular and functional studies we then compared EDL muscles dissected from adult and aged mice, the latter divided in 2 groups: control and exercised in wheel cages for 15 months. The results collected revealed that: i) ageing caused STIM1 and Orai1 to be accumulated in TAs; ii) exercise significantly reduced formation of TAs; iii) aged EDL exhibited a faster decay of contractile force than adult muscles, likely caused by their inability to recruit extracellular Ca^{2+}; and iv) exercise restored the lost capability to use external Ca^{2+} of aged EDL muscles by promoting maintenance/rescue of CEUs. Our results suggest that exercise prevents improper accumulation of STIM1 and Orai1 in TAs during ageing, restoring the capability of aged muscle to use external Ca^{2+} via SOCE.

Store-operated Ca^{2+} entry as a key mechanism in

Duchenne Muscular Dystrophy

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Keywords: Ca^{2+} signaling, *mdx*, muscular dystrophy, Orai1 channels, skeletal muscle, STIM1

Duchenne muscular dystrophy (DMD), an X-linked disorder caused by loss-of-function mutations in the dystrophin gene, is characterized by progressive muscle degeneration and weakness. Enhanced store-operated Ca^{2+} entry (SOCE), a Ca^{2+} influx mechanism coordinated by STIM1 sensors of luminal Ca^{2+} within the sarcoplasmic reticulum (SR) and Ca^{2+}-permeable Orai1 channels in the sarcolemma, is proposed to contribute to Ca^{2+}-mediated muscle damage in DMD. To directly determine the impact of Orai1-dependent SOCE on the dystrophic phenotype, we crossed *mdx* mice with tamoxifen-inducible, muscle-specific Orai1 knockout mice (*mdx*-Orai1 KO mice). Both constitutive and SOCE significantly increased in flexor digitorum brevis fibers from *mdx* mice, while SOCE was absent in fibers from both Orai1 KO and *mdx*-Orai1 KO mice. Compared with WT mice, fibers from *mdx* mice exhibited (1) increased resting myoplasmic Ca^{2+} levels, (2) reduced total releasable Ca^{2+} store content, and (3) a prolonged rate of electrically evoked Ca^{2+} transient decay. These effects were partially normalized in fibers from *mdx*-Orai1 KO mice. Intact extensor digitorum longus muscles from *mdx* mice exhibited a significant reduction of maximal specific force, which was rescued in muscles from *mdx*-Orai1 KO mice. Finally, during exposure to consecutive eccentric contractions, muscles from mdx mice displayed a more pronounced decline in specific force compared with that of WT mice, which was also significantly attenuated by Orai1 ablation. Together, these results indicate that enhanced Orai1-dependent SOCE exacerbates the dystrophic phenotype and that Orai1 deficiency improves muscle pathology by both normalizing Ca^{2+} homeostasis and promoting sarcolemmal integrity/stability.

Use of the immunomodulatory Sertoli cells to treat Duchenne muscular dystrophy

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Keywords: cell therapy, Duchenne muscular dystrophy, immunomodulation, Sertoli cells, xenograft

Duchenne muscular dystrophy (DMD) is a genetic X-linked disease due to mutations in the DMD gene coding for dystrophin resulting in a lack of functional protein. Absence of dystrophin translates into rupture of myofibers during contraction leading to progressive muscle damage and fibrous and adipose tissue accumulation, culminating in patient death due to cardiorespiratory failure. DMD muscles are characterized by chronic inflammation, which has a major role in the progression of the pathology so the gold standard therapy for DMD is represented by glucocorticoids. Sertoli cells (SeC) of the seminiferous tubules secrete a multitude of immunoregulatory and trophic factors to provide immune protection, and assist in the orderly development of germ cells, being responsible for the well-known immuneprivileged status of the testis. Grafts of naked or encapsulated SeC have been proved to represent an interesting therapeutic approach in a plethora of experimental models of diseases. The intraperitoneal (i.p.) injection of SeC encapsulated into clinical-grade alginate microcapsules (MC-SeC) in an animal model of DMD results in amelioration of muscle morphology (reduced myofiber necrosis, inflammatory infiltrate, and fibrosis) and performance in the absence of pharmacological immunosuppression thanks to restriction of muscle inflammation and upregulation of the dystrophin paralogue, utrophin, at the sarcolemma level. Thus, engraftment of MC-SeC might represent a universal (i.e., efficacious irrespective of the DMD mutation) therapeutic approach for DMD patients and patients suffering from autoimmune or inflammatory diseases.

The retina as a tool to study the neurovascular physiology

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Keywords: neurovascular unit; neurodegeneration; neuroprotection

In the central nervous system (CNS), neurons, glia and vessel are coordinated into a unique functional entity, the neurovascular unit (NVU). The study of molecular and cellular processes involved in neurovascular coupling has gained significant interest as its alteration is increasingly emerging as a common denominator of many neurodegenerative disorders. However, the high level of complexity of canonical CNS organs, such as brain and spinal cord, limits an effective experimental accessibility to both neural and vascular compartment of NVU, thus hindering the knowledge about the mechanisms underpinning neurovascular coupling. The retina is a displaced component of the CNS displaying several structural and functional analogies with other regions of the CNS. In this respect, the retina displays a hierarchical organization of neural circuitries providing the integration of input signals into an output delivered through the optic nerve towards the brain. The neural activity in the retina is dynamically coordinated with the vascular function, with a strict control of local blood flow in response to neural metabolic demands mediated by glial cells. However, unlike other regions of the CNS, the morpho-functional organization of the retina opens the possibility of an experimental control on both neural and vascular components thus allowing to manipulate NVU. Herein, the view of the retina as a tool to study NVU physiology will be explored by providing examples of experimental approaches mimicking physiological and pathological conditions.

Bioinformatics for computational genomics: characterizing prokaryotes and eukaryotes

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Keywords: Bioinformatics; Genomics; Computer Science

The support of algorithms and computer science to biology became evident towards the end of the 1990s: the organization and analysis of the enormous amount of sequencing data made it necessary for biologists to acquire computational skills. Informatics had been applied to biological data since the 1960s, but it was mainly with the advent of second and third-generation sequencing technologies that this discipline solidified over time. It is crucial not only to understand which methods to use based on the researcher's interests and needs, but also to approach the study in a way that allows for a linear acquisition of all necessary information.

During the seminar, the main applications of bioinformatics in the genomic field will first be presented, exploring the genomics of eukaryotes and prokaryotes, transcriptomics, and metagenomics. Subsequently, guidelines will be provided to understand which software tools (e.g., bash, Python, R), hardware (e.g., supercomputers), and databases (e.g., SRA) are worth learning, even self-taught, to learn the main computational techniques. Finally, practical applications of bioinformatics will be presented: for example, the characterization of the human microbiota in health and disease conditions using Machine Learning methods, the characterization of archaeological finds, and the support of genomics in the characterization of livestock phenotype.

Next-generation proteomics: an overview of techniques and computational approaches

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Keywords: proteomics; protein identification; protein quantification

Proteomic technologies have become a mainstay for studying global protein expression profiles across a variety of samples, leading to applications in different domains of biological sciences. Mass spectrometry (MS) represents the main technique capable of deeply characterizing protein content, copy number, and post-translational modifications (PTMs) in biological samples. Recent technological advances in instrumentation and computational techniques allow for the characterization of thousands of proteins in a single instrument run from limited sample amounts. New data acquisition methods have also improved the reproducibility of MS, facilitating the analysis of hundreds of samples in a short time and expanding its analytical capabilities towards clinical proteomics. In this talk, we will analyze novel MS approaches and the computational challenges that remain in the field of MS proteomics, presenting several applications in different fields of life sciences.

Systematic reviewlution:

artificial intelligence for evidence synthesis

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Keywords: evidence synthesis, systematic review, artificial intelligence, natural language processing

Systematic reviews serve as the cornerstone of evidence-based practice, offering comprehensive analyses that inform decision-making across diverse fields. The sheer volume of evidence, estimated at over 4000 health research articles published daily, presents a significant challenge for systematic reviews to keep pace with this immense literature production. This, however, raises concerns about outdated clinical guidance, particularly in scenarios where the most recent evidence is crucial, such as during pandemics. The process of collecting, extracting, and synthesizing data for systematic reviews is widely recognized as highly manual, error-prone, and labour-intensive. Natural Language Processing, a subfield of Artificial Intelligence, is increasingly utilized to derive insights from vast amounts of textual data. Its application in systematic reviews effectively reduces workload and enhances efficiency, expediting the screening process not only in clinical research but also in other domains such as industry or engineering, leveraging supervised models for improved performance. Currently, there exists a variety of machine learning-based tools designed to assist reviewers in synthesizing evidence. These tools cover the entire review process, from screening to work selection, quality assessment, and quantitative analysis of results. They support researchers' work and, in some cases, maintain the active status of a review, as exemplified by living systematic reviews.

Poster session

Host location by vibrational interactions in the ecto-parasitoid *Sclerodermus cereicollis* (Hymenoptera: Bethylidae)

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Keywords: *Sclerodermus cereicollis*; vibrations; host location

Sclerodermus cereicollis (Hymenoptera: Bethylidae) is an ectoparasitoid of xylophagous beetles, mainly belonging to the families Ptinidae and Cerambicidae. Bethylid species are often useful in biological control programs of various pests and it has been proved that these insects use chemical signals to locate their host. Often, alongside olfaction, insects also use visual and mechanical cues in host searching behavior. For this reason, in our work we investigated the role of vibrational signals in the host location process of *S. cereicollis*. For the tests were used the xylophagous larvae of *Hylotrupes bajulus* (Coleoptera: Cerambicidae), which during their trophic activity produce vibrations that could be perceived by the parasitoid. The larvae of *H. bajulus* were kept inside wooden blocks (*Pinus sylvestris*) (25 x 50 x 15 mm). For behavioral bioassays, two wood blocks, one with and one without larva, were placed in two opposite chambers inside a circular arena divided into four quadrants and the larval vibrations were recorded using laser vibrometer (VibroGo®). Mated females of *S. cereicollis* (naive), were released on a plastic lid placed on the circular arena and strictly in contact with the two wood blocks. The parasitoid tracking behavior was recorded using a digital camera (Koppace KP-2100-AMA050, China) for 10 minutes. Based on the preliminary observations, it appears that *S. cereicollis* adults spend more time in the quadrant where the wooden block is infested by cerambycid larva. These results support the hypothesis that *S. cereicollis* is attracted to the host's vibrations cues.

Unveiling the Egg Glue production sites of *Nezara viridula L.*
(Hemiptera: Pentatomidae)

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Keywords: Egg Glue, *Nezara viridula*, Ovariole, Ultrastructure Microscopy

The egg glue produced by insects during oviposition plays a critical role in adhering eggs together and to the substrate, thus providing protection and stability. In most insect species, the glue is secreted by the accessory glands in the female reproductive tract, known as the "collaterial glands". However, in *Nezara viridula L.* (Hemiptera: Pentatomidae), the collaterial glands are absent so the precise location of the egg glue production has remained elusive. Therefore, in this study, we conducted a histological analysis of the tissue and ultrastructure of the female reproductive system using light microscopy, scanning electron microscopy, and transmission electron microscopy. Our findings indicate the presence of a distinct layer of glue between the follicular cells and the chorion of the egg starting from the vitellarium region and continuing towards the proximal part of each ovariole. Furthermore, the ultrastructural analysis revealed the follicular cells containing numerous vesicles releasing the secretions into the space between the egg chorion and the follicular cells. This implies that inside the ovarioles of each ovary, follicular cells surrounding the eggs are responsible for secreting the egg glue following the chorion synthesis. This egg glue at the time of oviposition is released with the egg, hardens, and forms a cement layer for attaching eggs to the substrate. This study together with further investigations aiming to characterize the mechanical and chemical properties of the egg glue of *N. viridula* enhances our understanding of the reproductive biology of this harmful insect and also has practical implications for pest control strategies and innovations in materials science.

Functional comparative analysis of environmental *Legionella pneumophila* strains

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Keywords: Functional diversity; Environmental strains; Genomics

Legionella pneumophila (L.p.) strains colonizing anthropogenic water environments can differ in their functional characteristics, e.g. their proliferation ability in water systems and virulence.

This work aims to determine the functional diversity of L.p. environmental strains isolated from water systems of academic buildings for improving the health risk assessment concerning the presence of different genotypes.

The growth of the strains at different temperatures was investigated. Each L.p. strain revealed a temperature-dependent growth trend, with specific growth rates and maximum cell densities.

The virulence potential of the strains was assessed through infection assays on human macrophages. Differences in replicating in the host and killing them are evident and change according to the time of infection and the quantity of *Legionella* used.

Moreover, the comparative analysis of the genome sequenced revealed the presence of different patterns of virulence genes that can be linked to the characteristics observed *in vitro*.

Functional-Groups and Morph-Functional Groups as tools for phytoplanktonic assessment in Lake Trasimeno

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Keywords: phytoplankton assemblages, shallow lake

Ensuring the long-term sustainability and health of lakes requires monitoring and managing algae growth. The functional group approach has been utilized in various ecological research fields. In this approach, species that are ecologically similar or share similar morpho-functional traits are grouped together. The concept of functional group emerged as a popular approach in phytoplankton ecology in the early 1980s. Reynolds identified 14 algal groups by analysing the seasonal periodicity of lake phytoplankton. This system was subsequently updated and integrated with other groups that exhibit similar morphological and physiological characteristics. The morpho-functional group approach, pioneered by Salmaso and Padisak in 2007, has also been investigated for its effectiveness in deriving water quality indexes. The criteria used to define morpho-functional groups play a crucial role in predicting the most competitive species under diverse environmental conditions. Additionally, consolidating taxonomic information into functional units reduces data complexity and facilitates the simplification of phytoplankton identification. The functional approach can be more effective in detecting environmental changes and assessing water quality compared to analysing individual taxa. This is because species coexist in a shared environment and exhibit common responses to environmental changes. We assessed changes in the phytoplankton community structure in the shallow Lake Trasimeno throughout one year, using two classification systems: the Morpho-Functional Groups (MFG) and Reynolds Functional Groups (RFG) approaches. We found that colonial and filamentous cyanobacteria dominated during late spring and summer. Non-filamentous colonies and mixotrophic flagellate groups, maintained a constant presence from winter to summer.

Wastewater-based assessment of antimicrobic resistance circulation in large cities and rural areas

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During the recent pandemic, sewage surveillance contributed to the assessment of SARS-CoV-2 spread and variants, leading to increased interest in Wastewater Based Epidemiology (WBE). The potential of WBE in monitoring antimicrobic resistance (AMR), has been recently recognized by the proposed revision of the EU Urban Waste Water Treatment Directive (UWWTD) and the Italian National Action Plan on Antimicrobial Resistance (PNCAR), both including surveillance programs based on urban sewage. This work reports the circulation of AMR genes among the population inhibiting large cities and rural areas through the analysis of samples from municipal wastewater treatment plants (WWTPs) of different sizes and locations. AMR occurrence was evaluated by bacterial cultures (i.e. WHO Tricycle Protocol for ESBL-E. coli), as well as qPCR of AMR-genes (i.e. intI1, sul1, tetA, mecA, blaKPC, blaCTX-M, vanA). IntI1, commonly used as indicator of anthropogenic impact, was the most abundant gene followed by sul1 and tetA, ARGs conferring resistances frequently detected in the environment. Indeed, these targets did not show significant differences among WWTPs. On the contrary, clinically relevant targets, namely mecA, blaKPC, blaCTX-M, and vanA, showed much lower concentrations. Nevertheless, blaKPC, and vanA seemed to be enriched in samples coming from WWTPs serving larger urban areas, particularly those including large hospitals. This work highlights how AMR genes recovered from municipal wastewater may provide useful information to assess AMR circulation among the population.

Rendena cattle: a genetic perspective of one Dolomite valley

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Keywords: Rendena cattle, complete mitogenome, phylogenetic analysis

Rendena cattle is a small native dual-purpose breed (milk and meat) raised both in mountain and in plain environments of Val Rendena, a Dolomite valley. Historical sources suggest an ancient origin of the Rendena breed, tightly linked to the history of the human population of the valley and its surroundings. Its first documentation dates back to 1700 but, until the early '70s, the Rendena cattle suffered different significant demographic declines and introgressions from other breeds. Thus, the molecular analysis will allow us to genetically trace its origin and spread and provide a reliable tool for an efficient management of genetic resources of this local breed.

The complete mitogenome (mtDNA) sequencing of 140 samples from Rendena cattle allowed the identification of 93 different haplotypes classified in four haplogroups (Q1, T2, T3 and T5). As expected, the most frequent haplogroup was T3 (91%), but also the remaining haplogroups showed frequencies comparable to other Italian breeds. These data place the Rendena cattle within the general mitogenome context of Mediterranean breeds with specific lineages (Q1, T2 and T5) deserving further investigations. Furthermore, all Rendena mtDNAs were compared to ancient and modern mitogenomes from East-European and North-African cattle breeds, showing some haplotypes shared with Balkan and Podolian bovines, thus highlighting a genetic input from Eastern Europe.

Considerations on the spatial distribution patterns of architect cicada larvae (*Guyalna chlorogena*, Hemiptera: Cicadidae) at the Museu da Amazônia (MUSA),

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The *Guyalna chlorogena* (Walker, 1850), a cicada native to the central Amazon rainforest, at the beginning of the last year of its larval life, builds from a vertical well a turret of clay mixed with its own urine, which rises 20-40 cm out of the ground. For this peculiar characteristic, this *Guyalna* has been called the "cicada architect," and the biology of the larva, as well as the dynamics of the turret-building process, have been investigated; almost completely neglected, however, are the ecological aspects, such as the effect of soil characteristics on the insect and the specificity of the species for host plants.

A census of turrets was carried out within MUSA (Manaus) from 10 June 2023 to 30 September 2023, through a postgraduate traineeship implemented through an agreement between the University of Perugia (DCBB and CAMS) and the Universidade Federal do Amazonas. Our preliminary investigations focused on the relationship between soil pH, a key factor for the life of soil organisms, and the spatial distribution of 'tots'. The pH of the soil does not seem to influence the distribution of the turrets, which instead appears positively correlated with the presence of tree species of the genus *Sclerolobium*. The root sap of these plants had indeed been hypothesised as the main food source for *Guyalna* larvae in previous investigations. This type of insect-plant relationship, moreover, linked to larval feeding, is a frequent phenomenon in the family Cicadidae.

Astrocyte Derived Small Extracellular Vescicle Hinder Glioma Growth

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Glioblastoma (GBM) is the most common and lethal form of brain tumors with a very poor prognosis, despite the combined treatment of radio- and chemotherapy following surgical removal. The main reason for the poor prognosis is the tumour recurrence, due to tumour cells actively invading the peripheral zones of the healthy brain parenchyma to originate a new tumor mass. In this spreading process the ability of GBM cells to regulate their volume and shape is fundamental and, in this context, a key role is played by ion channels, mainly the volume-regulated anion channel (VRAC) and the Ca2+-activated K+ channel of intermediate-conductance (IK). In recent years, the peritumoral cellular microenvironment has been identified as an essential factor in GBM malignancy. Indeed, GBM comprises a complex mixture of tumor and nonmalignant stromal cells, with astrocytes being the major nontumoral cell type. In brain tumors, extracellular vesicles (EVs) are responsible for the bidirectional crosstalk between GBM cells and astrocytes, the latter assuming a protumoral or antitumoral role depending on the stage of the tumor progression. Herein, we investigated the role of small EVs derived from healthy (antitumoral) astrocytes (ADEVs) in GBM malignancy. We showed that ADEVs, through the specific action of miR124, hinder in vivo tumor growth in mice. These effects reside in the ADEVs/miR124 ability to reduce cell volume regulation and, by consequence, cell migration/invasion, by lowering the functional expression of both VRAC and IK channels. In conclusion, our results suggest ADEVs/miR124 as a potential therapeutic tool for GBM treatment.

Circulation and seasonality of respiratory viruses during five consecutive years, 2019-2023

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Respiratory viruses have always represented an important public health problem due to their contagiousness. The most common viruses that can cause Severe Acute Respiratory Infection are Influenza virus (FLU) and Respiratory Syncytial Virus (RSV), but there are many other viruses responsible for burden of disease. These viruses have a specific seasonality pattern that depends on geographic zone and climate condition. Generally, in Italy, the outbreaks of viral respiratory infection occur during winter whereas in summer the activity of these viruses is reduced. The arise of SARS-CoV-2 in 2020 and the non-pharmacological interventions adopted to contrast its spread has led to a profound change in the normal circulation and seasonality of respiratory viruses. This study aims to investigate the circulation of respiratory viruses between 2019 and 2023 at Perugia Hospital and evaluate the impact of the pandemic on the seasonality of respiratory viral infections. Samples, collected from people who accessed Emergency Room or hospitalized at Perugia Hospital, have been analyzed by multiplex RT-PCR. During the study period 11,337 samples have been collected. FLU reported a major reduction in circulation with the complete disappearance in 2021 and the out-of-season circulation in summer 2022. In 2021, the RSV circulation started since September and with a greater number of infections than other seasons. On the other hand, Rhinovirus circulation has decreased while maintaining its seasonality. The evaluation of these changes, monitored by surveillance systems as RespiVirNet, could represent an important weapon in the prevention of these infections to plan the therapeutic and preventive measures.

SARS-CoV-2 ORF3a protein is a water permeable channel

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Keywords: lysosomes, ORF3a, SARS-CoV-2, water transport

Open reading frame 3a (ORF3a) is among the most expressed viral accessory protein of SARS-CoV-2, the pathogen responsible of the last pandemic. ORF3a mainly targets lysosomes of the host cell and promotes lysosomal inactivation through their deacidification, an essential step for lysosomal exocytosis necessary for virus egress. However, the exact function of ORF3a is still unclear. While seminal studies suggested ORF3a functioning as a viroporin, a recent work confuted this conclusion. To unravel the possible function of ORF3a, here we employed a multidisciplinary approach including molecular dynamics (MD), molecular biology, and electrophysiology. Our preliminary structural analyses ruled out that this protein is a viroporin. This initial conclusion was confirmed by electrophysiological results showing that ORF3a did not generate any additional ion current, when expressed in HEK293 cells. Conversely, both MD and videoimaging experiments for the assessment of cell volume changes, demonstrated that ORF3a mediated the transmembrane transport of water. We also identified a putative selectivity filter indispensable for the passage of water molecules. Mutation of an asparagine belonging to the selectivity filter to leucin, or tryptophan, abolish water permeation. Finally, ORF3a expression in HEK293 cells determined lysosomal volume increase (swelling), mitochondrial damage, and accumulation of intracellular membranes. Collectively, our data identify a new function for the ORF3a protein as a water permeable channel. The ORF3a-mediated water transport across lysosome membrane might promote lysosomal swelling and deacidification and, by consequence, inactivation, a key step to promote virus egress from the cell.

Sutjeska National Park and Blidinje Nature Park (Bosnia and Herzegovina): freshwater species identification and population status assessment.

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Keywords: freshwater fish; Balkans; conservation

The Balkan Peninsula is one of the Mediterranean regions with the highest levels of freshwater endemism, which are still little studied and subject to different anthropogenic pressures. Two protected areas in Bosnia and Herzegovina (Sutjeska National Park and Blidinje Nature Park) were investigated with the aims to: i) increase the knowledge on freshwater biodiversity; ii) assess the ecological status of native fish and crayfish populations; iii) perform genetic characterization of trout populations. The presence of 10 fish and one crayfish species was recorded, among which three species are at high extinction risk (IUCN red list): *Squalius tenellus* (EN), *Aulopyge huegelii* (EN) and *Astacus astacus* (VU). Genetic analyses were able to identify the species when the morphological identification was not conclusive and enabled to assess the level of introgressive hybridization with the alien genome for native trout. It was possible to estimate the density and standing crop of riverine fish and crayfish populations, and the length-weight regression and the von Bertalanffy growth equation were estimated for the more abundant species. The populations recorded were all in good ecological status, with the primary concerns being the presence of the invasive Atlantic trout (*Salmo trutta*) and the overharvesting of crayfish. This research allowed to expand the knowledge on freshwater biodiversity in an understudied region.

Welcome home, *Selysiothemis nigra*! A new report at Lake Trasimeno (Umbria) after 62 years

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Keywords: dragonfly, colonization, Lake Trasimeno

Selysiothemis nigra (Vander Linden, 1825) is a Libellulidae classified as a Least Concern species in Europe and Italy, with its populations considered to be increasing. Across its range extending from Morocco through Iraq and Central Asia, the species is generally scarce, with a patchy distribution mainly restricted coastal localities. *S. nigra* occurs in the whole Mediterranean and is an excellent flyer, capable of covering great distances in search of new habitats to colonize. In recent years, the species has been newly recorded in multiple areas particularly in temporary standing waters and man-made water bodies. In 1960 and 1961 Felice Capra documented the presence and the reproduction of the species at Lake Trasimeno, but since then, no new reports had been received. In September 2023, during the annual census of a natural population of *Ischnura elegans*, a juvenile individual of *S. nigra* was randomly captured at the Sant'Arcangelo Ichthyology Center. Since then, daily censuses have been conducted along a transect of about 250 meters along the banks of fish spawning ponds during the hottest hours of the day. The observation of adults in flight lasted for 7 days. Biometric measurements were taken from all six captured individuals, comprising both sexes, with the aim of comparing them with data collected by Felice Capra. Currently, it remains uncertain whether the observation represents a stop of a swarm migration, or the species return to a biotope it inhabited years ago. This is a welcome return that only continued monitoring efforts will confirm.

Honeybees as bioindicators of heavy metal contamination

in the Umbria region

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Keywords: *Apis mellifera*; bioindicators; heavy metals; steelworks

Apis mellifera ligustica (Spinola, 1806), commonly known as the Italian honeybee, is renowned for both its use in apiculture and its role as a pollinator. Furthermore, this little insect can be used as a bioindicator of environmental pollution. Data collected from bees and their products can provide insights into various environmental conditions. The study focused on heavy metal pollution by assessing the content of these pollutants in bees, honey, and beebread from different sites around the main metal pollution source in the Umbria region: Terni's steelworks. Three areas were selected: the Vasciano site, located 12.5 km away from the steelworks, in a rural environment; the Perugia site, characterized by an urban setting and many human activities; the Alviano Lake site, a rural and esteemed naturalistic area. The study focused on the presence of six selected heavy metals in worker bees, honey samples, and beebread samples collected from hives located in these three areas. These metals, which are considered the most used in stainless-steel production, are Cr, Ni, Mn, Fe, Pb, and V. The differential accumulation of these pollutants through the body axis of the insects was also tested. Results confirmed the bioindicator role of honeybees. Vasciano was the most affected site by the steelwork pollution in all three matrices tested. All tested elements accumulated more in the bee's abdomen. Honey was the least polluted matrix in all the areas for all the metals.

Visual and chemical cues in the host plant selection of the melon ladybird *Chnootriba elaterii* (Coleoptera: Coccinellidae)

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Coleoptera are the most numerous insects; in particular, the family Coccinellidae includes many species of known economic interest. Among them, zoophagous species have been extensively investigated as biological control agents against phytophagous insects and mites. Less well-known is the tribe Epilachnini, constituted by phytophagous pest insects. Phytophagous insects use various sensory cues, such as visual, olfactory, gustatory, and tactile stimuli, to orient and locate host plants. In the present study, the host location process of the melon ladybird (*Chnootriba elaterii*) was investigated by behavioral assays, using both a Y-Tube olfactometer and an open Y-Track olfactometer. The results show, for the first time, that females of *C. elaterii* need to integrate visual and olfactory cues during the host location. Visual cues constituted by green colors are of higher relative importance in the location process compared to olfactory cues emitted by the host plants. In particular, green dummy plants made of cardboard, act as a superstimulus for *C. elaterii* females. The results of the present study can shed light on the host location process of phytophagous Coccinellidae and may help to develop biological control strategies, such as visual or chemical traps, with a view to environmental sustainability and biodiversity conservation.

The Wolf in Umbria (Central Italy) and new management perspectives

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The grey wolf (*Canis lupus*) has reoccupied most of its former ranges in Umbria (Italy), leading to new interactions and encounters with humans and with their activities in the region. Servizio Faunistico of Provincia di Perugia and of Provincia di Terni and the Osservatorio Faunistico Regionale collected critical encounters reported by citizens. From 2008 to 2017, we collected reports of 19 critical encounters between wolves and citizens in anthropic or natural areas, for example nearby towns. We consider as critical encounters, those in which humans and wolves are at close range, "face to face". In addition, we investigated the numerical trend of 86 dead wolves in Umbria and their causes of death, examined by the Istituto Zooprofilattico Sperimentale of Umbria and Marche.

Discussing our results, we should take into consideration local factors that affect wolf-management and the relation between humans and wolf: the expansion of wolf population, the absence of a new National Action Plan for the wolf, the lack of control on poaching due to the suppression of local police services (Polizia Provinciale), the chronic absence of public compensation funds for predations on livestock and for preventing further attacks.

A new framework for photo-trapping data management

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In the last years, photo-traps have represented one of the best proficient and inexpensive methods to monitor wildlife populations. The basic aim of the study was to develop a framework for a systematic organization of photo-trapping images for operators involved in wildlife management. In particular, the specific targets are: i) to reduce the time required to managers to archive the essential information collected by cameras; ii) to provide a set of standard procedures for the elaboration of photo-trapping data, and iii) to investigate the patterns and the habits of wildlife species. As a primary method for data elaborations, we chose the R computational environment because it is open source, cross platform, and widely used in ecological research. We designed a series of functions starting from the exif metadata reading to the species pattern elaboration. The elaboration procedure requires less than one second to process each picture and a glimpse on the distribution of the shots over time is provided. At the present time the framework is limited to the elaboration of photo files, but it is under study the possibility to extend the project to video files as well.

Ultrasound deterrents devices test for Ungulates in Umbria

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As a result of repeated reports of damage to high-income crops, OFR provided to purchase and install some ultrasound deterrents made by Natech at two farms of the Alto Tevere Umbro. These devices are battery operated, with continuous output within 24 hours and require periodic battery replacement and recharging. These devices were used on crops already in the field, during ripening and harvesting, in areas with a high density of roe deer and wild boar. The first devices installation was carried out on 29 December 2017 and involved three different plots at the same time, two of which cultivated with "radicchio" (*Cichorium intybus*) and one with "fave" (*Vicia faba*). Where the maintenance and control of the equipment was daily, there were no interruptions of the protection, ensuring a complete harvest.

At the moment Natech is producing new generation devices with a solar panel for power supply and passive infrared sensors (PIR) for activation that make them much more versatile and practical in the field. Their development and diffusion, if supported by economic measures such as "Piano di Sviluppo Rurale" and adequate Ungulates removal plans will allow a new phase of relationship between farmers, hunters and conservationists.

Wildboar Management in urban areas: a challenge in the city of Perugia, Umbria (Central Italy)

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The city center of Perugia develops in a sprawling shape on a series of converging ridges, separated from each other by deep incisions covered by dense vegetation of trees and shrubs, used by wildboars, roe deers and wolves as useful ecological corridors to re-establish themselves in a unused spaces throughout the year. From 2017 onwards, the social instances that ask for removal intervention of wildboar have multiplied due to the presence of the species close to houses and public urban parks, both at night and in broad daylight, with the risk of road casualties. During 2020, 38 wild boars were removed, 50 in 2021 and 127 in 2022. In 2022 the techniques used were trapping (40% of the animals removed), shot by firearm by points (32%), research with one specialised dog (28%). Unfortunately, the maintenance of these "new" natural areas used by wild boars is a weak link in the chain of management useful for limiting the presence and movement of wild boars, since they represent a heavy cost for both public bodies and private citizens.

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