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ORADORES CONVIDADOS: NUNO MONTEIRO - CIBIO, Universidade do Porto KATHARINA HIRSCHENHAUSER - University College of Education Upper Austria

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XVICONGRESSO
DA SOCIEDADE
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ORGANIZING AND SCIENTIFIC COMMITTEE

ORGANIZING COMMITTEE

Ana Cristina Gomes, Beatriz Saldanha, Cristiana Marques, Gonçalo Cardoso, Marta Soares, Paulo Silva, Rita Freitas, Sandra Trigo & Sónia Cardoso @CIBIO - Grupo de Ecologia do Comportamento.

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XVI CONGRESSO DA SOCIEDADE DA SOCIEDADE PORTUGUESA DE ETOLOGIA

Morning

- 09:00 Registration
- 09:30 Opening Remarks
- 09:45 Plenary 1: Katharina Hirschenhauser Taking ethology to the classroom – field studies on pupils' learning behaviour
- 10:30 Coffee break
- 11:00 Renata Alves Maternal separation impact on depressive mothers and the risk for adolescent offspring
- 11:15 Sophie von Merten Bold in the city Urban shrews show a bolder personality than rural ones
- 11:30 Eduardo Sampaio Neurally immature cuttlefish newborns exhibit complex social learning
- 11:45 Flash talks

Ana Vieira de Castro The effects of dog training methods on companion dog welfare and dog owner bond

Joana Carvalheiro Helping behavior in rats (Rattus norvegicus) when an escape alternative is present

Maria Pereira May dogs be mirroring us? A closer look at the association between owners' and dogs' anxiety

Miriam Casaca Effects of a rehabilitation protocol on feral dogs' sociability with humans

12:15 Lunch break / Poster walk

Afternoon

14:00 Plenary 2: Nuno Monteiro

If you think seahorses are cool, wait until you hear about pipefish

14:45 Flash talks

Eve Otjacques Ocean acidification does not affect fish ectoparasite survival

Rita Freitas Too cold to shine? Does temperature influence sex differences in a plastic social ornament?

Flávio Oliveira Metabolic and behavioural adaptations of greater white-toothed shrews to urban conditions

- 15:15 José Paula Presence of cleaning mutualisms affect reef fish distribution forecasts
- 15:30 Lígia Cascalheira Cleaner fish cognitive response to marine heatwaves

15:45 Coffee break / Poster walk

- 16:30 Isabel Damas Fortune favours the bold: behaviour of an invasive lizard
- 16:45 Jakob Friis The allometry of sound frequency bandwidth across songbirds

17:00 Flash talks

Beatriz Pereira Does sound variability of Meagre (Argyrosomus regius) provides acoustic cues on fish size, age, sex and context?

Manuel Vieira Boat noise impacts mating behaviour in a vocal fish

Rita Carriço Sounds from the deep: monitoring fish biodiversity in a protected seamount in Azores

Miguel Santos Behaviour and Ecotoxicology

- 17:30 Vitor Almada award Poster award
- 18:00 General Meeting of the Portuguese Ethological Society
- 19:30 Conference dinner

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INVITED TALKS



Katharina Hirschenhauser

University College of Education Upper Austria (PH OÖ)

Katharina Hirschenhauser's research background is the behavioral endocrinology of pair bonding and agonistic behavior in birds, fish and other vertebrates including humans. Currently, she is dedicated to interweaving biological concepts with educational contexts, such as looking at stress and learning of school children through the lenses of biophilia and attachment theory.

Taking ethology to the classroom – field studies on pupils' learning behaviour

There are various valuable ways of employing concepts and methods from behavioural biology in surveys of teaching and pupils' learning behaviour. For example, the interaction between stress and learning is complex. Development takes place in the context of relationships, where social support and exploration interact as antagonists. The significance of social and emotional aspects of learning is revealed in studies on the efficacy of animal-assisted interventions at school. Moreover, experiences with ethologists at school showed long-term effects on the pupils' factual knowledge, their development of personal interests and potentially, a knowledge transfer beyond the object. The talk aims at inspiring students and colleagues upon the potentials of ethological concepts for other disciplines, such as ethics, social sciences and education.



Nuno Monteiro

CIBIO, Universidade do Porto

Researcher at CIBIO/InBIO and Invited Professor at the Faculty of Sciences, University of Porto. He investigates various aspects of the evolutionary process and, lately, has been particularly focused on the interplay between adaptation and sexual selection. Most of his research involves syngnathids (seahorses, pipefish and seadragons), and he is now a IUCN specialist for this fish group.

If you think seahorses are cool, wait until you hear about pipefish

The fish family Syngnathidae, which comprises seahorses, pipefishes and seadragons, is best known for a truly exceptional feature: male pregnancy. Embryonic development takes place in intimate contact with a male's specialized brooding structure where, besides protection and aeration, nourishment and immune priming occurs. This evolutionary novelty, alongside a curious appearance and serene behavior, has long converted seahorses into iconic and charismatic species especially beloved by children. The monogamous mating system of seahorses, which inevitably entices ideas of fidelity, also helps sprout appreciation from older admirers. Pipefish, on the other hand, despite the family-ubiquitous male pregnancy phenomena, display a much more diverse array of mating systems, with the added 'bonus' that some species also exhibit sex-role reversal. Thus, at least from an evolutionary biology standpoint, pipefish can be seen as much more interesting models to address questions related with sexual selection. In this talk, I will present some of my results gathered when working with seahorses and pipefish, from exposing the dark side of male pregnancy to showing how we can use syngnathids to forecast the impacts of climate change.



ORAL COMMUNICATIONS

MATERNAL SEPARATION IMPACT ON DEPRESSIVE MOTHERS AND THE RISK FOR ADOLESCENT OFFSPRING

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Postpartum depression is a psychiatric condition that has detrimental effects on the mother that can put at risk the mother-infant bound, and consequently the offspring development. The present study used rats with different vulnerability to depression (Wistar and Wistar Kyoto (WKY)) to investigate the impact of prolonged maternal separation (MS) on mothers and their offspring. For this, mother–litter Wistar and WKY rats were subjected to 3h/day of MS from postnatal day 2-14. MS resulted in different qualitative increase of maternal behavior in Wistar and WKY. WKY dams increased the contact behavior, whereas Wistar dams spent more time in higher quality behavior (licking and nursing the pups). Moreover, all mothers subjected to MS showed no anxiety differences during the MS period, however WKY MS mothers exhibited lower anxiety levels after weaning. Anxiety results are supported by the higher oxytocin levels in the hypothalamus observed in the WKY MS mothers, but suggest a dysregulation in the oxytocin system in WKY when considering the observed poorer maternal behavior. In addition, MS impacted WKY adolescent animals worsening their spatial learning. Prone to depression animals (WKY) showed lower resilience to the negative effects of MS.

BOLD IN THE CITY – URBAN SHREWS SHOW A BOLDER PERSONALITY THAN RURAL ONES

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Urban environments differ in many aspects from natural ones and thus pose challenges on many animal species. These challenges, including increased levels of anthropogenic disturbances and light/sound pollution, lead to the prediction that individuals of urban populations are bolder and possibly more aggressive than their rural conspecifics. We tested this hypothesis with two European shrew species with strongly differing life-history strategy: The fast-paced *Sorex araneus* (trapped in and outside Poznan, Poland) and the slow-paced *Crocidura russula* (trapped in and outside Lisbon, Portugal). We conducted repeated tests of boldness and aggression to assess the differences in personality. As expected, urban shews were on average bolder than rural ones. Additionally, *S. araneus* was bolder than *C. russula*, following the framework of Pace-of-life syndromes. Neither between habitats nor species we found significant differences in aggression. However, individual levels of aggression were highly repeatable, independent of the species or habitat, suggesting aggression as an important personality trait in shrews. We also found repeatabilities in boldness. Their strength, however, differed between species and sites, depending on the analysed parameters. A bolder personality can be advantageous for animals living close to humans, as the more frequent anthropogenic disturbances might prevent natural daily activities of shy individual.

NEURALLY IMMATURE CUTTLEFISH NEWBORNS EXHIBIT COMPLEX SOCIAL LEARNING

Eduardo Sampaio^{1,2,3}, Catarina S. Ramos¹, Bruna L. M. Bernardino¹, Maela Bleunven¹, Marta L. Augustin¹, Érica Moura¹, Vanessa M. Lopes¹, Rui Rosa¹

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Learning can occur through self-experience with the environment, or through the experience of others. The latter allows for adaptive behavior without trial-and-error, and generally maximizes individual fitness. Given their mostly solitary lifestyle, cuttlefish have seldomly been tested under observational learning scenarios. Here we tested if and how neurally-immature *Sepia officinalis* hatchlings (up to 5 days) incorporate social information into their decision-making, when observing conspecifics perform a task where modulation (inhibition) of predatory behavior is learned. Results show that more observers than demonstrators learned the task, while also reaching learning criterion in fewer trials. We further registered that pre-exposure to stimulus did not alter learning rates. Moreover, observers always reported less attacks, and higher latency time to attack during trials (which was inverted, under reverse control conditions). Our findings reveal the vicarious capability of cuttlefish newborns to learn inhibition of predatory behavior, potentially through emulation (i.e. affordance learning). Despite ongoing changes on neural organization during early ontogeny, cognitively-demanding forms of learning are already present in cuttlefish newborns, facilitating behavioral adaptation at a critical life stage, and potentially improving individual fitness in the environment.

PRESENCE OF CLEANING MUTUALISMS AFFECT REEF FISH DISTRIBUTION FORECASTS

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Cleaning mutualisms are key ecological components in marine ecosystems, where cleaner fishes feed on and remove their client fishes' ectoparasites. In fact, long-term cleaner fish removal experiments (with cleaner wrasse, *Labroides dimidiatus*) in small patch reefs have shown associated declines in reef fish diversity and abundance. Yet, there is no knowledge on how the presence of cleaning mutualisms may influence present and future (climate change-driven) forecasts of their client fishes. Here, we present the first global forecast of reef fish species distributions (136 client fish species; 46 locally-dependent on cleaner presence) under three different end of the century scenarios (RCP2.6, RCP4.5 and RCP 8.5) using an ensemble of ecological niche models (with cleaner wrasse distribution also as a predictor). We found that forecasts using cleaner wrasse distribution were significantly different from those without it, and such a trend was more evident in species known to be locally dependent on cleaner's presence. Thus, we argue that the forecasting of these interspecific interactions is crucial to fully understand the future impacts of climate change on the structure and health of tropical reef ecosystems.

CLEANER FISH COGNITIVE RESPONSE TO MARINE HEATWAVES

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Heatwaves are becoming more frequent and intense mostly due to global warming. Coral reefs are one of the ecosystems in the frontline of these impacts. Cleaner fishes (*Labroides dimidiatus*) play a crucial role in these ecosystems, as they influence client abundance and diversity. During their cooperative interactions with other fishes (so-called "clients"), cleaners eat parasites and dead tissue, providing direct physiological benefits to their clients. Previous studies have shown that after heatwaves, changes in fish densities can alter cleaner fish cognitive sophistication. However, how direct exposure to heatwaves affect fish cleaner fish cognition is still unknown. We exposed cleaners to three different heatwave scenarios (categories1, 2 and 4) during 55 days. These take as baseline the heatwave occurred in 2016 at the Great Barrier Reef (Cat.1) and projected intensities with climate change (Cat.2 and 4). We tested cleaner fish ability to solve a pattern association task during the heatwave and after a 30-day recovery period. Here we show that exposure to all different heatwave scenarios, but cognitive impairments can be recovered. These results suggest that exposure to extreme events can directly affect cognition of cleaner fishes, with potential effects cascading to the rest of the ecosystem during heatwaves.

FORTUNE FAVOURS THE BOLD: BEHAVIOUR OF AN INVASIVE LIZARD

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Biological introductions are a global problem with major ecological and economic implications. Many species are regularly transported outside their native range, yet few species manage to successfully invade and adapt to new locations. What determines a species' invasion success is important for conservation efforts. Behaviour is believed to play a key role in the success of invasive species, although the mechanisms are still unclear. Lizards of the genus *Podarcis* show high variability in their invasive potential and are thus a well-suited model system to study the role of behaviour in biological invasions. We used a globally invasive species – *Podarcis sicula* – from an introduced population in Lisbon to quantify behavioural traits that may influence its invasive success. We found invasive *P. sicula* to be more exploratory, bold, and neophilic than the sympatric, native *Podarcis virescens*. Importantly, while the native species showed high repeatability in its behaviours, the invasive species was more inconsistent, which might indicate behavioural plasticity. Remarkably, we found exploitative competition occurring between the two species – the invasive *P. sicula* was first to arrive at food, consumed more food, and gained more weight than the native species. These experiments highlight the importance of examining behaviour for understanding biological invasions.

THE ALLOMETRY OF SOUND FREQUENCY BANDWIDTH ACROSS SONGBIRDS

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It is well documented that body size is negatively related to the sound frequency of vocalizations across avian species, due to small body size constraining effective production of low-frequency sounds. It was never investigated whether body size also constrains the extent of the sound frequency bandwidth. Theory predicts that extending sound frequency downwards is more limiting than upwards, relative to the optimum frequency for a given body size. As vocal communication occurs - and is more effectively transmitted - within a specific frequency range, a corollary prediction of this theory is that large-bodied species should be able to use larger bandwidths (comprising low and high frequencies), compared to small-sized species (who should use only the high frequencies). We tested this prediction comparing sound frequency bandwidth of songs across >500 oscine passerine species. We found a significant negative allometry (i.e. negative association with body mass), so that larger species typically has larger bandwidths. Further analysis indicated that this is due to the allometry of minimum frequency being stronger than the allometry of maximum frequency. These results suggest that body size should be assessed as a confounding factor, when studying acoustic communication and comparing measures of frequency modulation across species.

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FLASH TALKS

THE EFFECTS OF DOG TRAINING METHODS ON COMPANION DOG WELFARE AND DOG OWNER BOND

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The scientific evidence on the effects of different training methods on companion dog welfare and dog owner bond is limited. The present study aimed to fill this gap. In Study 1, companion dogs trained with reward-based methods (Group Reward, n=17) and with aversive-based methods (Group Aversive, n=17) participated in a Strange Situation Procedure. The presence and absence of owner and a stranger in a room with the dog was manipulated over six episodes. Dogs' behavior was then analyzed for attachment related behaviors: proximity-seeking, separation distress and secure-base effect. A secure attachment tended to be more consistent in Group Reward. Study 2 combined a short- and long-term assessment of the welfare of companion dogs trained with reward- (Group Reward, n=42) and aversive-based methods (Group Aversive, n=50). For short term welfare assessment, the frequency of stress related behaviors during training, and salivary cortisol levels at home and after training were analyzed. For long term welfare assessment, dogs underwent a cognitive bias task. Group Aversive displayed a higher frequency of stress-related behaviors during training, higher increases in cortisol after training and poorer affective states in the cognitive bias task. In summary, our results show that aversive-based methods compromise companion dog welfare and may also affect attachment to owner.

HELPING BEHAVIOR IN RATS (RATTUS NORVEGICUS) WHEN AN ESCAPE ALTERNATIVE IS PRESENT

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Prosocial behavior in rats is known to occur in response to a familiar rat's distress, but the motivations underlying prosocial behavior remain elusive. In this study, we adapted the experimental setting of Ben-Ami Bartal, Decety, and Mason (2011) to explore different motivations behind helping behavior in adolescent rats. In the original setting, a free rat is placed in an arena where a cagemate is trapped inside a restrainer that can only be opened from the outside by the free rat. Here we added a dark compartment to the experimental setting that allowed the free rat to escape the arena and the distress evoked by the trapped cagemate. As a control, we tested rats in the same arena but with the door to the dark area closed. Our results showed that in the escape condition, rats took significantly longer to open the restrainer to the cagemates when compared with rats that could not escape. Importantly, behavioral analyses revealed that struggling behavior (i.e., distress) in the trapped rat did not affect door-opening, whereas exploratory behavior (i.e., proactive/positive behavior) in both rats contributed to shorter times. These results shed new light on the importance of positive emotional states in prosocial behavior.

MAY DOGS BE MIRRORING US? A CLOSER LOOK AT THE ASSOCIATION BETWEEN OWNERS' AND DOGS' ANXIETY

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Based on previous research showing a 'personality fit' between owners and their dogs, this study further explored the association between owners' anxiety and anxiety in their dogs by testing for mediating and moderating factors. Two hypotheses were tested: that dogs may respond to their owners' anxiety directly through emotional contagion, or that owners' anxiety may affect dogs' indirectly via owners' protection behaviors.

A cross-sectional approach was followed using an online questionnaire designed to measure owners' trait anxiety and dogs' anxiety-related behavior problems, as well as owners' protection behavior, and dogs' empathic trait. Data was obtained from 1172 self-identified dog owners. Results showed a significant and positive correlation between owners' anxiety trait and severity of anxiety in dogs. No evidence was found for mediation of this relationship by owners' protection behavior. However, results showed that above a particular score in dogs' empathic trait, the link between owners' anxiety trait and anxiety in the dogs became stronger, thus pointing to moderation.

Owners' anxiety trait may contribute, to some degree, to the development of fear and anxiety in their dogs. This is relevant from a clinical perspective and in line with a One Health approach to human and animal health.

EFFECTS OF A REHABILITATION PROTOCOL ON FERAL DOGS' SOCIABILITY WITH HUMANS

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Feral dogs live in a free state with no direct resources intentionally supplied by humans. When captured these dogs tend to permanently stay at kennels, as their low sociability towards humans contributes to the lack of interest by potential adopters. As so, the aim of the present study is to determine if applying a rehabilitation protocol can develop the social skills of feral dogs, needed for companion dog human interactions, thus increasing their prospects of a successful adoption. Six feral dogs (Experimental Group) and 12 non-feral dogs (Control groups 1 & amp; 2), housed at a kennel, participated in the study. All groups were tested for their sociability with humans before and after the implementation of a 40-day rehabilitation protocol, which was only applied to the Experimental Group and Control Group 1. This protocol consisted essentially of desensitization and counterconditioning procedures, together with procedures of positive reinforcement of desired behaviours. The video recordings of the sociability test were then analysed by 3 observers in order to rate the dogs' level of sociability, to evaluate the effect of the rehabilitation protocol. To the best of our current knowledge this study is the first to address social rehabilitation of feral dogs.

OCEAN ACIDIFICATION DOES NOT AFFECT FISH ECTOPARASITE SURVIVAL

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On the Great Barrier Reef, gnathiid isopods are known as one of the most common fish ectoparasites, having substantial effects on settlement-stage larvae or very young juveniles, and repeated attacks also affect fish growth. In order to cope with these ectoparasites, reef fishes tend to engage cooperative interactions with cleaning organisms, like shrimps or fishes, where they benefit from the ectoparasite reduction and thus stress relief. Ocean acidification is known to pose a range of threats to marine invertebrates, yet the potential effects of ocean acidification on fish ectoparasites remain unknown. We exposed cultured gnathiid isopods to high CO2 in single vials and measured their survival rate under isolation. Our results showed that high CO2 did not have any effects on the gnathiid survival rate at any life stages. We advocate that, as ectoparasites would not be affected by ocean acidification, cleaner organisms would still play a significant role in coral reefs and thus, future studies are necessary to assess the effects of ocean acidification in such organisms.

TOO COLD TO SHINE? DOES TEMPERATURE INFLUENCE SEX DIFFERENCES IN A PLASTIC SOCIAL ORNAMENT?

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Some signals or traits in birds may have plastic characteristics. Sex differences in secondary sexual traits in species with conventional sex roles are usually thought to be genetically fixed but, instead, they may be due to environmental factors. Recently, it was found that the common waxbill (Estrilda astrild) has a sexually dimorphic social ornament (the red bill) whose sex differences are entirely plastic. It was suggested that those plastic sex differences may be due to environmental causes, such as climatic conditions. The aim of my work was to test experimentally the hypothesis that ambient temperature may be responsible for changes in bill colour saturation in the common waxbill, and how temperature affects female and male bill colour. For this, I manipulated temperature inside bird cages with electric warming devices and maintained other cages at ambient temperature in an outside aviary during the non-reproductive season (winter), and then I switched the temperature treatments. I found that feeding and movement rates changed when changing temperature treatments, confirming that temperature manipulation had physiological effects. In females, I found that warming temperature during winter, reduced oscillations in bill colour saturation, while in males, it had no effect. This was a sex-specific effect, in which females benefited from favourable warmer temperatures to express consist bill colour saturation, also suggesting that female investment in ornamentation is more sensitive than males' to environmental conditions, namely energetic stress and ambient temperature.

METABOLIC AND BEHAVIOURAL ADAPTATIONS OF GREATER WHITE-TOOTHED SHREWS TO URBAN CONDITIONS

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Studies investigating behavioural differences between rural and urban populations often report an increase in risk-taking behaviours in urban animals. According to the most common energy management model (the performance model), behaviours that increase access to resources, such as aggression and boldness, and behaviours that consume net energy, like locomotion and stress responses, are both positively correlated with basal metabolic rate (BMR). Consequently, we expect urban populations to not only exhibit a higher level of risk-taking behaviours but also a higher BMR. We analysed this hypothesis in the greater white-toothed shrew (*Crocidura russula*). Shrews were trapped in rural and urban areas near Lisbon and brought to captivity where we measured BMR, boldness and exploration rate three times in each individual. Our findings revealed that, at the among-population level, urban shrews were indeed bolder and more explorative, but contrary to our expectations, their BMR was lower than in rural shrews. This relationship remained similar at the within-population level. In conclusion, *C. russula* does not employ the performance model. We attribute this behaviour to the contrasting environmental conditions of these two habitats, such as higher ambient temperatures and/or lower prey availability found in cities.

DOES SOUND VARIABILITY OF MEAGRE (*ARGYROSOMUS REGIUS*) PROVIDES ACOUSTIC CUES ON FISH SIZE, AGE, SEX AND CONTEXT?

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Many fish taxa produce sound in social and in alarm contexts but knowledge on the full acoustic repertoire is lacking for most species. Yet, this knowledge is critical to enable monitoring of fish populations in nature through acoustic monitoring. In this study we characterized the sounds emitted in alarm and social contexts by captive juvenile and adult meagre, *Argyrosomus regius*. In the case of adults, the acoustic activity and characteristics of sounds produced during social contexts in relation with spawning events (monitored by the collection of eggs from the tanks) were also characterized. For comparison, we also registered meagre sounds in the Tagus estuary, Portugal, from unseen fish during the breeding season. The present dataset demonstrates for the first time that in this species dominant frequency is inversely related to fish size, and that sounds vary according to sex, context and throughout ontogeny. Sounds produced by captive meagre during the breading season showed a richer repertoire than previously observed. Sounds from captive breeding adults were comparable to sounds recorded in the field.

BOAT NOISE IMPACTS MATING BEHAVIOUR IN A VOCAL FISH

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Noise-generating human activities have altered marine soundscapes around the globe and is now recognised as global and chronic pollutant. In estuarine shallow waters one of the most prevalent sources of anthropogenic noise are motorboats and ferries. In this study we compared the effects of boat noise and ambient noise playbacks on calling activity of breeding Lusitanian toadfish males (*Halobatrachus didactylus*), a species that relies on advertisement calls for mate attraction. Four sets of 12 concrete nests were exposed for a fortnight to either control or playback of boat noise on two mating seasons (2016 and 2017). Noise treatment mimicked the passage of 10 ferries and of 4 small boats per hour during 18 hours, similar to what fish experience in Tagus estuary. During experiments each nesting male vocalizations were continuously recorded to a datalogger and offline monitored with an automatic recognition methodology based on the Hidden Markov Model. Preliminary analysis indicates that calling rate decreased in males exposed to boat noise both during and outside the playback period. These results suggest that exposure to anthropogenic noise may impact reproduction in vocal fish. Ongoing investigation is addressing how boat noise impacts reproductive success and the development of toadfish early-life stages.

SOUNDS FROM THE DEEP: MONITORING FISH BIODIVERSITY IN A PROTECTED SEAMOUNT IN AZORES

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Long-term marine biodiversity monitoring is paramount for Ocean conservation. Passive Acoustic Monitoring (PAM) is a cost-effective method to monitor sounds from marine organisms and therefore biodiversity, and is especially relevant in long-term surveys of inaccessible sites such as the deep sea. Using PAM, we evaluated seasonal and diel patterns of putative fish sounds identified in a recently protected deep-sea Azorean seamount, the Condor. We also compared abundance and diversity of fish sounds before and after establishment of the protection, and compared acoustic data with biodiversity of deep-water demersal fish assemblages surveyed by longline fishing.

We found increased number of sounds in winter compared to the rest of the year. There was a decrease in the mean abundance of sounds from 2008 to 2012 while the number of sound types kept stable even after the reserve establishment in 2010 (a higher diversity was only observed in 2010). Although we expected an increase in fish sound type abundance and diversity, the observed trends were in line with the fish biodiversity estimated by fishing. This study shows that monitoring fish sounds with PAM is a valid and promising tool for fish biodiversity assessment in the deep sea.

BEHAVIOUR AND ECOTOXICOLOGY

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There is an increasing number of observations indicating that several environmental pollutants, particularly endocrine disrupting chemicals, are able to alter key behaviour endpoints in an array of different taxa. This includes changes on anxiety and alterations to normal shoaling behaviours, sexual behavior, aggression levels, spatial distribution, among others. Hence, behaviour- based endpoints can hypothetically be used as proxies for the identification of the toxic effects of several environmental chemicals, many of which could be of ecological relevance. Here, we will discuss the use of behaviour endpoints in the frame of toxicity testing, highlighting in particular the usefulness of behaviour responses for high-throughput toxicity testing and hazard assessment.

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ANAESTHETIC EFFICACY AND AVERSION: COMPARING 2 COMMON ZEBRAFISH PROTOCOLS WITH A COMBINATION OF PROPOFOL/LIDOCAINE

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Zebrafish as a laboratory animal model has been continuously rising in interest. This evolution in usage asks for more refined protocols such as anaesthesia, one of the most commonly used procedures. Thus, we aim to study the clinical efficacy as well as fish aversion to equipotent dose of propofol/lidocaine, MS222 and clove oil. After determination of the equipotent doses, i.e. concentrations that induced the loss of equilibrium at similar times, 54 mixed-sex adult AB zebrafish were randomly assigned to MS222 (150mg.L-1), propofol/lidocaine (5mg.L-1 propofol + 150mg.L-1 lidocaine) and clove oil (45 mg.L-1) group. Latency to lose response to a painful stimulus and to recover was assessed. To study aversion to these anaesthetic protocols, the conditioned place aversion test was used, where aversion was measured by the latency to go to the conditioned place, where animals previously experienced the anaesthetic. HCl (pH= 3) (n=6) was used as the positive control.

Clinically, all protocols seem to be effective, with slight differences. Based on our present data, all protocols seem to be acceptable for adult zebrafish with MS222 showing no aversion contrarily to what was expected, but more research is ongoing to clarify potential side-effects regarding physiological, cellular and behavioural alterations.

ASSESSMENT OF DOGS AND CATS' WELFARE DURING HOSPITALIZATION: A PRELIMINARY STUDY

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Animal's welfare has a crucial importance in veterinary practice, since distress is detrimental for animals, impairing the recovery of those that are unwell. In order to evaluate dogs and cats' psychogenic distress during hospitalization in veterinary hospitals (VH), we decided to evaluate the presence of stress related behaviours/signs (SB) during hospitalization.

Specific stress related SB scoring systems for dogs (scores from 0 - 18) and cats (score from 0 - 7) were applied to 30 second videos, recorded during hospitalization. In this preliminary study dogs (n=15) from the same VH presented SB scores lower than 5 (mild distress) but interestingly, by evaluating older videos from other VHs (n=3) we found out that applying the same scoring system the outcome was different, with higher scores (between 7-10, moderate distress). Regarding cats (n=8), we were only able to evaluate videos from one VH; their overall SB scores ranged from 2 to 7, with 37,5% of cats reaching a score of 5 (moderate distress).

This preliminary data suggests that these SB scoring systems may be useful tools not only to classify dogs and cats' level of distress, but also to differentiate between VH that invest in stress-free environments and others, that need to improve their practice.

BEHAVIOR OF SMALL-SPOTTED CATSHARK IS NOT AFFECTED BY MARINE HEATWAVES

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Sudden warming events, designated as marine heatwaves (MHWs), have been occurring around the globe. As the climate changes, these warming events are becoming more frequent and intense. The exposure to a short-term MHW already showed a strong effect on small-spotted catshark's physiology. However, the effect that a MHW may have on these animal's behavior has not yet been studied. Hence, our work focused on the effects that a MHW ($\Delta 3 \, ^{\circ}$ C) could provoke on juvenile temperate shark's behavior (*Scyliorhinus canicula*). To address this question, sharks were divided in two treatments: control (18 $\,^{\circ}$ C) and a category II MHW (21 $\,^{\circ}$ C) for 15 days. After this acclimation period, their behavior was video recorded for 24h. Then, a blind observer analyzed the time spent i) swimming, ii) resting, iii) performing social interactions, iv) hiding, and v) their reaction to the daynight switch. Each shark was individually observed for all the endpoints listed. According to the results of our study, a 15-day long category II MHW had no effect on these shark's behavior. These results suggest that small-spotted catsharks' behavior will not be impacted by a category II MHW.

CLIMATE-DRIVEN OCEANIC DEOXYGENATION LEADS TO TOP PREDATOR HABITAT COMPRESSION MORE PRONE TO OVERFISHING

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Reduced oxygen levels may have dramatic consequences for ecosystems and marine animals. Studies indicate ongoing expansion of oxygen minimum zones (OMZ) may constrict available habitat for tropical pelagic fishes such as sharks. Shark populations have been declining at a worldwide scale due to overfishing and lack of international catch quotas. Using advanced satellite telemetry techniques, we were able to track blue and mako sharks, providing important information on these species' movement patterns, spatial dynamics and behaviour when inside and outside the Eastern Tropical Atlantic OMZ. When within the area OMZ, habitat was generally restricted to near-surface depths, apparently modifying their behaviour in response to the characteristics in the oxygen structure of the water column and prey vertical distribution. We observed that there is a larger fishing effort in the OMZ, increasing the risk of these species within this area. By examining the drivers of habitat compression, this study aims to assess the consequences of climate change in the vulnerability of sharks to fisheries. There is an urgency of protecting sharks from overfishing as some species are now at a fraction of their historical biomass, where recovery may be difficult or not be possible even if fishing pressure is removed.

DO NOSEWORK ACTIVITIES IMPROVE LABORATORY DOG WELFARE?

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The kennel environment in which laboratory dogs are maintained presents a potential welfare risk. Consequently, refinement strategies in the form of environmental enrichment should be employed. The goal of the present study was to investigate the impact of nosework activities on the welfare of laboratory dogs. Twelve beagle dogs were exposed to a 12-day period of nosework activities (experimental condition) and a 12-day period of heelwork activities (control condition), with a 21-day washout period in between, during which no intervention was performed (order of conditions counterbalanced between subjects). During the intervention periods, a 5-minute daily session was performed: in the nosework period, the animal was trained to use olfaction to find food hidden in boxes, and in the heelwork period, the animal was trained with food to walk side by side with the experimenter. In order to evaluate welfare, cognitive bias tests were conducted before any type of intervention and after each period of intervention. Results showed that dogs tended to be more 'optimistic' (to show better welfare) after the nosework period, but not after the heelwork period. This study thus suggests that nosework activities can present an easy and efficient way to improve laboratory dog welfare.

FOOD HABITS AND PREY SELECTION BY WOLVES IN A RECOLONISED MEDITERRANEAN AREA

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Large carnivores are increasing throughout the western Holarctic, re-colonising their former ranges. Ecological and socio-economic consequences of this process need to be monitored, to identify suitable management/conservation actions. We studied food habits and selection of main prey by wolves, in a Mediterranean protected coastal area (c. 70 km2 of the Maremma Regional Park, central Italy, April 2016-March 2018). Potential prey includes wild boar, fallow deer, roe deer, livestock and several species of meso-mammals. Wild ungulates dominated the diet with the fallow deer being the main prey (AO: 55%; V: 44%; B: 50-53%) followed by the wild boar (AO: 44%; V: 32%; B: 30-32%). Livestock was rarely used (2%, both AO and V; B: c. 5-7%); the coypu (AO: 8%; RO: 6%; V: 6%; B: 4-6%) was another important food item. Fallow deer were selected, wild boar were used according to their availability, whereas roe deer were under-used. Prey selection was probably driven by the greater accessibility and detectability of fallow deer, which are gregarious and attended mainly open habitats, in respect to wild boar/roe deer. Most likely, availability of a diverse spectrum of meso-large prey and a comparatively lower accessibility of livestock are key-factors to limit livestock consumption.

GENETIC ARCHITECTURE OF SOCIAL AND ASOCIAL LEARNING IN DROSOPHILA MELANOGASTER

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Social learning – the process by which animals learn about their environment by observation or interaction with other animals or their products – represents an advantage in complex and changing environments, often reducing the time and energy costs associated with asocial learning.

Although social and asocial learning are two distinct behavioural phenotypes, an essential question for eco-evolutionary and neuroscientific fields is whether these behaviours share common (generalpurpose) or distinct (special-purpose) cognitive modules.

To answer this question, a subset of the Drosophila Genetic Reference Panel (DGRP) will be behaviourally characterized and a bioinformatics analysis will disclose whether there are common or distinct groups of social and asocial learning-associated genes. Then, with a loss of function genetic approach using *D. melanogaster* transgenic lines, we will characterize the neuronal circuits underlying the general or special-purpose mechanisms of social and asocial learning.

Although not a highly social insect, the fruit fly is able to learn socially and asocially in distinct functional domains, such as mating, foraging and egg laying. Moreover, it is currently the species with the most powerful genetic and neuronal tools available, making it the ideal model organism to address this question.

INTERCEPTIVE CUES AND THE EVOLUTION OF SIGNAL DISHONESTY

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Signals are considered specialized traits of senders that transmit intentional social information to receivers and whose adaptive function is the specialized responses by the receivers. In contrast, social cues derive from actions and interactions of senders that inadvertently transmit information to bystanders. As social cues did not evolve to convey information to others, neither can be manipulated by the sender, they are considered honest information and highly reliable. However, here we acknowledge that inadvertent social information can also derive from signals when these are intercepted by bystanders. We draw attention to this shift of a signal into a social cue, to which we call "interceptive cue". By recognizing this new type of a social cue, a new corollary becomes evident: social cues are not always honest, because interceptive cues derived from dishonest signals will also contain dishonest information. Consequently, bystanders will engage in erroneous decision-making. This way, we propose the hypothesis that signal dishonesty is an evolutionary product of the interactions between all parties involved in a communication network and suggest possible evolutionary outcomes dependent on the costs and benefits to the sender and to the bystander.

MOUSE PRE-WEAN MORTALITY: DO WE KNOW IT?

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Mouse pre-weaning mortality is highly variable and frequently happens within the first 48hrs postpartum (pp). Thus, a good portion of the mice generated for research die before they even get to be used in science. Mouse mortality must be reduced in breeding facilities to allow for complying with the 2010/63/EU directive, while lowering an estimated yearly breeding cost in at least 6€ million in the EU. However, detecting mortality is challenging, as pup counting procedures vary among breeding facilities and very often pups do not get counted within the first 48hrs pp. This study compared an experimental procedure of counting pups daily with historical data records from two large breeding facilities in the U.K. (B1) and Portugal (B2), where pups get counted only once a week. Results revealed that historical data overestimated litter survivability by 8.5% (B1) and 6.3% (B2). Counting pups daily did not affect number of pups weaned/litter compared to historical data from the same breeding mice used in the experiment but in different parities. Instead, daily counting of pups allowed for a more accurately detection of the number of pups born/litter. Breeding facilities have been underestimating the amount of pups born and overestimating their survivability rates.

NO NEED TO TURN THE LIGHTS ON! - PREY AND RIVAL IDENTIFICATION BY JUMPING SPIDERS UNDER DIM LIGHT

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Jumping spiders (family Salticidae) are known for their elaborate vision-based behaviour during encounters with prey and conspecifics. However, most research on their visual performance has been carried under bright light levels. Here, we investigated the capacity of two salticid species, *Cyrba algerina* (Portugal) and *Cyrba ocellata* (Kenya), to perform two specific visual tasks under low ambient light levels: 1) prey identification – using lures made from spiders and midges and, 2) rival identification – response to their mirror images (virtual conspecifics). Experiments were implemented under dim light levels ranging between 234 cd m-2 and 0.24 cd m-2 (equivalent to a cloudy dull day and twilight, respectively). In most instances, *C. algerina* and *C. ocellata* were proficient at performing both visual tasks when ambient light was 234 cd m-2 (loudy dull day) and 1.35 cd m-2 (mid dusk). A minority of spiders performed these tasks at 0.54 cd m-2 (late dusk), but none succeeded when the light level was 0.24 cd m-2 (twilight). Both *C. algerina* and *C. ocellata* were able to perform vision-based discrimination tasks under ambient light levels previously only associated with nocturnal species.

OVERCOMING EXPLOITATION BARRIERS: TIME TO REMODEL FORAGING THEORIES? SMALL-SIZED BEES ADDRESSING TO HUMMINGBIRD-EVOLVED SALVIA SPECIES

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Foraging behavioural plasticity shapes a continuum of rapid overcoming of constrains to access newly available resources. Learning abilities allow resource exploitation, even in modes that evolutionary theories consider unfeasible. Theories predict that plants evolved floral traits according to the selection driven by the most fitted pollinator: mainly wind or animal as insects, birds or mammals. These adaptations were summarised and described long ago: through the concepts of pollination syndromes or flower classes. Therefore, for example, bees should be physically or timely prevented to visit flowers with structures/resources evolved for birds or mammals. However, exceptions have already been observed and reported.

We recorded the peculiar behaviour of bees belonging to the genus *Lasioglossum* visiting flowers of two Mexican *Salvia* species, *S. blepharopylla* and *S. greggii*. The sages grow at the Ghirardi Botanic Garden (Italy), and present flower characteristic of bird-pollinated species: corollas especially deep, abundant nectar, and a staminal lever mechanism preventing access. Possibly driven by chemical cues indicating nectar presence, the small-sized bees were forcing the flower "barriers" to collect the nectar, even at the cost to result trapped in. We will show details of this behaviour and correlate its evolutionary importance in the perspective of exotic plant trades.

PASSIVE ACOUSTIC LOCALIZATION OF SONIFEROUS FISH

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Numerous fish species communicate through acoustic signals. These sounds can be used to characterize animal presence, diversity and behaviour in aquatic environments, especially in situations where direct visual observation is difficult or long-term monitoring is required. Passive acoustic monitoring (PAM) has been demonstrated as a powerful, cost-effective tool to monitor vocal fish in the field. Although position and movements are important aspects of fish behaviour, to date only a limited number of studies have used PAM to characterize fish position and movements. Here we present a system capable of estimating the movement of individual vocal fish. The system uses 4 hydrophones mounted on a cubic steel frame connected to a multichannel recorder (96 kHz / channel, 16 bit). Position and movement is estimated from the differences of time of arrival of fish sounds to each hydrophone. This tool was tested by estimating the bearing of playbacks emitted from known positions. Preliminary results show that accurate bearing estimates can be obtained up to a distance of at least 25 m. Ongoing investigation is addressing how to localize individual soniferous fish in 3D, such as the meagre, to assess the impact of boat passages on their behavior during the mating season.

PERSONALITY AND LOCOMOTION IN WALL LIZARDS - ALTERNATIVE STRATEGIES OF COLOUR MORPHS

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Personality can determine individual fitness. Bold and highly mobile individuals may have better chances of encountering resources, food and mates, but they may also suffer higher predation risk. The trade-off between reproductive success and predation mortality can shape the frequency of each personality in natural populations. Wall lizards, Podarcis muralis (Squamata, Lacertidae), show ventral colour polymorphism involved in sexual signaling. Analyses of genomes of three distinct colour morphs detected differential genetic underpinning among white, yellow, and orange morphs. Variations in the levels of pigments such as carotene, pterin and melanins were found to be the reason for existence of the three colour morphs; and new genes underlying the color polymorphisms had been revealed. Detected color genes have likely pleiotropic effects on behaviors associated with color morphs. Higher local morph diversity is related to stronger sexual selection, but the distribution of morphs is also driven by differentiation in geography and ecology, suggesting complex interactions between sexual and natural selection. Here, we test the hypothesis that lizards showing divergent color morphs expressed alternative personalities and different locomotory strategies. We address the hypothesis using an open field test and animal video tracking to quantify locomotory behaviors and boldness/shyness in individuals of each morph. We use generalized mixed modeling to compare personality and locomotion to test whether different color morphs employ different behavioral strategies, concordant with pleiotropic hypothesis of color genes effects.

WAXBILLS' BILL COLOR SIGNALS PLASTIC SEX DIFFERENCES AND NON-PIGMENTARY ANTIOXIDANTS

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Carotenoid-based coloration is widespread in animals, and is assumed to be a condition-dependent signal of quality. It is assumed that carotenoids are a limiting factor and that they can only be allocated to coloration or physiological maintenance. However, this direct link between coloration and carotenoids availability is still on debate and has been proposed that coloration is signaling the individuals' antioxidant status, revealing the ability of non-carotenoid antioxidants, as vitamin A and E. We tested this hypothesis in the waxbill (Estrild astrild), a passerine with a carotenoid-based dynamic trait: the color of the bill. We manipulated the availability of carotenoids and a nonpigmentary antioxidant (vitamin E) in the drinking water. If the antioxidant properties of vitamin E protects carotenoids, we predict that birds supplemented with vitamin E should have redder bills than birds not supplemented. If carotenoids are a limiting factor, we predicted that birds supplemented with carotenoids should have redder bills than birds not supplemented. We found that increased availability of carotenoids enhanced the expression of a carotenoid-based social trait in females but not in males. Besides, taken into account the effect of contamination between supplementations, we found that the availability of a non-pigmentary antioxidant also enhances the expression of bill coloration in females but not in males. Taken together, these results indicate that the availability of resources are more important for females that males, and that both supplements have a role in the expression of bill coloration in waxbills.

SOCIAL COMPLEXITY IN THE LUSITANIAN PINE VOLE (MICROTUS LUSITANICUS)

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Social complexity can be thought of as a continuum ranging from solitary to eusocial species. Although the occurrence of eusociality outside insect colonies has been questioned, some support that it has also evolved in mammals. Among rodents, high sociality levels are usually associated with harsh environments (e.g. eusocial naked mole-rats from Kenya), potentially as a way to overcome habitat aridity and low food availability. As such, with the key components of social systems in mind, we propose to perform a long-term study to characterise the level of social complexity of the Lusitanian pine vole (*Microtus lusitanicus*), a fossorial rodent species whose social system has not yet been described. This species is known to build complex underground systems, form large social organisation, ii) social structure, iii) mating system and iv) care system. We hope to understand if *M. lusitanicus* fossorial habits and origin in the Iberian Peninsula constitute a different and possibly important step in the evolution of social complexity.

SOUND VARIABILITY IN A FISH SPECIES OF HIGH COMMERCIAL VALUE: GEOGRAPHICAL VARIATION OF CALLS IN A PASSIVE ACOUSTIC MONITORING PERSPECTIVE

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Sciaenidae, commonly called croakers and drummers, are species of high commercial and conservational value. Acoustic communication, typically occurring during spawning aggregations, has been documented in 18 of the 270 existing Sciaenidae species, where sound production is the result of superfast contractions of extrinsic muscles surrounding the swimbladder. In this study, sounds production of the meagre (*Argyrosomus regius*) was monitored before and during spawning in two rearing facilities, in Portugal and in Greece. The meagre was found to emit two types of sounds, i.e. knocks (1 to 3 pulses, recorded only in Portugal) and grunts (more than 3 pulses, recorded in both populations). In both population, grunts were characterised by a higher number of more quickly repeated pulses during spawning. Grunts were made of up to hundreds of pulses but presented higher number of pulses and faster pulse rate in Portugal. These differences could be explained by the effect of water temperature on the neuromotor system driving the contraction rate of sound production muscles, and by the effect of fish size variability. Results are discussed in a framework which takes into account the potential of Passive Acoustic Monitoring to protect wild spawning meagre populations on a European wide scale.

SPATIAL PATTERNS OF THE VOCAL FISH COMMUNITY IN THE SOUNDSCAPE OF THE AZORES

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Fish sounds are an important component of Azorean soundscapes. Passive Acoustic Monitoring is a useful tool to assess biodiversity patterns from an acoustic community.

Here we intended to characterize geographical variations in vocal fishes from Azores and uncover the main driving factors (oceanographic and anthropogenic) that may play an important role in fish acoustic communities. We compared fish sound abundance and diversity in a protected (Formigas, protected since 2003) and a non-protected seamount (Princesa Alice). Fish sound abundance and diversity were compared among four seamounts: Condor (190 m), Gigante (190 m), Princesa Alice (36 m) and Formigas (35 m). We observed a higher abundance and diversity of fish sounds in the shallower seamounts. Formigas presented the highest sound abundance, possible due its low depth and to its protection status. A higher sound abundance was also found at dawn and dusk for Condor and Gigante, and at dusk and night for P. Alice and Formigas. Sound type richness was higher in the shallower seamounts, especially in P. Alice. P. Alice also showed a higher equitability and diversity of the sound types. The deeper seamounts presented similar trends for these biodiversity indices.

THE INFLUENCE OF LIGHT ON FOOD PREFERENCES AND BEHAVIOUR OF THE GREATER WHITE-TOOTHED SHREW, *CROCIDURA RUSSULA*

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Light pollution is one of the consequences of urbanization. To persist in urban areas, animals need to cope with artificial light at night (ALAN). The Greater white-toothed shrew is a very small, nocturnal, insectivore that is common in gardens and parks of urban areas of southern and central Europe. We aimed to assess how this species copes with ALAN under laboratory conditions. We hypothesized that under artificial light, shrews minimize perception of risk by displaying behavioural plasticity and/or changing food preferences. First, we evaluated food preferences (mealworms, fly pupae, minced meat) under dark conditions. Second, we tested the effect of light by offering the preferred food item (mealworms) simultaneously under dark and light conditions. Third, we offered the preferred food item under light conditions and the second preferences (e.g. increase the consumption of less preferred food items). These results are in accordance with reports of increased nocturnality of urban animals and changes of food habits. Future studies should disentangle the consequences of such modifications on the fitness of urban shrews.

THREATS SIGNALS ALTER SURVEILLANCE BEHAVIOR IN EUROPEAN MINK (MUSTELA LUTREOLA)

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Signal of predators and competitors, as well as the existence of human disturbances, can modify the behavioral responses of carnivores such as the European mink. These responses involve decisions that consider the costs and benefits of implementing certain behaviors. This trade-off may be affected in captive animals, due to the absence of threats, restraining some behaviors that would otherwise be instinctively executed in the natural habitat. The objective of this study was to determine the variation in the surveillance rate of 25 European minks, observing their behavioral responses by simulating an intraspecific encounter (visual signal: without and with a mirror), the presence of a potential predator (olfactory signal: owl and dog feces) and an anthropic threat (acoustic signal: traffic noise and community noise caused by human voices). Both sexes decreased the rate in the presence of a mirror as it was a direct visual signal. Likewise, the rate drastically decreased during community noise caused by human voices in both sexes probably due to habituation to roads, but not to a high human presence. The surveillance rate decreased in the presence of predator feces and road traffic noise before the mirror. However, this one was no relevant during the human voices.

ETHOLOGY OF *ARATINGA LEUCOPHTHALMA* STATIUS MULLER, 1776 OF FREE-LIVING, IN PALOTINA, PARANÉ, BRAZIL

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Periquitão-Maracanã, (*Aratinga leucophthalma*), are parrots known to have green color on most of their body, red feathers on the lateral faces of the head, neck and wings. The species occurs in almost all Brazilian territory and part of South America. The objective of this study was to observe the behavioral habit of a group occurring in the city of Palotina - PR, Brazil. This was held near the Palotina Municipal Lake, located in Palotina - PR. A group of approximately 20 individuals was observed. Observations occurred on alternate days, totaling 21 hours of sampling effort. It was used as an aid instrument a camera and a recording containing the vocalization of the species. An ethogram was created to assist in the representation of the collected data, being divided into groups composed by the main activities (flight, foraging, cutting and reproduction, aggression, rest and social). It was observed that during flight or at rest they used vocalization (90% of the time). Flight was their main action (60%), followed by rest (12%) and social interactions between themselves (9%). Few studies are conducted regarding the behavior of these birds, which, due to their wide occurrence, could serve as a fundamental tool for the enrichment of the literature.