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SPE 2012

O 9º Congresso da Sociedade Portuguesa de Etologia tem lugar, este ano, na Faculdade de Ciências da Universidade de Lisboa, nos dias 12 e 13 de Abril de 2012, com organização do Centro de Biologia Ambiental.

Os Congressos Nacionais de Etologia cumprem as funções de reunir e atualizar a crescente atividade de investigação feita em Portugal na área do comportamento animal e de proporcionar um contacto produtivo entre cientistas e alunos.

Reunindo contribuições nas várias vertentes desta área do conhecimento – Fisiologia, Ecologia, Evolução, Psicologia, Neurociências – este Congresso pretende apresentar uma visão integrativa do comportamento animal, em homenagem a Niko Tinbergen e Konrad Lorenz, fundadores desta disciplina científica e dos seus quatro fundamentos básicos – mecanismo, desenvolvimento, função e evolução.

A Sociedade Portuguesa de Etologia é uma associação cultural e científica sem fins lucrativos fundada em 1987 e visa promover o estudo do comportamento animal em Portugal. As atividades da sociedade incluem a publicação da revista científica *Acta Ethologica* (em colaboração com o Instituto Superior de Psicologia Aplicada e a Springer-Verlag), a organização de reuniões científicas, a promoção de relações internacionais com sociedades congéneres e a divulgação da Etologia a nível do ensino secundário.

Agradecemos pela sua participação e desejamos-lhe um excelente Congresso!

A Comissão Organizadora.

PROGRAMA

QUINTA, 12 DE ABRIL

08:30 Coffee and Registration

09:20 Introduction: Organizing Committee SPE 2012

SESSÃO: Endocrine control of behaviour I

Moderador: Paulo Fonseca

09:30 **PLENÁRIA: Eduardo Nuno Barata**

Pheromones and modulation of social behaviour in fishes: origin, mechanisms and functions

10:20 **João Saraiva** Narcissus on fins: searching for dominance pheromones in Tilapia

10:40 **Tina Keller** Dominance pheromone(s) in Mozambique Tilapia males

11:00 Coffee break

SESSÃO: Endocrine control of behaviour II

Moderadora: Clara Amorim

11:20 **Olinda Almeida** Effects of castration on reproductive behaviour in males of the Cichlid fish *Oreochromis mossambicus*

11:40 **Gonçalo Oliveira** Threat appraisal mediates the effect of opponent familiarity on testosterone response

12:00 **Silvia Costa** Endocrine control of female sexual behavior in the Peacock Blenny, *Salarias pavo*

12:20 Lunch

SESSÃO: Messages of colour

Moderadora: Rita Ponce

14:00 **PLENÁRIA: Juan Carlos Senar**

Messages of colour: the evolution of plumage coloration as a multiple ornament

14:50 **Ana Leitão** The role of UV in female mate choice of European Serin

15:10 **Sandra Trigo** Are carotenoids really that important?

15:30 Coffee break

SESSÃO: Associative learning, cognition and social modulation**Moderador: António J. Santos**

- 15:50 **PLENÁRIA: Leonel Garcia-Marques**
Bayesians for the wrong reasons: Hypothesis-testing, contingency perception and associative learning models in humans, animals and machines
- 16:40 **Ligia Monteiro** Social characteristics of low social engagement preschool children
- 17:00 **Inês Peceguina** Explorations of a model of social competence for preschool children: testing the moderating effects of age, sex, and culture
- 17:20 **João Daniel** Preschool affiliative networks and social withdrawal in preschool children
- 17:40 **Joana Bessa** Auditory contagious yawning in domestic dogs (*Canis familiaris*): first evidence for social modulation

18:00 Posters & Wine / SPE Meeting

SEXTA, 13 DE ABRIL**SESSÃO: Brain and behaviour****Moderadora: Leonor Galhardo**

- 09:00 **PLENÁRIA: Susana Lima**
Mate choice in mice
- 09:50 **Catarina Bacelar** Brain areas involved in the navigational process: the role of odors
- 10:10 **Rui Oliveira** Rapid changes in brain transcriptome in response to social interactions in Zebrafish: the role of cognitive appraisal

SESSÃO: Stress and Welfare**Moderador: Paulo Gama Mota**

- 10:30 **Leonor Galhardo** Psychological stress in fish and implications for welfare
- 10:50 **Marta Soares** Tactile stimulation lowers stress in fish

11:10 Coffee break

SESSÃO: Habitat choice**Moderadora: Rita Covas**

- 11:30 **Sara Magalhães** Do plants delude their bodyguards? Behaviour of *Phytoseiulus persimilis* towards odours from plants with or without prey or competitors

- 11:50 Joana Martins Should I stay or should I go? Individual preferences of *Lipophrys pholis* adult males for specific shelters
- 12:10 Ana Gonçalves Homing in rocky intertidal fish. *Lipophrys pholis* L., as a biological model to study navigation

12:30 Lunch

SESSÃO: Behaviour and evolution
Moderadora: Susana Varela

- 14:00 **PLENÁRIA: Étienne Danchin**
Non-genetic inheritance of behaviour

SESSÃO: Sound production, variation and function
Moderador: Gonçalo Cardoso

- 14:50 Paulo Fonseca Sound production in closely related *Okanagana cicadas*: *O. canadensis*, *O. rimosa* and *O. bella*
- 15:10 Clara Amorim The role of agonistic sounds in territorial defence in a small Goby
- 15:30 Daniel Alves Detection of complex conspecific sounds in the Lusitanian toadfish (*Halobatrachus didactylus*)
- 15:50 Ana Mamede The pattern of inter-annual song variation in the Serin (*Serinus serinus*) is limited

16:10 Coffee break

SESSÃO: Deducing behaviour of dinosaurs
Moderadora: Joana Jordão

- 16:30 Octávio Mateus Deducing behaviour of dinosaurs: reproduction
- 16:45 Christophe Hendrickx Deducing behaviour of dinosaurs: Spinosaur theropod feeding
- 16:55 Emanuel Tschopp Deducing behaviour of dinosaurs: Sauropods migration, herding, and feeding

17:05 Questions

18:00 Posters & Coffee

AWARDS: Organizing and Scientific Committees

Award for the best oral presentation from a student (Springer-Verlag);
Award for the best poster from a student (CBA)

19:10 Closing: Rui Oliveira (SPE) & Juan Carlos Senar (Acta Ethologica)

20:30 Social Dinner

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Oradores convidados

Eduardo Nuno Barata (Centro de Ciências do Mar do Algarve e Universidade de Évora)

Eduardo Nuno Barata is Assistant Professor at the Biology Department of the University of Évora and researcher in the comparative molecular and endocrinology group at the Centre of Marine Sciences of Algarve. 'How, for what and why do animals smell what they do' has been the framework guiding his research published in various international scientific journals. Within such framework, his attention has been devoted to chemical communication in fishes and the role of pheromones in the modulation of their social behaviour.

Plenária: **Pheromones and modulation of social behaviour in fishes: origin, mechanisms and functions**

Animals release pheromones conveying chemical information about a sender's identity (e.g. species, sex) and physiology (e.g. reproductive condition). In fish, it is commonly accepted that sex hormones and its metabolites excreted to the water are involved in synchronizing the reproductive behaviour and physiology within a species (hormonal pheromones). Even so, fish may use pheromones beyond such traditional paradigm. In this talk I discuss that the chemical nature of fish pheromones may extend beyond the hormonal pheromones, and that fish have ways to release pheromones when and where appropriate in contexts beyond the chemical communication between sexes.

Étienne Danchin (Laboratory "Évolution et Diversité Biologique", CNRS, University "Paul Sabatier", Toulouse, França)

Dr. Étienne Danchin is head of research at the CNRS (the French National Centre for Scientific Research). He works in Behavioural Ecology and has dedicated almost twenty years of his career to the study of group living, namely to the study of the evolution of coloniality in birds. From his experience with colonial birds, he found out that animals interact and communicate between each other not only through intentional signalling, but also through the observation of several other types of social cues (inadvertent social information) about their conspecifics' reproductive success, which affect the habitat and sexual selection decisions of the observing individuals. It was through this finding that Étienne Danchin became increasingly interested in the subject of animal decision-making through the process of information sharing and animal cultural evolution and on how such a non-genetic transmission/inheritance of behavioural traditions across generations may ultimately influence the evolution of species. Étienne Danchin has published many papers on both the subjects of coloniality and animal cultural evolution and is the co-editor and co-author of the reference book in Behavioural Ecology: Danchin É, Giraldeau L-A & Cézilly F. 2008. Behavioural Ecology. Oxford University Press. Oxford, New York.

Plenária: **Non-genetic inheritance of behaviour**

Many Biologists call for an 'extended evolutionary synthesis' that would 'modernize the modern synthesis' of evolution. Many of these claims are based on recent findings related to non-genetic inheritance. Biological information is typically considered as being transmitted across generations by the DNA sequence (i.e. genes) alone. However, there is accruing evidence that interactions between genetic and non-genetic inheritance can deeply affect evolutionary

outcomes. Epigenetic, parental, ecological and cultural informations are examples of non-genetic mechanisms of inheritance that affect the behaviour of animals across generations, with momentous effects on phenotypic evolution, mostly through development. I will formalise the quantitative dynamics of non-genetic inheritance in order to propose methods that quantify the relative contributions of genetic and non-genetic inheritance on phenotypic resemblance among individuals. I will highlight the role of behaviour on evolution, as it is at the origin of a new inheritance system. It is only by adopting an inclusive view of inheritance, that would explore the multiple– genetic and non-genetic – dimensions of the evolutionary processes, that we will be able to accelerate and achieve the long sought construction of an extended theory of evolution.

Juan Carlos Senar (Evolutionary and Behavioural Ecology Research Unit, Natural History Museum of Barcelona)

Dr. Juan Carlos Senar is head of research at the Natural History Museum of Barcelona and Secretary of the Spanish Ethological Society. He has been working on the evolution of plumage coloration for the last twenty years, mainly focusing on the functional meaning of different colorations and on the evolution of multiple signals.

Plenária: **Messages of colour: the evolution of plumage coloration as a multiple ornament**

Most plumage coloration results either from structural feather properties (e.g. white, blue or UV) or from pigment molecules, mainly carotenoids and melanins, embedded in the feathers (e.g. red, yellow or black). A current debate on the evolution of mate choice through plumage coloration centres on whether different shades of colour convey different kinds of information. In this talk I analyse the different meanings of carotenoids and melanins, in species simultaneously displaying both kinds of colours. Results show that carotenoid coloration is mainly related to the ability of the individual to find out resources meanwhile melanin-based coloration is related to general aggressivity and hence to dominance and territory and nest defence in front of competitors and predators.

Leonel Garcia-Marques (Centro de Investigação em Psicologia, Faculdade de Psicologia da Universidade de Lisboa)

Leonel Garcia-Marques is Full Professor at the Faculty of Psychology of the University of Lisbon. His research interests cover several areas in the domains of Social Cognition (for instance person memory, stereotypes, and spontaneous trait inferences), Memory, and Judgments and Decision Making under Uncertainty. He has published in the top journals of the Social Psychology field (such as the *Journal of Personality and Social Psychology*, *Journal of Experimental Social Psychology*, *Social Cognition*), and he was recently the editor-in-chief of one of these journals (*European Journal of Social Psychology*). He regularly collaborates in projects funded by the Portuguese FCT, often as principal investigator, and he is the steering committee member for Portugal at the *European Social Cognition Network*, funded by *European Science Foundation*.

Plenária: **Bayesians for the wrong reasons: Hypothesis-testing, contingency perception and associative learning models in humans, animals and machines**

An important part of the research in psychology on how humans learn and form knowledge focused on understanding how they test hypotheses. According to the literature on hypothesis-

testing, the individual is either seen as someone with bounded rationality who, using precarious testing procedures, invariably ends up confirming any hypothesis under test (Snyder, 1981), or is seen as someone who adjusts his/her degree of belief in a certain proposition by seeking and relying on crucial observations (i.e., as an intuitive Bayesian; e.g., Trope & Liberman, 1996). However, this literature has ignored the close conceptual relationship between hypothesis-testing and associative learning or contingency perception for a long time. Importing what we already know about contingency perception and associative learning in animals, humans, and machines allows us reinterpreting the results obtained in the hypothesis-testing literature (e.g., Garcia-Marques et al., 2001) as well as predicting effects unknown to the present date.

Susana Lima (Programa de Investigação em Neurociências da Fundação Champalimaud, Instituto Gulbenkian de Ciência)

Susana Lima is Principal Investigator at the Champalimaud Neuroscience Programme from the Champalimaud Foundation, Portugal. She has a diverse background in neuroscience, from *Drosophila* genetics to auditory system in rats. In the last 3 years she has started an independent research path focusing on female sexual behaviour with a particular focus on the study of mate choice.

Plenária: **Mate choice in mice**

Along with finding food and avoiding predators, selecting sexual partners is one of the primary functions of the central nervous system. Choices serve a variety of functions, from avoiding familial inbreeding to avoiding inter-species mating, all of which generally serve the goal of maximizing the fitness of the resulting offspring and thereby providing the best investment of one's genes.

Our goal is to understand the neural mechanisms underlying this fascinating behavior. Much is known at the behavioral and neural level about what kind of features are used for choosing. But much less is known about how the criteria for mating are represented in the brain, how the decision-making process works, how it is influenced by internal state, and other key factors. In order to study those processes it would be ideal to reproduce mate choice in the laboratory under controlled, repeatable conditions. This would allow experiments with electrophysiological recordings and genetic manipulations, for example.

In this lecture I will introduce a new behavioral paradigm we have developed to study mate choice in the house mouse, in laboratory conditions, taking advantage of inbred strains of mice. I will also present some of our first insights into the rules governing this fascinating decision process.

RESUMO DAS COMUNICAÇÕES ORAIS

(POR ORDEM DE APRESENTAÇÃO)

QUINTA, 12 DE ABRIL

Sessão: Endocrine control of behaviour I

Narcissus on fins: searching for dominance pheromones in tilapia

João Saraiva (jsaraiva@ualg.pt), Tina Keller, Peter Hubbard, Eduardo Barata, Adelino Canário

Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Algarve, Portugal

Chemical communication is a widespread phenomenon in the animal world. In teleosts the existence of pheromones is still a matter of debate. In this work we present a research based on aggression towards mirror images that aims to 1) validate the existence of a dominance pheromone in the Mozambique tilapia and 2) raise questions into personality and responsiveness of fish fighting their own image on the mirror.

Experimental males were randomly selected from stock pools and each male went through a gradual process of social isolation (7 days only with females + 7 days in total isolation). On the last day, a partition concealing a mirror was lifted in the isolation tanks and fish were observed interacting with the mirror image. Olfactory stimuli were delivered during mirror exposure only to the fish that reacted to the mirror: a) control water, b) dominant male urine and c) synthetic analogues of two olfactory active steroid isomers found in male urine (see communication by Tina Keller et al). Only 50% of tested males responded to their image on the mirror. Males stimulated with dominant male urine seem to attack less than control males. Surprisingly, males stimulated with the synthetic compound seem to escalate more in aggressive behaviours towards the mirror than control males. While the question why 50% of males do not respond to the mirror remains open for debate, we propose that the complete pheromonal message should not come only from these two steroid isomers, but instead possibly from an array of signals in male urine.

Dominance pheromone(s) in Mozambique Tilapia males

Tina Keller (tinakeller.tk@googlemail.com)^{1,3}, Peter C. Hubbard¹, Christian Paetz², Yoko Nakamura², Bernd Schneider², Eduardo N. Barata^{3,1}, Adelino V. M. Canário¹

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²*Max Planck Institute for Chemical Ecology, Jena, Germany*

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Mozambique tilapia (*Oreochromis mossambicus*) males establish dominance hierarchies and females mate preferentially with territorial dominant males. Urine signals mediate aggressive interactions between tilapia males; dominant males increase urination frequency during aggressive disputes and courtship. The olfactory system of both sexes is highly sensitive to dominant male urine. Bioassay-guided fractionation of male urine using HPLC and recording of the electro-olfactogram (EOG) revealed one urinary fraction with strong concentration-dependent olfactory activity. The objective of the current study was to identify this putative dominance pheromone(s) present in this fraction and to assess its olfactory potency using synthetic analogues.

Mass spectrometry analysis of the most active urinary fraction unveiled two compounds of identical molecular weight and sum formula [C₂₇H₄₄O₉]. Using NMR spectroscopy, we identified

them as two isomeric steroid glucuronides, differing from each other only by the stereochemistry of one hydroxyl group.

The olfactory system of both sexes was sensitive to synthetic analogues of both glucuronidated steroids, but not the unconjugated forms. Stimulation of the olfactory epithelium with increasing concentrations of the two glucuronides (10^{-10} M to 10^{-5} M) produced well-defined sigmoidal concentration-response curves. Cross-adaptation suggested that both steroids share the same olfactory receptor(s) but with different affinities, as one of the two steroids has a significantly lower apparent EC_{50} . In conclusion, the Mozambique tilapia uses at least urinary steroid glucuronides as chemical signals to advertise social status. Currently, we are investigating the behavioural effects of these steroids and other urinary fractions (see communication by João Saraiva et al).

Sessão: Endocrine control of behaviour II

Effects of castration on reproductive behaviour in males of the cichlid fish *Oreochromis mossambicus*

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Gonads have been shown to be a major source of sex steroids and pheromones that are directly related to reproductive behaviour. In the Mozambique tilapia territorial males have higher levels of circulating androgens, express reproductive behaviour and use a urine-borne pheromone to signal their status towards conspecifics. Here we investigated the effects of gonadectomy on the circulating sex steroid levels, and expression of territorial and reproductive behavior (nest building, courtship behavior, nuptial coloration, and territorial behaviour. Castrated, sham and urine bladder damaged males (this treatment was included because the castration procedure damages the urine bladder) were visually exposed to a group of females (during 8 days) and to a neighbor male (day 9). According to our predictions gonadectomy lowers dramatically the circulating levels of androgens measured 4, 8 and 9 days post-castration and abolishes the expression of nest building, courtship behaviour and nuptial coloration but has no effect on the expression of aggressive behavior. These results demonstrate that gonads are effectively the main source of androgens in this species and are essential for the expression of reproductive behaviours. The expression of aggressive behavior seems to be decoupled from gonads suggesting the action of independent central mechanisms.

Threat appraisal mediates the effect of opponent familiarity on testosterone response

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It has been proposed that the testosterone (T) changes found after a competition are not simply related to the outcome, but could result from the interference of psychological variables. With this experiment we aim to test the role of familiarity and appraisal on the T response expected after a competition. Participants competed in pairs, against a same sex opponent using the Number Tracking Test as a competitive task. Pair ratings for opponent familiarity were equally

distributed across both sexes. Losers rated the task as more threatening than winners. Females in the loser condition, 20 minutes after the end of the competition, display higher levels of T than female winners. No T differences were found for men in both conditions. Cortisol increased throughout the experiment. No differences were found for DHEA. Threat, familiarity of the opponent and T response were associated only in the female loser group. Mediation analysis suggests that for female losers the appraisal of the competition as threat completely mediates the effect of opponent familiarity on T.

Endocrine control of female sexual behavior in the peacock blenny, *Salaria pavo*

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In fish, the endocrine regulation of female reproductive behavior has been hypothesized to be determined by the mode of reproduction employed. Sex steroids have been implicated in the regulation of female sexual behavior in internal fertilizers while in external fertilizers this role has been attributed to prostaglandins. However, the functional roles of both sex steroids and prostaglandins have not been studied in the same species, nor this has been studied in species where females assume a more active role during the breeding episodes, presenting elaborate and stereotyped courtship displays. In a sex-role reversed population of peacock blenny, *Salaria pavo*, females present elaborate sexual displays. In this study we tested the effect of the gonadal hormones and of the prostaglandin, PGF2 α , on the sexual behavior of the peacock blenny females. After two weeks, ovariectomized females presented a reduction of both the frequency of courtship displays and the time spent in nuptial coloration, when compared to sham-operated females, but not a complete abolishment of reproductive behaviors. Estradiol had a modest effect on the recovery of nuptial coloration and courtship displays. Courtship levels in ovariectomized females injected with PGF2 α did not differ from sham females. The results suggest that the regulation of brain circuits underlying the expression of female sexual behaviors is at best modestly influenced by gonadal steroids and that PGF2 α is a good candidate for synchronizing oocyte maturation with sexual behavior. Nevertheless, the significant percentage of ovariectomized females still exhibiting courtship behavior two weeks after ovariectomy suggests that other molecular partners might be involved in the expression of female sexual behavior in fishes.

Sessão: Messages of colour

The role of uv in female mate choice of European Serin

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Conspicuous ornamental colouration exhibited by males of many bird species are one of the most common signals of sexual selection. The expression of colourful plumages is in many species honest signals of individual quality, since its development is generally costly and condition dependent. Recently, avian colour vision and colouration has received a great attention, particularly the significance of Ultraviolet (UV) plumage that most birds can perceive. This capacity suggests that sexual selection operates also in these wavelengths.

In this study, we sought to understand whether visible and ultraviolet spectrums of males' plumage are sexually selected by female choice. The species in study, the serin (*Serinus serinus*) is sexually dichromatic, with males showing a more intense yellow plumage with a peak in the ultraviolet region, typical of carotenoid pigmentation plumage. We conducted two rounds of mate choice tests: one to test female preference for yellow colour saturation and a second to test the importance of UV-coloration by applying an UV-blocking chemical directly in the feathers of the more colourful male.

We confirmed that the yellow carotenoid colouration is sexually selected, as females were more responsive to it. However when we blocked the UV wavelength of males, females stopped exhibiting any preference. Either males lose their attractiveness in the absence of UV or, yellow colouration corresponds to a combination of yellow and UV, and in the absence of the UV, yellow is perceived as another colour.

Are carotenoids really that important?

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In birds, carotenoid-based coloration is one of the most important sexual signals. An immunostimulatory and antioxidant function has been attributed to carotenoids. However, in recent years these functions have been questioned. Here, we experimentally manipulated carotenoid intake and measured the effects on the carotenoid-based plumage coloration, immune system, blood carotenoid levels, physical condition and female mate choice. Males with carotenoid supplement had higher levels of blood carotenoids, higher immune response (PHA-P and SRBC immune challenges) and were brighter than males with no supplement. These results confirm the hypothesis that plumage coloration can function as quality signals.

Sessão: Associative learning, cognition and social modulation

Social Characteristics of Low Social Engagement Preschool Children

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Peer interactions and relationships play critical roles in becoming socially competent and socially anxious or withdrawn children tend to be characterized as less socially competent than more engaged peers and are at risk for internalizing problem behaviors over time. Although most studies of social anxiety/withdrawal have focused on school age children, similar challenges to the development of social competence are faced by preschoolers. This study examined the social competence and temperament/personality outcomes associated with low rates of peer engagement for preschoolers.

The participants were 425 children between 3 and five years old. As part of the assessment battery, observations of initiated interactions and receiving visual attention from peers were collected (with low scores indicating low levels of engagement). Additional measures included Q-sort observations (2 Q-sets, 172 items) and sociometric acceptance and dislike interviews. Low engagement was negatively associated with sociometric peer acceptance (but not with disliking), ego-undercontrol and self-esteem (scored from the Q-sorts). Low engaged children had fewer reciprocated friendships (i.e., being chosen by peers whom they had chosen themselves as "liked") and displayed lower levels of positive affect in the classroom. Additional analyses of the Q-data revealed that many individual items (67 to 40 across the three samples) had significant

correlations with low social engagement. Furthermore, the majority of significant correlates were reproduced in all four samples. Positive item correlates of low engagement included items concerning inhibition, social distance, and anxiety. These findings suggest that low social engagement is a valid indicator of social anxiety and social withdrawal for preschool children.

Explorations of a Model of Social Competence for Preschool Children: Testing the Moderating Effects of Age, Sex, and Culture

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There is a widespread consensus in the developmental sciences to the effect that social competence is a critically important attribute (or attainment) of preschool children because social competence is presumed to underlie later interpersonal adjustment and school readiness. The primary goal of this study was to examine further the assumptions and conclusions reached in an earlier structural analysis of the social competence construct for preschool age children. Two equal size samples of children (total N= 446; one from Portugal, one from US samples), approximately matched for age and sex proportions, were included in the analyses.

Assessments included direct observations of interactions (e. g. frequencies, rates of social exchange) that also included a judgment of the hedonic tone of the interactions (based on expressed affect by one or both interaction partners). Additional observations were made concerning the distribution of visual attention to peers and receiving visual regard from peers. Another team of observers provided Q-sort descriptions of the children, based on 20+ hours of observation in each classroom. A third team conducted sociometric interviews of individual children. Exploratory and confirmatory structural analyses were also computed. Results showed few mean or pattern differences across sample, age, and sex breakdowns. Exploratory factor analyses revealed that a three-factor structure was a good fit for the observed data. Confirmatory factor analyses showed that the hypothesized structure of the social competence construct fit the data and was invariant across sample and age in both its measurement and structural aspects. The structural model was not invariant across sample and sex. These findings both replicate and extend results reported in the original study.

Preschool affiliative networks and social withdrawal in preschool children

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Social withdrawal is defined as the child isolating him/herself from the peer group through the consistent (across situations and over time) display of solitary behavior in the presence of familiar and/or unfamiliar peers (Rubin, 1982; Rubin & Asendorpf, 1993). Our Research demonstrates how the individual focused and group focused traditions of research and explanation may be bridged (Santos & Strayer, 1997; Santos, Vaughn, & Bonnet, 2001; Santos & Winegar, 1999; Santos, Vaughn, Daniel & Bost, 2008). The main objective of the present study is to compare subgroups in terms of Social withdrawal. N=316 children; 162 girls; 154 boys. Children were recruited from Portuguese preschool programs and observed as 3, 4-year-olds and 5-year-olds. Frequency of association was obtained using nearest neighbor techniques. Sequential focal sampling of individual children was conducted for each classroom group. A minimum of 200

observational rounds per class were conducted. Dyadic co-occurrence matrices were then tabulated and analyzed through complete linkage clustering algorithms. An arbitrary level of within-cluster similarity (i.e., average within-cluster correlation coefficient at the conventional level of significance, $p < .05$) was first chosen to identify Subgroups vs. Ungrouped cases. In order to verify if children included in the same subgroup also selectively associated with co-members, chi-square analyses were used to examine the relative density of association among subgroup members. Results permitted distinguishing children in “low mutual proximity” (LMP) subgroups from members of “high mutual proximity” (HMP) subgroups in terms of selective association between co-members. Children in the low mutual proximity groups revealed higher values of Social withdrawal.

Auditory contagious yawning in domestic dogs (*Canis familiaris*): first evidence for social modulation

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Dogs' capacity to catch human yawns has recently attracted the attention of researchers in the field of animal cognition. In light of recent studies suggesting that contagion yawning in humans (and some other primates) is empathy related, some authors have considered the possibility that the same mechanism may underlie contagious yawning in dogs. To date, however, no positive evidence has been found and more parsimonious hypotheses have been put forward.

The present study aimed at exploring the so-called ‘contagion only’ hypothesis. Twenty nine dogs of different breeds and ages were tested in order to determine whether a mere sound of a human yawn could be sufficient to elicit yawning in domestic dogs (*Canis familiaris*), in a way that is unaffected by social-emotional factors.

Surprisingly, results showed an interplay between contagion and social effects. Not only dogs were found to catch human yawns by hear, but also they were found to yawn more at familiar than unfamiliar yawns. Although not allowing for conclusive inferences about the mechanisms underlying contagious yawning in dogs, this study provides first data that renders plausible empathy-based, emotionally connected, contagious yawning in these animals. Considering the increasing involvement of dogs with human activities, assessing contagion yawning might be a useful complementary tool for selecting the most appropriate dogs for specific tasks. Future research is needed.

SEXTA, 13 DE ABRIL

Sessão: Brain and behaviour

Brain areas involved in the navigational process: the role of odors

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Atmospheric trace gases (odors) have been proposed as the main source of navigational information used by vertebrates during the navigational process (Navigational hypothesis). However, recent behavioral studies have shown that odors rather than provide navigational information, they may activate brain areas involved in the navigational process (Activation hypothesis). In the present research we used young homing pigeons (well known by relying on route based information) and manipulated the access of birds to olfactory information during the

displacement to the release site (route information). A natural air group was exposed to natural air from the outward journey, a synthetic air group was exposed to a sequence of artificial odors introduced into filtered air (i.e. natural air deprived of natural odors by means of active charcoal filters), and a no odors group exposed to filtered air. Moreover to ensure that the only olfactory information processed was just that given during the displacement, after arriving at the release site all birds were made anosmic. Birds were released and after arriving at the home loft pigeons were euthanized and brains processed according to an Immunocytochemical protocol (e.g. c-fos). Brain areas like the olfactory bulbs, the piriform cortex, the hippocampal area and the cerebellum are being searched for neuronal activation in order to provide definitive evidence or not of an olfactory activation of brain regions typically involved in process navigational information. An activation role of odors may have many other implications such as in the onset of some human neurodegenerative diseases.

Rapid changes in brain transcriptome in response to social interactions in zebrafish: the role of cognitive appraisal

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In social living species individuals have to adjust their behavior according to previous social experience and to social context. This behavioural flexibility depends on neural plasticity of the circuits underlying social behavior, which is hypothesized to be achieved by social regulation of gene expression in the brain. Here we will present data on social regulation of gene expression in zebrafish that shows how short-term behavioral interactions (staged agonistic interaction) have a significant impact on the brain transcriptome. We have also tested the hypothesis that the response to a social interaction involves cognitive appraisal of social stimuli. For this purpose a group of individuals that fight their own image on a mirror was also studied, since mirror fighters do not experience winning or losing despite expressing aggressive behavior. Cluster analysis of changes in gene expression correctly grouped all the individuals according to their social experience (i.e. winners, losers, mirror fighters and controls). An analysis of differentially expressed genes using the control group as reference identified a set of genes differentially expressed in males fighting a real opponent but none were detected in mirror fighters. These results suggest that it is not the objective structure of the event that triggers a genomic response to social interactions but rather the appraisal that the individual makes of the event. For males fighting a real intruder losing had a higher impact on changes in gene expression than winning suggesting that it has a higher salience to the individual, and a stronger impact on biological processes.

Sessão: Stress and Welfare

Psychological stress in fish and implications for welfare

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In fish, the physiological and behavioural aspects of stress are considerably well known phenomena and show striking similarities to those of other vertebrates. However, the psychological component is not well known. Some authors deny mental experiences to fish on the basis of their lack of neocortex. Nevertheless, recent studies have shown neuroendocrine, cognitive and emotional processes in fish that are not only equivalent to other vertebrates, but also allow inferring some forms of mental representation. The integration of psychological

elements in fish stress physiology is insufficiently studied, but there is already indirect evidence to admit that some form of stimuli appraisal can take place in fish. This fact has profound implications on the regulation of the stress response, as well as on fish welfare and its management. An adjusted level of environmental complexity, control and predictability, with the concomitant learning opportunities, are very important factors to keep both the mental and physical mechanisms within the boundaries of an acceptable allostatic load. These are likely to be among the most important components to contribute for the animals' positive mental attitude towards their environment and hence to their good welfare.

Tactile stimulation lowers stress in fish

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In humans, physical stimulation, such as massage therapy, reduces stress and has demonstrable health benefits. Grooming in primates may have similar effects but it remains unclear whether the positive effects are due to physical contact or to its social value. Here we show that physical stimulation reduces stress in a coral reef fish, the surgeonfish *Ctenochaetus striatus*. These fish regularly visit cleaner wrasses *Labroides dimidiatus* to have ectoparasites removed. The cleanerfish influences client decisions by physically touching the surgeonfish with its pectoral and pelvic fins, a behaviour known as tactile stimulation. We simulated this behaviour by exposing surgeonfish to mechanically moving cleanerfish models. Surgeonfish had significantly lower levels of cortisol when stimulated by moving models compared with controls with access to stationary models. Our results show that physical contact alone, without a social aspect, is enough to produce fitness-enhancing benefits, a situation so far only demonstrated in humans.

Sessão: Habitat choice

Do plants delude their bodyguards? Behaviour of *Phytoseiulus persimilis* towards odours from plants with or without prey or competitors

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Predators of herbivores benefit from rapidly finding patches with prey. For plants, however, having predators before prey arrival will minimize future herbivore damage. Here, we studied the olfactory response of the predatory mite *Phytoseiulus persimilis* to volatiles of plants (cucumber, bean or rose) that were either clean (i.e., empty), occupied by their prey (*Tetranychus urticae*) or by their conspecific competitors. We found that, relative to clean air, predators were attracted to clean plants, and also to plants with prey. On cucumber and bean, predators preferred plants with prey over clean plants. This was not the case on rose plants. This is clearly to the benefit of plants, but not of predators. However, after 24 hours of experience with rose plants, predators preferred rose infested with *T. urticae* to clean rose, suggesting that the plant benefit is limited to the early stages of the interaction. Predators avoided all plants with prey and conspecifics, even

without experience, suggesting that this discrimination involves other cues. Attracting predators to clean plants may favour biological control, as plants may become better guarded from herbivores, but it may also be detrimental, as predators may starve on those plants.

Should I stay or should I go? Individual preferences of *Lipophrys pholis* adult males for specific shelters

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Lipophrys pholis is the most common intertidal fish in the Portuguese rocky shores. The males of this blenny breed in vertical walls inside holes or crevices from December to May. During this period, males take care of the developing eggs remaining in their nests even during the low tide when both adults and eggs are emersed. Fish have been followed during low tide for a year in Cabo Raso, Portugal. In order to study the individual preferences of adult males for specific shelters, electronic tags were surgically inserted in more than 50 individuals (total length > 10 cm). The observation of new individuals in the intertidal area was observed with the onset of the reproductive season which may agree with previous reports that adult *L. pholis* are subject to large displacements with the tide. Contrarily to expectations, the rate of recapture was surprisingly high, suggesting that the number of adults in this population is relatively constant at each site even along consecutive reproductive seasons. Although other authors have showed that pool communities are highly resilient, our findings showed that adult males are highly attached to a specific area year around. In constant changing environments, like the intertidal zone, staying in well known areas can be highly adaptive allowing individuals to minimize risks of predation or dehydration. Conversely, the availability of holes and crevices can be scarce and regular visits of specific shelters year around could enhance the reproductive success in the following breeding season.

Homing in rocky intertidal fish. *Lipophrys pholis* L., as a biological model to study navigation

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In the intertidal zone, fishes from the family blenniidae are mentioned to have a good geographic position sense. The morphological characteristics and behavioral adaptations of *Lipophrys pholis* suggest that this species is an outstanding biological model to study homing behavior. In the present study we developed a new assay to experimentally test the navigational abilities of *L. pholis*. Individuals were captured and tested *in loco*, in our assay, at two different locations in the west coast of Portugal. Three experiments were performed with a total of 87 individuals. In experiment 1 and 2 ontogenetic differences (i.e., Immatures vs. Adults) and sex differences (i.e., Males vs. Females) were tested, respectively. In experiment 3 true homing abilities of adult *L. pholis* individuals were tested. The findings showed that motivation for homing is just exhibited by adult fishes independently of their sexes. In experiment 3, the translocated group (i.e. the group was removed from their original place and tested at an unfamiliar place) showed consistent orientation that coincided with the expected home direction. Altogether, these findings provided robust evidence that adult fish displayed homing behavior and that they are able to perform true homing navigation, which in turn points to *L. pholis* as an excellent biological model to clarify which cues are used during the navigational process.

Sessão: Sound production, variation and function

Sound production in closely related *Okanagana* cicadas: *O. canadensis*, *O. rimosa* and *O. bella*

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Mate attraction in singing cicadas is usually mediated by a stereotyped male calling song. The basic song pattern is produced with powerful timbal muscle contractions that apply the force to the timbal plate through an elastic ligament, the timbal apodeme. The *Okanagana* species in this study produce a series of sound pulses, one per rib buckled, during timbal muscle contraction, while the outward timbal recovery is essentially silent. Tensor muscle contraction, responsible by strong song amplitude modulations in other species, appears to have a minor effect in these species, while the extension and vertical abdominal movements have a marked effect on sound output. Simultaneous monitoring of the activity of the two timbal motoneurons during singing, elicited as an after effect of brain electrical stimulation, revealed a bilateral alternating stable pattern of timbal muscle contraction in both *O. rimosa* and *O. bella*, but a surprising variability in *O. canadensis*. In the latter, single or doublet timbal motoneuron action potentials may present modifications in their bilateral time pattern suggesting a certain degree of bilateral independence of the central pattern generation (CPG) network. Moreover, and as a response to self-produced sound, we observed an activity in the auditory nerve with a too short latency to be attributed to the response of sound receptors, as reported years ago by Franz Huber and collaborators. We also recorded a strong response to the almost silent sound generated during the outward timbal release. This sensory feedback may play a role in CPG functioning.

The role of agonistic sounds in territorial defence in a small goby

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Many fish species emit sounds in agonistic contexts including during territorial defence but experimental evidence demonstrating the role of sounds in avoiding fight escalation or winning disputes is scarce. Here, we studied sound production during territorial defence in captive painted gobies, *Pomatoschistus pictus*, and related acoustic parameters with male traits. Territorial males emitted drumming sounds during displays that involved darkening the chin and fins, spreading fins and quivering the body. Drums were trains of low frequency pulses (≈ 23 pulses) repeated every 27 ms, usually lasting less than a second. Drums were produced in bursts (sequences of sounds). All acoustic parameters differed significantly among males. Drum and burst duration, and drum number of pulses increased significantly with male size. We further tested the role of drums (with no visual signals presented) as a keep-out signal during nest defence with playback experiments where drums or a control (white noise or silence) were played back upon an approach of an intruder male to a nest. Nest avoidance was significantly higher when drums were played-back than during control playback, but nest occupation was similar regardless of the playback treatment. Our results suggest that in painted gobies agonistic sounds

may contain information that can be used during mutual assessment in contests over territories. Further, acoustic signals seem to be important to initially deter intruders to enter a male's nest but the actual presence of a nest-holder is needed.

Detection of complex conspecific sounds in the Lusitanian toadfish (*Halobatrachus didactylus*)

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Many fishes use acoustic signals to communicate and it is believed that the temporal structure of their sounds is the main information carrier, but little is known about the minimum detectable pulse interval, an important parameter in communication. Moreover, males of many fish species emit mate attraction sounds in choruses, imposing a challenge for females to distinguish the call of one male within the chorus. Consequently, the auditory system of fish should, be able to resolve the temporal structure of vocal communication signals, and to detect sounds in a biologically noisy environment. We have two objectives: 1) to describe hearing temporal resolution in the Lusitanian toadfish and 2) to determine the minimum sound amplitude difference allowing the detection of a mate attraction call over a chorus. Auditory evoked potentials were measured to characterize the fish response. Our results, using a gap detection paradigm, suggest that the toadfish may detect silence gaps as short as 0.5 ms but a correct representation of a natural sound's temporal structure is only possible if the pulse period is longer than 4 ms. Moreover, a mating call with an amplitude 1-2 dB above the background chorus appears to be well represented within the auditory response. The Lusitanian toadfish vocalizations are relatively complex and some acoustic features may be key in social interactions. Therefore, the evaluation of the auditory temporal resolution and details of signal representation in the auditory system may give hints on how a highly vocal fish obtains biologically-relevant information from the acoustic environment.

The pattern of inter-annual song variation in the Serin (*Serinus serinus*) is limited

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The patterns of age-related changes in singing behaviour after the first year in passerine birds are poorly studied in comparison to song learning during the first year. In general, bird species were divided into two categories of open-ended learners and closed-ended learners, depending on whether songs continue to be modified after the first year or not. The degree and type of age-related modifications in song raise interesting developmental and evolutionary questions. Serins sing very complex songs with large repertoires but delivered in a very rigid way with little structural modification, but to date it was not known how modifiable is Serin's song with age. Here we recorded male Serins for two years and show that there is a very limited variation between years, with just about 8% of new syllables included in the repertoire. Most new syllables replaced older ones in the same locations within the song. We identified for the first time new syllables that resulted from fusion or splitting of already existing ones. We also show that syllables were produced with great consistency. Sequences of syllables were very stable between years, revealing that adult serin song has very limited plasticity after the first year which may be a consequence of selection for greater performance.

Sessão: Deducing behaviour of dinosaurs

Deducing behaviour of dinosaurs: reproduction

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Although all non-avian dinosaurs are extinct, their reproductive evolutionary strategies can be deduced from the extant phylogenetic brackets (crocodiles and birds) and from the fossil record, in which nest, eggs, and embryos, are often preserved. But dinosaurs are very diverse in size, metabolism, ecological niches, and biological strategies, therefore also their reproductive behaviour also varies extensively.

The mating strategies of extinct dinosaurs are almost unknown, but conspicuous and apparently restrictive crests, frills, feathers, and sails are common in archosaurs which suggest the use for sexual display. Fossilized nests show different structures: linear dug grooves (in sauropods?), paired nesting (probably due paired oviduct; in oviraptorid theropods), circular eggs arrangement (in sauropods), and common nesting (as in *Lourinhanosaurus*).

The nest attributed to the Portuguese theropod *Lourinhanosaurus antunesi* shows about 100 eggs, which suggest a communitary nesting. In this example, three eggs attributed to crocodylians were found in the nest of Paimogo, Lourinhã, which could be a case of commensalism, in which the crocodylian would benefit the protection, but would be neutral for the theropod dinosaur. The presence of gastroliths and an adult theropod tooth suggests that adults spent time near the nest before hatching. No well-defined nest structure is visible for *Lourinhanosaurus* theropod dinosaurs.

Deducing behaviour of dinosaurs: spinosaur theropod feeding

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Spinosauridae encompass the largest terrestrial predators that ever existed. They were highly specialized bipedal dinosaurs having elongated crocodile-like skulls, conical teeth and a hypertrophied manual ungual.

Investigation on behaviour and parental care in these dinosaurs is highly problematic due to the scarcity of fossils and absence of eggs and juvenile specimens, but feeding and ecological behaviours are relatively well-known and revealed that members of this group were unusual dinosaurs.

Oxygen isotopic composition on spinosaurid material suggests that these predators had semi-aquatic lifestyles, spending a large part of their daily time in water, like extant crocodylians or hippopotamuses. Finite Element Analysis and morphological analysis of the skull support an ichthyophagous diet, but stomach contents and other direct evidences demonstrate that spinosaurids had a very broadly based diet, feeding on fish, but also on small herbivorous dinosaurs and pterosaurs. Morphofunctional analyses of the jaw articulation revealed that derived spinosaurids were able to swallow large preys such as living pelicans, and the recent discovery of a neurovascular cavity within the cranium of these spinosaurids seem to suggest the presence of pressure-receptors at the extremity of the snout, giving a tactile function, useful to catch swimming preys without relying on sight.

Spinosaurids possessed elongated neural spines sometimes forming a large bony sail on the back, and this structure has been interpreted either as a dorsal crest for thermoregulation and display or a hump of a quadrupedal animal capable of long-distance pursuit.

Deducting behaviour of dinosaurs: Sauropods migration, herding, and feeding

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Sauropods are among the most enigmatic dinosaurs. Their particular body plan minimizes the use of extant animals as behavioural models. However, recent studies revealed significant components of sauropod behaviour. Oxygen isotope analyses of sauropod teeth reveal that sauropod dinosaurs migrated over long distances on the search for food. Parallel trackways indicate herding, and similar size of the footprints, or of skeletons found at the same site imply age segregation in some sauropod taxa. On the contrary, the brachiosaur-like *Europasaurus holgeri* is known from a herd with 18 individuals of four well-defined size classes (1.8 m long juveniles to 7 m long adults), suggesting multi-age herding. The discrete size-classes, rather than continuous growth, indicates individuals of different years of age, and thus a well-defined reproduction season.

Computer analyses of distal caudal vertebrae in sauropods with so called whiplash tails (e.g. *Diplodocus*), or tail clubs (e.g. *Shunosaurus*), allow the modeling of their movement, and an evaluation of their utility as weapons.

A lack of molar-like teeth in sauropods eliminates the time needed for mastication, and in some taxa, gastroliths aided the digestion, forming a gastral mill as in some extant birds. In Portugal, e.g., gastroliths were found in more than 80% of the sauropod specimens preserving significant dorsal portions.

Different snout shapes and semi-circular canals of diplodocoid and camarasauromorph sauropods indicate varying feeding strategies: square-snouted diplodocoids like *Nigersaurus* probably grazed large areas of low-level vegetation. Camarasauromorphs had more rounded snouts, implying a more selective feeding strategy than diplodocoids.

RESUMO DAS COMUNICAÇÕES EM POSTER

Tema: Brain and behaviour

[P1] Identifying neuromuscular abnormalities in RhoBTB3 deficient mice using the SHIRPA protocol

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RhoBTB3, an atypical RhoGTPase of unknown function, is highly expressed in the central nervous system. RhoGTPases are ubiquitous and essential binary molecular switches that regulate cellular responses to a variety of extracellular stimuli, including activation of integrin and growth factor receptors, or chemotactic stimulation, among many others. The generation of genetically modified laboratory mice allows to investigate the roles of such molecules at organism level. Using the SHIRPA protocol (SmithKline Beecham, Harwell, Imperial College and Royal London Hospital Phenotype Assessment) we have started to characterize potential neuromuscular and sensorial phenotypes present in RhoBTB3 knockout mice. Tests will be carried out in mice with three different genotypes: homozygous (KO) mice (-/-), heterozygous mice (+/-) and wild-type (WT) mice (+/+), at three developmental stages: P30days (d) to P40d, P90d to P100d and at P180d. Preliminary results obtained from P30-P40d mice show that KO mice have a significant reduction in body weight compared to the two other genotypes (WT and Heterozygous mice); decreased response in touch escape compared to WT mice, and accelerated heart rate compared to heterozygous mice. In addition, limb grasping capacity is totally absent in both KO and heterozygous mice. Future experiments will now characterize the phenotypes at later developmental stages.

[P2] Social modulation of adult neurogenesis in the neural network underlying aggressive behavior in zebrafish

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In social systems, animals adjust their social behaviour according to available information in the social environment. The “core” neural circuits underlying social behaviour are composed of a network of forebrain and midbrain nuclei with reciprocal connections, the social behaviour network (SBN). This brain network is differentially activated with singular behaviours leading to distinct patterns of response across the nodes, which ultimately lead to behavioral plasticity. Recent evidence suggests that adult neurogenesis is a driving force for this plasticity and that social interactions can change adult neurogenesis in vertebrates and invertebrates species. For example exposure to an acute psychosocial stressor decreased number of newly generated cells in the hippocampus. In the present work we investigate the influence of social experience in the SBN and how changes in SBN can modulate the expression of different behaviors, with particular focus on adult neurogenesis. For this purpose, we studied agonistic interactions to establish social dominance in zebrafish. A simple protocol was used with pairs of males where animals could experience winning the interaction, losing the interaction, have an unsolved interaction or experience no interaction. In a first set of experiments, each node of the SBN was microdissected

and the expression of several neurogenic genes, including Wnt3, BDNF, and NeuroD1 were characterized in the different behavioral conditions. In a second set of experiments animals were injected with BrdU immediately after the interaction in order to characterize proliferation and short-term survival of the newborn cells in the same nuclei (SBN). For short-term survival BrdU immunohistochemistry was also combined with immunolabeling against DXC and high-throughput confocal stereology was used to determine the number of new born cells and the number of cells undergoing neuronal commitment. This study will establish the quantitative response of neuronal plasticity in the SBN network to social changes. Data from the gene expression study will help establish the important modulators of neuronal recruitment in response to social interactions in zebrafish.

[P3] What drives sociability in zebrafish? Neuronal mechanisms underlying shoaling behavior

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Social species continuously adjust their behavior as a function of the available social information. This suggests that specific evaluation mechanisms may have evolved to allow appropriate behavioral and affective responses to social stimuli.

It is our goal to investigate these mechanisms. In particular we are using zebrafish (*Danio rerio*) to explore the neuronal mechanisms driving and influencing shoaling behavior (grouping of fish). We hypothesize that shoaling with conspecifics has rewarding properties to zebrafish and is modulated by specific neuronal systems. To this end we are currently establishing a conditioned place preference paradigm, using the shoaling behavior as the unconditioned stimulus (that evokes a natural response) and we are exploring the relevance of two different sensory pathways (visual and olfactory) to this preference. These results will serve as the starting point to explore this hypothesis both at the behavioral and neuronal levels. In the future we will investigate if the drive to shoal is influenced by specific monoamine and nonapeptide systems. We will also address to what extent it is hardwired and modulated from early stages of development by the systems previously explored.

[P4] Neuropeptide regulation of interspecific cooperative behaviour in cleaner fish

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Mutualistic interactions are particular social interactions where individuals of different species cooperate in order to gain benefits. A textbook example of mutualism is provided by the Bluestreak cleaner wrasse (*Labroides dimidiatus*) which feeds on so called clients' ectoparasites, mucus, scales and dead or infected tissue. Although much is known about the ultimate functions of cooperative behaviour, studies on their underlying proximate mechanisms are scarce. The nonapeptides arginine vasotocin (AVT) and isotocin (IT) and mammalian homologues are well known for their role in the modulation of several social behaviours. Surprisingly, their effect on cooperative behaviour has remained largely unexplored. We investigated the effects of a single AVT, Manning (an AVT-V1a receptor antagonist), IT or saline injection on female cleaner wrasses

in their social interactions with clients and conspecifics. AVT decreased the cleaners' likelihood to interact with clients whereas Manning compound had the opposite effect. In contrast, AVT increased the frequency of conspecific social interactions, such as paired close swimming events and received tactile stimulation events. Both AVT and Manning decreased the quality of the service provided to clients. Our results demonstrate that AVT pathways may play a differential key role in the regulation of mutualistic and conspecific social interactions. Furthermore we hypothesize that this neuropeptide has been co-opted in the evolution cooperative behaviour in an interspecific context.

[P5] Female sexual behavior: neuronal pathways for arousal termination

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Similar to other motivated behaviors, sexual arousal has a beginning and an end during the normal flow of sexual behavior which is critically influenced by the central nervous system. Although in males ejaculation leads to termination of arousal, in females this process is much less clear.

Our hypothesis is that female sexual arousal is controlled by a neuronal system that integrates sensory stimulation until an internal threshold is reached, ending sexual drive and initiating a refractory period.

To unravel the mechanisms that trigger the refractory period, we first need to understand and describe accurately mouse sexual behavior. To do so we have developed a paced mating paradigm where, as in nature, females can control their interaction with males and the amount of sexual stimulation they receive. We are currently using wild derived mouse strains (PWD/K) that display a much complex mating behavior when compared with the classical C57BL/6 laboratory strain.

Secondly, in order to identify which brain areas receive genital sensory input we are using pseudorabies virus as a neuronal retrograde trans-synaptic marker. Virus injection in the vagina or the uterus allowed us to identify several brain areas including Medial preoptic area (MPOA) and Paraventricular nucleus (PVN), described as being related to sexual arousal and consummatory events. To determine if these brain areas are reciprocally connected to the genital organs, we are currently injecting the same virus in the MPOA and PVN.

Tema: Habitat choice and use

[P6] Ecophysiological traits vs. behavioral interference: insights from two species of Podarcis lizards

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Ecophysiological traits determine habitat use and geographic range of ectotherm vertebrate species. In particular, lacertid lizards are constrained by temperature and humidity. Among the Iberian representatives of the genus *Podarcis*, the predominance of parapatry over sympatry suggests that interspecific interference may take place. Here, we test the effects of the behavioral interference between two *Podarcis* species in an ecophysiological context.

Preferred body temperature (Tp) and water loss (Wl) were determined in two locally sympatric *Podarcis* species: *P. liolepis*, widely distributed in NE Iberia but restricted in SE France, and *P. muralis*, restricted to mountains in Iberia but widespread across the rest of Europe. The Tp was measured in photothermal gradient at 10 time intervals and the Wl rates in sealed chambers during 12 hours. Tests in thermogradient were conducted individually and for intra- and interspecific interaction.

Although (pregnant) females attained lower Tps, no differences between species were detected, while *P. muralis* displayed higher Wl than *P. liolepis* in the second half of the experiment. *P. liolepis* (but not *P. muralis*) shifts in Tp support both intra- and interspecific asymmetric competition. Overall, even if both species isolately differ mainly in their water physiology, when together, behavioral interference has consequences on the thermal physiology of *P. liolepis*. The biogeographic implications of these results are discussed.

[P7] Sleeping tree choice by *P. troglodytes verus* in Lagoas de Cufada Natural Park, Guinea-Bissau

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Tropical forests are under severe threat and disappearing at alarming rates as a result of destructive human activities such as agriculture, logging, and mining exploitation. *Lagoas de Cufada* Natural Park (LCNP), Guinea-Bissau, has been severely affected by these human activities. During 2008, LCNP biodiversity was further put at risk by criminal logging, seriously compromising *Pan troglodytes verus* habitat. In 2011, a local project for large-scale exploitation of *Dialium guineense* fruit started, this tree species being one of the most consumed seasonally by chimpanzees (February-June). The overall goal of this study is to assess the impact of land-use change on the nesting patterns of LCNP chimpanzees. Nesting patterns were studied along 11 systematically placed linear transects, by walking transects several times within an interval of 15 days, ensuring that no nests had disappeared between each visit. We found that chimpanzees used 17 tree species for nesting (n = 248 nests). Most of the nests were observed in closed forest (64.52%), followed by savanna-woodland (24.60%), and open forest (10.89%). Although chimpanzees used a range of different tree species for nest building, nests were typically constructed at approximately the same height (ca. 14 m), independently of habitat type and tree species. Preferentially, chimpanzees chose *D. guineense* for nesting (52.42%). These results suggest nesting tree species preference but for a better understanding of nest-tree selection by chimpanzees, tree species density as well as other variables (e.g., distance to waterlines and to villages) will be included in further approaches.

[P8] Amphibian larvae temporal patterns in pond communities in a Mediterranean transitional landscape

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In order to understand species traits and factors affecting community assembly rules we need to study the spatial and temporal patterns of community structure. This knowledge should also contribute to good conservation practices. Amphibian pond communities play an important role in ecosystem services and nature conservation and are threatened by land use change and climate change. They also provide good ecological and behavioral models to study community structure rules. This study objective was to investigate whether there is an intra-annual pattern and which factors influence phenology in amphibian larvae communities in ponds in a Mediterranean transitional landscape. We collected data on the monthly occurrence of larvae and eggs of ten species in eight ponds during two and a half years. Species richness and larvae abundance showed a circa-annual pattern. Larval periods varied among species. Seven species presented phenology flexibility. Number of species was positively correlated to pond depth. Precipitation affected seven species and temperature affected four species. Five species were influenced by pond depth. Our findings support the existence of an intra-annual pattern in pond use by amphibians. They also suggest that surveys need to cover the entire year. Different species strategies (temporal segregation) may contribute to the maintenance of diverse communities. However, because most species are affected by climate and hydrology, climate change and manipulation of pond hydrology may influence community structure and stability. Nevertheless, phenology plasticity might favor the persistence of communities in unpredictable environments as the Mediterranean region.

[P9] Substratum preferences of rocky intertidal fishes (Pisces: Blenniidae)

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The main goal of this work is to contribute for the investigation of patterns of movement of the specie *L. trigloides*, through the analysis of time by occupation of different substrates – Rock, sand and gravel.

For this purpose, four individuals from the rocky intertidal platform Avencas – Bafureira were captured and observed for 23 hours of focal individual observations, divided into two periods, one in the morning between 9h-12h AM and the other in the afternoon between 3h-6h PM.

Differences were observed on the substratum choice, *L. trigloides* clearly prefer rocky to sandy and gravel substratum. This behavior confirms the previously published descriptions for blenniideos.

[P10] The role of habitat choice in ecotype formation in the flat periwinkle *Littorina fabalis*: an experimental approach

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Reproductive isolation can more easily occur in allopatry, but it can also evolve in the presence of gene flow, as a consequence of divergent natural selection. Taxa experiencing the same divergent selection pressures over a large geographic range may lead to similar ecotypes independently in different areas, a process called “parallel evolution” that may cause ecological speciation.

Recent studies on *Littorina* species (marine intertidal gastropods) have highlighted their potential for studying ecological speciation. In particular, *L. fabalis*, for which three ecotypes were described for the Iberian Peninsula associated with different algae/seagrass genera, is among the most interesting species for studying the genetic and behavioural mechanisms involved in ecotype differentiation.

In particular, the role of habitat choice in ecological speciation has been largely underappreciated. When animals are faced with more than one type of habitat, they may decide based on many types of information, either genetic or non-genetic, either passively acquired or by directly interacting and learning from the environment. The information provided by the performance of conspecifics on each habitat type (social information) has the potential to considerably influence one’s choices, with implications to animal dispersion and to the levels of gene flow across habitats, influencing the probability of ecological speciation.

Within a framework of a recent project, aiming to understand the relative contribution to ecological speciation of *L. fabalis* genetic and socially acquired habitat preferences, we conducted a first series of laboratory experiments of which we will present the results and discuss their possible evolutionary significance.

Tema: Mate choice and mating systems

[P11] ***Syngnathus abaster* genetic mating system: a quest for the mother phenotype**

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Pipefish are interesting model species to study sexual selection related subjects as males become pregnant and, in some species, there is an explicit sex role reversal, with females competing for male access. However, understanding how sexual selection works depends on a deep knowledge of a species mating system. Thus, several studies have been conducted on the determination of pipefish mating system, most of which using molecular markers, given their intrinsic power and sensitivity. Nevertheless, no study has yet tried to identify genetically putative mothers in the wild, which might be of great importance for questions related with mate choice. In the black striped pipefish, *Syngnathus abaster*, previous observations point to the presence of multi-maternity (we were able to find clusters of eggs in distinct development stages within a male’s brood pouch), thus hinting at a polyandrous mating system, but there is still no empirical determination of the actual genetic mating system. Taking this into consideration, our goals were to: 1) Determine the genetic mating system using microsatellites markers; 2) Quantify the rate of multi-maternity; and 3) Identify putative mothers from within our samples. Our results indicate that the black striped pipefish presents a polygynandrous mating system (males received eggs from distinct females that were able to distribute eggs among different males) and a high degree of multi-maternity. We were also able to identify, for the first time, four putative pipefish mothers from within our samples.

[P12] Do male sand gobies (*Pomatoschistus minutus*) advertise their quality during courtship calls?

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Female mate choice based on variability in one or more male attributes is key in sexual selection theory. Acoustic signals are good examples of sexually selected traits predominantly used by females of several taxa to identify, locate and choose among potential mates. Sand goby females (*P. minutus*) are known to use cues such as male size and courtship rate in mate choice but whether they also use acoustic cues remains unknown. The males of this species have been described to produce drums to attract females to their nest. Therefore, the aim of this study was to test the hypothesis that acoustic courtship signals advertise male sand goby quality. All fish were collected during the breeding season (May-June 2011) in Sweden (Kristineberg, 58°14'N, 11°26'E). During the experiments a resident male was allowed to interact with a gravid female. The following acoustic parameters were measured for each subject male: calling rate and calling effort (% of time calling), sound duration, number of pulses, pulse period, pulse repetition rate and dominant frequency. Preliminary results showed that calling rate and calling effort were significantly correlated with male condition (lipid content) (Spearman correlation, $r=0,5$, $P<0,05$) suggesting that these can signal male quality. Additional sound features, the degree of nest cover with sand, and visual courtship cues, currently under analysis, may be putative measures of male quality. We suggest that courtship acoustic signals can advertise male quality in the sand goby and might be used by females to choose their mates.

[P13] Personality effects on mate choice in the serin

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In many animal species, individuals exhibit consistent individual differences in their behaviour, such as in aggressiveness, activity, exploration, risk-taking, fearfulness among others. Although these behavioural traits can be circumstance susceptible, some individuals are consistently more exploratory, aggressive or active than others and this individual stability of correlated behavioural traits is often defined as personality traits. While the upsurge of studies in the last decade, the evolutionary origin of personality and its maintenance is still unknown.

In this study we subjected male and female serins to several contexts to measure behavioural responses – tonic immobility, neophobia, exploratory activity - that could be used to access proximate-level explanations to personality and relate it with mate choice tests.

Here we described differences in behaviour of serins in different contexts that have not previously been studied in this species. We could discriminate individuals in two dimensions related to their behaviour: bold-exploratory and shy-inactive. Also, this is one of the first investigations to test effect of personality traits in the performance of individuals in mate choice tests. We found differences in behavioural responses, as bolder and exploratory individuals were also more active during the mate choice trials in detriment of shy and inactive ones.

The influence of personality on ecology and evolution of species increases the importance of continued studies to better understand the role of personality variation in populations and between species.

[P14] Why do spider mite females mate with several males if there are no reproductive benefits?

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Polyandry is an evolutionary puzzle, since it commonly entails major costs for females. Yet, it is a widespread phenomenon, hence it should provide some selective advantages, which is the case in several species. However, in species for which only the first mating event is effective and sufficient to fertilize a lifetime egg production, the occurrence of polyandry is still puzzling. This is the case in the two-spotted spider mite, *Tetranychus urticae*, a haplodiploid species in which the second male only stands a chance to sire some eggs if it mates with a female within 24 hours after the first mating event.

The aim of this study was to investigate the consequences of polyandry for spider mites in terms of reproductive fitness, depending on the frequency and timing of the mating events (within or after 24 hours). We found that females that mated with several males did not have higher survival or fecundity than females mated with a single male, irrespective of the frequency or timing of the subsequent mating events. Moreover, when the second mating event occurred 24 hours after the first, females produced offspring with a higher male frequency than when mated with a single male. Hence, not only is the sperm from the second male not used to sire more eggs, as it even leads to a reduced proportion of fertilized eggs. Hence, the adoption of this mating pattern in *T. urticae* remains a mystery and further investigation addressing this subject is needed.

[P15] Reproductive interference between two spider mite species: how can it affect the outcome of competitive interactions?

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Reproductive interference (RI- any kind of sexual interaction decreasing the fitness of one or both species) can play an orchestral role in species distribution. However, its occurrence might frequently have passed unnoticed, as studies on species interactions focus mostly on resource competition.

We investigated the occurrence of RI between wild populations of two haplodiploid spider mite species (*T. urticae* and *T. evansi*) and a laboratory strain of *T. urticae* (London Strain). We tested (a) whether fertile hybrids were produced, (b) whether females preferred to mate with conspecific males and (c) the consequences of mating with the wrong species for subsequent conspecific mating events.

Although hybrids were not found, (i.e., heterospecific matings produced only haploid males), females did not mate preferentially with conspecific males. However, mating with heterospecific males did not have any consequences for spider mites that subsequently mated with conspecific males, both in terms of fecundity and sex ratio of the progeny. Therefore, heterospecific mating events is likely to have little influence on the dynamics of these populations interacting, provided conspecific males are not limiting. . However, the consequences of the *Wolbachia* infection found in wild *T. urticae* in this results demand further investigation.

[P16] **Mate-choice copying in *Drosophila subobscura*: socially learned mate preferences between geographically isolated lineages**

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Animals require information to make decisions about fitness-affecting resources, such as mates. They extract information about potential mates based on their individual preferences, but also based on social information from conspecifics.

One direct consequence of using social information for mate selection is the copying of conspecifics choices. Mate-choice copying (MCC) by females may reduce uncertainty about male quality, allowing more adaptive decisions. Moreover, by consistently modifying selection pressures for certain male traits, MCC may promote the hybridization of isolated populations. This has implications for adaptation to novel environments, because a population can more rapidly respond to the challenge of environmental change if its standing genetic variation is enhanced by introgressive hybridization. On the other hand, local adaptation may be constrained by such facilitated gene flow. The final outcome will depend on the relative importance of these two opposite evolutionary forces.

Experimental studies have shown that MCC occurs in several species of fish, birds and mammals, and more recently in *Drosophila melanogaster*. We are interested in studying MCC in another invertebrate species, *Drosophila subobscura*, and specially the role of MCC in the hybridization frequency between populations with different degrees of genetic isolation. Our aim is to study the extent to which socially learned mate preferences can counter-balance female previous individual preferences for males of their own lineage, increasing the opportunity for introgressive hybridization to occur. Here we present preliminary results of a study of MCC involving laboratory populations of *D. subobscura* founded from the extremes of the European distribution range.

Tema: Sound production, variation and function

[P17] **Effect of anthropogenic noise on serin song, *Serinus serinus***

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Acoustic communication in birds is extremely important for its survival and reproductive success (Bradbury and Vehrencamp 1998). Noise has a detrimental effect on this type of communication, which can have short term and long term consequences. It is expected that animals cope with noise through changes in the way, timing of producing it, or even changing its form. In several bird species, it has been shown that males respond to anthropogenic noise by increasing the lowest frequencies in order to avoid overlapping from noise. We wanted to determine if birds of the species *Serinus serinus* adjust their singing behaviour when singing in noisy environments.

We compared recordings made under conditions of different noise levels and found that the minimum frequency increased when background noise was higher. There was no change in any other song traits considered. The analysis of temporal variation in the adjustment of the minimum frequency indicated that this is done rapidly and is reversible. Our results reveal the existence of considerable vocal plasticity in these birds' singing in response to changes in environmental conditions.

[P18] **Aggressiveness is in the message. Male serins change song structure when being aggressive**

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Birds song has a dual function of attraction and stimulating the females to mate, but it is also used in intra-sexual competition. In birds with learned song, some song features are used only in the context of intra-sexual competition between males and they are associated with increased aggression before a physical attack. Song overlapping is one of these song features that is usually related to the vocal signalling and as aggressive signal.

The objective of this study was to find if and how the European serin (*Serinus serinus*) answer to songs overlapping by applying an interactive experiment in the field and analysing some songs traits and the behavioural changes of the subjects tested. This study attempted to understand the value of the overlapping playback in this non-territorial species and to increase the knowledge about serin's singing behaviour. The experiment involved three phases of recording: 1st) "free-singing" before the playback; 2nd) interactive playback, where all the subject's song are overlapped by the stimulus; and 3rd) "free-singing" after the playback.

We found that subjects replied to playback by shortening the duration of songs and by increasing the peak frequency of songs. Furthermore, the individuals that approached the speaker, replied with shorter songs. These results show that song overlapping causes significant changes in male song in *the serin* which occur both in the temporal and the frequency domains, and suggest that song overlapping is interpreted as an aggressive signal.

[P19] **Do sexual ornaments decrease on islands? The case of bird song**

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Islands have played a central role in the development of ecological and evolutionary theories, being often called natural 'laboratories'. Surprisingly, however, few systematic studies have been conducted to test the validity of the patterns associated with insularity syndromes and the processes underlying these adaptations. One pattern that has recently emerged is a tendency of island species worldwide to exhibit decreased sexual dimorphism and expression of sexually selected traits, such as plumage colouration, suggesting that sexual selection is weaker on islands. Birds are well known for using song or other vocalizations in sexual displays and hence, a systematic study of patterns and underlying processes of sexual selection on islands requires a thorough assessment of variation in bird song. Impoverished or lower quality songs may arise from decrease sexual selection pressure, decrease the necessity for species recognition or simply as a result of founder effects or drift. Here we use song recordings for island-mainland pairs of related species to describe the general pattern of song in island birds, taking into account several ways in which birdsong can be elaborated by sexual selection.

[P20] Sounds of Silence: auditory cues play a major role in social transmission of fear

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Communication of danger between con-specifics is present across different taxa of animals. Extensive studies have characterized the private communication channels used in social transmission of fear (STF), such as alarm pheromones and calls. However, despite the great advancement in the understanding of the neural basis of fear learning, very little is known about the mechanisms underlying STF. The Norway rat, a social species used as a model in Neuroscience has been shown to display STF. However a study of the cues that trigger fear in the observer rat is lacking. Hence, we developed a behavioral paradigm to study STF in rats, in which a demonstrator rat displays fear responses triggered by the presentation of a tone to which it was previously conditioned in the presence of an observer cage-mate. We found that observers freeze while witnessing a demonstrator displaying fear responses, provided they had prior experience with shocks. Next, we tested which sensory cues are important for STF. We found that visual cues and contact between rats are not necessary, but that auditory cues play a crucial role in this process. Alarm calls were neither sufficient nor necessary for STF. However, a transition from sound of movement to silence is a strong cue that is both sufficient and necessary for STF in rats. Therefore, although a multitude of cues can mediate STF, sudden silence, which likely reflects a pervasive fear response in animals—freezing—provides a public cue of danger that can be sensed by animals in the same ecosystem.

[P21] Acoustic divergence in the calling song of *Tettigetta* sp. (Hemiptera: Cicadidae) in Portugal

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Cicadas are famous for the ability of males to produce distinctive acoustic signals used as the most important method of intraspecific communication. The first step in the mating sequence consists in the production of a calling song by males in order to attract females, which, in turn, detect the sound and use it to locate and approach them. Calling songs are typically regularly patterned and species-specific and function as a premating isolation mechanism. In this study, we recorded the calling song of several males of the genus *Tettigetta* in different Portuguese localities and analysed them in time and frequency domains. The genus includes four known species in Portugal. *T. argentata* distributed throughout the mainland, *T. estrellae* found in north of Portugal and *T. josei* and *T. mariae* only found in south of Portugal (Algarve). An acoustic divergence was found in the calling songs of *T. argentata*, with specimens from south of Portugal grouping apart from those of center and north of Portugal. The echeme repetition rate was the variable that contributed most for this separation. This acoustic differentiation is also in agreement with molecular data under investigation. To our knowledge, *T. argentata* is the only species which occurs in sympatry with other species of the genus. The remaining species of the genus seem to be allopatric with one another. This apparent acoustic divergence in southern populations might be the result of strong selection on the response to sympatric heterospecific similar calling signals.

[P22] **Effects of swimbladder deflation on hearing in the Lusitanian toadfish *Halobatrachus didactylus***

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In fish with morphological adaptations for hearing (*i.e.* hearing specialists) the swimbladder plays an important role in hearing sensitivity enhancement. In contrast, in species that lack such morphological adaptations (so called hearing generalists), such as the Lusitanian toadfish, the swimbladder is considered unimportant for hearing improvement. Since there is little available information regarding the role of this structure in these fishes' hearing, we used the auditory evoked potential (AEP) technique to investigate the effects of the swimbladder deflation on the hearing sensitivity of the toadfish. Preliminary results indicate that deflation significantly affects hearing sensitivity causing an increase of 6-12 dB in auditory thresholds at frequencies below 100 Hz, which are among the most biologically relevant frequencies in this species. These data may suggest a so far unsuspected and important role of the swimbladder in sound reception of hearing generalist fishes.

[P23] **Physiological constraints on sound production of the Lusitanian toadfish *Halobatrachus didactylus*: an ontogenic perspective**

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The Lusitanian toadfish (*Batrachoididae*) is a vocal fish, with an unusually large acoustic repertoire. During the reproductive season males use long sounds, the boatwhistles, to attract females into their nests for mating. Batrachoidids produce sounds by contracting a pair of sonic muscles intrinsic to the swimbladder walls. Boatwhistles have a fundamental frequency of c.50-60Hz while grunts (a stress sound produced by both gender) have a fundamental frequency of c.100Hz). To enable such high contraction rates, sonic muscle fibers present several adaptations such as abundant sarcoplasmic reticulum with numerous Ca²⁺ pumps that allow fast calcium transportation. In the breeding season territorial males have larger sonic muscles than females, with higher glycogen and mitochondria content to meet the seasonal increase in vocal activity. In this study we are measuring the ability of sonic muscle contraction of males in different ontogenic stages, and in the winter vs. the breeding season. We're using a force transducer to measure the movement of the swimbladder and are recording muscle action potentials during sonic muscle contractions produced by electrical stimulation of the sonic nerve. We will also couple these observations with histological characterization of the sonic muscle fibers and the state of gonad development. We aim to identify from which ontogenic stage there is a clearly physiological and histological adaptation of the sonic muscles to the breeding season. So preliminary results show that adult males present an unusual sustained contraction of the sonic muscle during sound production besides the individual contractions.

[P24] FishTalk: a new underwater speaker to test the function of fish sounds

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Many fish communicate with acoustic signals mostly in mating and agonistic contexts. These signals are often pulsed low frequency sounds with fast transients. Playback experiments, a widespread tool to study the function of animals' acoustic signals, have been hampered in fish due to limitations of commercially available underwater loudspeakers, which do not reproduce fish sounds appropriately. In fact such devices have typically a strongly irregular frequency response, generate damped oscillations (resonance) being inappropriate to reproduce fast transients in a signal, are affected by water level (pressure), cannot be used beyond a depth of a few meters, and are usually relatively large. We developed a device that overcomes these limitations that is small sized, has a smooth frequency response in the range 10-20 Hz up to above 1000 Hz (typical frequencies of fish sounds) and can reproduce underwater sounds with a high fidelity. Preliminary tests with *Pomasthoschistus* point to a behaviour modulation in response to conspecific sound playbacks. Examples of the device performance and of such behavioural responses will be given.

[P25] Men and women cannot assess men's features only by hearing their voices: research in a portuguese sample

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There is significant literature about assessing people's features through the hearing of their voices, especially through the male's voice, and what voice parameters are involved in, although no general agreement is established.

To test this hypothesis, we conducted a study in the Faculty of Sciences of the University of Lisbon using men's voices and both men's and women's judgements of them. We were interested in testing men and women abilities to assess other men's features. We asked to 100 listeners (50 men and 50 women) to guess features from 7 men voices randomly selected from a sample of 31 speakers. The features used were masculinity, age, weight, height, body condition according to Body Mass Index and success with women.

Results provide evidence that men and women seem to have no ability to assess other men features through the hearing of their voices, except when the degree of masculinity of the voices was moderate, but not when voices with low, moderate and high masculinity were considered at the same time. The human male voice thus seems not to correlate with an individual's features when those characteristics can't be seen by the observer, but may be useful, as we hypothesize, to distinguish among speakers with resembling visible phenotypes. The evaluation of one's features should depend on a complex of traits, and not only on a single feature such as voice parameters.

Tema: Cooperation

[P26] Coordination in rats: the use of game theory to study the proximal mechanisms of Cooperation

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Animals must survive in an ambiguous world governed by shifting probability landscapes that are intricately linked with their own behavioural choices. This is most evident in social interactions in which the outcome of one's decision can be influenced by the decisions of other animals. Through the use of game theory we aim to unravel how two interacting rats make adaptive behavioural choices in the face of a probability landscape that changes as a function of their combined choices. An automated double T-maze for testing behaviour in 2x2 social dilemma games has been developed. We have employed a simple coordination task with pellet rewards corresponding to a Stag Hunt (SH) game that are both discernible and desirable to rodents. By fixing the strategy of one rat (stooge) to a pseudo random distribution and observing the choice of a freely behaving rat (agent) it was found that rats rapidly engage in coordination. This suggests that rats are able to understand the economic terms of the SH game and optimize their behaviour accordingly. This finding paves the way to the use of game theory to study coordination in rodents. We will then observe the behaviour of two agents in a SH game. Importantly in this game there are two Nash equilibria, mutual cooperation and mutual defection, where mutual cooperation yields the highest reward but it entails the highest risk. In this circumstance rats have to make two decisions, whether to choose first and whether to cooperate or defect. We propose that choosing to cooperate first may be used as a measure of 'trust'.

[P27] Maternal effects and life history trade-offs in the cooperatively breeding sociable weaver *Philetairus socius*

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In cooperatively breeding species mature individuals called 'helpers' forgo independent reproduction and assist others with their breeding efforts. Why do these 'helpers' assist other birds? It is often the older offspring of the breeding pair that are retained as 'helpers', suggesting that kin selection (i.e. increasing one's fitness indirectly by assisting the reproductive effort of close relatives) may play an important role in the evolution of helping behaviour. This, however, pre-supposes that the helpers do indeed contribute to increasing the reproductive success of the breeding pair, which is not always the case. Nonetheless, parents often reduce their work loads when helpers are present, which may improve parental survival. In addition, females may decrease their investment in producing eggs, thereby saving energy for future reproductive events and enhancing their own survival, since the additional food provided to the chicks by the helpers may compensate for the smaller or poorer quality eggs. We investigated these 'cryptic' helper effects in a southern African passerine, the sociable weaver *Philetairus socius*. We found that females lay smaller eggs when assisted by helpers, but found no clear differences in the egg contents (e.g. lipids, proteins or carotenoids).

[P28] Human cooperation is affected by cultural traits

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Human cooperation is still a defying subject to evolutionary theory, particularly when we are facing cooperation between unrelated individuals. Some games, such as the theory of games, the ultimatum game and the dictator game, have been used to try to model and understand in which circumstances cooperation occurs and which variables are most relevant to it. Recent studies suggest there is cultural variation in the way humans respond to some of the challenges, indicating we do not follow a fixed strategy to play the cooperation game.

We decided to test whether there is cultural variation in the way people play cooperation games, using a sample of 246 university students, studying in Coimbra, from Portugal, Brasil, Cape Verde, Guinee and Angola. Players tended to be more cooperative if the opponent was a familiar to them, irrespective of being kin. We found some differences in the amounts offered in the dictator and the ultimatum games in relation to religion and nationality. The results indicate that social norms and eventually the type of environment where individuals grew, had effects on their propensity to cooperate.

Tema: Parasite/hosts and predator/prey relationships**[P29] Well-behaved and misbehaving plasmids in bacteria – The role of toxin-antitoxin loci**Rita Ponce (anaritaponce@gmail.com)¹, Eduardo Rocha²*¹Centro de Biologia Ambiental (CBA), Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal**²Institut Pasteur, Microbial Evolutionary Genomics, Paris, France*

Within a bacterial cell, besides the bacterial chromosome, there are frequently shorter non-essential DNA molecules that are called plasmids. The relationships between plasmids and bacteria are complex, ranging from parasitic to mutualistic. Bacteria may grow faster without plasmids, but plasmids can contain genes that can confer an advantage in certain circumstances, such as antibiotic resistance genes. In addition, plasmids are capable of horizontal gene transfer, and the difference of inheritance between chromosomal DNA and plasmid DNA adds a stress in their relationship.

Toxin-antitoxin (TA) loci are genes that can be located in bacterial chromosome and in plasmids and whose expression can have an important effect in that relationship. TA loci are composed by a toxin gene, that encodes a bactericid or bacteriostatic protein and can kill the cell that expresses it, and an antitoxin gene, that can prevent the effect of the toxin. When they are located in a plasmid, TA loci maintain the plasmid in the populations by killing the cells that loose plasmid during cell division; but when located in the chromosome they may counteract this effect. Herein, we will summarize and debate the current knowledge on the role of TA loci in the relationship between plasmids and their bacterial host.

[P30] Use of aromatic plants in Blue Tit nests – does it matter?Bárbara A. Pires (barbara.afonso83@gmail.com)¹, Anabela F. Belo^{2,4}, João E. Rabaça^{3,4}*¹ Departamento de Biologia, Universidade de Évora, Évora, Portugal**² Laboratório de Botânica, Departamento de Biologia, Universidade de Évora, Évora, Portugal**³ Laboratório de Ornithologia (LabOr), Departamento de Biologia, Universidade de Évora, Évora, Portugal*

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The Nest Protection Hypothesis suggests that some birds add aromatic plants to their nests in order to repel or kill ectoparasites. This behavior has been described for several species, namely the Blue Tit (*Cyanistes caeruleus*). We have studied a total of 26 Blue Tit nests (in nest boxes) in forested areas of *Quercus* spp. and *Pinus pinea* located in the 'Parque Florestal de Monsanto', the largest park of Lisbon. We collected data on the reproductive performance of each breeding pair and the frequency of aromatic plants in the nests was compared with its frequency in the study area. Results showed that the three most frequent aromatic plants found in the nests (*Dittrichia viscosa*, *Lavandula dentata* and *Calamintha baetica*) were used more than expected from their availability in the study area. However, we could not reject the null hypothesis that the nest survival rate is independent of the presence of aromatic plants in the nest.

[P31] **Face your fears: cleaning gobies inspect predators despite being stressed by them**

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Social stressors typically elicit two distinct behavioural responses in vertebrates: an active response (i.e., "fight or flight") or behavioural inhibition (i.e., freezing). Here, we report an interesting exception to this dichotomy in a Caribbean cleaner fish which interacts with a wide variety of reef fish clients, including predatory species. Cleaning gobies appraise predatory clients as potential threat and become stressed in their presence, as evidenced by their higher cortisol levels when exposed to predatory rather than to non-predatory clients. Nevertheless, cleaning gobies neither flee nor freeze in response to dangerous clients but instead approach predators faster (both in captivity and in the wild), and interact longer with these clients than with non-predatory clients (in the wild). We hypothesise that cleaners interrupt the potentially harmful physiological consequences elicited by these aversive stressors by becoming increasingly proactive and by reducing the time elapsed between clients approach and the start of the interaction process. The activation of a stress response should nevertheless be responsible for the increase of cleaning service prolongation provided by these cleaners to predatory clients in the wild. Future experimental studies should reveal similar patterns in other social vertebrate species when, for instance, individuals approach an opponent for reconciliation after a conflict.

[P32] **Individual differences in antipredatory and exploratory behavior in an iguanid lizard**

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There is increasing interest in understanding the consequences of behavioral syndromes in different animal taxa. Behavioral syndromes are suites of correlated behaviors expressed either within or across different contexts. In addition, behavioral syndromes can be correlated with morphological and physiological characteristics of the individuals. One established relation is

between antipredatory and exploratory behavior. It has been shown that more active and explorative individuals tend to be more bold and aggressive in relation to predators. We tested this relationship using males and females of the iguanid lizard, *Liolaemus nitidus*. We studied their exploratory behavior observed in an unfamiliar environment and their behavioral responses to simulated predator attacks. The variables measured in association with predation included length of tonic immobility, bite force, and vocalizations. We also measured load of ectoparasites, since parasite infections have the potential to decouple behavioral syndromes. We did not find strong correlations between the measured traits, and our results do not support a behavioral syndrome in this species. We discuss two alternatives to explain our results: 1) there is a decoupling of the different traits, possibly associated with parasitism, or 2) the measured traits used were inadequate to establish a behavioral syndrome in this species.

Tema: Behavioural patterns

[P33] Behavioural patterns and group characteristics of common bottlenose dolphin (*Tursiops truncatus*) in São Tomé (São Tomé e Príncipe)

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Since 2002, an effort has been made in order to study the occurrence, distribution and behaviour of cetaceans in São Tomé waters. For the present study, we present data from 2002-2006 of bottlenose dolphin's sightings in order to study the behavioural patterns and its relation with group size and type. A total of 210 boat-based surveys were conducted, during which GPS positions, group composition, size, behavioural pattern and photographic identification were registered. The behavioural patterns considered were: travelling, feeding, socializing, resting, travelling/feeding and socializing/travelling. Sightings were grouped according to group characteristics, such as size (1-30, 31-60, >60 individuals) and type (adults, adults/juveniles, adults/juveniles/calves). Travelling was the behavioural pattern most observed (20 sightings), followed by feeding (18 sightings) and social behaviours (5 sightings), resting was never observed. All three group sizes were observed during travelling and feeding, being the smallest the predominant. The group size was smallest during social behaviours and largest in feeding/socializing. There was no association between behavioural activity and group size or behavioural activity and group type. Groups of only adults were observed during traveling, feeding and socializing. Groups with calves were observed in all behavioural categories, except socializing and in all three group sizes, and groups with only adults were smallest. Data suggest that there is an association between group size and group type ($p < 0,05$). In an area where little is known about cetacean behaviours and ecology, this study shows first results on bottlenose dolphins populations in São Tomé.

[P34] **Patterns of behaviour and residency of coastal common bottlenose dolphins off the Arrábida shores: A first approach**

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Considering the occurrence of coastal populations of common bottlenose dolphins (*Tursiops truncatus*) and other cetacean species in the waters adjacent to Sado estuary, which is home to a resident population of bottlenose dolphins, it becomes critical to develop a continuing study to evaluate the presence of coastal populations and interactions between them and the resident population. This study is a first approach to the residency and behavioural patterns of the common bottlenose dolphins' population in this region. Between 2007-2011 a total of 102 boat-based surveys along the coast between Cape Espichel and the Sado estuary, as well as the marine and coastal area of the Peninsula of Tróia, were conducted and pre-defined transects were used since 2010. On each sighting behavioural activities, such as travelling, feeding, socialising and resting, were recorded. Data analysis indicates that travelling was the most frequent behavioural activity; feeding occurred preferentially on waters shallower than 50 meters deep; and presence of calves is significantly more common within feeding groups ($p < 0,05$) than in travelling groups. Photo-identification for individual recognition was the primary technique used to obtain results and to conduct the subsequent analysis of bottlenose dolphins' interactions, resulting in an association index for the individuals. Preliminary results suggests that only about 11 individuals (in a total of 128 identified coastal bottlenose dolphins) shown considerable level of residency in consecutive years and one pair and two trios of adult animals had shown high association indexes. Knowing these population's behaviour patterns will help define critical areas for its conservation.

[P35] **A first description of behavioural activities of common dolphins (*Delphinus delphis*) in three areas of Portugal mainland coast**

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The short-beaked common dolphin (*Delphinus delphis* Linnaeus, 1758) is the most frequent cetacean species in Portugal mainland coast. From January 2007 to October 2011 a total of 145 boat-based surveys were conducted between January from which resulted 82 observations of common dolphins and 73 independent observations of behavioural activities, including travelling, feeding, socializing and resting. Travelling was the most frequent activity (55%), followed by feeding (27%), socializing (14%) and resting (4%). Groups between 1-10 individual were the most frequently sighted (45%), followed by groups of 11-20 (22%), 21-50 (21%), 51-100 (10%) and more than 100 (1%). Calves were present year round, reworking on the idea that the reproduction of

common dolphins is nonseasonal. From the events of socializing, aerial behaviour was frequently registered within our study area, such as the “pitch poling”. Also, sexual behaviour was photographed and video recorded and included persecution, belly-to-belly contact and copulation, occasionally involving the repetitive intercourse of the same female by different males. Mixed-groups of common dolphins were also recorded, namely with striped dolphins (*Stenella coeruleoalba*). For these first results it seems that common dolphins use the study area to conduct all the activities of its daily life in the study area. Further research on the subject will allow us to infer not only about inherent species behaviour issues but also about patterns of habitat use and species’ ecological niche.

[P36] **One day as a *Chioglossa lusitanica***

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Chioglossa lusitanica is the only referred species of *Chioglossa* gender and is endemic of the Iberian Peninsula. This species has no functional lungs and, therefore, needs a habitat with running water. This particular characteristic makes it difficult to do direct observation of their behavior and is probably responsible for the lack of research work in this area. This work focuses on the behavior of this salamander in its natural environment, in this case at a gold mine in Valongo, near Porto. With the help of an infrared camera installed in the mine without the presence of the researchers, a video record was made, for a 24 hours cycle, captured in two different days, during 12 hours each day. This register allowed elaborating an ethogram of all the salamanders’ occurred behaviors as well as knowing the major periods of their activity during day and night. As far as it is known this is the first time that the behavior of this particular species is studied during a 24 hour cycle.

[P37] **Mallard behavioural adaptations to winter: a study in Laje river**

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The Laje river, located at the Estoril coast in Portugal, is a hotspot for many bird species, both in the Summer and in the Winter. We investigated the behavioural adaptations to winter of the mallard ducks, *Anas platyrhynchos*, the most abundant species at the Laje river, in order to understand how can they survive to wintering in a rainy and cold habitat instead of migrating. Behaviours related to energy budget, preference for dry sites and gregariousness were studied in December 2010, by means of the scan sampling method. In Portugal, 2010 was the year with higher rainfall of the decade and in December there was a negative anomaly in air temperatures. Our data suggest that mallard preference for land and dry places, instead of being on the water, is their main adaptation to winter, which agrees with previous findings in other mallard populations. On the other hand, energy-spending activities and resting behaviours were equally common in the population, as well as the frequency of their solitary and gregarious behaviours. Since gregariousness is an effective strategy to avoid loss of heat in birds, our findings suggest that winter temperatures in the Laje river are not as severe as in other locations, despite the 2010 abnormal rainfall and cold temperatures.

In a future study, the same behaviours should be recorded throughout a whole year in order to better characterize mallards' typical behavior during the winter and to reliably determine if their winter adaptations are totally absent from Laje river or not.

[P38] **Interaction of activity patterns and tide rhythms in the species *Lipophrys trigloides***

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This study aimed to investigate the existence of an endogenous stimulus in *Lipophrys trigloides* species related to differences of tides controlling their periods of activity. According to some of the bibliography found for this study, *L. trigloides* is a species with nocturnal activity pattern. Random individuals of this species were filmed in separated aquariums for three consecutive days with constant illumination. After these three days, the footage acquired was analysed and we used statistical methods for treatment of the data collected from the observations. The results have shown that differences in the activity of *L. trigloides* from day to night are not statistically significant contrary to the bibliography found. We realized that the species increased activity during high tides and lowered activity during low tides. We also noticed that these differences diminish over the three days of observation which led us to conclude that the species *L. trigloides* has an internal clock adjusted by the different tides. The work presented here has profound implications for future studies of tidal rhythm in *Lipophrys trigloides*.

[P39] **Demography, environment and the origin of individual differences in behaviour**

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It has been hypothesized that differences in behaviour evolve or are maintained due to variation in ecological factors, and demographic differences among populations. In this study, I characterized variation in behaviour among individuals and asked if it conformed to behavioural differences consistent across situations. I assessed for sex and age differences in behaviour, and whether behavioural differences were related to individual quality. I then asked if behaviour varied geographically and, if so, whether that variation was explained by demography and/or ecology. In addition, I studied a candidate gene (DRD4) to help distinguish between plasticity from genetically-based differences in behaviour. To test these different hypotheses, I used a recent biological invasion—the invasion of continental Portugal by common waxbills (*Estrilda astrild*).

I performed 4 behavioural tests in the field to obtain indexes of 1) boldness towards predator, 2) social behaviour, 3) exploratory behaviour, and 4) fear.

In short, I found few sex/age effects on behaviour, no relation with condition, weak evidence for a broad scale behavioural syndrome, but strong geographic variation in behaviour associated with climatic variation related to seasonality. And also no evidence for genetically-based behaviour.

Habitats typified by higher precipitation and temperature seasonality and, associated with this, on average hotter and drier, contained populations that behaved more attentively and less actively towards social stimuli, and explored less in a new environment. Above all, I demonstrate that no fixed behavioural variation is to be expected. In particular, I show that spatial and temporal variation in environmental conditions can be on the origin of behavioural diversity.

[P40] Ethogram of the behaviour of Labrador Retrievers in aquatic environment

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Labrador Retriever is a breed of domestic dog (*Canis familiaris*) developed to perform tasks related to the aquatic environment. However, the behaviour of these animals during interaction with water has never been described or included in any ethogram of the specie.

In this study, an ethogram of the behaviour of Labrador Retrievers in aquatic environments was developed. Previous ethograms of the domestic dog were reviewed to produce an inventory of typical dog behaviours. Thirteen Labrador Retrievers were observed, videotaped and photographed in two kinds of aquatic environments for a total of 118 min, in order to identify and obtain detailed descriptions of the observed behaviours.

A total of 55 behaviours were catalogued and classified into seven categories. These included exploratory behaviour (six entries), water play (25 entries), approach behaviour (three entries), comfort behaviour (seven entries), emotional cues (four entries), physiological behaviour (five entries) and retrieving behaviour (five entries).

The resulting ethogram offers a practical tool for quantitative research and further studies on the influence of these environments to this breed. Furthermore, it enriches the knowledge about the behaviour of the domestic dog and aids to the progress of developing a complete ethogram for this specie.

[P41] General ethogram on common otter (*Lutra lutra*, Linnaeus 1758) kept in captivity

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The Common Otter or Eurasian Otter (*Lutra lutra*, Linnaeus 1758) is considered a “Near Threatened” species according to the International Union for the Conservation of Nature and Natural Resources (IUCN, 2008). In Portugal, the status of this species is “Least Concern” (Cabral et al, 2005), so there is a bigger responsibility to understand the biology and ecology of the species (Trindade et al, 1998) in order to contribute to the world conservation.

Two adult otters *Lutra lutra* (Linnaeus, 1758), a female and a male, together in captivity in the “Aquamuseu do Rio Minho” (Zoo Licence number 21/2009) at Vila Nova de Cerveira, Portugal, were observed. The study comprised 2800 minutes of naturalistic observation and two ethogram on Common Otter kept in captivity were developed: one with individual behaviours and another with the interaction behaviours.

The ethogram on individual behaviour had 12 entries: smell, sleep, feeding, dig, swim, sprait, rub, grooming, run, clapping, shake, still, stare and yawn. The ethogram on interaction behaviour had 22 entries: smell each other, run together, scent marking, swim together, touch, social rubbing, avoiding approach, steal, paw, play, mating position, avoiding mating, escape, deviate, chase, approach, approach with open mouth, expression antithesis, together, sleep together and no interaction.

The present work presents the first ethograms on *Lutra lutra* in captivity and the first approach on Common Otter behaviour and thereby we suggest that these ethograms should be used as a basis to the study of this species in other enclosures and in nature.

Tema: Cognition and social modulation

[P42] Social interaction of goats (*Capra hircus*) after the introduction of unfamiliar individuals in a stabilized group

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In intensive farm animal management, groups are continuously being formed at various stages of production. This creates a competitive environment which triggers social aggression. In goat species, frequent social conflicts due to their hierarchy can negatively affect feeding and resting behaviours, which might contribute to the reduction of milk and meat performance in low-ranking goats. Therefore, the knowledge of the goat behaviour is essential to the development of more appropriate and efficient management techniques. The purpose of this project of applied ethology was (1) to study the effects of the introduction of unfamiliar members into a stabilized group and (2) to study the effect of the time of the day when that introduction was made (morning or afternoon). Two months before the experimental date, 28 goats were divided in four treatment groups: two groups with 9 goats (morning and afternoon stabilized groups) and two groups with 5 goats each (unfamiliar animals). Each group was observed and video-recorded during 24 h before, during and after the introduction of the unfamiliar animals into the established groups. The social structure was disrupted by the introduction of the unfamiliar members, resulting in significant increases ($P < 0.05$) in agonistic behaviours, especially in frontal clash and threat. The frequency of aggressive behaviour decreased with time after the introduction of the unfamiliar members. The results also showed that morning introductions generated more aggressive behaviours.

[P43] Affect expression and social competence in preschool children

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Although the facial expression of emotions in infants and young children and their perception of affect expressions in others dominated developmental research on affect/emotion during the 1980s, current developmental treatments of affect tend to emphasize cognitive (Saarni, Campos, Camras, & Witherington, 2006) and temperamental (Rothbart & Bates, 2006) factors when characterizing links between affects and life-outcomes. Expressiveness *per se* is rarely studied independently from knowledge and/or regulation. We assessed affect expressiveness for 150 preschool children at preschool and a common set of social competence (SC) indicators. These included two Q-sort descriptions (California Child Q-sort [CCQ], Block & Block, 1980; Preschool Q-set [PQ], Bronson's adaptation of a Q-sort originally used by Baumrind, 1967), direct observations of initiated interaction and visual attention to peers (Bost et al., 1998), and two sociometric interviews (3-like, 3-dislike nominations; paired comparison sociometric). All measures were collected by independent observers, previously trained and reliability reached percent agreement levels of 80% or higher.

Correlations indicated that dyadic positive affect was associated with peer acceptance, visual attention received from peers, rate of initiating positive interactions. We believe that our results also pose a challenge for current treatments of affect expressiveness, especially the expression of positive affects, from developmental science. Our findings suggest that affect regulation does not fully explain the effects of either positive or negative affect experience. Rather, measures of both experience and regulation of affects are needed to explain the impact of affect on children's adaptation to the peer group.

[P44] **Been there, done that: how prior-experience modulates social transmission of fear**

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In natural conditions social cues are often used to avoid danger. This transmission of information can account, in part, for the success of social species, as an animal does not need to self-experience all scenarios to learn about them.

Studies in the laboratory showed that prior experience with shock is necessary for robust social transmission of fear in rats.

What happens during prior experience with shock that makes an animal respond to the distress of others? One possibility is that experience with an aversive event sensitizes the rats to any cues that might signal danger. Alternatively rats can learn to associate their own behavioral responses with footshock and, thus, become responsive to these same responses displayed by their conspecifics.

Using an Immediate Shock Deficit – ISD paradigm, where the animal is shocked immediately upon being placed in an unfamiliar context ensuring an aversive experience with no learning, we found that these animals do not display vicarious fear, suggesting that self-experience results in an associative learning event that facilitates the social transmission of fear.

To investigate what is being learned during prior-experience we conditioned rats to fear a context without allowing them to express fear responses in that environment. We found that these rats show low levels of vicarious fear during the social interaction, and that this is rescued if the rat is allowed to express freezing to the conditioning context in the absence of shock before the social interaction.

[P45] **How social interaction modulates fear expression**

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Fear responses are innate defensive behaviors exhibited by animals in response to aversive stimuli. During fear conditioning an animal can learn to fear a neutral stimulus when it is paired with an aversive one. This form of learning is crucial, as it allows animals to use cues associated with aversive events to avoid future threats. Interestingly, social buffering experiments provide evidence that social interactions can buffer fear responses to fearful stimuli. As we are interested in the neural mechanisms of social buffering, we developed a paradigm to elucidate the effect of social buffering on fear conditioned rats. We conditioned rats to fear a tone by pairing it with footshocks. We then re-exposed them to the tone in the absence of shock and found that the rats that were tested for fear of the tone in the presence of their cage-mate showed less freezing than if tested alone. Moreover, it is known that repeated exposure to the tone in the absence of footshocks leads to a decreased fear response. This decrement in freezing is a result of animals either forming a new memory, or an update of the old memory. When tested, we found that rats that were exposed to the tone in the presence of their cage-mate and then tested alone a month

later, exhibited lower levels of freezing compared to rats that had been exposed alone. Our study shows that social buffering has long lasting effects on fear, which may contribute important knowledge pertaining to underlying causes of anxiety disorders, and possible treatment applications of these disorders.

Tema: Cognition, social attachment and emotions

[P46] Parental care in chimpanzees (*Pan troglodytes*): age, sex and kinship effects between mothers and infants. Study in a captive population at the Lisbon's Zoo under environmental enrichment

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Great ape infants are dependent on adults for a long period of time, during which they learn how to live in complex social groups. When in captivity, environmental conditions are less stimulating and the way parental care is provided to the infants may then change. Environmental enrichment focuses on trying to recreate conditions that encourage species-typical behaviours, as they generally occur in the natural environments.

This study, conducted in the Lisbon's Zoo, analyses parental care in chimpanzees (*Pan troglodytes*) according to features such as the kinship degree among females and infants, age of the infants and parental experience of the females. We started collecting data in November 2011 and will continue for a period of eight months. Focal continuous sampling has been applied to all the chimps of the Zoo population (n=17) giving a total of ten hours of observation each. The observation time was divided in three different periods, according to the typical environmental enrichment protocol: base-line, enrichment and end-line periods. We chose to enrich the chimps' environment with physic/occupational stimuli.

Based on previous studies, we predict that older and hence more experienced mothers would interact more with their infants; the interactions would be more frequent with younger infants and alloparental interactions would be less frequent than mother-infant ones. Environmental enrichment is expected to increase the time and frequency of the mother-infants interactions, contributing to a better development of the infants within the social group, with positive implications to the welfare of the whole population.

[P47] Development of a questionnaire for evaluating social attachment in dogs

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Social attachment can be expressed during the life of an individual in various forms (filial, parental and pair bonds). It is a complex process defined as a selective and enduring social bond. According to the operational definition by Ainsworth it is characterized by proximity seeking and

separation protest to and away from the attachment figure which is considered a safe haven and a secure base from which to explore the environment. Differential behavioural responses to the presence/absence of social stimuli are used to infer social bonds and attachment. Deriving from attachment theory several survey instruments have been developed and extensively used to quantify attachment relationships between owners and their pets. Nevertheless, despite the central role of attachment in group coordination, individual development and the expression of behaviour problems, there is no such instrument to assess attachment of pets to owners. We proposed to develop a questionnaire for evaluating social attachment in dogs. Although this method relies on the ability of the owner to correctly report its dog behaviour, several authors have demonstrated its reliability and accuracy for assessing behaviour and temperament traits in dogs. The design of the instrument is based on the four components in attachment behaviour. Owners answer questions regarding their dog's behaviour towards other dogs/persons (strange/familiar) and in a range of circumstances (e.g. isolation, strange situations), with the frequency of the behavior shown rated on a 1-5 scale. Preliminary data will be presented together with a discussion on the design and validation process of this new tool.

[P48] **Similarities and differences in the quality of attachment to father and mother**

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According to Bowlby and Ainsworth theory during the first year of life infants form an attachment relationship with a primary caregiver, usually the mother, being that the quality of parental behavior is viewed as playing a central role in the organization of children's secure base behavior. Other figures, namely the father, interact with the child in the family context, assuming in the present society a new and important role. Using Bowlby/Ainsworth's attachment theory, this study analyses the ways in which the child uses both mother and father as a secure base, as well as, the concordance (or not) of these attachment relationships. 85 child/mother and child/father dyads participated in the study. Children's ages range between 29 and 38 months ($M= 31.91$, $SD= 2.56$). Teams of two independent observers carried out home observations of the child interacting with the mother and with the father, separately, using the Attachment Behavior Q-Set (AQS, Waters, 1995). Observers were previously trained and interrater agreement range from .85 to .91. The mean security scores for mothers is .45 and .41 for fathers. No differences were found between average scores for security with mothers and fathers, showing that the child is able to use both parents as secure base. Significant differences were found in the Proximity and Physical Contact scales between parents, suggesting that mothers and fathers may have different communicative styles. Smooth Interaction, Proximity and Physical Contact scales are significantly correlated with the AQS scores for both parents. A significant correlation was found between security scores for mother and father. The results are discussed using the attachment theory.

[P49] **Preschoolers' Attachment quality and Emotional Understanding**

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Secure attachment may be viewed as a positive pattern of adaptation allowing children to observe and encode information about emotional expressions and situations. Moreover, the use of emotion language and positive emotional responsiveness were demonstrated to predict positively emotion

understanding (Denham, 1994). Children who can anticipate others' feelings and behaviours are more prompt to behave in a socially adapted way and when identified as securely attached, they are also characterized by a warm, open, flexible and generally positive interpersonal style leading to a better acceptance from their peers. Those conditions were being considered important determinants for school and social adaptation. The goal of this study is to analyze the contemporaneous association between attachment quality and the understanding of emotions in preschool children. Participants were 160 children, 64 girls and 96 boys, from Lisbon, Portugal. Children's age ranged between 41 - 73 months ($M = 57,72$; $DP = 8,45$); all Caucasian. All families were from middle class in terms of income level, according to the standards of the local community. Attachment Representations were assessed using the Attachment Story Completion Task - ASCT (Bretherton et al, 1990), 3 independent coders, blind to other information, coded the stories. Inter-coder agreement average was 0.81 for security and 0.85 for Coherence. The Affect Knowledge Test developed by Denham (1986) was used to measure emotional understanding. Cronbach's Alphas were 0,55, 0,66 and 0,83 respectively. Finally, no correlations were found between the security scores and language competence (WPPSI). Emotional understanding was significantly correlated with security ($r = 0,35$; $p < 0,01$) and also with coherence ($r = 0,33$; $p < 0,01$). Results will be discussed within the context of attachment theory.

[P50] **Internal working model of the self and Quality of attachment**

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Attachment theory suggests that notions about the self and representations of the attachment relationships become internalized over the time within a matching mode, with early interactions performing a crucial role on this process. Nevertheless, few studies looked at the associations between self-concept and attachment quality during the preschool period, as this study aims to do. Our sample were 75 Portuguese children (37 boys and 38 girls) Mean age was 73,4 months. Quality of attachment representations was assessed through the Attachment Story Completion Task - ASCT (Bretherton *et al*, 1990). All the 5 stories were videotaped and rated, by three blinded trained coders, on a 8-point scale for Coherence and Security (Heller, 2000). Interrater agreement reached, respectively, 0.81 and 0.85. Self representations were assessed using a using the Puppet Interview (Cassidy, 1986) to evaluate the internal working model of the self. Results show the relation between attachment relationship established with mother and the organization of the child self internal working model. Children with a secure attachment model showed a more positive internal model of the self. In this sense, we may conclude that the quality of attachment. Our results support the presence of connections between the quality of the attachment representations and the global representation of the self. In the future it would also be important to diversity our sample, presently a non-risk one, so that different patterns of association between self-concept and attachment representations can be explored.

[P51] **From emotional expression to attachment security during the pre-school period or the other way around**

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The notion that secure attachment relationships promote children's capacities to regulate negative emotional arousals while contributing to uphold positive affect expression in different

relational contexts and across development has been supported by research (de Rosnay, 2002; Kerns, 2007; Kochanska, 2001; Raikes, 2005). This study goal is to assess possible connections between the quality of the narratives, namely differential attachment strategies (Ainsworth, Blehar, Waters & Wall, 1978), and emotional expressiveness in the context of IWM's elicitation. Participants were 70 Portuguese preschoolers (33 boys and 37 girls) attending 1 private day care center in Lisbon. All children came from middle and upper-class families. The Attachment Story Completion Task (Bretherton, Ridgeway & Cassidy, 1990) identifies individual differences in the way children tend to enact a variety of attachment related situations. Stories were videotaped and security was rated on a four-point-scale with the Dusseldorf System (Gloger-Tippelt & König, 2000) by blinded trained. ASCT performance was also rated, by three independent trained coders, blind to the Dusseldorf scores, in terms of Positive and Negative Child overt Emotional Expression, Characters' Positive and Negative Emotional Displays and Inappropriate Emotional Expressiveness. Each variable was scored on a 7 point scale, from highly uncharacteristic to highly characteristic, according to clearness, frequency, intensity and duration (Hinde, 1997; Martin, 2007). Intensity of Expression was rated on a 4 point scale from restricted to intense. Interrater agreement was satisfactory for all variables. Significant associations were found between stories security, valence of emotional displays and appropriateness of overt emotional expressions. Cluster analysis indicate that a differential emotional expression organization among groups is related to the use of distinctive strategies, namely minimization or intensification of the problem presented by each story (Abe & Izard, 1999; Kochanska, 2001; Miljkovitch, Pierrehumbert & Halfon, 2007;Thompson, 1998).

[P52] **Can humans read emotion in animals' facial expressions?**

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The ability to recognize human facial expressions has been vastly documented, yet little is known about the human ability to interpret other species' facial expressions. We report a study that aimed to investigate if humans differ in the recognition of human, chimpanzee and dog emotional facial expressions from video-clips, and in their emotional reactions to those same stimuli. Participants watched randomized series of clips (essays) recorded previously in semi-naturalistic non-invasive conditions and portraying emotional behavior in the face. After each clip, the participant selected, from an onscreen menu, the emotion label thought to better describe the video. Throughout the experiment participants' skin conductance was recorded to measure emotional reaction and signals were acquired with a Biopac System. Data from 139 volunteer participants (average age 23.4yrs) mostly comprised of university students, was subjected to an exploratory crosstabs analysis, which showed that participants correctly labeled the emotion in 50.9% of essays and correct labeling differed significantly according to species face ($X^2=131,03$; $p=0$; $df=2$): dogs' were best decoded and chimpanzees least. At a non-conscious level, however there was no such distinction in emotional response: Skin conductance response did not differ according to the stimuli species. Results indicate that people correctly identify an animal emotional facial expression, and because they do it more efficiently if it is a dog than a human or chimpanzee, familiarity may be a key factor in recognition, but not the only one; as results may be related to other aspects of stimuli, such as intensity.

[P53] **“Do you know what I’m feeling?” Children’s perception of animal emotions**

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Children’s perception of emotions (POE) has received considerable attention in research, so there is abundant information about how children see other people’s emotions, but little is known about children’s perception of animal emotions (PAE).

We are currently not aware if the factors that affect POE of other humans overlap with those affecting PAE. In an attempt to contribute to filling this gap, the main goals of this project is investigating perceiver’s demographic and individual factors, as well as contextual and environmental factors likely to influence children’s perception of animal emotions, with an emphasis in the setting in which animals are observed (park vs. zoo). Another goal is applying findings to the improvement of zoo design/management, since can highlight situations that elicit positive/negative emotions in the animals, and correct/incorrect interpretations in the children, who are a major target of zoos. So the project is designed so that PAE is the dependent variable, and experience, age, trait empathy, emotional context and setting are independent variables; gender will be treated as a moderator variable.

This project comprises two studies. The first study will be conducted in sanctuaries and zoos representing different types of environmental design. Chimpanzees will represent the zoo/park animal for *in situ* assessment of PAE. A structured interview will be conducted whenever a child (3-6 years-old) witnesses an emotional situation in the chimpanzee’s group. The second study will be conducted in kindergartens measuring trait Empathy – Emotional-empathy (EE) and Empathy toward animals (impossible to obtain in the field), and the others variables.

Tema: The evolution of behaviour

[P54] **The role of phylogenetic analysis in the study of parental care evolution in fish**

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There is a large body of literature on the evolution of parental care in teleost fishes. Transitions between no care and different forms of care were traditionally analysed using taxonomic units like families and orders. However, the taxa used were often not independent because some forms of parental care originated in ancestors of several species groups. In this study we show, using a phylogeny of the gobiesocoid / blennioid fishes that male parental care arose only once in the ancestral of the entire clade, so that several families and a large number of genera and species represent a single event of parental care evolution.

[P55] **Testing the “Commodity Selection Framework”: can conspecific cues be the key to the evolution of coloniality in birds? (ongoing project)**

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Colonial breeding is a widespread phenomenon in animals, especially in birds, where 13% to 19% of the species live in colonies. The classical hypotheses predict colony formation when the benefits, like reduced predation and enhanced food finding, at least balance the costs of breeding in aggregations. This approach has not, however, provided general answers to the origins of coloniality.

As an alternative, the Commodity Selection Framework (CSF), states that coloniality has not been selected *per se*. It is rather a by-product of fitness-affecting decision-making processes during habitat selection. Because it is physically impossible for each individual to evaluate all the fitness-affecting commodities of one habitat while prospecting for a breeding site, animals may use social cues that indicate the reproductive success of conspecifics, and choose to breed near the most successful ones. This would lead to the formation of breeding aggregations, thus secondarily generating costs and benefits of group living that lead colonies to grow or not.

The scope of this project is to test the CSF using Pagel's general method of comparative analysis for discrete, multistate and continuous variables that tests correlations of trait evolution along phylogenetic trees. We will test correlations between breeding dispersion and various types of conspecific cues related to breeding success and to their level of detectability. We predict that solitary avian species are more prone to evolve colonial breeding if they have traits that make their reproductive success more easily detectable by conspecifics.

[P56] Microsatellite characterization and marker development from massive sequencing data of the blenny *Salaria pavo*

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The number of studies using next-generation sequencing is fast-growing and a new focus has been given to the development of microsatellites from cDNA due to their potential in targeting candidate genes (type I markers). When the microsatellite polymorphism is of interest, developing microsatellites can become time-consuming due to the numerous primer pairs to be tested for polymorphism. Assemblies have a new potential not yet fully explored for microsatellite mining and evaluation, which can help improve the polymorphism rates obtained. Therefore, in this study the transcriptome assembly obtained with pyrosequencing for the blenny *Salaria pavo*, was mined for microsatellites and their polymorphism manually evaluated *in silico*. Two strategies emerged for microsatellite selection and application in a sample of 26 individuals from the islands of Culatra, Formentera and Borovac. Microsatellites were selected based on their *in silico* polymorphism and annotation results (first strategy) or based only on their repetition length (second strategy). From a set of 63 microsatellite loci isolated in *Salaria pavo* sequences, 28 were validated plus one microsatellite from *Lipophrys pholis*. All microsatellites, except 5, revealed to be polymorphic on the 20 individuals genotyped from Culatra Island, the focal population of study. With the results obtained in this work, the second strategy revealed to be more efficient in yielding polymorphic microsatellites than the first strategy (average number of alleles was 6.5 and 3.54 respectively). Nevertheless, merging these two strategies in future studies may help improving the polymorphism results and at the same time develop type I markers.

Tema: Animal testing and Welfare

[P57] Non-invasive measurement of steroid hormones in zebrafish holding water

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Zebrafish (*Danio rerio*) has recently been used in neuroscience research and is a promising model species in neuroendocrine and behavioral studies. Often, the concentrations of steroids in the blood are used to study the endocrinological status of individuals and their response to physiological, social and environmental factors. However, due to the small size of these fishes, blood sampling to quantify hormone levels is not possible without sacrificing individuals.

In this study, it was validated a non-invasive method of extracting cortisol from zebrafish holding water. Briefly, individuals were transferred to 0,5 L of water for one hour, then holding water was filtered and passed through an activated solid phase extraction cartridge. The steroids elution was made with ethanol and the extraction of free hormone fraction was made with diethyl ether. Since steroids are released into the water through the gills, we were able to measure steroid hormones (namely cortisol and 11-ketotestosterone) of water samples and compared the results to steroids in plasma, using enzyme immunoassays (EIAs). Results demonstrate that cortisol released to holding water correlates to plasma levels of this steroid (males: $p = 0,050$, $r = 0,345$, $n = 33$; females: $p = 0,002$, $r = 0,698$, $n = 17$), while 11-ketotestosterone levels do not correlate to plasma levels. Individuals were subjected to ACTH challenge which was responsible for a significant increase of cortisol holding water levels. Finally, the developed method was applied to the study of acute stress agents.

[P58] Swabbing for DNA: The best of two worlds?

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During the last decade, the introduction of molecular techniques allowed to resolve, with unprecedented detail, issues related with parentage. However, the application of these tools depends on our ability to obtain good quality DNA. Invasive techniques, such as tissue collection, provides high amount of DNA, but can also lead to death or damage able to affect reproductive fitness. Non-invasive techniques, although causing no damage, often provide few or poor quality DNA. Ultimately, the choice of the most suitable technique for DNA recovery will depend on the studied species.

Nerophis lumbriciformis is a small pipefish that can be viewed as an interesting model species to study sexual selection due to pronounced sex-role reversal and male pregnancy. We are working on the definition of this species mating system and thus depend on the ability to obtain parental DNA for kinship analysis without harming the fish. Since fin clipping is not adequate (this pipefish has only a small dorsal fin, crucial for locomotion), we opted to collect DNA from the outer surface epithelial layer, using swabs. We show that is possible to collect a fairly amount of undegraded DNA (we provide the results of DNA extractions using freshly collected or ethanol conserved fish), suitable for kinship analysis using microsatellites, without causing harm to the sampled fish (apart from the stress related to capture and handling). We believe that the use of swabs provides a highly promising method for the determination of the mating system of fish species that are extremely sensible to physical damage.

[P59] Ethological observation as a tool for environmental enrichment in birds of prey (Strigiforms)

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Environmental enrichment is assumed as a tool to optimize rehabilitation and promoting ethological study of wild birds in rehabilitation centers. This study focused on physical and social enrichment as a potencial source of stimulus to trigger normal behaviors and provide welfare for individuals in recovery. This work was conducted at the CERVAS (Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens) inserted in Natural Park of Serra da Estrela. The target species were Tawny Owl (*Strix aluco*) and Barn Owl (*Tyto alba*) and enriched a total of 22 individuals, observed by video surveillance, in order to express their natural behavior. Intended to compare the behavioral patterns exhibited by animals in the control vs. enrichment phases. In addition, the implementations of structures, the enrichment protocol also assumed to approach the sociability, thus, were placed juveniles among adults of the respective species. Through the results we see that the predominant activity during the daytime was the “repose” and night time was “vigilance” as agreement with the case in nature for these owl species. In terms of preference structures, we observed that the resting boxes are indicated for the species, occupying the highest and farthest structures from de entrance of facilities. To analyze the variation of daily activities and preference for perches between the control and enrichment periods was accomplished Mann-Whitney test ($P = 0.05$). Observation using the video surveillance allows for a large volume of data, it is extremely important in the ethological study of Strigiforms.

[P60] Effects of environmental enrichment on behaviour and welfare of sheltered dogsCatarina Espanca Bacelar (catarina_bacelar@hotmail.com)¹, Gonçalo da Graça Pereira², Leonor Galhardo¹¹*ISPA-Instituto Universitário, Lisboa, Portugal*²*Faculdade de Medicina Veterinária, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal*

Many dogs spend variable periods of time in kennels for diverse reasons. Usually, kennels are characterized by austere and stressful conditions to dogs and their welfare can be seriously compromised. To prevent or diminish development of physiological and behavioural pathologies that kennel's environment is likely to produce, environmental enrichment is widely used. Long-term housing generates behaviours associated with chronic stress, such as excessive vocalization and auto-grooming or stereotypies. Therefore, behaviour is an excellent tool for measuring animal welfare, has all the advantages of a non-invasive method, and has been used to assess welfare problems in dogs, and other species, in several studies. However, in Portugal there are few studies relating behaviour with welfare of dogs in kennels or with environmental enrichment. The present study aims to find practical and economic ways (such as blankets, cardboard boxes, dog walking, human-dog interaction) to increase sheltered dogs welfare by means of environmental enrichment, using behaviour as a measure of dog welfare. In Canil Municipal de Lisboa, dogs were observed and tested with different environmental enrichments, in order to choose which were the most appropriate for each dog. Then dog behaviour was registered and analysed for signs of stress, before, during and after the environmental enrichment. Results show that during environmental enrichment a significantly decrease in behaviours associated with stress, like stereotype locomotion, yawning, total activity and alert behaviour. In conclusion, simple, practical and economic environmental enrichment seem to be sufficient to reduce stress related behaviours and improve animal welfare.

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