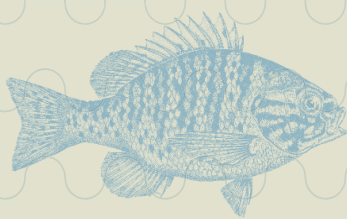


CIBIO Centro de Investigação em Biodiversidade
e Recursos Genéticos - Vairão, Portugal

SPE 2014 

XI CONGRESSO DA SOCIEDADE PORTUGUESA DE ETOLOGIA

09 & 10
OUTUBRO



ORADORES CONVIDADOS:

Nadia Aubin-Horth
(Université Laval, Québec)

Adam Jones
(Texas A&M University)

Marta Moita
(Chamalimaud Neuroscience Programme, Lisboa)

László Garamszegi
(Estación Biológica de Doñana, Sevilla)



INFORMAÇÕES: www.ispa.pt/spe

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PROGRAM

THURSDAY, 9TH OCTOBER

08:30		Registration	
09:30		Opening remarks	
09:50	Session 1	Nadia Aubin-Horth	Ecological genomics of behavioural variation and its underlying mechanisms
	10:40	Sandra Trigo	What does females' carotenoid-based plumage colouration signal?
	11:00	Clara Amorim	Agonistic sound cues for male-male assessment in the Lusitanian toadfish
11:20		Coffee break	
11:40	Session 2		
	11:40	Susana Varela	Negative public information in mate-choice copying helps the spread of a novel trait
	12:00	Diana Carneiro	The relationship between facial coloration, gonads and female size in the sex-role reversed worm pipefish
	12:20	Cristiana Marques	Signalling role of avian barred plumage
12:40		Lunch break	

PROGRAM

THURSDAY, 9TH OCTOBER

14:00	Session 3	Marta Moita	You are not alone: Fear in the context of social interactions
	14:50	Magda Teles	Cognitive appraisal drives neural and behavioural plasticity in Zebrafish
	15:10	Salomé Clemente	Asymmetric reproductive interference among two spider mite species
15:30	Coffee break		
16:00	Poster Walk		
18:00	Session 4		
	18:00	João Messias	The role of dopamine on cleanerfish decision making
	18:20	Àlex Cortada	Causes of intraspecific variation in lizard's antipredatory behaviour
	18:40	Filipa Saraiva	Nonapeptide regulation of helping behaviour in a cooperatively breeding cichlid fish
	19:00	Renata Lopes Alves	Post-conflict affiliative interactions between bystanders in Japanese macaques.
19:20	Plenary Meeting of the Portuguese Ethological Society		
20:30	Dinner		

PROGRAM

FRIDAY, 10TH OCTOBER

09:30	Session 5	Adam Jones	Genomic insights into sexual dimorphism and selection in male-pregnant seahorses and pipefishes
	10:20	Mário Cunha	Quality beyond size: multiple traits combined reflect female reproductive potential in real time
	10:40	Maria Joana Ferreira da Silva	Hunting pressure and sex-biased dispersal: a comparative socio-genetic study of Guinea baboons (<i>Papio papio</i>)
11:00		Coffee break	
11:20	Session 6		
	11:20	Emily Rose	Identifying target genes responsible for mating system variation in two pipefish species using whole brain transcriptomes
	11:40	Leonor Rodrigues	Sperm precedence and potential sexual conflicts in the spider mite <i>Tetranychus urticae</i>
	12:00	Daniel Alves	Estimation of communication ranges in the Lusitanian toadfish (<i>Halobatrachus didactylus</i>)
	12:20	Eliana Soukiazes	Multiple ornaments and sexual selection in a passerine bird: the Serin
12:40		Lunch break	

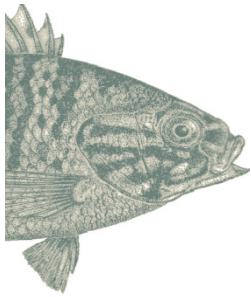
PROGRAM

FRIDAY, 10TH OCTOBER

14:00	Session 7	László Garamszegi	The evolutionary significance of behavioural plasticity: from within-individual variation to among-species patterns
14:50		Ana Cristina Gomes	Speciation involves changing ornamentation, not increased sexual selection
15:10		Gonçalo Faria	The role of mate-choice copying in hybridization
15:30		Coffee break	
16:00		Poster Walk	
18:00	Session 8		
18:00		Júlia Pinho	Different mechanisms involved in fear learning in Zebrafish
18:20		Caterina Funghi	Similar preferences for ornamentation in opposite- and same-sex choice experiments
18:40		Sara Magalhães	The evolution of sexual conflicts in <i>Tetranychus urticae</i>
19:00		Paulo Fonseca	Mating success and parasitic spawning in the vocal Lusitanian Toadfish, <i>Halobatrachus didactylus</i>
19:20		Vítor Almada award Poster award	



ABSTRACTS



ORAL COMMUNICATIONS

SESSION 1

Ecological genomics of behavioural variation and its underlying mechanisms

Nadia Aubin-Horth

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One of our goals as biologists is to understand the proximal and ultimate causes of biodiversity and its consequences. Behaviour often shows a high diversification and can differ between species, populations, individuals and life stages of an individual. This variation has tremendous consequences on fitness, social rank, life history, habitat use and evolution. Studying the mechanisms leading to behaviour divergence in nature allows us to understand how such an incredible diversity is produced and illuminates its evolution. Ecological genomics aims to study genes in natural population in order to determine if the candidate signalling molecules identified by work in laboratory model systems are also implicated in natural variation in behaviour of ecological model species and to uncover convergent evolution at the molecular level. In this presentation, I will illustrate this ecological genomics approach using studies from my laboratory that have looked at behaviour variation between species, populations of a species, and individuals, in two model systems: the three spine stickleback and the African cichlids.

SESSION 1

What does females' carotenoid-based plumage colouration signal?

Sandra Trigo, Ana M. Leitão, Paulo Gama Mota

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 Department of Life Sciences, Faculty of Sciences and Technology of the University of Coimbra, Portugal

It is unclear whether female ornamentation is maintained as a sexually selected signal, a social selected signal, a naturally selected signal, or a nonfunctional by-product of selection on male ornaments. European serins have dichromatic carotenoid-based plumages, with females presenting a much drabber colouration than males, but still exhibiting some yellow colouration and between individual variability. We tested for a possible function of female plumage colouration by performing male mate choice trials and social competition tests for access to limited food. Our results reveal that none of the variables we considered were predictors for female plumage colouration. We found no evidence for male sexual discrimination on carotenoid-based plumage colouration of females. Furthermore, although females established steep hierarchies in groups, dominance over food was not related to yellow colouration. These results suggest that in this species, female plumage colouration is the result of genetic correlation of a male selected trait.

SESSION 1

Agonistic sound cues for male-male assessment in the Lusitanian toadfish

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Acoustic signals may be used in mutual assessment during animal contests but the role of agonistic sounds in fish is poorly understood. Lusitanian toadfish males defend nests using agonistic boatwhistles, which present the main energy in either the fundamental frequency or its harmonics. Here we investigated if agonistic boatwhistles signal male quality features relevant to resolving disputes. We carried out territorial intrusion experiments and related residents' boatwhistle features, with male features, including energy reserves and steroid levels. We found that the main frequency decreased with sonic muscle lipid content but its variability increased with sonic muscle lipid content and relative sonic muscle mass. This suggests that males with higher sonic muscle condition can call with a lower frequency and exhibit higher plasticity in the main frequency of boatwhistle, which may be important in social interactions. Higher mean pulse period (i.e. lower fundamental frequency) reflected higher levels of 11-ketotestosterone (11KT), the main teleost androgen. 11KT was significantly related with male condition (relative body mass and glycogen content). Taken together, this study suggests that agonistic sounds may signal male features that are key in fight outcome.

SESSION 2

Negative public information in mate-choice copying helps the spread of a novel trait

Susana A. M. Varela¹, Mauro Santos² and Margarida Matos³

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Numerous field and laboratory experiments have shown that many species have the capacity for social learning, including mate-choice decisions that can be influenced by witnessing the mating decisions of others. Here we develop a numerical model of mate-choice copying that follows the population genetics tradition, consisting in tracking allele frequencies in a population over time under various scenarios. In contrast to previous evolutionary models, we consider both positive and negative social information because many mating systems are driven by males in pursuit of a mate, and female refusal of copulation may provide negative social information. The inclusion of negative social information to mate-choice copying helps the spread of a novel trait, even if female innate mate-choice preference is biased towards the common male-type. We argue that the presence or absence of copying might simply mirror the associated cost-benefit relationship of the mating system of a given species, and suggest how to test this prediction.

Keywords: Copying, mate choice, negative information, polyandry, social learning.

SESSION 2

The relationship between coloration, fecundity and mating success in a sex-role reversed pipefish

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In sex-role reversed species, and according to sexual selection theory, correctly assessing mate attractiveness is especially important in males. In sex-role-reversed pipefish, coloration was shown to serve as an amplifier of female body size, but other layers of information might have been overlooked. Here, the relationship between the sexually dimorphic facial markings, female size, fecundity and mating success is explored in the sex-role reversed worm pipefish *Nerophis lumbriciformis*. A parentage analyses conducted in a wild population during a full breeding season, using DNA retrieved from embryos and body mucous from adults, revealed that the most colorful females were able to monopolize matings, presenting higher reproductive success. Also, we observed that facial coloration was positively correlated with gonad development. Lastly, we detected that mating episodes influenced the expression of facial markings. We believe that, in the worm pipefish, facial coloration is indeed an honest trait, possibly under sexual selection, able to signal in real time female reproductive status and fecundity. Positively discriminating a female with increased coloration over another of equivalent size but with a less developed pigmentation might be a safer bet for males in terms of potential fitness gains.

Keywords: mate choice, coloration, sex-role reversal, sexual selection, reproduction

SESSION 2

Signalling role of avian barred plumage: a test with common waxbills

C. Marques^{1,2}, H. R. Batalha² and G. C. Cardoso²

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²CIBIO, Research Center in Biodiversity and Genetic Resources, University of Porto

Pigmentation patterns are thought to function as camouflage, because pigmentation patterns are less conspicuous against heterogeneous backgrounds. Barred plumage consists of alternating bars of lighter and darker pigmentation transversal to feathers, and it was suggested that at close range its regularity might indicate plumage quality and therefore simultaneously function as a sexual signal. We tested for the first time this hypothesis. If the regularity of barred plumage is a sexual signal of quality, it is predicted that males and adults develop more regular barred patterns than females or juveniles, as typical of sexual ornaments, and that the regularity of barred plumage correlates with aspects of individual quality. We used a novel method to quantify the regularity of barred plumage in common waxbills, *Estrilda astrild*, and show that, as predicted, there adult males had higher regularity than females or juveniles. Also, we found that higher regularity of barred plumage indicates better body condition. These results are consistent with the hypothesis that the regularity of barred plumage has a communication function, indicating individual quality, and that it evolved at least in part by sexual selection.

PLENARY LECTURE

THURSDAY, 9TH OCTOBER

SESSION 3

Marta Moita

You are not alone: Fear in the context of social interactions

Champalimaud Neuroscience Programme, Fundação Champalimaud

Animals regulate their defense responses using cues from the social environment. They can signal danger to other individuals either actively (e.g. alarm calls) or passively (e.g. display of defense responses). Social interactions have, on the other hand, also been shown to be anxiolytic, a phenomenon known as social buffering, suggesting that animals can also provide safety cues.

In our lab we are studying both danger signaling and social buffering by studying the effects of social interactions between a fear-conditioned rat and its cage-mate. We trained one rat to fear a tone cue and the next day tested its fear of the cue in the presence of its cage-mate. We found that the cage-mate froze upon the display of freezing by the conditioned rat, provided it had prior experience with shock. This suggests that learning from self-experience with an aversive event is important for rats to respond to freezing displayed by others. In addition, using the recorded sound of a rat exploring a box we found rats perceive the cessation of movement-evoked sound (caused by freezing of the conditioned rat) as a signal of danger and its resumption as a signal of safety. Conversely, we found that the presence of a naive cage-mate during exposure to the tone down regulates freezing by the conditioned rat in a long lasting manner. In a complementary set of experiments, we found that conditioned rat dams freeze when tested alone, but switch to active defensive behaviors in the presence of their pups.

SESSION 3

Cognitive Appraisal Drives Neural and Behavioural Plasticity In Zebrafish

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In social species animals tend to adjust their social behaviour according to the available social information in the group. In order to give the appropriate behavioural response, animals need to evaluate the stimulus and the output response will depend on the “meaning” that the stimulus has for the animal in that moment in time rather than its objective (intrinsic) structure. The neural substrate for social behaviour has been identified as the social decision-making (SDM) network. This network is composed by two interconnected neural circuits that are reciprocally connected and regulate a variety of social behaviours. In the current work we microdissected the brain nuclei of the SDM and measured the mRNA levels of the immediate early genes (IEG) *cfos* and *egr1* as a proxy for neuronal activity, and candidate genes related to behavioural plasticity (*bdnf* and *npas4*, *wnt3* and *neurod* and *ngln1*, *ngln2*). For this purpose we used zebrafish (*Danio rerio*) males to study the effects of acute social interactions in rapid changes in the brain. A behavioural paradigm under which male zebrafish consistently express fighting behaviour was used to investigate the effects of different social experiences: winning the interaction, losing the interaction, or fighting an unsolved interaction (mirror image) to unravel the role of perception. Our results indicate that rapid changes in the SDM occur in response to acute social interactions and different social experiences promote distinct neuronal states.

SESSION 3

Asymmetric reproductive interference among two spider mite species

Salomé Clemente, Leonor Rodrigues, Ana Rita Ponce, Cristina Cruz, Sara Magalhães

Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa

Incomplete species recognition can lead to the occurrence of reproductive interference (RI) - reproductive interactions between species resulting in (a)symmetric fitness losses.

We investigated the occurrence and magnitude of RI between *Tetranychus urticae* and *Tetranychus evansi*, two closely related haplodiploid spider mite species that often coexist. Incomplete specific recognition occurs among these species: heterospecific matings were observed, although no hybrid progeny was produced.

Behavioural assays showed that (1) only *T. urticae* females and *T. evansi* males prefer to mate with conspecifics; (2) regarding latency to copulation individuals behave as virgins after mating heterospecifically; (3) *T. urticae* females copulate for a shorter period with heterospecifics than with conspecifics.

Results for fecundity and sex-ratio revealed that (1) heterospecific crosses did not affect egg viability; (2) *T. evansi* females had higher (haploid) fecundity when mating with a heterospecific. (3) *T. urticae* females that mated with a heterospecific male after a conspecific had lower fecundity and less female offspring.

The results obtained point to the occurrence of asymmetric RI, with *T. evansi* females benefiting from mating with heterospecifics, whereas *T. urticae* pay a cost. These results may affect the coexistence of these species, a hypothesis requiring further testing.

SESSION 4

The role of dopamine on cleanerfish decision making

João P. Messias¹; José R. Paula¹; Alexandra Grutter²; Redouan Bshary³; Marta C. Soares^{1,3}

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Presenting author: João P. Messias, CIBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Porto, Portugal

Cleaning interactions are a classical example of mutualistic interactions where both parties benefit from it: cleaners remove ectoparasites and harmful debris off the clients' surface, and consequently contribute for the improvement of reef fish health condition and overall ecosystem welfare. From a functional point of view these interactions are widely studied, however the causal mechanisms underlying these behaviour are still not well understood. Dopamine (DA) is a neurotransmitter involved in the regulation of social behaviour and heavily influences decision-making. As such, it can have an important role in modulating cleaner fish behaviour towards client reef fish. DA agonists and antagonists targeting two different receptors (D1 and D2) were exogenously injected in wild cleaner wrasses (*Labroides dimidiatus*) and their behaviour was then recorded. D1 blockade prompted cleaner wrasses' cooperative investment to rise without increasing cheating events, probably due to impairment of their decision-making competence. D2 blockade also increased cooperative investment but not to the same magnitude, and overall DA stimulation had no effects. This shows that functional DA transmission, rather than high DA levels, have an important role in behaviour modulation and decision-making capabilities on a cleaner wrasse.

SESSION 4

Causes of intraspecific variation in lizard's antipredatory behaviour

Alex Cortada, Miguel A. Carretero

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Lacking defensive devices, lacertid lizards commonly use escape when detected by predators. Nevertheless, an optimal predation entails not only escaping successfully, but also accounting for the associate costs of fleeing. If predation risk and fleeing costs are properly assessed and weighted in the decision-making process before and following escape, a maximized fitness could be reached. In heliothermic lizards the use of refuges carries a substantial thermoregulatory cost, making the recovery behaviour another critical component of the antipredatory behaviour.

In the present work, we analysed factors modifying both escape and recovery behaviours in lizards. We conducted field experiments on populations of the lacertid *Podarcis bocagei* from NW Portugal subjected to divergent predation regimes and habitat types. During days with suitable conditions for lizards' activity, we recorded lizard traits (sex, size class), environment (microhabitat, refuge) and behavioural responses when simulating a predator attack for 80-100 lizards/population.

Of the factors analysed, predation intensity rather than microhabitat accounted for most of variation between populations while sex had the strongest influence within each population. The complex variation observed appears to be in agreement with the predictions of the escape theory.

SESSION 4

Nonapeptide regulation of helping behaviour in a cooperatively breeding cichlid fish

Filipa Cunha Saraiva and Michael Taborsky

Ethologische Station Hasli, Department of Behavioural Ecology, University of Bern, Switzerland

Neurtransmitters from the nonapeptide family translate social stimuli into behavioural responses, thereby regulating social interactions. Arginine vasotocin (AVT) and isotocin (IT) expressed in fish brains are homologs of arginine vasopressin (AVP) and oxytocin (OT) in mammals. Previous studies of these homologs revealed important regulatory functions for a variety of social behaviours, including parental care.

Neolamprologus pulcher is renowned for its remarkably complex social system, which involves an impressive diversity of social behaviours and communication signals. Social groups consist of a breeding pair and up to 20 helpers. Subordinate group members help the dominant breeding pair to defend the territory and to raise offspring. Here we tested for potential effects of AVT and IT on territory defence against predators, and on social behaviours exhibited among group members. We exposed the test fish to different ecological challenges, and studied the effect of social rank. Our results show that isotocin decreased the fear response towards predators, while vasotocin influenced the maintenance of the group by reducing aggression levels and increasing group cohesion. This cohesion was lost when applying Manning compound, an AVT V1a receptor antagonist, independent of the ecological challenge.

SESSION 4

Post-conflict affiliative interactions between bystanders in Japanese macaques.

Renata Lopes Alves¹ and João Rodrigo Daniel^{1,2}

¹ ISPA- Instituto Universitário

² Unidade de Investigação em Psicologia Cognitiva, do Desenvolvimento e da Educação

Conflicts can potentially affect the behaviors and the social relationships, not only of the individuals involved in the confrontation, but also of bystanders. The aim of this study was to characterize bystanders behavior in a captive group ($n = 15$ adults) of Japanese macaques (*Macaca fuscata*). We tested if bystanders responded to conflicts by affiliating with other bystanders, and whether these post-conflict affiliative interactions depended on the quality of their dyadic relationships (value, compatibility and security). Also, we tested if after a conflict bystanders increase their levels of anxiety (self-directed behaviors), and whether affiliative interactions with others bystanders helps to decrease anxiety to baseline levels. We collected 5 min post-conflict focal samples of a bystander and compared them with matched-control observations ($n = 156$). Results show that bystanders after conflicts increase their levels of anxiety and seek affiliative interactions with others bystanders. After these interactions, their anxiety levels return faster to baseline levels. Bystanders tend to seek mostly individuals with whom they have a valuable and insecure relationship. This suggests that these interactions can be a way to preserve relationships that are potentially more affected by the disruptive effects of conflict, but from which bystanders obtain benefits.

PLENARY LECTURE

FRIDAY, 10TH OCTOBER

SESSION 5

Genomic insights into sexual dimorphism and selection in male-pregnant seahorses and pipefishes

Adam Jones

Texas A&M University

New genomic approaches offer unprecedented opportunities to investigate sexual selection and its effects at the level of the genome. These approaches produce a wealth of data, but it can be difficult to choose appropriate questions and to interpret the huge data sets generated by next-generation sequencing. Here, I discuss our efforts to use genomic approaches to understand sexual dimorphism and sexual selection in seahorses, pipefishes, and seadragons. These species are particularly interesting from a sexual selection standpoint, because females transfer eggs to males during mating, and the “pregnant males” carry the brood until it is born. In particular, I will discuss what we know so far about sexual dimorphism in this group at the level of the whole organism and the level of transcriptomes. I will also discuss how genomic data can be used to test evolutionary hypotheses regarding the rapid evolution of genes involved in reproduction.

SESSION 5

Quality beyond size: multiple traits combined reflect female reproductive potential in real time

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Multiple ornaments can evolve due to intra-sexual competition or mate choice. The information conveyed by these morphological characters can complement others or add new layers of information on individual quality. Nevertheless, not all characters have the same plasticity in expression, either due to mechanical and/or physiological constraints, and they are deemed to differ in their ability to provide honest information in real time. Although several examples have already been shown in species where the male is the more competitive sex, information on sex-role reversed species is scarce. Here we use the sex-role reversed black-striped pipefish, *Syngnathus abaster*, where size is known to be a good indicator of female quality, to test if other morphological characters (namely those related with colouration) are able to convey extra layers of information on female quality. Specifically, we tested if the striped pattern, colour and trunk broadness do translate immediate female reproductive potential. Also, we tested the plasticity of these characters by assessing changes before and after mating events. We conclude that although size is generally a good predictor of female quality, the immediate reproductive female potential is better translated by the joint information conveyed by more plastic traits, namely those related with the striped patterns that name this pipefish.

SESSION 5

Hunting pressure and sex-biased dispersal: a comparative socio-genetic study of Guinea baboons (*Papio papio*)

Maria Joana Ferreira da Silva^{1,2,3}, Gisela Kopp⁴, Catarina Casanova^{3,5}, Raquel Godinho², Tania Minhós^{1,4}, Rui Sá^{1,6}, Dietmar Zinner⁴, Michael W. Bruford¹

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Hunting potentially influences dispersal decisions in animal populations and can contribute to intra-specific variation in sex-mediated gene flow. Although context-dependent plasticity in dispersal has been demonstrated in various taxa, studies addressing how these practices shape sex-biased dispersal are rare in the large number of species threatened by hunting. We compared two populations of the Guinea baboon at the same geographic scale but under contrasting levels of hunting: Guinea-Bissau (GB, high hunting pressure) and Senegal (SEN, low hunting pressure) to assess the effect of hunting in the patterns of sex-biased dispersal and in the composition of social units. We found a clear difference in the extent and direction of sex-biased dispersal. The female-biased pattern displayed in SEN was not found in GB, where gene flow was mediated by both sexes. In GB, we found a lower proportion of males and unrelated individuals in social units and the origin of dispersing males was predominantly from genetically differentiated units. We propose that male immigration is the result of evasion from areas of higher mortality risk and/or attraction to male-depleted areas, where reproductive opportunities are greater. We demonstrate that sex-biased dispersal patterns can vary between populations inhabiting similar environments with different levels of human interference.

SESSION 6

Identifying target genes responsible for mating system variation in two pipefish species using whole brain transcriptomes

Emily Rose and Adam G. Jones

Institution: Texas A&M University, College Station, Texas USA

The family Syngnathidae, which includes seahorses, pipefishes, and seadragons, provides an excellent system for studying mating system variation with species ranging from monogamous to polyandrous and polygynandrous. We chose to study changes in gene expression levels in the brains of the monogamous Messmate pipefish, *Corythoichthys haematopterus*, and the polyandrous Gulf pipefish, *Syngnathus scovelli*, to identify target genes responsible for mating system variation. Using Illumina next generation sequencing, we sequenced whole brain transcriptomes from females, non-pregnant males and pregnant males to determine the differential expression levels of genes in the brains of these two pipefish species. We looked at gene expression differences across the two species and between sexes within each species, as well as between brooding and non-pregnant males within and across species. I will be presenting gene expression patterns for two target genes, arginine vasotocin and isotocin, that have previously been shown to affect partner preference formation, pair bonding, and paternal care in other fish species.

SESSION 6

Sperm precedence and potential sexual conflicts in the spider mite *Tetranychus urticae*

Leonor Rodrigues^{1,2}, Salomé Clemente¹, Susana A M Varela³, Rita Ponce¹, Isabelle Olivieri² and Sara Magalhães¹

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First male precedence is thought to occur in the spider mite *Tetranychus urticae*. However, males attempt to copulate with mated females. Possibly, such behavior is linked to the inability of males to distinguish mated from virgin females. Alternatively, the second mating event may be beneficial to males and/or females. To test this, we first performed paternity tests using susceptible and pesticide-resistant strains. We showed the existence of incomplete first male precedence. Next, we performed male choice experiments. Males preferred virgins over mated females. Moreover, latency and duration of copulation with mated females were longer and shorter compared to those with virgins, respectively. Male survival was not affected by the number of females they mated with. However, males placed with mated females survived longer than those placed with virgins. Hence, the cost of mating with mated females is reduced (but so is the benefit). Extra-matings are not costly for females, as the fecundity and offspring sex-ratio of once and multiply-mated females did not differ. In conclusion, males may benefit from mating with mated females, whereas females do not benefit from remating. This may entail sexual conflicts due to disagreement over optima for mating frequencies, a hypothesis that requires further testing.

Key-words: *Tetranychus urticae*; sperm precedence; sexual conflicts; remating

SESSION 6

Estimation of communication ranges in the Lusitanian toadfish (*Halobatrachus didactylus*)

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Acoustic communication is fundamental for successful reproduction in the Lusitanian toadfish *Halobatrachus didactylus*, since calling rate is a good predictor of clutch size. Besides of calling rate the characteristics of the male's call may be used by females in mate choice and by other males in rival assessment. Our aim is to estimate the maximum distance at which this fish is able to perceive correctly features of its mating call in the presence and absence of anthropogenic noise, as anthropogenic noise is a possible source of disruption for underwater acoustic communication.

We used the AEP technique to characterize the physiological response of individuals to sounds recorded in natural conditions at different distances from the sender, with and without masking noise. Our results estimate a maximum communication range in shallow waters varying between 6 and 13 meters, depending on the frequency content of the fish advertisement call (the boatwhistle). These data will allow us to better understand the ecological limits and the impact of human activities on the acoustic communication and reproduction of this highly vocal fish.

SESSION 6

Multiple ornaments and sexual selection in a passerine bird: the Serin

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A wide range of animals have more than one sexual ornament and much attention has been devoted to understand how they evolved. Frequently, birds exhibit simultaneously, a large variety of acoustic and visual ornaments, providing a multiplicity of signals with informative content. The Serin, *Serinus serinus*, is a small monogamous sexually dimorphic passerine bird, where males court females singing and exhibiting their colouration. It has been shown that song and colouration are involved in mate choice in serins, as females showed preference for higher frequency songs and for more colourful males. In order to understand if these traits signals the same qualities of individuals and the extent to which ornament expression reflects individual condition and behaviour, we studied the relationship between song and colouration and indicators of male quality: body condition, body size, immune capacity (PHA-P) and physiological response to stress (breath rate). We found that colour saturation predicted body condition and varied with age, whereas age has a relation with body size. Our findings meet the multiple message hypotheses, and suggest that multiple ornaments may convey information on different aspects of male quality.

SESSION 7

The evolutionary significance of behavioural plasticity: from within-individual variation to among-species patterns

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Estacion Biológica de Doñana, Sevilla

One of the most spectacular aspects of animal behaviour is that it varies considerably even within the same individual in a very short period of time. However, most studies in ethology and behavioural ecology focus on individual-specific behaviours (i.e. mean behaviour) while neglecting within-individual variation. I will argue that such an approach may be misleading, because disregarding such components of variation may lead to loss of important biological information. I will provide a simple statistical framework based on mixed modelling to demonstrate how questions about the evolutionary importance of behavioural plasticity and consistency can be tackled when multiple measurements are available within individuals. Furthermore, this framework will be extended to higher hierarchical levels to allow inferences for the between-population and between-species patterns of variation in behaviour. The concept will be supported with illustrative examples. Field data from the collared flycatcher (*Ficedula albicollis*) will be used to show the biological relevance of within-individual variation in song post exposure. In addition, results from phylogenetic comparative approaches and meta-analyses will also be given to demonstrate how higher level of variations (i.e. between-population and between-species) in behaviour can be studied.

SESSION 7

Speciation involves changing ornamentation, not increased sexual selection

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Sexually-selected ornamentation mediates species recognition and reproductive isolation but, collectively, comparative work has failed to demonstrate a relationship between the strength of sexual selection and speciation. Since evolutionary changes in ornaments often involve repeated gains and losses, rates of phenotypic evolution may not increase with the strength of sexual selection, because alternation of gains and losses becomes rarer. Therefore, we propose that stronger sexual selection may not increase speciation rates, even if changes in sexually selected ornamentation do promote speciation. To test these ideas, we studied colour ornamentation in estrildid finches. Increased ornamentation (as measured by maximum or mean plumage colour saturation, achromatic contrast, extent of ornamental coloration, or bill colour) was not related to the quantity of speciation through the phylogeny, or to the time since the most recent speciation. Most ornamental traits fit a model of speciational evolution better than a model of gradual evolution, meaning that changes in ornamentation are associated with, and perhaps promote, speciation. Together, these results indicate that diverging ornamentation, rather than the strength of sexual selection per se, is implicated in speciation. Past work relating the strength of sexual selection to speciation may have greatly underestimated the importance of sexually-selected ornamentation in speciation.

SESSION 7

The role of mate-choice copying in hybridization

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Mate-choice copying (MCC) by females occurs when they obtain information about the performance of a male, increasing or decreasing their preference for that male, accordingly. It has been proposed that MCC can lead to reproductive isolation between populations from the same species, since it can be responsible for a cultural background of sexual choices. However, if invader individuals copy the choices of natives, this can also lead to a hybridization event. We studied this hypothesis using different populations of *Drosophila subobscura*, originated from the extremes of the species European latitudinal cline: Portugal (PT) and the Netherlands (NL). Half of the females received positive social information about a male from their own population and the other half about a male from a control (lab) population. At generation six in the lab, we saw that females had an innate preference for the males of their own population, and that they did not copy. After four more generations of convergent adaptation in the lab, PT females started to mate-choice copy. This suggests that MCC may be selected in nature only under specific ecological and social contexts and it supports the hypothesis that MCC is most advantageous when males are phenotypically similar.

Keywords: Copying, mate choice, social learning, hybridization, *Drosophila subobscura*.

SESSION 8

Different mechanisms involved in fear learning in Zebrafish

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Animals have the ability to learn by trial-error or observing conspecifics. The observational learning has less cost and risks, being biologically more rentable.

The aim of this work is to understand the mechanisms behind different types of fear learning in Zebrafish: classic conditioning, social conditioning and social facilitation.

In classic conditioning, a light (conditioned stimulus, CS) was delay-paired (10 seconds) with alarm cue (unconditioned stimulus, US) (3 trials/day during 3 days) inducing erratic movement and freezing. In social conditioning, a naïve fish observed a pre-conditioned demonstrator which is responding to CS (3 trials/day during 3 days). In social facilitation, a pre-conditioned fish demonstrates fear response after CS but the observer fish was sacrificed after the first fear response.

Behaviorally, classic conditioning and social facilitation presented a similar pattern (erratic movement followed of freezing). However, the potency of response learned by observation is weaker. In social conditioning, the observer fish in the trial test has already extinguished the response.

Looking at the expression of several immediate early genes (IEG), the olfactory areas were differently activated within the types of fear learning described. We observed a decreased of expression of IEG in classic conditioning and an increase in social facilitation that is abolished in social conditioning.

In conclusion, Zebrafish are able to learn classically or by social facilitation involving differential mechanisms.

SESSION 8

Similar preferences for ornamentation in opposite- and same-sex choice experiments

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Selection due to social interactions comprises competition over matings (sexual selection) plus other forms of social competition and cooperation. Sexual selection explains sex differences in ornamentation and in various other phenotypes, but does not easily explain similarities between the sexes. Understanding such similarities requires knowing how phenotypes influence non-sexual social interactions as well, which can be very important in gregarious animals, but whose role for the evolution of sex differences and similarities has been overlooked. For example, ‘mate choice’ experiments often found preferences for ornamentation, but have not assessed whether those are strictly sexual or are general social preferences. Using choice experiments with a gregarious and mutually-ornamented finch, the common waxbill (*Estrilda astrild*), we show for the first time that opposite-sex preferences for ornamentation also extend to same-sex interactions. Waxbills were able to respond differently to opposite- and to same-sex individuals, but most preferences for colour traits were similar when interacting with either sex. Similar preferences in sexual and non-sexual associations may be widespread in nature, either as social adaptations or as by-product of mate preferences. In either case, such preferences can set the stage for the evolution of mutual ornamentation and of various other similarities between the sexes.

SESSION 8

The evolution of sexual conflicts in *Tetranychus urticae*

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In haplodiploids, males always benefit from female-biased sex allocation, whereas for females the optimal sex ratio ranges from highly female-biased under strict local mate competition (LMC) to even in panmixia. Therefore, sexual conflict over sex ratio should occur in panmixia, which might select for manipulative traits in both sexes. Although this sexual conflict is straightforward in theory, its evolutionary consequences have not been investigated experimentally. We addressed this issue by crossing spider mites that evolved under varying levels of sexual conflicts, generated by manipulating the intensity of local mate competition, to mates from isogenic lines. Under high levels of sexual conflicts, both males and females evolved manipulative traits to turn the sex ratio to their own advantage, whereas no manipulative traits evolved in absence of conflicts. In females, sex ratio control was achieved through changes in egg size. This study highlights the extraordinary potential of LMC theory to address sexual conflicts over the sex ratio, and demonstrates that males can have some control over sex allocation in haplodiploids.

Keywords: sexual conflicts, local mate competition, experimental evolution, *Tetranychus urticae*

FRIDAY, 10TH OCTOBER

SESSION 8

Mating success and parasitic spawning in the vocal Lusitanian Toadfish, *Halobatrachus didactylus*

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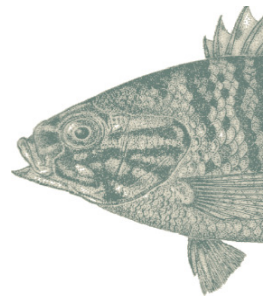
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Calling rate in Lusitanian toadfish reflects male quality and appears to predict male reproductive success assessed by egg number, but this proxy may not be adequate if sneaking or nest takeovers occur. Here we estimated the percentage of sired eggs by toadfish nest-holders and the probable number of egg-laying females using 5 microsatellite polymorphic markers. Males were allowed to mate in two setups in the Tagus estuary that differed in the degree of exposure to sneaking and nest takeovers. We further related the number of sired eggs, number of egg-laying females and the number of eggs per female with male quality. The percentage of paternity and the number of egg-laying females was similar in both setups (88% vs 82% sired eggs and 2.3 vs 2.2 females) suggesting that extra paternity is mainly due to parasite fertilizations by type II sneaker males. Regression analyses showed that the number of sired eggs, number of egg-laying females and the number of eggs per female were negatively associated with androgens. Male condition was also a positive predictor of the number of females spawning in the nest in line with previous studies that associated male condition with mate choice.

ABSTRACTS



POSTER COMMUNICATIONS



POSTER WALK 1

1

Validation of the light-dark test as a model of anxiety for rats

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The light-dark (LD) model is widely used with mice, and to a lesser extent with rats for anxiety evaluation, however it is only validated for mice. In order to test the value of the LD in rats as a model of anxiety three sets of validity criteria were analysed. Thus, behavioural, neurochemical and hormonal evaluations in the presence or absence of an anxiolytic drug (diazepam) were performed, and a comparative analysis of these evaluations in the LD and in the elevated plus maze (EPM), the most used test to evaluate anxiety, was established. Results showed that the EPM and LD tests induce similar behavioural and neurochemical responses. This generalization of responses in both behavioural tests constitutes an evidence of the constructive validity of the LD model. The hormonal data showed no increase in corticosterone after exposure to the EPM or LD, which points to the absence of face validity. Although diazepam has no anxiolytic effects in behavioural responses, it induces neurochemical alterations in the hippocampus consistent with anxiolytic effects in the EPM model. In the LD diazepam had no efficacy. These results impaired the predictive validity of the model. The present work argues against the use of the LD model in rats as a model of anxiety, however some aspects of the procedure may influence the results and are discussed.

POSTER WALK 1



Sex differences, mate choice and personality in the European Serin, *Serinus serinus*

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Animal personality has increasingly been the focus of studies to understand the evolution and consequences of stable behavioural differences among individuals. While several hypotheses have been considered, sexual selection has been scarcely studied although it may play an important role in the origin and maintenance of personality differences. Using a gregarious species, *Serinus serinus*, we tested for consistent behavioural differences among individuals of females and males in different behavioural contexts – tonic immobility, new object, exploration, and mirror tests. Additionally, we investigated the role of personality traits in female mate choice. We showed that males and females differed in their behavioural responses and in their consistency across the different tests. Furthermore, we found that individual's personality affected their performance in the mate choice tests. Our results stress the importance of looking for sex differences in personality and of considering the influence of personality in mate choice contexts.

POSTER WALK 1

3

An experimental study of interference behaviour in thermoregulating lizards

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Many lizards use behavioural thermoregulation to achieve and maintain a narrow range of activity body temperatures. Thermoregulating lacertids shuttle between hot and cold areas and in between exhibit many other types of behaviour, namely foraging, avoiding predators, and having interactions, either reproductive or agonistic. We investigated relationships between thermoregulation and behavioural interaction in two potentially competing sympatric lizards. We used a realistic experimental design allowing two males to thermoregulate and interact on a restricted sun spot. Lizards were filmed for 24 minutes and recordings were examined to determine the frequency of interactions and body temperatures were estimated from lizards' positions along the thermal gradient. Significant variation in the frequency of both behaviours by species, test (conspecific, heterospecific) and side of the body was detected. *I. horvathi* approached and touched the other lizard only if it was a conspecific and with a higher intensity. *P. muralis* more frequently approached or touched heterospecific males. *P. muralis*' interactions were associated to warm areas, while *I. horvathis*' were associated to hot areas. Touches were usually directed to the head, while approaches were mostly directed to the side or the back in both species. Results reinforce the asymmetric ecological interaction proposed for these species pair.

POSTER WALK 1

4

Aspects of the behavioural ecology of a group of gray woolly monkeys, *Lagothrix cana*, living in an ex-situ conservational area, in Central Amazonia, Brazil

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Lagothrix cana is an endangered species and information on their general ecology is still limited. We analysed the habitat use, diet and social behaviour of eighteen *L. cana* living in an *ex-situ* conservational area, in Central Amazonia. Fieldwork was conducted over wet-season months, from February to June 2013. The animals have been rescued from the illegal animal trade, thus they were provisioned twice a day. Behavioural observations were conducted through scan sampling and focal animal methods. The group spent most of their time in the peripheral forest around the provisioning area, where they obtained about 79% of their daily diet. They complemented it with local resources such as fruits (65%), leaves (21%), sprouts (12%), and some invertebrates. The group spent 25% of its time foraging, 23% travelling and 22% interacting. Grooming interactions occurred mostly between mother and infant. Play-related behaviours were most frequent among young males. Aggression was more commonly directed from adult males to adult females. Agonistic interactions were also observed between the group and two primates species in the area. Comparing the behavioural ecology of this population to other populations of *Lagothrix* may help establishing an effective strategy to prepare these monkeys to wild life.

POSTER WALK 1

5

Unusual Behaviour in Gray Woolly Monkeys (*Lagothrix cana*): Females Breastfeeding Adult Males

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To our knowledge, breastfeeding between adult individuals has never been reported in non-human primates. From March to June 2013, we conducted a study on a group of 18 gray woolly monkeys, *Lagothrix cana*, living in an ex-situ conservational area in the Brazillian Amazonia. Through ad libitum observations, five breastfeeding events between adults were reported in detail. The events involved two adult females and two adult males and ranged from 10 to 90 seconds. In all situations the males were the initiators of the breastfeeding while the females involved were relaxed and didn't try to push the males away. The other individuals of the group continued in the activities they were conducting, i.e. foraging, moving, playing and resting. Our findings are in line with some of the allonursing hypotheses, though the age of the nurse far exceeds that typically found in the allonursing literature. A likely explanation for these cases is that the female woolly monkeys use breastfeeding to move up the hierarchy, through alliances with top ranking males. Further systematic and long-term observations of this and other populations of woolly monkeys would help to bring additional insights about the evolution of breastfeeding behaviour between adult, non-human primates.

POSTER WALK 1



Feral Cats (*felis catus*): Differences between ecological niches and food availability

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The aim of this study was to realize how feral cats' behaviors can change at different environments and at different times of the day depending on the distribution and abundance of resources. With this goal three different populations were studied, in three urban zones (near the city center, near the beach and near a disused factory) having each one different food resources. A video capture was made at three moments of the day [10:50, 12:50 and 17:20] for all groups during some weeks. The frequency and duration of cats' behaviors were analyzed with the Observer software. Results for the three cats' populations and the three different moments of the day were compared with a Kruskal-Wallis test. Significant results were obtained for the moment 12:50 when compared either with 17:20 or 10:50. Some behaviors also showed significant differences regarding cats' population belonging, namely Eat and Drink that was more frequent in the population that have food in abundance (factory environment). According to these results one may expect that this cats' population need less to hunt/search for food than the other ones do.

POSTER WALK 1



One year with *Chioglossa lusitanica*

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During 10 months, the behavior of *Chioglossa lusitanica* was recorded using an infrared video surveillance camera. Those recordings were captured in a gold mine at Valongo, near Porto, with the presence of this species during mating season. With this recording an etogram was made and we analyzed the differences in the behavior along a 24 hour and 10 months periods. Aggressive behaviors, sexual interference (that we start to think is satellite behavior) and another interactions that seems to be acknowledgment/communication between individuals were observed. The frequency and duration of behaviors was analyzed through the Observer program from Noldus Information Technology.

POSTER WALK 1

8

Does interspecific competition affect the plant colonization behavior of invasive and resident herbivorous spider mites?

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Invasive species, lacking natural enemies in invaded areas, are likely to exert a strong competitive pressure on native species. *Tetranychus evansi*, originating from South America, recently invaded Mediterranean countries. It is specialized on Solanaceae plants, with tomato as preferred host. The resident spider mite *Tetranychus urticae* also attacks tomato plants, hence both species are likely to compete. Unlike *T. urticae*, which up-regulates tomato anti-herbivory defenses, *T. evansi* down-regulates them. Moreover, in laboratory studies *T. evansi* out-competed *T. urticae* on tomato. Here, we tested whether within- or between-plant distribution of these mite species is affected by interspecific competition. When colonizing clean tomato plants, both species preferred younger leaves but lost this preference when such leaves were infested with heterospecifics. Additionally, *T. evansi* preferred older leaves when these were infested by *T. urticae*. Finally, *T. urticae* did not discriminate between clean and infested plants, whereas *T. evansi* preferred plants infested with heterospecifics.

The within-plant avoidance of *T. evansi* by *T. urticae* may result from interspecific competition. *T. evansi* did not avoid *T. urticae* possibly because it can out-compete the latest on this host. This study shows that the presence of heterospecifics on tomato affects differently the colonization behavior of these spider mites.

Keywords: Invasive species; inter-specific competition, plant colonization, spider mites.

POSTER WALK 1

9

Larval behavior of *Mytilus galloprovincialis* in laboratory condition

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Many marine invertebrates and fish have a pelagic larval stage in their life cycle. Larval dispersal patterns can determine local population size and the degree of connectivity between sub-populations, providing crucial information for Marine Protected Areas (MPAs). Larval behavior is a key component of larval dispersal. Vertical migrations between layers flowing in opposite directions have been reported as a mechanism able to regulate larvae transport along the coast and control offshore dispersal. Bivalve vertical migrations are greatly influenced by responses to physical or hydrographic variables (haloclines, thermoclines and food available), are highly variable, specific to each species and dependent on the stage of development (veliger/pediveliger).

We performed laboratory experiments manipulating salinity gradients (24/35‰; 30/35‰ and 34/35‰) to understand how the presence of physical discontinuities, can interfere in *Mytilus galloprovincialis* larvae behavior at two different developmental stages (veliger and pediveliger). The veliger larvae showed a tendency to swim to the surface in the absence of haloclines, and stay below or at the discontinuity when the surface salinity showed values under 34‰. However pediveliger larvae showed a tendency to remain in the mid-bottom depth even in the absence of haloclines

Keywords: Veliger, Pediveliger, *Mytilus galloprovincialis*, vertical migrations, larval dispersal, larval behaviour

Does behavioural avoidance play a role in spider mite defences' against bacteria?

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The spider mite *Tetranychus urticae* is unable to mount an immune response to bacterial infection. It is thus highly vulnerable to infection, with bacteria imposing high mortality upon spider-mite populations. Hence, strategies to avoid contact with bacteria are expected to be selected.

Here, we tested if *T. urticae* is able to avoid the bacteria *Escherichia coli* or *Pseudomonas putida*, the latter being a known *T. urticae* pathogen. To this aim, we offered infected and uninfected pigmented liquid inside parafilm bubbles to the mites for 48 hours. We then measured the percentage of individuals that fed on the bubbles, by counting the amount of pigmented mites. For both bacteria tested, fewer mites fed on contaminated than on uncontaminated bubbles. Next, we tested whether odours were involved in this avoidance behaviour using a Y-tube olfactometer. Mites did not seem to discriminate between bacteria odours and clean air.

Our results suggest that spider mites are able to avoid bacteria, but olfactory cues do not seem to trigger this behaviour. How effective this behaviour is in preventing infection in natural populations is an open question.

POSTER WALK 1

11

Vigilance behaviour of *Oryx gazella* and *Aepyceros melampus petersi* on the presence of visitors in the Lisbon Zoo

Inês Pacheco, João Castelo Branco, Rita Ramos, Wilson Vieira

Faculdade Ciências da Universidade de Lisboa em colaboração com o Jardim Zoológico.

Vigilance behaviour in animals is very important to detect and avoid predators. However, under human care, animals are not exposed to this factor. They may be exposed to human presence, but it does not represent a risk for them. It would be expected that they would lose their vigilance behaviour, but there are studies that show that wild animals in non-natural environments often maintain it. With this in mind, it was intended to know whether zoo conditions can affect this behaviour in ungulates by choosing two different species and measuring frequency of vigilance behaviours to verify if zoo's visitors can affect it. Moreover, by comparing the two species vigilance frequency, it may be possible to understand if there are any differences in the species vigilance behaviour. This study reveals that both species maintain, under Zoo conditions, a certain level of vigilance despite having different vigilance frequencies. Indeed, *Aepyceros melampus petersi* (Black-faced-impala), is more susceptible to have that behaviour than *Oryx gazella* (Gemsbok), and to change its frequency according to the number of visitors. This could be explained due to different ecologies of the species, where the first one is more preyed than the second, increasing the frequency of vigilance behaviours.

POSTER WALK 1

12

Effect of *Wolbachia* removal in mating discrimination among populations of *Tetranychus urticae*

Inês Santos, Leonor Rodrigues, Flore Zélé, Sara Magalhães

Wolbachia is a widespread endosymbiotic bacterium, commonly found in populations of the two-spotted spider mite *Tetranychus urticae*. These bacteria are known to induce reproductive manipulations in hosts and consequently affect host populations dynamics. We investigate the effect of *Wolbachia* in mating behavior using populations of naturally-infected *T. urticae* collected from the field. Female mate choice tests were performed three months after sampling. Females from one population (TuDF) were given the choice between individuals from their own and those of five other populations, in pairwise tests. When infected, TuDF females preferred to mate with males from their population only when presented with males from one other population (TuLou). Patterns of mate choice could not be explained by differences in fecundity resulting from such crosses. After six months sampling, tests were repeated with infected and uninfected TuDF and TuLou mites. This time, no preference was observed irrespective of the presence of *Wolbachia*. Loss of preference may be due to laboratory adaptation of both populations to the same rearing conditions. Currently, *Wolbachia* does not seem to play a role in mate choice in these populations. Further tests will allow gathering knowledge of the dynamics of mate choice, in populations with or without *Wolbachia*.

POSTER WALK 1

13

Mice preferences for different types of nest material

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The type environmental enrichment has an impact on the health and well-being of animals and may cause biased experimental results. In this context, the demand for environmental standardization in laboratory animals is emergent. Requirements for enrichment materials, particularly those regarding animal comfort are poorly supported by experimental data. In this study, we tested the preference of balb/c mice for cheap nest material (Eggs box, paper tubes and paper) commonly used as environmental enrichment for mice. The preference test system consisted of two type II cages, provided with sawdust and ad libitum food pellets and tap water, connected with a PVC tube. One of the test cages was additionally furnished with tubes or an eggs box. To assess the strength of preference, both nest materials were also tested against a highly preferred nesting material. Preferences were established as a measure of time spent in two choice compartments. The results seem to indicate a preference for eggs box. Differences between eggs box and paper tubes are discussed regarding their attractiveness to mice. Affording a cage with a preferred nesting material can be a simple method to supply the well-being of laboratory mice. This worked was funded by FCT – Fundação para a Ciência e a Tecnologia - project ref: PTDC/MHC-PAP/5304/2012

POSTER WALK 1

14

Grouping behaviour in red deer: an anti-predator strategy?

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Grouping behaviour is a common phenomenon in animals, which is frequently addressed as an anti-predator strategy. However, the existence of intraspecific variation in grouping behaviour suggests that other aspects like density, availability of resources, habitat heterogeneity and social interactions may also play an important role. We used direct observations of animals to understand the capability of the anti-predator strategy in explaining the grouping behaviour of red deer *Cervus elaphus* in a Mediterranean mountainous environment. We also analysed the influence of local population density and land cover. The results showed temporal variations in group size and composition, as well as an influence of local population density and land cover. A more pronounced increase in the number of groups than in group size was observed in areas with a higher population density. As expected, in open areas red deer forms larger groups. The results suggested that grouping behaviour in red deer is not only a response to the presence of predators but also influenced by density and habitat type. Our results provided support for the anti-predator strategy, indicating that even in populations without or with low levels of predation, the natural anti-predator response continues to modulate the grouping behaviour in red deer.

POSTER WALK 1

15

First report of cleaning behaviour by the white seabream *Diplodus sargus* (Sparidae)

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Cleaning behaviour, with the *cleaner* species being responsible for the removal of ectoparasites or injured tissue from the *client* species, is characterized either by obligatory or facultative/complementary mutualism. This last category encompasses all the examples known for fish. The most common cleaner fish species belong to the Gobiidae and Labridae families, however cleaners can also be found in other families. Cleaning behaviour was never described for the omnivorous species *Diplodus sargus*, although previous studies have reported the existence of ectoparasites in their stomach contents. Here we report this behaviour for the first time which was exclusively performed by juvenile *D. sargus* less than 2 years old (2.5-7.5cm TL). This behaviour was mainly but not exclusively directed towards adult grey mullet (Mugilidae) within sheltered areas and near the surface. It was surprising that it was never described before because group scan observations revealed frequencies as high as 23 successful interactions and 85 failed interactions per hour. Occasionally, cleaning was “requested” by the *client* but most frequently it is followed by active escape. Failed interactions also reveal uncertain willingness of the potential *client* to be cleaned and may help explain why only juvenile *D. sargus* are allowed to clean other fish.

POSTER WALK 1

16

Different mechanisms involved in fear learning in Zebrafish

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Animals have the ability to learn by trial-error or observing conspecifics. The observational learning has less cost and risks, being biologically more rentable.

The aim of this work is to understand the mechanisms behind different types of fear learning in Zebrafish: classic conditioning, social conditioning and social facilitation.

In classic conditioning, a light (conditioned stimulus, CS) was delay-paired (10 seconds) with alarm cue (unconditioned stimulus, US) (3 trials/day during 3 days) inducing erratic movement and freezing. In social conditioning, a naïve fish observed a pre-conditioned demonstrator which is responding to CS (3 trials/day during 3 days). In social facilitation, a pre-conditioned fish demonstrates fear response after CS but the observer fish was sacrificed after the first fear response.

Behaviorally, classic conditioning and social facilitation presented a similar pattern (erratic movement followed of freezing). However, the potency of response learned by observation is weaker. In social conditioning, the observer fish in the trial test has already extinguished the response.

Looking at the expression of several immediate early genes (IEG), the olfactory areas were differently activated within the types of fear learning described. We observed a decreased of expression of IEG in classic conditioning and an increase in social facilitation that is abolished in social conditioning.

In conclusion, Zebrafish are able to learn classically or by social facilitation involving differential mechanisms.

POSTER WALK 1

17

On personality, energy metabolism and mtDNA introgression in bank voles.

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Animal personality is emerging as an important determinant of a wide range of life-history traits and fitness. Individual behaviour, however, may be constrained by among individual variability in energy metabolism and may become unstable due to intrinsic and extrinsic stressors. Here we test the relationship between personality and physiology using wild caught bank voles, *Myodes glareolus*, that vary according to mtDNA type (original or introgressed from *Myodes rutilus*). Personality traits and their within-individual consistency were assessed using an open field test and basal metabolic rate (BMR) was measured in an open-flow respirometer. A significant relationship was found between individuals' consistent (repeatable) personality trait (principal component analysis score reflecting individual differences in proactivity) and their consistent (repeatable) residual BMR (body mass corrected); however, this association depended on mtDNA type and sex. Particularly, the males with original mtDNA showed a positive relationship between proactive behaviour and BMR, which supports the increased-intake model, stating that BMR is positively related to the capacity to engage in costly behaviours. However, this relationship was disrupted in introgressed males, and showed a negative trend in females, suggesting the alternative compensation model. Consistent differences in behavioural patterns and mtDNA types promote variation among individual in energy metabolism.

POSTER WALK 1

18

A collaborative and distributed computational tool for behavior analysis: OpenCSP

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The development of software for behavior analysis is expensive, requires time and talented human resources to develop, maintain and operate. Is not uncommon that these software are wasted after being published because there is no one to support them. For small labs, the spend of resources in experimental software is an exotic luxury and for the ones that can afford it, in not rare cases they run into reinventing the wheel. In an effort to reduce these issues and increase the exchange of knowledge between labs and therefore improving the quality of the developed behavior analysis software, we propose the use of the Open Computational Scheduler Platform (OpenCSP). OpenCSP is a web based computational tool that centralizes the maintenance and distribution of the software. Once installed or updated the software becomes immediately available for every user with access to the tool. Users have access to the software through a web interface where they can schedule applications to run with no need of any installation or advanced skills to do it. Also because the OpenCSP operates under a computational grid several analyses can be made in parallel and in a scale that would not be possible with individual workstations. Labs will have their generated knowledge kept and maintained in the OpenCSP, and their budget for powerful workstations may be redirected to other areas, because the grid optimizes very well its resources. The OpenCSP has already available video tracking, spikes sorting and brain alignment algorithms that have been used in the context of neurosciences, in which has been proving to be a great tool for researchers and developers.

Keywords: Tracking algorithms, Development of scientific software in collaboration, Data analysis, Distributed computation, Resources optimization.

POSTER WALK 2

19

Behavioural Study of European-Otter (*Lutra Lutra*) In Captivity

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The European-otter is an aquatic mammal distinctive of the Portuguese territory. Despite its observation in the wild to be complicated due to the fact of being elusive and having a low life expectancy is still possible to do some studies, such as evidence of their presence through footprints or even their diet by analysis of the excrements. However behavioural studies are scarce and easiest to do in captivity.

The aim of this study is to elaborate a detailed ethogram for this species and understand how the behaviors observed in captivity contribute to animal welfare. To accomplish this purpose, footage with ten minutes each of one male and one female with her three cubs were performed in Gaia Biological Park. All of the behaviors observed were organized in an ethogram and ultimately evaluated in terms of frequency / duration. The analysis of behaviors may indicate whether an animal is in a stress situation or instead a situation of well-being.

This study will help to improve an understanding of the habits of this species and to know what must be done in order to make the experience of captivity as healthy as possible.

Keywords: European-otter; captivity; ethogram; animal welfare

POSTER WALK 2

20

Peafowl social behaviour

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Peafowl is a favourite subject for the study of sexual selection, mate preferences or lek behaviour but it is poorly known in its social behaviour, kin relationships and cognition.

Observations made during 2013 and 2014 in feral *Pavo cristatus* (5 ♂s, 9 ♀s adult, 24 chicks and immature) indicate that peafowl has a complex social behaviour that may explain contradictory results on, for instance, mate preferences [1], [2]. Peafowl behaviour show several traits present in social species of birds and mammals such as male and female hierarchy independent of physical conditions (males present work, females [3]), but dependent perhaps on age, individual recognition, kin recognition and group exploratory behaviour (see also [4]), selective and non selective grouping behaviour (see also [5]), association between mothers and share of broods, adoption of orphaned brood and allopreening; they emit also several types of vocalizations encoding different kinds of information [3]; females transmit information on edible items to the chicks by postural and acoustic signals.

[1] Dakin, R., Montgomerie, R. (2011), *Animal Behaviour*, 82(1): 21-28; [2] Takahashi, M., et als. (2008), *Animal Behaviour*, 75:1209-1219; [3] Yorzinski, J.L. (2014), <http://dx.doi.org/10.1121/1.48613>; [4] Petrie M., et als. (1999), *Nature*, 1999, 401, 155–157; [5] Rands, M. R. et als, (1984), *Animal Behaviour*, 32: 830 – 835.

POSTER WALK 2

21

Modulation of acoustic signalling and territorial behaviour by androgens in a vocal fish

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Steroid hormones are rapidly released into the plasma in response to social interactions or to acute stress and regulate behaviour including sound production. Numerous teleost fish species produce sounds during agonistic and reproductive behaviour but the regulation of vocal mechanisms by steroids is still poorly understood in this taxon. Breeding Lusitanian toadfish males (*Halobatrachus didactylus*) use sounds (boatwhistles) to defend nests from intruders. Previous studies with territorial intrusion experiments suggest that boatwhistles (BW) are the main 'keep-out signal' but whether boatwhistles features are modulated by steroid hormones remains to be studied. Here we experimentally tested if 11 ketotestosterone (11KT), the main teleost androgen, can modify territorial behaviour and BW production (number of signals and sound features) by staging territorial defence experiments using males with experimentally elevated 11KT through intraperitoneal implants (1 mg/1000g 11KT dissolved in coconut oil). Preliminary data suggest that 11KT implanted males produced less BW but suffered fewer nest takeovers than control (sham-implanted) males indicating that BW of 11KT males maybe more effective as keep-out signals. BW features of 11KT and sham males will be compared to ascertain this hypothesis.

POSTER WALK 2

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The “woman in red” effect: can a pregnant male influence embryo development at the sight of a sexier female?

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Life history theory predicts that investment in reproduction should mirror the expected fitness benefits. Thus, it seems reasonable to expect that both sexes should be capable of modulating investment in reproduction according to male attractiveness, either prior or even after fertilization. Although several post-copulatory sexual selection mechanisms acting during embryonic development have already been described in viviparous species, these are currently circumscribed to females. In the family Syngnathidae, however, pregnancy occurs within the male’s body, a fact that converts this fish group into a unique model to assess previously undescribed post-copulatory sexual selection mechanisms occurring within the male’s body. Knowing that pipefish males prefer larger partners, a male already carrying eggs from a small low quality female, was presented (visually and chemically) to a large sexier female. Juvenile length was measured as a proxy for fitness. These results were then compared to those emerging from pregnancies where the male was kept observing the same small female responsible for egg deposition. This allowed us to test our prediction that the glance of a more attractive female (here representing a potential future reproductive episode), would suffice to impact the current male investment, indirectly affecting the fitness of lesser quality rivals.

Cooperative modulation of brain neuropeptide levels in a cleaner fish

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Social behavior and its underlying mechanisms have been the subject of several studies in the past few years. In teleost fish, the neuropeptides arginine vasotocin (AVT) and isotocin (IT) play an important role in the modulation of social interactions. However, it is not completely clear how AVT and IT affect cooperative activities. In the present study, we used the Indo-Pacific bluestreak cleaner wrasse *Labroides dimidiatus*, as to study the effects of different contexts (interspecific and intraspecific) on brain AVT and IT levels. To do so, cleaners were placed in five different contexts, a) Cleaner vs Conspecific b) Cleaner vs heterospecific (*Naso elegans*), c) Isolated cleaner with visual stimuli of a heterospecific, d) Isolated cleaner with visual stimuli of Conspecific, and , e) Cleaner with a geometric object (ball - control). Following 60 min of observations, the brain areas of each focal individual were collected and the different levels of the neuropeptides analysed. Our preliminary results show that, both neuropeptides (AVT and IT) change in accordance to context. Our data suggest that alterations in behavioural contexts (interspecific vs intraspecific) are capable of mediating fast neuropeptide changes, which vary across different brain regions.

Latitudinal patterns in the barber goby *Elacatinus figaro* feeding habits.

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Fish are able to show considerable diet plasticity and feeding strategies, which largely depend on behavioral and physiological flexibility associated with phenotypic plasticity. Species of cleanerfish mostly depend on ectoparasites, mucus and scales of client fish to their nutrition and may present great variability on their diets depending on local conditions. The cleaner fish *Elacatinus figaro* is endemic to the Brazilian coast and occurs along the entire coastline, which makes this species a appropriate model to study the effects of large spatial scales on their diet. The objective of this work is to compare the diet of *E. figaro* in three localities of the Brazilian coast spanning 2200km (Maragogi, Salvador and Arraial do Cabo). Across the three sites, the main item was client-gleaned material, especially ectoparasites. On the other hand, the secondary food item (plankton and free-living copepods) differed among localities, being very representative of each locality. These results confirms the status of *E. figaro* as an obligatory cleaner fish, demonstrates its functional importance on reef systems along the Brazilian coast, but also highlights the feeding plasticity exhibited by this species.

POSTER WALK 2

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**A comparative study of cleaning activity by two facultative cleaner fish species
Coris julis and Thalassoma pavo of São Miguel island, Azores**

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The behaviour of facultative cleaner fish species in temperate waters is poorly known thus more studies are needed to fill this gap. Here we aimed to find out more regarding the cleaning activity of two facultative cleanerfish species found in Azores Archipelago (*Coris julis* and *Thalassoma pavo*) and of other visiting fish (known as clients). We observed cleanerfish behaviour at their home territories (e.g. cleaning stations), analyzed stomach content of each cleaner species caught randomly and the ectoparasite load of different species (that included client species). We found that the specific diversity of these cleaners clientele was not different while the species *Symphodus caeruleus* (endemic specie of the Azores) was the most observed at cleaning stations. Diet of the two cleaners differed significantly while gnathiid isopods were solely found on *C. julis* stomachs. Client fish species were also found to carry ectoparasites (mostly caligid copepods) while cleaners appear to be selectively foraging on gnathids copepods. Our data reveals that these cleaner fish have a positive impact on ectoparasite control of other visiting fish which have a crucial and positive ecologic role on the health of coastal fish communities of the Azores.

Impairment and recovery of fish swimming performance following waterborne mercury exposure and association with accumulated levels in brain and eyes

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Mercury (Hg) compounds have triggered major environmental and human health concerns. Still, there is a knowledge gap on fish behaviour impairments related with Hg accumulation in brain

and neurosensory structures, as well as regarding the recovery of behavioural trials in a post-exposure period. This study presents the swimming performance of the white seabream (*Diplodus sargus*) together with Hg accumulation in brain and eyes. Fish were exposed to realistic levels of inorganic Hg in water (2 µg L⁻¹) during 7 (E7) and 14 days (E14). After that, fish were allowed to recover for 28 days (R28). The Fulton condition and hepatosomatic indexes did not vary significantly between control and exposed fish along the experiment. At E7, exposed fish exhibited a significant decrease of the first swimming distance, as well as a lower time for refuge. In parallel, Hg levels were significantly higher in brain and eyes. At R28, previously exposed fish still swam a smaller distance in the first run, exhibited a lower resistance against the water flow (measured as time for the immobility) and lower latency immobility. Accordingly, Hg levels in eyes and brain did not decreased during the recovery period. Realistic levels of waterborne Hg can alter fish swimming performance without a rapid reversibility. Such impairments could have repercussions in organism's fitness and survival.

Keywords: Inorganic mercury; Swimming behaviour; Fish brain; Eyes; Bioaccumulation

What happens when a strange neighbor sings? The effect of a heterospecific intruder in the song activity of the blackcap *Sylvia atricapilla*

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Interspecific competition has been studied in many animal species with overlapped distributions, still the research has been focused mostly on ecological subjects as niche occupancy or food resources sharing. In this work, we aimed to evaluate the effect of a heterospecific intruder in the song activity of a passerine, the blackcap *Sylvia atricapilla*, during their main period of territorial defense. During spring 2014, we selected two areas in Portugal with high densities of the blackcap and differences in the presence of the robin *Erithacus rubecula*, which was the selected species to act as the intruder. In each blackcap territory, we trapped and banded the male and then mapped the territory. The experiment was developed within each territory, and was divided in three parts: recording of spontaneous song, interactive playback using a heterospecific song and recording of bird reply. Song rate and song length were the parameters analyzed to compare the effect of playback experiments. To compare the effect of playback experiments we analysed the song rate and length. We discuss the results based in acoustic avoidance and heterospecific rival recognition. The results can contribute to our understanding of community structure and evolution and of the coexistence between potentially competitive species.

Sharing a room? Yes, but with benefits - the case of an arid island gecko

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Two types of aggregations are recognized in lizards, ecological and social, i. e. related to the attraction to a site or to conspecifics, respectively. As most lizards are territorial, aggregations increase, among other things, the likelihood of aggressive interactions among individuals as agonistic behaviour is density dependent. We hypothesize that lizards with high intraspecific density and scarcity of optimum refuges have increased likelihood of presenting ecological aggregations and mechanisms to avoid agonistic interactions. To test this hypothesis, we have conducted a field-based study with the endemic Cape Verde nocturnal gecko *Tarentola substituta*. To describe the incidence, size and composition of aggregations and to study the effect of gecko and refuge density, plus refuge quality on refuge sharing, we sampled 48 transects and 40 10x10 m quadrats on São Vicente Island. We found that this species forms aggregations around 30 to 40% of the time, and that refuges are almost always shared by a pair of individuals of different sexes. We also observed that refuge sharing is dependent on its quality, as medium-large (thermally optimum) rocks are shared much more frequently than small ones, and directly related to the density of geckos and inversely to the density of optimum refuges. We found no relation between the body temperatures of geckos and refuge sharing, suggesting that these are not social thermally mediated aggregations. Hence, our results suggest that in *T. substituta* aggregation incidence is mainly driven by an ecological factor (scarcity of optimum refuges) and its intersexual composition by social factors (avoidance of agonistic interactions by males, and possible increased reproductive success of the pair). This study sheds some light on the little explored gecko aggregation behaviour and other studies should follow to investigate this further.

POSTER WALK 2

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New findings in the sociality of the Eurasian otter: further evidence that solitary carnivores may be highly flexible in their social behaviour

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Eurasian otters (*Lutra lutra*) are described in the literature as solitary, with the two sexes interacting only during mating. Data on otter sociality are rather scant, however, especially in Mediterranean regions, and the group formation documented in temperate zones has suggested some social plasticity. We investigated the sociospatial organization of a Mediterranean population of Eurasian otters by analyzing static and dynamic interactions and diurnal resting site use in 15 animals radiotracked within a time span of 3.5 years in Alentejo (Southern Portugal). Contrary to what is described in the literature and expected for solitary animals, otter dyads showed positive interactions, with individuals associating more often than expected by chance. Moreover, otter movement patterns were correlated. Finally, otters shared diurnal resting sites more often than expected, and, surprisingly, adult males and females with cubs overlapped spatially and temporally, even sharing resting sites when the males had no paternity. Our results contradict several statements in the literature on European otter sociality and reproductive behavior, as well as confirm the classic mustelid intra-sexual territoriality and a polygynous mating system also for a Mediterranean population. We conclude that Eurasian otters are more social than previously thought, adding further evidence that social behavior in solitary carnivores may reveal significant flexibility.

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A first assessment on inequity aversion in domestic dogs (*Canis familiaris*) using social reinforcement

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The study of inequity aversion in non-human species has been attracted increasing attention with domestic dogs (*Canis familiaris*) recently emerging as a new model species. Studies have tested whether dogs perceive and respond to unfairness within the context of human-dog interactions, using food reinforcement to create inequity situations. Curiously, despite the highly social nature of these animals and the unique features of their relationship with humans, no attempt has yet been made to assess inequity aversion using social reinforcement and compare results with similar studies using food as rewards.

Based on the above, a study was planned as to test whether dogs show inequity aversion in situations involving social rewards instead of food ones. Using an adapted test of inequity aversion which looks at advantageous and disadvantageous inequity, this study investigated the behaviour of 15 dogs. A subject dog and a control dog approached two persons in turn: one who rewarded them equally for sitting on command (using verbal praise and petting) one who rewarded them unequally. After familiarization with these two persons, a preference test was conducted as to assess which person the subject dogs chose to approach by themselves.

Results showed no evidence for inequity aversion reactions, as opposed to what has been obtained in studies using food reinforcement.

Effects o environmental enrichment on social behavior of balb/c mice

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Environmental enrichment¹ is frequently considered to improve caged animal welfare. However little is known about the influence o enrichment on social behavior of balb/c mice. The aim of this study was to evaluate the effects of environmental enrichment on social behaviour of these mice. Subjects were socially reared during 15 days under enriched or standard rearing conditions. The enriched environment consisted of an assembly tunnels and balls that the mice could move into and out of or climb over, in addition, objects that they could scratch and chew were also used. The objects were rotated systematically through the cages in way that for each cage, the same objects were never presented during subsequent switches. The subjects were tested in three situations: home cage spontaneous social activity, Social Approach Test procedure (a model of sociability), and Resident/intruder test (a model of aggression). The results suggest that there were some differences in the social behaviour. The implications of these social differences were discussed in terms of animal welfare

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Age and parasites predict carotenoid-based plumage colour on male European serin

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A fundamental assumption of theories on the evolution of sexual signals is that they should be costly to produce and honestly signal the quality of the sender. The expression of carotenoid-based plumage signals are thought to be condition-dependent traits, due to carotenoids function as pigment and health modulator. We explored carotenoid-based plumage colouration in a free living population of male European serins (*Serinus serinus*) during breeding season. We captured, measured and took colorimetric measurements of male serins, in a 4-year field study in order to evaluate the signalling value of colouration in relation to body condition and level of parasites. We started by evaluating the differences between two colorimetric approaches, the tristimulus variables, based on human colour perception, and physiological models of avian colour vision, and found that they were highly correlated. We investigated the information provided by plumage colour and we found that plumage colour expression could be predicted by age and ectoparasite load. We concluded that the colour expression of the plumage of the serin is an age dependent trait and an honest signal of the ability to cope with parasitic infection.

Neurogenomics of alternative reproductive tactics in a blenniid fish

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In the peacock blenny *Salaria pavo* males can follow two developmental pathways, mimic the females' morphology and courtship displays in order to approach the nests of larger males and parasitically fertilize eggs, or skip reproduction in the first year to invest in growth and become nest-holders in the next breeding seasons. These alternative reproductive tactics (ARTs) are sequential as sneakers later develop into nesting males, a phase where males aren't reproductively active (transitional males). So far, only the neuroendocrine correlates and environmental cues that trigger the expression of these ARTs have been characterized for this species. Hence, we used RNA-seq to identify genes associated with reproductive plasticity in the peacock blenny on a genomic scale. First, a normalized multi-tissue cDNA library was sequenced on the 454 platform, in order to create a reference transcriptome. Then, RNA was isolated from whole brain tissue for each one of the four morphotypes (sneaker, nesting male, transitional male and female) for high-throughput sequencing using Illumina HiSeq 2000 platform. A *de novo* assembly was performed using Trinity assembler, which was functionally annotated against Uniprot database. Expression analysis between the four groups was made in order to identify the up and down-regulated genes.

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The use of salivary cortisol to assess stress in domestic dog pups

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Cortisol levels are increasingly used as a physiological stress marker in animals, namely to assess welfare and social stress in a variety of species. High cortisol levels indicate elevated stress, and can be adaptive when the stressor is an immediate threat or challenge. In dogs, salivary cortisol is highly correlated with plasma values, being a less stressful alternative to venipuncture.

In this study we examined factors affecting stress and cortisol production in pups belonging to different breeds. 23 pups from 4 breeds were involved. Each pup was placed inside an arena, and subjected to a test battery adapted from the Strange Situation procedure. Saliva samples were collected with a synthetic swab (Salivette) 30 minutes before the tests (basal value) and 3 minutes after a series of negative (strange place, person, object and startling sound) and positive stimuli (reunion with owner and mother). Tests were repeated every two weeks for each pup, from 6 to 10 months of age. 142 samples were collected and frozen prior to analysis at the Laboratory of Faculdade de Ciências do Desporto e Educação Física (Coimbra University). Results suggest individual and breed differences; a deeper analysis of the variables that may be at play is presented.

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Variation in brain arginine-vasotocin and isotocin levels across species of labrid fish that differ on the expression of mutualistic behaviour

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Evolutionary and ecological comparative approaches provide unique knowledge into the consequences of social organization variation in brain and behaviour. Studies on neuropeptide influence (such as arginine vasotocin and isotocin) across different species of vertebrates are helping to unravel how changes in these neuromodulators associate with social behaviour complexity, however, when aiming to identify potentially conserved mechanisms across vertebrate evolution, teleost fish become the best suitable models to explore these links. Here, we show that differences in cognitive abilities related to cooperative behaviour were reflected in the brain levels expression of our two candidate neuropeptides; which varied significantly across species, both overall and at each specific macro-area. Our results confirm arginine vasotocin' relevance as a mediator of cleaning behaviour and identifies the cerebellum as a main character area (within the brain).

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Dispersal in the Desert: genetic diversity and population structure of the Mauritanian baboon (*Papio papio*)

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Dispersal is an important evolutionary force impacting the species' spatial structure and global persistence. Dispersal strategies are frequently sex-biased in mammal species with polygamous or promiscuous mating systems. Although males usually disperse at higher rates than females the reversed pattern has been identified before for several species including the Guinea baboon (*Papio papio*). Guinea baboons occupy a small distribution area in West Africa and have been mostly studied in Senegal and Guinea-Bissau. Molecular data points to female dispersal and male philopatry in Senegal and historical female-biased dispersal pattern over the entire range. However, intra-specific variation in the extent and direction of sex-biased dispersal was found in Guinea-Bissau, where gene flow was mediated through by both sexes. This study aims at investigating the dispersal strategies of Guinea baboons in Mauritania using non-invasive faecal samples and contributing for the conservation of these populations. In Mauritania, the populations are restricted to the southern mountains and considering the large distance between suitable areas for baboons, it is expected some degree of isolation between the populations of the mountains and between these populations and the ones in the core area of the species' range.

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