

Behavioural Biology in Animal Welfare Science

ASAB Winter Meeting 2018



Thursday 6 December

09.30-10.15 Coffee & set up Session A posters

Chair: Mike Mendl

10.15-10.25 Introduction: Suzanne Held and Mike Mendl

10.25-11.15 PLENARY: **Per Jensen**, *Linköping University, Sweden*
From genes to behaviour: on dogs and chickens

11.15-11.35 **Jennifer Weller**, Irene Camerlink, Simon Turner, Marianne Farish, Gareth Arnott
Does play fighting experience influence later life contests? A test with welfare-relevance using domestic pigs (*Sus scrofa domestica*)

11.35-11.55 **Tom Smulders**, Dan O'Hagan, Elena Armstrong, Timothy Boswell
Neural markers of animal welfare

11.55-12.15 **Clare Andrews**, Jonathan Dunn, Daniel Nettle, Melissa Bateson
Time drags when life's a drag? Time perception as a potential window into affective state

12.15-12.35 **Vikki Neville**, Liz Paul, Peter Dayan, Iain Gilchrist, Mike Mendl
Applying computational methods to animal welfare science

12.35-14.00 Lunch & Session A posters

Chair: Liesbeth Bolhuis

14.00-14.50 PLENARY: **Georgia Mason**, *University of Guelph, Canada*
Using phylogenetic comparative methods in animal welfare research

14.50-15.10 **Gareth Arnott**, Irene Camerlink, Simon Turner
Using principles from evolutionary biology to address aggression in animal welfare contexts

15.10-15.30 **Anika Immer**, Sasha Dall, Simon Griffith
Ecology of fear in the zebra finch: a useful window into welfare

15.30-15.50 **Emma Mellor**, Heather McDonald Kinkaid, Yvonne van Zeeland, Nico Schoemaker, Michael Kinkaid, Georgia Mason
Nature calls: Is there an ecological basis for abnormal behaviour and breeding problems in captive psittacines?

15.50-16.30 Tea & Session A posters

Chair: Georgia Mason

16.30-16:50 **Conor Goold**, Lisa Collins, Ruth Newberry
Psychometric measurement models for animal welfare science

16:50-17:10 **Andrew Crump**, K. Jenkins, Helen Kabboush, Emily Bethell, C.P. Ferris, Niamh O'Connell, Gareth Arnott
Cognitive bias in dairy cattle: do personality and pasture-access influence judgements about ambiguous stimuli?

17.10-17.30 **Jasmine Clarkson**, Matt Leach, Paul Flecknell, Candy Rowe
Handling method alters laboratory mouse sensitivity to reward loss and gain

17.30-17:50 **Camille Raoult**, Lorenz Gyax
Attention bias test in sheep with different induced mood

17:50-18:10 Jasmine Sowerby Greenall, Anne Maignot, Monica Padilla de la Torre, **Elodie Briefer**
Human recognition of vocal expression of emotions in domestic and wild ungulates

18.10 Wine reception sponsored by Wisepress & remove Session A posters

Friday 7 December

09.30-10.15 Coffee & set up Session B posters

Chair: Per Jensen

- 10.15-11.05 **PLENARY: Liesbeth Bolhuis**, Wageningen University, Netherlands
Friends or foes: impact of personality and social environment on animal welfare
- 11.05-11.25 **Lorenz Gyga**
Animal welfare cannot be equated with “eternal bliss”
- 11.25-11.45 **Kyriacos Kareklas**, Hansjoerg Kunc, Gareth Arnott, James Wilson, Rebekah McMurray
Noise affects contest dynamics in captive and urban animals
- 11.45-12.05 **Tanja Kleinhappel**, Tom Pike, Oliver Burman
Behavioural predictors of stress in groups of animals
- 12.05-12.25 **Lisa Collins**
Between the sexes: pseudoscience, fallacy and why sex differences matter in animal welfare
- 12.25-14.00 Lunch & Session B posters
- 14.00-15.00 **THE NIKO TINBERGEN LECTURE: Bart Kempenaers**, Max Planck Institute for Ornithology, Seewiesen, Germany
Always on the move – how sexual selection shapes activity patterns
- 15.00-15.30 Tea & Session B posters

Chair: Suzanne Held

- 15.30-15.50 **Sara Hintze**, Luca Melotti, Simona Colosio, Jeremy Bailoo, Maria Boada-Saña, Hanno Würbel, Eimear Murphy
All creatures great and small: towards a cross-species judgement bias task to assess emotional valence in animals
- 15.50-16.10 **Andy Higginson**, Tim Fawcett
Emotions and physiology as memory: a dynamic optimisation approach
- 16.10-16.30 **Coline Poirier**, Melissa Bateson
Welfare and biomedical implications of stereotypic pacing in laboratory macaques
- 16.30-16.50 **Helen Lambert**, Gemma Carder
Do ear postures indicate emotional states in dairy cattle?
- 16.50-17.10 **Anne van den Oever**, Bas Rodenburg, Liesbeth Bolhuis, Lotte van de Ven, Bas Kemp
Effects of different nest designs on nesting behaviour in broiler breeders
- 17.10 Close

POSTERS

Session A: Thursday 6 December (Posters 1-20)

- #1 *Adriaense et al.* The influence of negative emotional contagion on behaviour and social interaction in chickens (*Gallus gallus domesticus*).
- #2 *Archimbaud et al.* An assistant robot improves animal welfare in poultry
- #3 *Armstrong et al.* Keel bone fractures are associated with suppressed adult hippocampal neurogenesis in laying hens
- #4 *Bidaisee & Keku.* Welfare of pets and people: an inextricable link
- #5 *Cenni et al.* Are stones being used as sex toys by Balinese macaques?
- #6 *Chapman et al.* The challenges of collecting behavioural evidence 24/7: Using a novel camera trap methodology to investigate nocturnal behaviour in captive flamingos
- #7 *Dutra & Young.* Assessing a Dog's Life
- #8 *Garnham & Løvlie* The effect of positive affective state on social interactions
- #9 *Greening.* Understanding sleep-related behaviour (and lack of) as a measure of welfare using the horse as model
- #10 *Haddy et al.* Validating a new social science questionnaire for working equid owners: The effect of owner attitude on equid welfare
- #11 *Havercamp & Green.* Monitoring sleep in captive primates to enhance welfare
- #12 *Hooper et al.* Enriching the lives of wolves (*Canis lupus*)
- #13 *Houdelier et al.* Environmental enrichment during laying influences Japanese quail offspring's phenotype
- #14 *Hunt et al.* Clicker versus non-clicker training: effect of training method on behaviour and heart rate in donkeys (*Equus asinus*)
- #15 *Jenikejew et al.* Interview with a rhino – How vocalisations reflect social interactions in the captive Southern white rhinoceros (*Ceratotherium simum simum*)
- #16 *Jordan et al.* Learning via exploration in captive capuchin monkeys?
- #17 *Knowles et al.* The effects of personality upon breeding success in Humboldt Penguins (*Spheniscus humboldti*)
- #18 *Kogan et al.* Veterinary behavior: assessment of veterinarians' training, experience, and comfort level with cases
- #19 *Kull et al.* Habituation to enrichment in kea (*Nestor notabilis*)
- #20 *Lemaire et al.* A computational ethological approach to filial imprinting in chicks (*Gallus gallus*)

POSTERS

Session B: Friday 7 December (Posters 21-41)

#21 *Londardo et al.* Cognitive offloading in pet dogs

#22 *Londoño et al.* Chemosensory enrichment as a simple and effective way to improve the welfare of captive lizards.

#23 *Lush & Ijichi.* A preliminary investigation into personality and pain in dogs

#24 *Marshall et al.* Perception of the ethical acceptability of live prey feeding to aquatic species kept in captivity

#25 *Mcloughlin et al.* Developing an automated system for monitoring poultry welfare via their vocalisations

#26 *Meyer et al.* Impact of three common blood sampling techniques on aspects of animal welfare in laboratory mice

#27 *Nemvalts et al.* Keepers' ratings of personality, but not behavioural tests, predict male reproductive performance in the critically endangered European mink (*Mustela lutreola*)

#28 *Norman & Nicol.* The effect of rearing complexity on navigational ability

#29 *O'Hagan & Smulders.* Assessing transient affective state using intracranial recordings of brain oscillations in poultry

#30 *Pallante & Versace.* Newly-hatched chicks (*Gallus gallus*) discriminate between sexes

#31 *Perkins & Sprague.* Octopus: A multi-armed approach to improving captive welfare

#32 *Sariava et al.* The CAREFISH project: establishing scientific welfare standards in fish farming certification

#33 *Schork et al.* Combining behaviour, personality and life history to evaluate the welfare of police horses

#34 *Spence et al.* Public attitudes to animal sentience and welfare

#35 *Stockin et al.* Mind the gap - Can AI be used to bridge the conservation-welfare fracture that occurs during human intervention at whale strandings?

#36 *Trevarthen et al.* Measuring affect-related biases in distractibility in mice

#37 *Van Dessel et al.* Spatial and social relationships in horses and their reaction upon separation

#38 *Versace et al.* Born to be asocial: Newly-hatched tortoises spontaneously avoid unfamiliar individuals

#39 *Whiteside et al.* The welfare of game birds destined for release into the wild: A balance between early life care and preparation for future natural hazards

#40 *Williams, E. et al.* Monitoring behavioural change in elephants: development of a novel welfare assessment tool

#41 *Williams, L. et al.* Hot or not? Monitoring behaviour as a welfare indicator following a change in heat provision

TALK ABSTRACTS (in alphabetical order by first author)

Time drags when life's a drag? Time perception as a potential window into affective state

Andrews, C., Dunn, J., Nettle, D. & Bateson, M.

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Non-human animals' affective states can only be inferred using physiological, behavioural and cognitive measures. A number of cognitive measures of animal affective state, such as judgement biases and sensitivity to reward losses or gains (successive contrast effects), have arisen from consideration of the cognitive signatures of human emotions or emotional disorders. Human perception of time is often distorted rather than perceived veridically, being modulated by emotional experiences and altered in psychiatric disorders such as anxiety and depression. Here, we consider whether time perception could offer novel measures of affective state in animals, and test for altered time perception in a cohort of European starlings (*Sturnus vulgaris*) found previously to display other depression- or anxiety-like behaviours following a manipulation of early life stressors. We start by reviewing evidence concerning how emotion and emotional disorders influence time perception in humans. Next, we briefly review how pharmacological manipulations of the dopaminergic system alter timing behaviour in humans and animal models. We then explore whether distortions of time perception could be used to assess animal affective states, and whether such measures might theoretically distinguish emotional arousal from emotional valance. Lastly, we ask whether 'depression-like' responses to successive contrasts or 'anxiety-like' responses to potential threat in European starlings predict individual variation in birds' perception of time measured using a temporal reproduction task (tri-peak procedure).

Using principles from evolutionary biology to address aggression in animal welfare contexts

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³Animal Behaviour & Welfare, Animal and Veterinary Sciences Research Group, Scotland's Rural College (SRUC), West Mains Rd, Edinburgh, EH9 3JG, UK

Aggression is a major animal welfare problem in many captive environments, including agriculture. Regrouping aggression in pigs is a particular problem as these animals form dominance hierarchies when unfamiliar individuals are mixed together, resulting in an intense period of aggression, which is a major welfare issue. Animal contest models from evolutionary biology and based on particular assessment strategies provide an opportunity to better understand the decisions used by pigs to resolve aggressive encounters. We have previously demonstrated the influence of aggressiveness, in terms of a stable personality trait, on contest dynamics, while also revealing that pigs require prior contest experience to become proficient at a sophisticated form of information gathering termed mutual assessment. In this conference we will provide results of an experiment using an early life manipulation, termed socialisation, during which adjacent litters of piglets were allowed to mix (between 14-28 days of age), with control litters remaining unmixed. Socialisation was hypothesised to equip individuals with enhanced social skills in terms of assessment ability in later life agonistic encounters. In resident-intruder tests at seven weeks of age socialised pigs had a shorter attack latency, while in dyadic contests at eight weeks of age they had shorter contests, with fewer skin lesions consistent with enhanced assessment. Furthermore, there was

evidence of a novel form of opponent assessment in the socialised group revealed by a positive relationship between winner fighting ability (body weight) and fight duration. Thus, early life socialisation equipped pigs with social skills that enabled more rapid establishment of dominance relationships at lower cost, offering an intervention that could be adopted on-farm to reduce aggression

Handling method alters laboratory mice's sensitivity to reward loss and gain

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³ Comparative Biology Centre, The Medical School, Newcastle University, Newcastle, UK

Mice are the most commonly used species for scientific research, and therefore refinements to their husbandry and use in scientific procedures are important for ensuring good welfare. The standard method of handling laboratory mice by the tail increases stress and anxiety, which can be reduced by handling them using a tunnel. Despite evidence that tunnel handling would improve the welfare of millions of mice worldwide, it has yet to be widely implemented. The aim of our study was to further understand the impacts of tail handling on the affective state of mice to enable informed changes to laboratory practice. We investigated if the method used to handle laboratory mice changed their sensitivity to both reward loss and reward gain. This is because humans with depression change how they process and evaluate information, which can be measured by their responses towards changes in reward. Typically, individuals in negative affective states are more sensitive to reward loss (i.e. show greater disappointment), and less responsive to reward gain (i.e. show less elation). Therefore, measuring how animals respond to changes in reward offers a useful technique to understand their underlying affective state. We handled groups of mice using their tail or a tunnel, and compared their hedonic evaluations of a rewarding sucrose solution following a shift in reward value. Mice initially received a sucrose solution, and then the sucrose concentration was subsequently reduced (Successive Negative Contrast) or increased (Successive Positive Contrast). Measurable changes in their drinking behaviour suggest that handling method may differentially affect mice's responses to reward loss and gain and provide insight into how both the positive and negative valence of affect is affected by handling method. We discuss the implications of our findings in relation to animal welfare and for scientific data collection for a number of in vivo paradigms.

Between the sexes: Pseudoscience, fallacy and why sex differences matter in animal welfare

Collins, L.M.

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The evidence around the existence of real, measurable sex differences in the brain that result in real, measurable differences in behaviour is rife with controversy and debate. Stepping away from the tabloid headlines and considering the underlying science and its potential implications for our interpretation of behavioural experiments – particular in the field of animal welfare – is critical. Sex is perhaps one of the most evolutionarily well-conserved factors to influence animal behaviour and cognition. Sex differences have been identified in stress-related psychiatric disorders, such as depression, generalized anxiety disorder, acute and chronic post-traumatic stress disorder, with a higher risk of development in females

than in males. Stress-related disorders are closely linked with cognitive alterations, and differing levels of performance in learning, memory and other cognitive tests.

In this presentation, I will consider the strength of evidence for meaningful sex differences that are relevant to behaviour and welfare, present new data from our recent investigations on the impacts of early environment on sex differences

Cognitive bias in dairy cattle: do personality and pasture-access influence judgements about ambiguous stimuli?

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Affect-driven changes in cognition, or cognitive biases, are increasingly used as indicators of animal emotions and welfare. The best-studied, judgement biases, describe the tendency of animals in positive affective states to interpret ambiguous information more positively (optimistically) than those in negative affective states. However, recent research suggests optimism is also a personality trait, i.e. relatively stable over time. In this study, we developed the first judgement bias methodology for adult dairy cows and investigated whether judgement biases indicated affective states or personality traits. Thirty cows were trained that a bucket at one location contained food, whereas a bucket at a different location was always empty. We then recorded latency to approach "probe" buckets at intermediate locations. Shorter latencies were classified as more optimistic. In a repeated-measures crossover experiment, we used daily pasture-access as a positive affect manipulation. Half the cows were tested eight times during three weeks of pasture-access, followed by eight trials during three weeks of continuous housing. This order was reversed for the other fifteen cattle. Response latencies were not significantly different in the pasture-access treatment, suggesting this affect manipulation did not induce an optimistic judgement bias. Instead, cows exhibited trait optimism, with latency to the probe locations significantly concordant across repeated probe location trials. This effect persisted when speed to the rewarded and unrewarded locations were accounted for, although it was not correlated with other personality tests measuring boldness, neophobia, and sociability. Overall, these findings suggest degree of optimism is a personality trait, as well as indicating affective state. Future studies should therefore investigate both the consistency and plasticity of judgement biases.

Psychometric measurement models for animal welfare science

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²Department of Animal & Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Norway

Which indicators best reflect animal welfare has long been a topic of discussion among animal welfare scientists. To measure animal welfare, one must be clear on the theoretical relationships between welfare (an unobserved construct) and its proposed cognitive, behavioural and physiological indicators. To enable this inference process, a suitable 'measurement model' needs to be employed before data is

collected and analysed. A measurement model makes predictions about the relationship between potentially unobserved variables and observable indicators, and can be tested statistically using observational data. However, they have been infrequently proposed in welfare research to date. This risks making welfare, as a scientific concept, ontologically ambiguous. In this presentation, three ‘psychometric’ measurement models originating in the human psychological sciences will be presented (formative, reflective and network), and their relevance to animal welfare science illustrated. Formative models assume that unobserved or latent variables are linear compositions of observed variables (i.e. welfare is an additive combination of potentially uncorrelated indicators). Reflective models, in contrast, assume that variation in the observed variables is caused by variation in the latent variable (i.e. welfare is a biological state that determines the observed expression of welfare indicators). Evidence for or against formative and reflective models can be tested using latent variable models, namely principal components analysis (formative) and structural equation modelling (reflective). Network models depart from formative and reflective models by defining unobservable constructs as emergent properties of dynamic and causal relationships between the observable indicators. Network relationships between observable indicators are discerned using causal graphical modelling. We illustrate how different interpretations of welfare can be assessed using these measurement models. We argue that greater awareness and comparison of these measurement models (or alternatives) can improve the theory of animal welfare, as well as the design of experiments and the analysis of welfare data.

Animal welfare cannot be equated with “eternal bliss”

Gygax, L.

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Recently, good animal welfare has been equated with continuously avoiding negative and promoting positive emotional states in animals (“eternal bliss”). Animal emotions are likely the mechanism that makes animals avoid threats (negative emotions), seek opportunities (positive emotions), and prioritise accordingly different behavioural options. In this sense, animals do what they “want”. Moreover, emotions signal whether a goal state of a behaviour has been reached (“liked” or not). In terms of functionality, such a control mechanism may specifically be successful if disliking leads to insistent (but variable) behaviour to reach a goal state. In contrast, liking should eventually lead to a reduced motivation to produce the current behaviour. This frees up time to follow another want (perform another behaviour) and does so most efficiently if liking is a fleeting state. Wanting in itself seems unlikely to lead to a positive affective state as seen in extreme form of addictions (where the goals of wants are disliked) and “liking” may be too short to do so. Therefore, eternal bliss does not seem to be reachable by purely avoiding negative and promoting positive emotions. Longer-term mood is another candidate to promote welfare. Yet, it seems that mood states are influenced most heavily by cumulative mismatches, i.e. experiencing discrepancies between expectation and what was actually reached. Without mismatches, the base emotional state seems to rather quickly adapt to some neutral level independent of how good or bad a situation may appear from an outside view. All in all, certain negative experiences and mismatches and the according deterioration of mood may be a necessary precondition for positive experiences and mismatches that help to improve mood. Instead of aiming at “eternal bliss”, a basis for good welfare could mean that an animal can do what it wants, can reach the goals that it likes and does so with varying success. In the long run, we might have found the perpetual motion machine of welfare if “liking” could be made a “want”. The starting point of whether and how that is possible could be the study of leisure time that seems to exist in humans and animals alike.

Emotions and physiology as memory: a dynamic optimisation approach

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Behavioural models often assume that animals are capable of sophisticated learning. If learning abilities are costly natural selection will favour simpler mechanisms that deal with with new conditions. In order to understand how animals cope with novel environments such as captivity, we need to understand the mechanisms underlying their responses. I will present two dynamic optimisation models exploring potential mechanisms. In a foraging context, we found that a mechanism based only on current energy reserves performs almost as well as a Bayesian learning rule. This mechanism exploits the fact that reserves are a source of information about recent food availability. Similar principles could apply in non-foraging contexts: any physiological or psychological state variable that is altered by experience might act as memory of past experiences. We explored a mating context in which a male must deter other males by being aggressive and attract females by courtship. We assume there is perceptual error meaning that they must accumulate information over time to respond appropriately. Behaviour is a product of the emotional state, which is captured in two state variables: valence and arousal. We predict how emotional state changes in response to stimuli when the animal has no other memory of past events. We compare the behaviour and fitness of this strategy to a Bayesian animal that keeps all information and updates beliefs optimally. Again, the simple mechanism performs almost as well as the Bayesian learner. The model makes several interesting predictions including: inertia in emotions such that they are slow to calm down, erroneous aggression towards females, and hysteresis in movements through emotional state space. Our conceptualisation of states as memories may help to predict how animals will respond to the dynamics of conditions in captivity.

All creatures great and small: Towards a cross-species judgement bias task to assess emotional valence in animals

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Judgement bias tasks are promoted as promising tools to assess emotional valence, an important measure of animal welfare. Due to the variety of task designs, however, it is difficult to compare results across studies. Moreover, current designs have practical limitations, including lengthy training and limits to how often ambiguous test cues can be presented. The current study aimed to establish a task design that works across species, is relatively quick to learn, fulfils aspects of internal validity, and allows for repeated presentation of ambiguous cues. Mice (n=48), rats (n=16) and horses (n=25) were trained on a spatial Go/No-go task in which the location of an open goal-box signalled either reward (Go trial) or non-reward (No-go trial). Animals learned to initiate each trial using an operant response, giving them control over trial intervals and allowing them the possibility to skip No-go trials by re-initiating. Once they had learned the discrimination (go to goal-box in Go trials; do not go or reinitiate in No-go trials), ambiguous test trials with open goal-boxes at intermediate locations were interspersed among Go and No-go trials. The Go:No-go response ratio was used as a

measure of judgement bias. Most animals successfully learned the discrimination (mice: 46/48, rats: 16/16, horses: 17/25) within relatively few sessions (mean (range); mice: 5 (3-12), rats: 4 (3-8), horses: 11 (5-19)). All three species showed a graded response across the different cues (GLMM; mice: $X^2_4=1489$, $p<0.001$, rats: $X^2_4=588$, $p<0.001$, horses: $X^2_4=477$, $p<0.001$), thereby fulfilling aspects of internal validity of judgement bias tasks. There was no evidence of learning about the outcome of ambiguous cues across the six test sessions (GLMM; mice: $X^2_1=2.48$, $p=0.12$, rats: $X^2_1=0.01$, $p=0.95$, horses: $X^2_1=0.36$, $p=0.55$). The task protocol developed here is therefore a promising step towards a practical task for research on judgement biases in different animal species.

Ecology of fear in the zebra finch: a useful window into welfare

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²Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

Welfare is an important issue in terms of ethics in itself and through its influence on the quality of research. The zebra finch is the most commonly used passerine bird in captive research. In addition, they are common pets and are widely kept in cages and aviaries, in domestic situations and also in pet shops and zoos. Surprisingly, to date, few studies have examined the welfare of these birds in captivity, with those that have examining measures such as stress hormones and song rate to evaluate well-being. Here, we have used the perspective of the 'ecology of fear' to understand how the behaviour of zebra finches in captivity responds to characteristics of the captive environment such as shelter, cover, and ground substrate. This approach describes how the decision to feed at a specific location is made through balancing the cost and benefits of feeding at that location (induced by the fear that is inherent in a prey species such as the zebra finch). We used giving-up densities (density of seeds remaining in a patch when a patch is left by a foraging bird) to measure the response to the experimental treatments. Our results show that zebra finches feel safer at the front part of cages rather than at the back and their perceived risk regarding the number of perches available was dependent on the duration for which they were exposed to the experimental treatment. We found the effects of the ground substrate and cover to be weak. Our results suggests that the *ecology of fear* perspective is a straightforward and relevant way to determine environmental features in which zebra finches feel safer, and this new approach can add an important new tool to explore welfare questions in captive animals, improving the environment and ultimately well-being.

Noise affects contest dynamics in captive and urban animals

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Humans are increasingly influencing the behaviour of other species and this could have implications on fitness-related processes, such as social interactions. Contests are one type of interaction that play an important role in resource distribution and life-history trade-offs. However, effects on the dynamics of animal contests are often overlooked and especially in regards to anthropogenic stressors that are more difficult to control. A prominent example of such stressors is noise, which can travel long distances and between media, such as from air to water, and has been found to affect both physiology and behaviour.

We examined the effects of noise on male-male territorial contests in two species that rely on such contests to ensure the survival of their offspring. The sample populations of the two species differ in their type of interaction with humans. Siamese fighting fish were bred and kept in captivity, whereas non-captive urban populations of the European robin share their environment with humans. The dynamics of contests in both species were affected considerably by noise treatments, with evidence that this might relate to the value they attribute to their territory. Our findings further show that the effects of noise on territory value also carry over to other important fitness-related processes like parental investment. We argue that when studying the welfare of animals, more consideration should be given to their behavioural ecology and the fitness consequences that arise from extensive interactions with humans.

Behavioural predictors of stress in groups of animals

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In many standard laboratory assays involving animals such as rodents and fish, individuals are tested in isolation. However, many of the species used live in groups, and social interactions are central to their normal behavioural profiles. As such, certain response-behaviours exhibited by individuals can only be observed when tested in groups, including changes in social structure, or social behaviours as a result of stress. Thus, group testing can provide a powerful tool for behavioural monitoring, revealing information not otherwise available from conventional single-animal tests - especially as the behaviour of one animal can influence the behaviour of all others within a group. Here, we aimed to determine if metrics describing the behaviour of grouping animals could be used to predict stress. Using zebrafish (*Danio rerio*) as a model, we observed replicated shoals in mildly stressful (i.e. novel) and non-stressful situations (i.e. familiar), and quantified standard behavioural measures commonly used to investigate stress-responses in single-animal tests, in combination with group-specific metrics, such as shoaling and social behaviours. We show that metrics describing grouping behaviour can significantly predict stress, and in many cases significantly outperformed individual-based metrics. Thus, the use of group metric data could allow for the refinement of behavioural protocols carried out in a range of research areas, by providing sensitive and rich data in a more relevant social context that would not be possible to obtain through the testing of isolated individuals.

Do ear postures indicate emotional states in dairy cattle?

Lambert (née Proctor), H¹ & Carder, G²

¹Animal Welfare Consultancy, London, UK

²World Animal Protection, London, UK

Farmers, veterinarians and welfare assessors need practical measures of animal emotions to assess and improve the welfare of their animals. We explored the suitability of ear postures in dairy cattle as indicators of emotional state. Our results show that cattle will perform certain ear postures in response to different treatments, acting as indicators of different emotional states. We used a positive-negative paradigm to elicit what we considered to be frustration and excitement. The 22 focal cows were first conditioned to anticipate the standard feed over four trials. This silage feed was considered to be a neutral stimulus as it did not affect their heart rate, and the cows had constant

access to it. They then received five trials of concentrates feed intended to elicit positive excitement, as this is a highly desired enriched feed. Finally, the cows received one trial of inedible woodchip to elicit frustration. Both the concentrates and the woodchip elicited a high arousal state as the mean heart rate was significantly higher than in the standard feed treatment ($F_{(1.89, 357.29)}=125.70, p < 0.001$). We found the treatments significantly affected the cows ear postures. Ear posture one (upright posture) tended to be performed when the cows were positively excited ($F_{(2, 40)}=19.75, p < 0.001$), whereas ear posture three (ear forward posture) was performed more when they were frustrated ($F_{(2, 36)}=16.07, p < 0.001$). These findings complement our previous work which found ear posture four (relaxed, drooping ear) to be associated with a positive, low arousal emotional state. Ear postures may therefore represent a quick and objective measure that can be used in practice by farmers and veterinarians, as well as in welfare assessments to improve the welfare of cows.

Nature calls: Is there an ecological basis for abnormal behaviour and breeding problems in captive psittacines?

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Psittaciformes are popular as pets, and as aviculture and zoo species. However, in captivity they show variation between species in susceptibility to problems such as stereotypic behaviours and poor breeding. For example, self-inflicted feather-damaging behaviour (e.g., self-plucking) is prevalent in African grey parrots, *Psittacus erithacus*, but rare in Senegal parrots, *Poicephalus senegalus*; while monk parakeets, *Myiopsitta monachus*, breed readily, yet blue-throated macaws, *Ara glaucogularis*, do not. Comparing species using phylogenetic comparative methods could help provide insights into the fundamental bases of such problems, thereby identifying species pre-disposed to be good pets and informing species' *ex situ* conservation management. This study therefore investigated relationships between various species-typical biological traits proposed to affect parrot welfare and three welfare-sensitive captive outcomes: feather damaging behaviours (FDB), other stereotypic behaviours (SB), and hatch rates (HR). Prevalences of FDB and other SBs were gleaned via a survey of pet parrot owners, yielding information on 53 species (~1,380 birds). Captive HRs (chicks hatched/breeding pair/p.a.) for 122 species were taken from Allen and Johnson (1990 Psittacine Captive Breeding Survey). Next, phylogenetic generalised least squares regressions were used to examine the predictive power of the following aspects of species-typical biology: sociality (maximum group size, communal roosting); foraging effort; ecological flexibility (diet and habitat breadth); intelligence (innovation rate, relative brain volume); and IUCN conservation status. Communal roosting ($T_{4, 33}=1.87, P=0.04, \lambda = 0.54$ [one-tailed]) predicted FDB, while higher foraging effort ($T_{3, 34}=-0.10, P=0.06, \lambda=0.65$ [one-tailed]) tended to predict FDB. Relatively large brain size predicted other SBs (whole body: $T_{3, 36}=2.90, P=0.01, \lambda=0.27$; oral: $T_{2, 38}=2.36, P=0.02, \lambda=0$). More threatened species had lower captive HRs ($T_{5, 75}=-2.12, P=0.02, \lambda=0.34$ [one-tailed]). These traits are thus species-level risk factors for poor parrot welfare, so helping to identify species well or poorly-suited for captivity, and suggesting evidence-based ways of tackling these specific problems.

Applying computational methods to animal welfare science

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Behaviour associated with poor welfare, such as 'pessimistic' decision-making, can arise from a number of different affect-induced shifts in cognitive function. For example, risk aversion can arise from an altered sensitivity to, or expectation of, rewards or punishers, and these processes can themselves be influenced by several environmental factors. By characterising the cognitive processes that generate behaviour, we can gain a better insight into the relationship between specific forms of adversity and welfare, and develop more targeted approaches to measuring and improving welfare. Computational modelling provides a means to extract parameters relating to different aspects of cognitive processing from behavioural data. The aim of this talk is to outline how computational modelling can be applied to animal welfare science. We have recently built computational models to describe and explain human and rodent decision-making on the judgement bias task; one which takes a Bayesian ideal observer approach and another which models decision-making as a partially-observable Markov decision process. Analyses using these computational models have indicated that experiencing rewards or punishers prior to testing alters both reward sensitivity and expectation during testing, and that surprising outcomes influence decision-making. As such, these models inform our understanding of the relationship between the environment, affect, and decision-making. The generative nature of computational modelling can also drive the formulation of new hypotheses by allowing researchers to observe the data produced as parameters are varied or added to the model. Thus, computational modelling can be a useful tool in the study of animal welfare.

Welfare and biomedical implications of stereotypic pacing in laboratory macaques

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Stereotypic behaviours are commonly observed in captive animals and are usually interpreted as a sign of poor welfare. Stereotypies have also been linked with brain abnormalities. However, stereotypies are a heterogeneous class of behaviours and mounting evidence indicates that even within one species, different stereotypies can have different causes, and can be linked to different affective states. To better understand the mechanisms underlying stereotypies and their relation with animal welfare, it is therefore crucial to investigate each stereotypic behaviour separately. Understanding the cause of stereotypies is particularly important in laboratory animals. Indeed, beside welfare concerns, if some stereotypies are the product of an abnormal brain, the use of stereotyping animals in research might compromise the validity, reliability and replicability of scientific findings. Laboratory rhesus macaques are one of the main animal models used to understand human brain mechanisms. In research facilities, macaques are often seen displaying stereotypies, the most prevalent one being pacing, when an animal walks repetitively in the exact same pattern. In this presentation, we will review what is known about pacing in rhesus macaques. We will also present new unpublished data showing that under some circumstances of acute stress, pacing can decrease, while another non-stereotypic behaviour, agitated locomotion, can increase. These results suggest either, that pacing as an indicator of acute stress is prone to false negative results, increasing in some stressful situations but not others, or that agitated locomotion has been mistaken for pacing in previous studies and that pacing is in fact unrelated to current acute stress. Both interpretations lead to the conclusion that pacing is unreliable as an indicator of acute stress in laboratory rhesus macaques. The

review of the existing literature reveals that we still do not know whether pacing individuals have a worse welfare than non-pacing ones and whether pacing is linked with brain abnormalities.

Attention bias test in sheep with different induced mood

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The measurement of affective states has become an important issue in animal welfare research in recent years. Mood (i.e. long-term affective state) is highly relevant for animal welfare because subjects in a more positive mood state seem to deal more easily with negative short-term experiences. However, easy applicable methods to assess mood in farm animals are lacking. Attention takes advantage of an animal's spontaneous reaction, and it is thought that, as in humans, a negative mood will lead to heightened attention toward negative compared to positive stimuli. In this study, we conducted an attention bias test using acoustic stimuli in sheep. We tested twice 32 female sheep, before and after inducing chronic stress in 16 sheep and a more positive mood state in the other 16 sheep. We used (1) a white noise played alternatively from one side or the other, and (2) animal vocalizations (i.e. barking dog as presumed negative and bleating sheep as presumed positive stimulus) of varying intensity (i.e. at simulated distances) played simultaneously from one side each. We measured the behavioral reactions of the sheep (e.g. head and ears positions) using an ethogram defining lateral attention in sheep. Results indicate that sheep were more attentive during the vocalizations phase than the white noise phase. As expected, we found an interaction between sheep vocalizations intensity, dog vocalizations intensity and mood. Specifically, sheep in a more negative mood focused their attention more toward the dog low intensity vocalizations when played simultaneously with sheep high intensity vocalizations. Therefore, the attention bias test seems to be promising to measure an animal's mood state without much prior training.

Neural markers of animal welfare

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Animal welfare is at its core about the subjective experience of the animals involved. However, these subjective experiences are per definition very difficult to assess. As affective states are generated and represented in the brain, we attempt to assess these by measuring different aspects of brain structure and/or function. In the lab, we are pursuing two separate approaches to developing neural markers of animal welfare. The first approach is to look for neuroanatomical correlates of chronic stress. In patients with Major Depressive Disorder, as well as in pre-clinical animal models of MDD, the number of newly-generated neurons in the hippocampus decreases. This is reversed upon recovery, be it through environmental enrichment or anti-depressant drug treatment. We are therefore quantifying the number of new hippocampal neurons as a biomarker of recently-experienced chronic stress. We found that chronic stress indeed reduces the number of newly-generated neurons in the hippocampus of both mice and

chickens. We have since applied this knowledge to assess the long-term welfare state of mice undergoing induction of chronic liver disease and laying hens with chronic keel bone damage. The second approach is to look for neurophysiological correlates of acute stress. We are focusing particularly on broiler chickens, and their experience during processing between barn and slaughterhouse. Under these conditions, behavioural indicators of acute stress are difficult to assess. We are in the process of validating neural (intracranial EEG) and other physiological measures of acute stress. I will present our preliminary results in this endeavour.

Human recognition of vocal expression of emotions in domestic and wild ungulates

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Vocalisations constitute promising ideal indicators of animals' emotions. Indeed, vocalisations have been shown to reflect the valence (positive versus negative) and arousal (intensity) of the emotion experienced by the producer across species. In addition, animal vocal expressions of emotions have the potential to be recognized by humans, as a result of conservation of emotion expression throughout evolution, domestication and/or experience with the species. Human-animal emotion recognition would allow pet/farm animal owners to gather instant knowledge about the emotion experienced by their animals, and hence to improve welfare by promoting situations inducing positive emotions and minimising those inducing negative states. Using an online questionnaire translated in 8 languages, we tested if humans were able to correctly rate the valence and arousal of the vocalisations produced by 6 species of domestic and wild ungulates in situations validated using physiological and behavioural indicators of emotions. We then tested if demography (e.g. gender and education), domestication (domestic versus wild), experience with the species (e.g. education/work related with the species and contact frequency), and/or empathic tendencies (Interpersonal Reactivity Index) could explain how accurately participants rated the valence and arousal of the vocalisations. We found that participants were better at recognising the emotions of domestic ungulates (56% correct), compared to wild ungulates in captivity (47% correct; $N = 2072$ participants). Participant's correct ratings also positively increased with experience with the species (contact frequency) and empathic tendencies (perspective taking, empathetic concern and fantasy). Finally, we highlighted several acoustic parameters that participants used to rate vocalisations according to their valence and arousal. Overall, our results show that humans are able to recognise the emotions of most species above chance levels using vocalisations, particularly when experienced, suggesting that educating people about animal vocal expression of emotions has the potential to improve animal welfare.

Effects of different nest designs on nesting behaviour in broiler breeders

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Floor eggs are a common issue in broiler breeder flocks, which are unwanted for two reasons: high economic costs and reduced animal welfare. The extra costs are caused by an increase of manual labour required, in addition to a reduced saleability and hatchability of floor eggs. The welfare of floor laying hens is reduced as the housing, in terms of nest attractiveness, is suboptimal. The goal of this study was to investigate which nest design is preferred by broiler breeder hens to lay their eggs. In a relative preference test four nest designs were provided to six groups of 100 females housed with 8 males during ages 20-33 weeks. The four designs had the following characteristics: nest with a partition wall, nest with a subtle air flow inside created by a ventilator under the nest, nest with wooden walls and control nest. Eggs per nest were collected daily. Videos were made for one day of each pen at ages 24-26 weeks and 27-29 weeks during 3-5h and 7-9h after lights-on. Behaviour inside and outside the nests was scored continuously during 5 minutes per half hour for point behaviours and scan sampled at a 10 minute interval for event behaviours. At 32 weeks of age the most preferred nest in each pen was closed to observe subsequent preference. We found a relative preference for the nest with wooden walls compared to the other nest designs as more eggs, nest inspections and nest entrances were recorded for that nest. Signs of crowding were recorded inside the nest with wooden walls in the form of increased aggression, displacement and piling behaviour. After closing the nests with wooden walls, the hens laid their eggs in the adjacent nest, independent of the design of this nest.

Does play fighting experience influence later life contests? A test with welfare relevance using domestic pigs (*Sus scrofa domestica*).

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Although play behaviour is commonly observed in a wide range of animal taxa, its evolutionary function has long been debated. Several theories have been proposed including, training for unexpected events, skeletal muscle differentiation, and the development of social skills. We investigated the effect of pre-weaning social play experience on post-weaning contest behaviour, predicting that individuals who experienced more social play would be more likely to win a later life encounter with an unfamiliar conspecific. Additionally we investigated the effects on contest dynamics and assessment strategies. In order to explore this, three measures of social play (play fighting experience, additional piglet directed play, and sow directed play) were recorded for 152 individuals (12 litters) on six days pre-weaning. Subsequently, post-weaning contests were staged at eight weeks of age ($n=76$) between randomly paired, unfamiliar pigs in a neutral arena (3.8m x 2.9m). Contest outcome analysis was performed using generalised linear mixed-effects models (GLMME) with a binomial distribution. The difference in competitor play experience, weight, and focal individual sex were included as fixed effects while batch, litter, and dyad ID were used as random effects. Play fighting experience was found to correlate with body weight (Spearman Rank Correlation: $r_s=0.21$, $p=0.02$) and further analysis was performed on a subset of weight matched dyads ($n=34$), allowing weight to be removed from the model. An interaction effect of play fighting experience and focal individual sex was observed (GLMME: $\chi^2_1=3.87$, $p=0.049$), with males being more likely to win a contest when they had the least play fighting experience within the dyad and females being more likely to win when they had the most play fighting experience. These results reveal a sex dependent effect of social play on later life fighting ability and suggest that pre-weaning play fighting experience may lead to enhanced social skills post-weaning.

POSTER ABSTRACTS (in numerical order = alphabetical order by first author)

#1

The influence of negative emotional contagion on behaviour and social interaction in chickens (*Gallus gallus domesticus*).

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Through emotional contagion, emotions carry-over from one animal to another which results in an emotional state matching between two subjects. The current main hypothesis proposes that such contagion facilitates information sharing and coordination in social groups. The scientific study of emotional contagion is of importance to the farming industry as contagion may negatively impact the welfare of many animals. Research on birds provides established evidence for complex social group structures and extensive parental care, suggesting an emotional sensitivity in birds towards conspecifics. While empirical examples of avian emotional contagion are scarce, recent experimental findings corroborate preceding behavioural observations. Studies on chickens demonstrate an empathic responsiveness between parents and offspring, as well as between groups of chicks. Our study investigates emotional contagion further by focusing on its underlying mechanisms and its suggested informative function. A total of 74 chicks participated in pairs in which a demonstrator was either exposed to a stressor or not, while an observer watched. We applied two methods to assess emotional contagion including a behavioural analysis (e.g. freezing) and a physiological evaluation (eye thermography). Prior to the study observers were exposed to the same stressor or not, allowing to disentangle the different modulations of emotional contagion. To examine the information sharing function and whether the demonstrator's response would inform and alter the observer's behaviour, we gave the observer access to the stressor's location and measured an avoidance or approach response. At the end of the experiment we explored a potential effect of emotional contagion on a subsequent social interaction between demonstrator and observer. While data processing is ongoing at the moment of abstract submission, preliminary results suggest that chickens are highly sensitive to the state of others and that a combination of the presented methods has a strong potential to investigate emotional contagion in birds.

#2

An assistant robot improves animal welfare in poultry

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Domestication causes behavioral changes that can affect equally the animal to the breeder. Thus, laying on the ground, caused by a too great stillness of birds, generates a decrease of the profits for the breeder and an increase in working time. In this context, the prototype TiOne robot has been designed, in order to stimulate locomotor behavior of animals. The purpose of this study was to determine the impact of the robot on the behavior and welfare of hens in livestock. Our results showed that immediate movement of the robot induces a density decrease of hens in the area and reduce the immobility. Moreover, the long-lasting presence of the robot had a positive impact on the quality of the plumage, as well as on the reduction of human fear. In addition, zootechnical data showed a significant decrease in laying on the ground. In conclusion, this first study showed the benefits of the robot in poultry rearing, both at the level of productivity as well as that of the animal welfare and of the breeder well-being.

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#3

Keel bone fractures are associated with suppressed adult hippocampal neurogenesis in laying hens

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Objective markers which integrate experiences over a long time period and in a valence-specific manner would contribute considerably to animal welfare assessment. In mammals, adult hippocampal neurogenesis (AHN) is suppressed by cumulative chronic stress and persistent pain, whilst being increased by experiences associated with improved mood, such as exercise, environmental enrichment and antidepressant treatment. Recent findings in our group indicate that AHN in poultry is similarly sensitive to cumulative chronic stress, but whether it similarly reflects the prolonged experience of pain is yet to be determined. Keel bone damage in laying hens is prevalent in commercial flocks, individually variable and likely associated with chronic pain and consequent poor welfare. In an existing longitudinal study, radiographs were taken from Lohmann Brown laying hens housed in a commercial multitier aviary at regular intervals between the ages of 21 and 60 weeks. Each X-ray followed 6 days of mobility data collection, which utilised a custom designed infrared tracking system to record transitions between aviary zones. Following scheduled post-mortem, hippocampal tissue was collected from 15 hens with multiple and/or severe fractures linked to a decrease in their activity levels, and 9 hens with minimal fractures. As expression of the protein doublecortin (DCX) provides a marker of immature neurons arising from AHN, immunohistochemistry was used to stain DCX-positive cell bodies for stereological quantification in serial sections. Whilst hens with severe keel bone fractures had lower densities of DCX-positive cells in the hippocampus than hens with minimal fractures, fracture severity scores also negatively predicted cell numbers on an individual level. These findings indicate that AHN is suppressed by chronic pain in laying hens, as in rodents, and support the potential application of AHN as a marker of lifetime subjective welfare in commercial poultry.

#4

Welfare of pets and people: an inextricable link

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Evidence suggests that pet ownership may have potential health benefits regarding reduced morbidity and mortality of chronic diseases in people. However, more evidence is needed to affirm this relationship, particularly in countries such as Grenada, where there is limited information available on the potential risks or benefits associated with pet ownership on human health as well as a high prevalence of chronic diseases. A cross-sectional study was conducted in Grenada to assess the physical health of local pet owners, who served as the target population, and non-pet owners as the control group. Surveys were used to assess participant demographic information, health history, and pet ownership information. The objective of this study was to determine if pet ownership is protective by reducing risk factors associated with chronic diseases such as diabetes, cancer, and cardiovascular disease. Risk factors such as body mass index (BMI), waste-hip ratio (WHR), exercise habits of the target and control groups were recorded and analysed for statistical significance. Data was collected from 300 participants across all seven parishes in Grenada and described using descriptive statistics. The study confirmed differences between the pet-owning and non-pet owning population. The percentage of pet owners with a body mass index within or above the overweight class (38%) was found to be less than that of the non-pet owning population (66%). Additionally, the prevalence of obesity in pet owning participants (12.5%) was found to be less than that of the non-pet owning participants (50%). Other notable finding include the complete absence of tobacco product use among the pet-owning group compared to 47% of non-pet owners who admitted to using tobacco products regularly. Pet ownership was identified as promoting healthy behaviour and reducing the burden of chronic diseases in people. The welfare of people and their pets are inextricably linked.

#5

Are stones being used as sex toys by Balinese macaques?

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Given that tool use is more functionally constrained than object play, the former should be more temporally structured (i.e., more predictable in its sequence) than the latter. Stone handling (SH), defined as the non-functional and culturally transmitted manipulation of stones in a playful context in several macaque species, is an ideal candidate behaviour for testing this prediction. Indeed, even though SH does not typically lead to any apparent advantage, it may have evolved into the functional use of stones. In this study, we analysed video-recorded data on SH behaviour performed by provisioned free-ranging Balinese long-tailed macaques (*Macaca fascicularis*), a primate species exhibiting sophisticated object manipulation abilities. We focused on two of the 36 SH behaviour patterns: "tap on groin" (TOG) and "rub on groin" (ROG), respectively the repetitive tapping and the rubbing of a stone onto the genital area. We proposed the "sex toy" hypothesis, which holds that TOG and ROG are two forms of auto-erotic stone tool use displayed in the context of object-assisted self-masturbation. We predicted that (1) SH sequences including TOG and ROG should be more temporally structured, and thus more predictable, than those without TOG and ROG, and that (2) SH sequences including TOG and ROG should be overall more temporally structured in sexually mature individuals than in sexually immature ones. We also predicted that (3) TOG and ROG should occur in temporal proximity with, and (4) precede erection in males, and that

(5) the stone-assisted stimulation of the genital area should last longer than that of other body parts. To do so, we compared the frequency, form, duration, and context of expression of TOG and ROG performed by individuals from all age and sex classes. Our study aims to elucidate how non-adaptive but culturally-maintained activities can form a basis from which functional behaviours emerge.

#6

The challenges of collecting behavioural evidence 24/7: Using a novel camera trap methodology to investigate nocturnal behaviour in captive flamingos

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Most behavioural research on captive wild animals takes place during daylight hours, meaning a wealth of data on their complete daily time-activity patterns are being missed. As zoos continue to develop an evidence-based approach to husbandry, such data are especially important for species with nocturnal behavioural performance. One such animal is the lesser flamingo (*Phoeniconaias minor*) that performs a range of nocturnal foraging activities. Using motion-detection cameras, this project aimed to record the behaviour and enclosure usage of a captive flock of lesser flamingos at WWT Slimbridge Wetland Centre. As a specialised species from a narrow ecological niche, lesser flamingos are an ideal subject for investigations into the responses of such species to their captive care. This flock at WWT has access to their entire enclosure during day and night, allowing observations on bird behaviour to be continuous across a 24-hour period. Three trail cameras were positioned around the indoor and outdoor enclosure to monitor bird activity from the end of January to the end of July 2018. Flock activity and enclosure usage (using the modified Spread of Participation Index) were calculated from the photographs taken. Advantages of this method include capturing behaviour largely unseen by staff and visitors, including nocturnal feeding and early-morning courtship displays. However, this method does have several limitations including difficulty in determining behaviour from photographs, technical issues such as water damage and an over-production of photos caused by wild birds or human presence incorrectly triggering cameras. Despite these challenges, this approach to behavioural data collection offers valuable insight into the activity profile of a captive species and shows how species fully use all available enclosure areas. Results produced can direct future research opportunities such as comparing between zoo animals and wild conspecifics and between zoo populations at different institutes.

#7

Assessing a Dog's Life

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We investigate whether 'apparent age', the difference between chronological age and biological age, can be used as a measure of the cumulative life-time experiences of dogs in terms of their welfare status. We chose domestic dogs as our model species because of the variety of lifestyles they can experience: pet, stray, shelter, laboratory, working, laboratory, amongst others. The dogs' apparent age was assessed by a machine learning process. We used the Computer Vision System Toolbox in Matlab R2018a to create a collection of image descriptors. Each descriptor consists of a subset composing features from the original image by the command "bagOfFeatures". A total of 1000 descriptors for each image were obtained using a custom extractor based on colour information. We trained the computer with a 90 pictures dataset divided in three categories of dogs' age: Young, Adult and Senior (30 pictures in each category). The resultant trained image classifier from age categories dataset had an accuracy of 56.7% with the Support Vector Machines classifier method. The most accurate category was Seniors with 72% of correct identifications from the dataset, followed by Young with 61%, and Adults with 37%. Adults were 33% classified as young which even for experienced dog handler could be easy to misclassify, however, the model was consistent in distinguishing Young from Seniors. Animals that are biologically ageing more quickly than their chronological age are prone to a variety of diseases and reduced longevity, hence the importance of differentiating young from senior. The next steps include increasing the dataset and model's accuracy, yet this model already can point that the Apparent Age Assessment, can useful and inexpensive and has the potential to be used in animal welfare assessment.

#8

The effect of positive affective state on social interactions

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The red junglefowl, *Gallus gallus*, is a social species in which individuals of both sexes duel to establish their position on dominance hierarchies. Positive affective state, where individuals behave more optimistically, could make individuals more likely to initiate duels and thus win. As a consequence, affective state could potentially affect social interactions and status.

Positive affective states have been associated with increased impulsivity; this in turn could also make individuals more likely to initiate duels by reducing time spent planning or considering potential negative consequences. Therefore, more impulsive individuals are also expected to win duels.

A cognitive judgement bias test was used to determine individual levels of optimism. Impulsivity was tested using a detour-reaching task, and duel outcome determined using staged duels between morphologically matched individuals. Only in females did optimism and impulsivity tend to affect social interactions. However, despite predictions, less optimistic and less impulsive females were the ones initiating and/or winning duels. In addition, birds were personality assayed and from this, we show that females that won duels tended to be more aggressive, and males that won duels were bolder. This indicates that both aspects of cognition and personality can affect social interactions, and in a sex specific way, in turn which can have potential welfare implications.

#9

Understanding sleep-related behaviour (and lack of) as a measure of welfare using the horse as model

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Horses sleep for an average of 3 hours inside of a 24-hour time budget, less than half of the time spent asleep by humans. As a large prey animal, the horse appears to have adapted to function optimally on comparatively little sleep. However, current knowledge pertaining to the importance of obtaining sleep, what is quantifiable as 'good quality sleep', and the consequences of depleted sleep opportunities is still lacking in terms of equine welfare. Using recumbent behaviour as a recognised marker for when horses most effectively rest and engage in Rapid Eye Movement sleep, this paper discusses factors known to alter equine sleep that may thus affect equine welfare. In particular, 1) The influence of equine husbandry practices on nocturnal behavioural profiles should not be underestimated. Straw bedding may be used to facilitate a nocturnal behavioural repertoire that promotes more biologically significant behaviours including greater than average recumbent behaviour in the stable. Recumbency is seen as an instinctive behaviour linked to rest across species although the extent to which prolonged duration may be considered a beneficial adaptation is yet to be discussed. 2) Significant differences in nocturnal behavioural profiles have been observed for horses acclimatising to overnight stabling from overnight turnout suggesting that horses may suffer from sleep deprivation as a result of changes in routine. Sleep duration has also been positively correlated with competition performance suggesting that the impact of novel overnight environments at competition location may require further consideration 3) Finally preliminary studies of nocturnal auditory enrichment within the stable indicate that this type of intervention may help to reduce behavioural switching during the nocturnal behavioural repertoire. Future research may consider the use of sleep deprivation may be a useful measure of the quality of sleep where indeed this has been linked to emotionality in previous research.

#10

Validating a new social science questionnaire for working equid owners: The effect of owner attitude on equid welfare

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It is now recognised that the quality of human-animal relationships plays a significant role in animal welfare and that human perspectives towards animals are affected by both culture and context. However, there is little research on working equid-human relationships, a context where this knowledge may have a significant impact on working animal welfare. This study field-tested a new protocol for assessing working equid welfare which included questions on the topics of owner perspectives towards their equids and the social transmission of equid management practices. The protocol was trialled on 62 working equid owners from communities in Portugal and Spain where, despite the decline in traditional agricultural practices and livestock keeping, donkeys remained used for agriculture and tourism. Some owners stated that the help that donkeys provided was invaluable, however, owners showed two differing perspectives towards their equids; primarily instrumental: viewing their equid as a working tool, or affective: incorporating an emotional connection with their equid. These two perspectives have been shown to differ based upon geo-

cultural context and this study showed a link between these perspectives and equid management practices. Equids of affective owners had a higher average body condition score than those of instrumental owners. Recognition of animal emotions also influences the treatment of domestic animals and in this study, the general health status of equids whose owners believed that they could feel emotions was significantly better than those whose owners did not. The findings suggest that the new protocol is feasible, providing insights into the traditional practices, attitudes and beliefs of equid owners and detailing how these factors may influence the welfare of their animals. Increasing understanding of the cultural context, social structure and attitudes within a community may, in future, help to make equid welfare initiatives more effective.

#11

Monitoring sleep in captive primates to enhance welfare

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Primates, like many other animals, spend more than half of their lifetime sleeping and resting, yet this behavior is almost completely unstudied compared to daytime activities. In their natural habitats, it might be difficult or impossible to directly observe nocturnal activities, but in captivity, where we are able to closely monitor both day and night activity, the opportunity exists to record, study and thus better understand individual and/or group sleeping behavior and patterns. In humans, Alzheimer's, cardiovascular disease, diabetes, obesity, stress, depression and even social isolation have shown significant and sometimes causal links to poor sleep (i.e. sleep loss, disruption). If such relationships exist in humans, sleep may also influence the behaviors and physical and mental health of our close primate relatives. Primate caretakers, researchers and staff working in a captive setting have the unique opportunity and responsibility to provide the best possible care to each individual. We suggest that monitoring sleep is an important step to allow for 1) the better understanding of individual needs and behaviors, and 2) the detection of changes in behaviors and potential prediction of welfare problems. We will present our independent research on monitoring the sleeping patterns of Rhesus macaques (Green) and chimpanzees (Havercamp) to better understand their sleep and other nocturnal activities, with the goal to work towards improving the mental and physical wellbeing of primates living in various captive environments. We argue that failing to address the sleeping behaviour of captive primates could be considerably limiting our understanding of these animal's biology.

#12

Enriching the lives of wolves (*Canis lupus*)

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The grey wolf (*Canis lupus*) is the most commonly kept canid species in European zoological collections however, there is limited research available on the influence of environmental enrichment on their behaviour and welfare. A survey was sent out to wolf keepers to gather an understanding of the types of enrichment currently being provided to captive wolves and keepers perception on the effectiveness of

some of these methods. 58 collections covering 11 subspecies of grey wolf responded and survey results found that naturalistic enclosure structures (44%), and food based enrichment (34%) were considered by keepers to be the most important. However, analysis of the survey results suggest a difference between the type of enrichment keepers perceived to be most important and which enrichment was most commonly offered to the wolves ($p=0.018$). Based on the survey responses seven different enrichment items were designed and offered to wolves at two separate UK collections. In line with results from the survey food enrichment was interacted with for longer than the other enrichment items ($p<0.03$). Also, the wolves returned to the food and the cognition-based enrichment more often than any other enrichment. Cognitive enrichment, based on the survey findings, was one of the least offered items suggesting the need for more research to provide a basis for an evidence based husbandry approach to the keeping of grey wolves.

#13

Environmental enrichment during laying influences Japanese quail offspring's phenotype

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Animal welfare is strongly influenced by characteristics of the animals' environment. Thus, enrichment of Mammals' and Birds' environments at early age modulates their behavioural development and can increase their ability to cope with their milieu, consequently improving the animals' welfare. However, individual behavioural development is strongly affected prenatally by maternal living conditions. Many authors consider these prenatal maternal effects to be prenatal adaptive mechanisms preparing offspring for their postnatal environment. In this context, our study investigated the effects of environmental enrichment (via foraging and structural enrichments) of laying Japanese quail females' living conditions on their offspring's general development. For that, 10 laying quail were kept in enriched cages (EC females) for 5 weeks and characteristics of their eggs and chicks were compared to those of 10 females housed in standard cage (SC females). After the treatment, EC females were heavier than SC females and produced eggs containing proportionally more yolk and less albumen than those of SC females. However, levels of egg hormonal contents (testosterone, androstenedione, progesterone) did not differ significantly between the two groups. Chicks of EC females (EC chicks) were globally heavier than chicks of SC females (SC chicks) from hatching to 5 weeks old and their sexual development was more precocious than that of SC chicks. Moreover, emotional reactivity of EC chicks, in behavioural tests, was lower and their social motivation was higher than that of SC chicks. So, our study shows that enrichment of laying females' environment modulates their offspring's morphological and behavioural development, probably in relation to characteristics of the eggs. Controlling prenatal maternal effects could be a way to improve animal welfare over several generations.

#14

Clicker versus non-clicker training: effect of training method on behaviour and heart rate in donkeys (*Equus asinus*)

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Studies in horses (*Equus caballus*) have shown the benefits of clicker training, such as a reduction in physiological indicators of stress and increased exploratory behaviours evidenced. Whilst not behaviourally the same as horses it is prudent to review if the same response is seen. Donkey assisted therapy is increasing in popularity in the United Kingdom and Ireland, especially as a role for rescued animals. Over an 8-week period, 24 donkeys (mean age 3.125 SD +/-2.52) were trained on three tasks. These were lifting hoof, stepping backwards and walking with a head collar and lead. Donkeys were randomly allocated to either the clicker training or non-clicker trained group before being trained using positive reinforcement methods. Heart rate was measured before, during and after training. Behaviour was recorded using a video camera and later reviewed using instantaneous sampling of state behaviours at one minute intervals and continuous recording of event behaviours. Donkeys trained using non-clicker training methods showed more avoidance behaviours ($p < 0.001$) and had a significantly higher mean heart rate ($p = 0.047$) than the clicker trained group. The clicker trained donkeys showed more investigative behaviours and more nip behaviour ($p < 0.001$). Activity budgets also showed that clicker trained animals spent less time stationary and approached the trainer more quickly in successive training sessions. Whilst based on a relatively small sample, the results indicate clicker training could be less stressful for donkeys than other methods indicating the need for further research on this topic.

#15

Interview with a rhino – How vocalisations reflect social interactions in the captive Southern white rhinoceros (*Ceratotherium simum simum*)

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White rhinoceroses (WR) are one of Africa's most endangered mammals and highly reliant on successful captivity breeding. Regrettably, these semi-social megaherbivores often reproduce only laboriously in captivity. Deficiency in socio-sexual behavioural patterns and unsuitable group composition are currently discussed as potential causes and raise the question of how these conditions can be monitored non-invasively. Bio-acoustic methods were currently investigated for animal welfare monitoring, as vocalisations are discussed to be indicators for social interferences and individual condition. Therefore, this study aims to examine the function of vocalisations in WR regarding socio-sexual behaviour and the quality of social interactions between group members. Behaviour and vocalisations of 32 Southern white rhinoceroses (*Ceratotherium simum simum*) were video- and audio recorded in seven European zoos using focal animal sampling. Vocal analysis focused on the three predominant call types: pants, threats and snorts. In order to clarify their function in social interactions, the context in which each call type was uttered and the respective receiver of the call were determined. The results show that the three call types have distinct functions. Pants are mainly uttered in affiliative interactions by males while approaching the females. Threats are mainly uttered by females towards males during agonistic interactions to displace the bull. Snorts are uttered by all individuals equally during various positive relieved contexts such as resting, feeding and affiliative interactions but not during agonistic interactions. It can be concluded that the three call types can be used to monitor sexual behaviour (pants) as well as the social quality of group composition (threats, snorts). Thus, in order to address the animals' natural requirements in captivity, attention should not only be paid to obvious signs of behavioural anomalies (e.g. stereotyped movements, escalated fights, health impairments), but also to vocalisation that can serve as a bio-acoustic tool for animal management.

#16

Learning via exploration in captive capuchin monkeys?

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Famous for their stone tool use, wild capuchin monkeys have been documented to explore novel objects and engage in object play (Westergaard & Suomi, 1994). Human children have been shown to learn about object properties via play (Sim & Xu, 2017), however, there is little information about whether object exploration can lead to learning in non-human primates. In this task we aimed to investigate whether capuchin monkeys could learn the affordances of objects via unrewarded exploration. Ten monkeys were given two sets of 10 balls (5 heavy, 5 light, coded by colour) in two unrewarded play sessions (of 2 minutes each), in which they could explore the balls alongside a collapsible-platform box. When heavy balls were inserted into the box, they caused the shelf to collapse, whereas the light balls did not.

Exploration sessions were separated by a *rewarded* session where the monkeys were given another set of 10 balls with a reward on the collapsible platform. Monkeys won rewards for inserting the heavy balls but not for inserting the light balls. Each session involved novel ball sets. They then underwent 15 forced choice trials in which the 30 balls from the previous phase were presented pairwise, and rewards were present in the box. All 10 monkeys engaged with the task during the *rewarded* sessions, but in contrast only 2/10 monkeys inserted balls into the box during the *exploration* sessions. At test the monkeys chose the heavy ball at chance levels. Detailed coding of the exploration behaviour is currently underway, but preliminary results suggest that in the absence of a direct food reward the capuchin monkeys were not motivated to explore the balls, even after experiencing the connection between inserting balls into the box and food rewards.

#17

The effects of personality upon breeding success in Humboldt Penguins (*Spheniscus humboldti*)

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Humboldt penguins (*Spheniscus humboldti*) are a vulnerable species commonly kept within UK zoological collections. However, in captivity, their breeding success is half that of their wild counterparts. Past research has tended to focus on determining optimal breeding conditions, however personality may also be a determinant of breeding success. The aim of this study was to determine whether personality affects breeding success in captive Humboldt Penguins. Personality profiles were created for 30 individual Humboldt penguins (15 pairs), housed at ZSL London zoo. Personality was assessed using keeper questionnaires considering interactions with conspecifics and human-animal interactions, novel object testing (via three separately presented novel objects: bamboo wind chime, glitter ball and an abacus) and behavioural observations. For the behavioural observations, each individual was observed for a total of two hours over a two-month period via continuous focal sampling. Breeding success was assessed in terms of number of eggs laid, number of eggs hatched and number of chicks fledged. Data on breeding success were obtained from the Zoological Information Management System (ZIMS) for 2014-2017. Principal component analysis was used to create personality dimensions, and individuals were rated as high/low on each dimension. Chi square tests were then used to determine if there was an association between each personality dimension and reproductive success. Three personality dimensions were identified: dominant, sociable and shy toward people. Penguins that were less sociable and shy toward people showed

greatest reproductive success and produced more eggs, chicks and fledglings. These findings suggest that personality can affect breeding success in Humboldt penguins and is a factor that should be considered in captive breeding programmes. Interventions such as providing more nest sites away from public access and reducing keeper interactions to a minimum may be beneficial in enhancing reproductive success in captive Humboldt penguins.

#18

Veterinary behavior: assessment of veterinarians' training, experience, and comfort level with cases

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Studies of US animal shelters consistently indicate that behavior is often a major reason for relinquishment and, thereby, euthanasia for millions of dogs and cats annually. Even though this is an area where veterinarians can intervene to support the human-animal bond, prior research has documented that they frequently do not bring up the topic during appointments. This study explored veterinarians' training in animal behavior and behavioral medicine, along with their level of comfort in treating common behavioral problems. An online survey of practicing veterinarians ($n = 1085$) found that only 42.8% felt they'd received a significant amount of training in this field during veterinary school, but the majority reported participating in continuing education sessions about behavior. Almost all respondents reported seeing patients with behavioral issues (99.6%), even when the initial appointment was made for other reasons. Participants felt most comfortable discussing inappropriate elimination and begging for food, but were least comfortable treating issues involving aggression. Most veterinarians treat their own behavior cases, using a combination of behavior modification techniques and medication. Only 22.1% refer cases needing behavioral therapy to a specialist. Given the prevalence of behavioral problems in companion animals, and the potential for early veterinary intervention to play a significant role in animal health, it is important for veterinary schools to include this topic in their curricula. At present, 73% of schools require a course in animal behavior. The release of the new Competency-Based Veterinary Education (CBVE) framework is anticipated to support a greater teaching emphasis in this area.

#19

Habituation to enrichment in kea (*Nestor notabilis*)

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Animals may be held in captivity for multiple reasons, for example in research institutions and zoos. Enrichment can be of great importance for the physical and mental health of captive animals, since it provides occupation and stimulates captive animals' minds. However, one of the problems with providing enrichment is the habituation to enrichment items, meaning the amount of responses towards the item decreases after repeated presentations. Kea parrots are inquisitive and social birds, known for neophilia, complex cognitive abilities and for their complex play behaviour. They are also potentially long-living and popular as aviary birds. Inclusion of enrichment is therefore essential for captive kea to ensure their wellbeing and to allow for wider behavioural repertoire. The experiments in this study were conducted with three populations of captive kea (two from a research facility and one from a zoo). It is investigated whether the time enrichment is available, or if addition of visual or olfactory stimuli influences kea's habituation to enrichment. Wooden cubes altering in colours or smell were used as enrichment items. Preliminary results show there is a difference in usage of enrichment depending on the presentation protocol. Kea used the enrichment with added visual or olfactory complexity more than without it. Findings like these could be used when designing enrichment items and schedule for captive kea.

#20

A computational ethological approach to filial imprinting in chicks (*Gallus gallus*).

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Filial imprinting allows animals to learn the characteristics of an object during a sensitive period. Imprinting has been investigated in domestic chicks using observational methods, by testing many animals for a short amount of time. A growing restriction of preference for what is familiar, associated with a preference for slight novelty at an early stage of imprinting, has been described. Little is known, though, about the exact time course of filial imprinting preferences for familiar and unfamiliar objects, and about sex differences. This study aims at investigating the preference for the imprinting object across 6 days composed of two phases (imprinting and testing phase) and the effect of sex of imprinting in chicks (*Gallus gallus*). We developed and tested an automatized set-up. Soon after hatching, chicks were individually placed in an apparatus with two opposite screens where stimuli (a green cylinder or a blue cube) were played. During the imprinting phase, chicks were reared with one stimulus and during the testing phase chicks were stimulated with both stimuli and we scored the amount of time spent at the familiar stimulus. Our method confirms the preference for the familiar object previously observed in the literature and is robust enough to describe the exact time course of imprinting preference and sex differences. We hence show how to use this method to fine tune the exploration of filial imprinting across time. The greater accuracy of this method compared to other paradigms has the effect to significantly reduce the sample size required in imprinting experiments, addressing an important ethical issue in animal research.

#21

Cognitive offloading in pet dogs

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Cognitive offloading is the spontaneous use of physical action to reduce the mental load of a task. For example, people often rotate their head to ease the recognition of a rotated image. Using cognitive offloading strategies improves performance in different domains and tasks, allowing us to overcome the intrinsic limitations of our cognitive systems. Until now, this phenomenon has only been investigated in humans. With the present study, we aim at assessing cognitive offloading in a non-human animal, pet dogs (*Canis familiaris*), for the first time. This comparison is useful to better understand the origins and phylogeny of cognitive offloading strategies. Just like humans, dogs could align rotated images by using cognitive processes (internal normalization) or physical action (external normalization). In this study we focus on dogs' head tilts as possible physical action to solve a mental rotation task. We first trained pet dogs to recognize rewarded and unrewarded geometrical shapes in an upright orientation and then tested them with the rotated versions ($\pm 45^\circ$, $\pm 90^\circ$, $\pm 135^\circ$) of the same stimuli. Both training and test followed a simultaneous two choice discrimination paradigm. Stimuli were presented on a touchscreen. This system automatically scores whether the response is correct or not for each trial. A movable chinrest opposite to the screen allowed dogs to start each trial keeping the head in a standardised position. This permits a rigorous offline coding of video recordings, in which the exact angle between the dogs' nose-forehead line and the vertical axis will be measured. If dogs share, to some extent, their mental rotation mechanism with humans, we expect wider rotations of the stimuli to cause higher internal demands and hence wider rotations of the head and lower accuracy.

#22

Chemosensory enrichment as a simple and effective way to improve the welfare of captive lizards

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Environmental enrichment has proven to be a useful and effective welfare tool in order to evaluate and enhance the well-being of captive animals, but only when it is based on detailed knowledge of each species' natural behaviour. Chemoreception is fundamental to many aspects of reptilian biology; however, sensory enrichment with chemical stimuli has rarely been applied to reptiles. In this study, we evaluate the use of chemosensory enrichment as a method to enhance the welfare of *Podarcis liolepis*. For seven weeks, we exposed field-caught males to scents from donor conspecific males collected on pieces of filter paper (i.e., "enriched" group, $n = 18$), and compared their behaviour to that of control males provided with unscented pieces of filter paper ($n = 18$). We measured the occurrence of normal (e.g., locomotion) and abnormal (escape attempts) behaviours each day for three weeks. In addition, we conducted two exploration tests and a visual barrier test. Compared to controls, enriched lizards showed a consistent long-term decrease (29%–38%) in the occurrence of escape attempts. During exploration tests, enriched lizards spent less time performing escape attempts and devoted more time to perching than controls. As expected, both control and enriched lizards showed a reduction of time in locomotion and an increase in the time spent perching between the first and second exploration test, but these changes were significantly more pronounced for enriched animals. Taken together, our results suggest improved welfare of enriched animals, as they spent less time engaging in abnormal behaviours, more time in normal behaviours, and showed signs of faster habituation to a novel environment. Chemosensory enrichment is a relatively simple enrichment strategy that could potentially be applied to improve the welfare of a wide range of captive lizards, and reptiles at large.

#23

A preliminary investigation into personality and pain in dogs

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Adherence to basic animal welfare standards involves effective monitoring and control of pain, especially in a veterinary setting. Assessment relies on behavioural and physiological indicators. However, individual differences in physiology mediate consistent individual differences in behaviour, referred to as personality (Koolhaas et al., 1999). Therefore, personality may confound measurements of pain (Ijichi et al., 2014). The current work is a preliminary investigation into whether Extraversion and Neuroticism are associated with differences in individual behavioural and physiological responses to pain. Twenty dogs were observed during recovery from routine castration in a clinical setting. Core temperature was recorded using Infrared Thermography (IRT) (Stewart et al., 2008) upon admission, 15 minutes post-extubation and every 30 minutes thereafter, until the subject was collected by their owner. Behaviour during recovery was scored using Short Form Glasgow Composite Measure Pain Scale (Reid et al., 2007) at the same intervals as IRT readings. Personality was measured using Monash Canine Personality Questionnaire- Revised (Ley et al., 2009) and owners rated their dog's tolerance to pain on a five-point Likert scale. Pain score did not have an association with eye temperature discrepancy or core temperature changes from control, indicating it may not predict affective response to pain. More highly extravert subjects had significantly higher pain scores ($p = 0.031$), despite experiencing similar tissue damage. More extravert subjects showed significantly greater right eye temperature ($p = 0.035$), suggesting hemispheric dominance. Neuroticism had no association with physiological or behavioural responses to pain. Finally, owners were not able to predict their dog's behavioural or physiological response to pain. These results indicate that personality may be a useful clinical tool for assessing individual differences in response to pain, whilst owner ratings of their dog's response is not reliable.

#24

Perception of the ethical acceptability of live prey feeding to aquatic species kept in captivity

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Previous research into public perceptions of live prey feeding has been focused on terrestrial animals. The reasons for this likely relate to the difficulty humans have in being compassionate to animals who are, phylogenetically, distantly related. To test these assumptions, the general public (two groups; one who had just visited an aquarium; and one group who had just visited a zoo), aquarium professionals in the UK/US and terrestrial zoo animal professionals would differ in their responses when asked about feeding various live aquatic animals to one another. Likert based surveys were used to obtain data face to face and via online social media. Demographics in previous research identified a lower acceptance of live prey feeding by females, however, in aquatic animals this was not reflected. Instead, separations in perception were seen to exist between participants dependent on whether they had just visited a zoo or aquarium or worked with animals.

#25

Developing an automated system for monitoring poultry welfare via their vocalisations

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To date, audio analysis for monitoring animal health and welfare has mainly focussed on the development of disease classification tasks, particularly those associated with respiratory illnesses. It is also known that physiology and emotional state can have a major influence over the vocalisations that animals produce. In chickens, vocalisations associated with body size, social isolation, fear, and pleasure have been identified through behavioural studies and acoustic analysis. Most of these studies have been carried out in controlled laboratory environments, and there is a need to study chickens in commercial farm settings. Here, we introduce the LiveQuest project, an ongoing collaboration between partners in the UK and China, to monitor poultry welfare via their vocalisations on intensive farms. Our research is being carried out in Guangxi province, China. We collected over 98 hours of flock recordings from an intensive farm. From these, we built a database of 160 recordings of high-volume individual vocalisations and 160 general recordings of barn and flock noise. High-volume individual vocalisations are used, because they can be recognised from the rest of the flock and can be identified in the animals' repertoire. Some of the sounds in the animal's repertoire correspond to states such as fear, or pleasure, that can inform us about the welfare of the animal. After labelling these recordings, we extracted acoustic features from them and used them to train a variety of classifiers such as Support Vector Machines (SVM), *k*-nearest neighbour algorithms, and classification trees. Out of all these models SVM's produced the most accurate results, correctly classifying 81% of the recordings.

#26

Impact of three common blood sampling techniques on aspects of animal welfare in laboratory mice

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In mice, several blood sampling techniques have been established and are in accordance with guidelines for laboratory animal handling. While the effects of these methods on blood quality and histological alterations at the sampling site are well studied, their influence on the animals' welfare has not yet been extensively investigated. Therefore, the aim of our study was to compare three commonly used blood sampling techniques regarding their effects on different indicators of animal welfare, including endocrine, behavioural and physiological parameters. For this purpose, young adult male C57BL/6J mice were subjected to blood collection from either the *vena facialis*, the retrobulbar plexus, the tail vessel or were allocated to the respective control condition. While all blood sampling techniques led to an acute increase in plasma corticosterone levels, the stress response was strongest in animals that underwent sampling

from the *vena facialis* and the retrobulbar plexus. Similar results were found when the time course of hormone secretion was monitored over a period of 24 hours by repeated measurement of corticosterone metabolites from faecal samples. Moreover, behavioural responses to the different blood sampling procedures were investigated, including pain measures, home-cage behaviour and nest building as well as exploratory behaviour and neophobia. Thus, by also taking the animals' perspective and the severity of the particular sampling method into account, our study could contribute to refinement and selecting the most suitable blood sampling technique for mice in biomedical research.

#27

Keepers' ratings of personality, but not behavioural tests, predict male reproductive performance in the critically endangered European mink (*Mustela lutreola*)

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The conservation of critically endangered species, such as the European mink (*Mustela lutreola*), relies on captive breeding. However, in this species, extreme aggressive/passive behaviour at mating compromises male reproduction, jeopardizing conservation actions and raising welfare concerns. Personality - assessed by behavioural tests - can affect reintroduction outcomes in this species; however, it is unknown if aggression/passivity at mating reflects long-term personality differences. Thus, we tested whether personality: i) affects mating success and, ii) can be assessed practically by keepers. Five 'blind' keepers scored 107 mink (49M:58F) on nine personality traits (10-point scale): bold, shy, aggressive, passive, curious, playful, social, patient, impatient. For 97 mink (45M:52F) four behavioural tests were performed: novel object test (home cage), novel arena test, mirror test (home cage and novel arena). Latency to leave nest box and contact object/mirror, as well as behavioural responses to object/mirror (e.g. biting) were recorded. Copulatory success was assessed for the 18 males bred following personality assessment by recording whether they copulated, and the ratio of mating attempts resulting in copulation. The strength of keepers' agreement varied with trait rated (strongest: bold, W:0.32, $p < 0.05$; weakest: playful, W:0.19, n.s.). The number of keeper-rated traits for further analyses was reduced to bold and aggressive (traits correlating with $R^2 > 0.8$ were considered to convey similar information). Principal Component Analyses on behavioural tests' variables revealed three personality types: bold, extrovert and social. Keepers' ratings and behavioural traits partially covaried: bold and aggressive-rated mink also behaved boldly (bold: $F_{1,93} = 8.05$, $p < 0.01$; aggressive: $F_{1,93} = 11.47$, $p < 0.01$), and bold-rated males also behaved more extrovertedly ($F_{1,40} = 8.35$, $p < 0.01$). Males rated by keepers as more aggressive were less likely to copulate (Chi-square=4.98; $p < 0.05$) and tended to have lower ratios of success when they did ($F_{1,14} = 3.45$, $p = 0.08$). Keepers' ratings, particularly of aggression, may be considered as a tool in the reproductive management of male European mink.

#28

The effect of rearing complexity on navigational ability

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UK farm animal welfare assurance schemes state laying hens reared in commercial systems must have access to perches by 10 days of age. There is some evidence that earlier perch provision, coinciding with periods of rapid brain development, may provide greater long-term benefits but the effects of early perch provision have not been fully characterised. The aim of the current study was to assess the effects of perch provision on chicks' physical and spatial abilities. Ninety-six day old chicks were reared in 16 pens. Chicks from half of the pens were reared with A-frame perches with 8 rungs varying from 10cm to 60cm high, with attached platforms (30cm by 30cm) and ramps (30cm by 40cm) at an angle of 40 degrees. The remaining pens were provided with no enrichment structures. All chicks were tested in two navigational tests: a detour test and jump test. To complete the detour test chicks had to navigate out of a U-shaped holding pen to reach companion chicks that could be viewed through wire mesh. The Jump test required the test chick to jump up to platforms (20cm and 40cm high) to reach the companion chicks. Preliminary analysis showed that the enriched group completed the test significantly more quickly than the control group ($F(1,65)=4.032, p=0.049$; enriched group $58.42s\pm 52.5$; control group $89.91s\pm 73.73$). There was no difference in the rearing groups' latency to complete the jump test. However, the proportion of chicks that completed the test successfully increased from 37% at 15 days of age to 83% at 28 days of age. The results suggest that physical ability at less than 4 weeks of age was not affected by the provision of additional enrichment structures, but that spatial navigation was improved. This may be important in commercial situations to ensure navigation throughout the complex laying systems

#29

Assessing transient affective state using intracranial recordings of brain oscillations in poultry

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The welfare of farmed animals is becoming an increasingly important and emotive factor for the public when determining what products to purchase. Additionally, concerns have been raised in regards to welfare and consequently the implementation and maintenance of welfare standards has been the focus of important legislation. Clearly animal welfare is a significant research area to both the public and government. Many welfare studies focus on the effect of chronic stressors on animals, however, it is also vital that we consider the stress which can be imposed by short term processes animals encounter throughout their lives. Traditional methods used to measure welfare such as stress hormone levels, behavioural observations or gene expression are difficult to implement for the assessment of an animal's transient affective state, during periods of restriction or inaccessibility. Broiler chickens are exposed to many short term processes prior to slaughter, e.g. capture, loading into crates, transportation and lairage, all of which restrict the animal's movements and the opportunity for a researcher to collect samples. Recording local field potentials from key brain regions (Nidopallium Caudolaterale and Nucleus Taeniae) suspected to be involved in processing affective state, using intracranial electrodes, could provide a method to measure transient affective state during these processes. I will be presenting data from a pilot study that aims to validate electrophysiological markers of affective state in 6 week old broiler chickens.

#30

Newly-hatched chicks (*Gallus gallus*) discriminate between sexes

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It is not clear when and how animals which are not sexually dimorphic start to tell apart males from females of their own species, and how they use this information to drive their social behaviour. Questions on early behaviour are conveniently addressed using precocial species such as galliformes, that can be controlled-reared and tested soon after hatching. For these reasons, we used chicks of the domestic fowl (*Gallus gallus*) that were observed with or without previous experience with conspecifics to test (1) whether soon after hatching male and female chicks preferentially approach individuals of same or different sex; (2) how age and experience with other chicks affect sex preferences. Looking at the time spent close to groups of males or females, we found that newly-hatched chicks could discriminate between males and females, and spent more time with females. However, exposure with conspecifics of same or different sex influenced the preferences: after four days of exposure to same sex peers, chicks initially aggregated with opposite sex siblings. No preference was detected for chicks reared with opposite sex peers. We discuss these results in the context of predispositions evolved for social behaviour and highlight how social predispositions may have crucial potential applications for animal welfare.

#31

Octopus: A multi-armed approach to improving captive welfare

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Since the inclusion of cephalopods in animal welfare research legislation, interest in determining good welfare parameters and baseline behaviours in this group of animals have increased. Using octopus as an example we will discuss whether changes in behaviour can be used to detect and predict welfare problems in cephalopods. A brief overview of how these changes may vary dependent on species and ontogeny will also be presented. By examining these behaviours, we hope to demonstrate the similarities and differences with other animals often seen in captivity, giving an overall perspective of how cephalopods fit within other animal welfare frameworks.

#32

The CAREFISH project: establishing scientific welfare standards in fish farming certification

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Many certification schemes appeared in recent years for environmental friendly, safe, ethical, and sustainable food production. Presently, there are at least 30 certification schemes relevant to aquaculture production, including schemes promoted by retailers, industry, governments, NGOs and other organizations. However, none of them include specific welfare requirements related to the coping of aquaculture conditions provided. The pillar for effective and relevant requirements should be the natural behaviour of each species and how the captive conditions influence its ethology. The CAREFISH project (2018-2019) uses this framework to develop specific fish welfare standards to be included in the Friend of the Sea (FOS) certification scheme. This project is based on the experience and knowledge of FishEthoBase, an open-access database that aims to assess current and prospective welfare of all farmed fish species. The core of the CAREFISH project relies on a series of visits to FOS certified fish farms worldwide to perform gap analyses, based on which a series of recommendations are proposed to address identified welfare issues. As a result, we aim to establish welfare criteria that are 1) feasible, 2) auditable and 3) make a difference to lives of fish. These are designed according to species-specific needs, rearing conditions and life stages. The results after visits to 24 farms of nearly a dozen species reveal a very low percentage of use of humane slaughter methods, lack of appropriate environmental enrichment, higher densities than recommended insufficient space and/or induction of spawning through invasive methods. In most cases there is no or insufficient training in welfare. Most farms, however, show interest in improving the welfare of their fish population. The CAREFISH project is funded by the Open Philanthropy Project (California, USA) and based on an agreement between Friend of the Sea (FOS) and fair-fish international.

#33

Combining behaviour, personality and life history to evaluate the welfare of police horses

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The welfare of an individual is related to different aspects of its life, but it is mainly associated with the ability to cope with change. Stabled horses have a predisposition to develop behavioural and health problems associated with the lack of control over their environment, which leads to poor welfare. In police horses, the occurrence of these problems is even greater, not only because of the type of training these animals receive, but also, due to the absence of a standard procedure for selecting suitable individuals for the activities. Personality traits actively contribute to an individual's adaptability and susceptibility to the development of disease, hence assessing horses' personality could be crucial for their welfare. In this study, we evaluated 46 police horses in a semi-confinement regime in Brazil using non-invasive methods to assess their behaviour, personalities and health. To this end, behavioural tests were conducted (Frustration test and Novel object test),

the personality of the horses was evaluated through questionnaires and a survey of veterinary records was done. Additionally, we sampled marks (e.g. bite marks, licking spots) found on the horses' stalls, as signs of abnormal behaviour. The data were analysed individually. It was possible to identify the occurrence of multiple abnormal behaviours related to food and lack of social contact. Moreover, the incidence of horses' diseases, such as colic, was directly associated with the marks found on the stalls. The results also showed that different personality traits correlated with the expression of abnormal behaviour and with the occurrence of health problems, elucidating the importance of this component when evaluating welfare. Horses described as passive, stubborn and confident seemed more suitable for police work. Classifying horses by their personalities could prevent the future development of behaviour and health problems and increase horses' welfare.

#34

Public attitudes to animal sentience and welfare

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Animal sentience may be defined as the ability of non-human species to perceive, feel or experience subjective states. This concept is a central component of animal welfare research on the basis that the capacity for suffering necessitates consideration of well-being. Similarly, an individual's belief in animal sentience is thought to influence public views on the ethical use of animals. However, there is little evidence of how the public constructs the concept of sentience itself. Improving scientific understanding of attitude formation regarding belief in animal sentience is therefore crucial. We investigated attitudes towards animal sentience as a method of understanding moral decision-making in relation to meat consumption and animal welfare. A modified Repertory Grid was used to investigate public perception as part of an online survey in which the public generated constructs relating to animal sentience. Participants (n = 5433) submitted a total of 26,542 constructs over a 3-month period. Analysis showed variation between demographic groups in their perceptions of characteristics considered critical for sentience, as well as characteristics subsequent allocation to differing species. The prominence of some characteristics (e.g. pain) indicates a widespread belief in the capacity for suffering across species boundaries, contrasting with industry/scientific standards, which are largely mammal-centric. Across demographics, intelligence was commonly designated as unimportant for animal welfare despite being one of the most frequently submitted constructs. These findings suggest that while clearly associated with increasing levels of sentience, intelligence may not be a critical factor in animal welfare decision-making processes among the public in comparison with the widely accepted constructs of pain. This study represents an important advance in our understanding of public attitudes to animal sentience and welfare, which is essential for future progress in ensuring the ethical use of non-human species within society.

#35

Mind the gap - Can AI be used to bridge the conservation-welfare fracture that occurs during human intervention at whale strandings?

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New Zealand has an international reputation not only for its high incidence of pilot whale (*Globicephala melas*) strandings, but also its extensive degree of public engagement at such events. On average, 2.4 mass strandings per annum occur, predominantly in the austral months of November to February. Between January 1978 and February 2017, a total of 132 pilot whale mass strandings occurred, involving an estimated 9,234 whales. Despite costly, logistically challenging efforts to 'rescue' live whales, there remains a disconcerting lack of empirical data to support such efforts. Furthermore, matters of animal welfare, including the impacts of human manipulation and the fate of 'rescued' individuals remain largely unknown. While the importance of understanding the outcomes for refloated animals has long been recognized, a lack of scientific evaluation, especially in relation to post-rescue monitoring, means the fate of individual whales remain undetermined. Historically, animal welfare science and conservation have been regarded as separate disciplines, with dissimilar objectives that often conflict. However, the newly emerging field of conservation welfare has started to align these disciplines more closely, with the aim of improving both animal welfare and conservation outcomes. In putting conservation science into practice, conservationists, scientists and managers alike may inadvertently induce significant detrimental impacts upon the welfare of the animals that they seek to conserve. However, the ethical and scientific foundations of decision-making regarding stranded whales, have yet to be explicitly explored in any detail. We explore how recent advances in Artificial Intelligence (AI) can be used to challenge perspectives and improve welfare outcomes during human intervention at strandings. Within the context of a record breaking 600 whale stranding event in Feb 2017, we discuss Ethical and animal welfare issues surrounding rescue attempts and offer considerations for future discourse, research and practice in the broader context of conservation welfare.

#36

Measuring affect-related biases in distractibility in mice

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Emotions can influence cognitive processes in humans and animals, resulting in measurable biases, thereby providing an indicator of affective (emotional) state. Because humans in a more negative affective state demonstrate greater sensitivity to negatively-valenced stimuli, we aimed to validate a protocol for measuring similar bias in mice by monitoring their sensitivity to positively- and negatively-valenced distractors. Sixty-two female mice (31 C57BL/6J; 31 DBA/2J) were individually trained to return to their home cage in a runway. Then, mice (pseudorandomly allocated) underwent an affective manipulation (AffManip) acutely inducing either a negative (tail-handling + 1-min isolation) or a comparatively more positive (normal tunnel handling) affective state before immediately being tested in

the runway with either a positively-valenced (familiar food) or negatively-valenced (flashing light) distractor. Mice were subsequently re-trained (no distractor) and re-tested with the distractor of the opposite valence. Effect of AffManip, distractor valence and interaction were analysed using linear mixed models, using each mouse's latency to approach each distractor and the ratio of their overall runway latency with distractor / latency without distractor to control for inter-individual differences in arousal. We expected negative AffManip mice to attend more (i.e. longer overall runway latency and longer latency to approach the distractor) to the negative than to the positive distractor, and vice-versa for positive AffManip. Mice took longer to approach the negatively-valenced distractor ($X^2_{(5)}= 17.80$, $p=0.003$), however approach latencies tended to be longer following a positive AffManip ($X^2_{(5)}= 10.05$, $p=0.074$). We found no significant effect of AffManip ($X^2_{(5)}= 2.07$, $p=0.840$), distractor ($X^2_{(5)}= 4.96$, $p=0.421$) or AffManip*distractor ($X^2_{(4)}= 2.04$, $p=0.728$) on the overall runway latency, although DBA mice took longer to approach the distractor ($X^2_{(4)}= 21.59$, $p<0.001$) and to complete the runway than C57 ($X^2_{(4)}= 31.37$, $p<0.001$). The results indicate that this protocol requires further refinement (e.g. alternative AffManips), but that strain differences should be considered when developing cognitive bias tasks.

#37

Spatial and social relationships in horses and their reaction upon separation

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Welfare in horses consists of good biological functioning, feeling well and having control over the expression of natural behaviour. We observed two herds to understand the spatial and social dynamics within the herds and the role of rank in relation to the expression of these behaviours. We also performed a behavioural isolation experiment in one group to look at the relationship between the stress responses and the natural group behaviour. A herd of Akhal-Teke horses (n=14 horses; 17.5 hours) located in Estonia and a herd of Icelandic horses (n=9; 22.5 hours) in Belgium were observed. The Icelandic group was exposed to a separation test during which each horse was physically and visually separated from its conspecifics for five minutes. Our results revealed a linear dominance hierarchy in both groups (Akhal: $h'=0.760$, $p<0.05$; Icelandic: $h'= 0.858$, $p<0.05$). Moreover, rank was significantly correlated to age (Akhal: $rs= -0.636$, $n=14$, $p<0.015$; Icelandics= -0.792 , $n=9$, $p<0.011$) and residence time (data only for Icelandic: $rs= 0.850$, $n=9$, $p<0.004$), but not to height or body condition. Agonistic behaviour consisted mostly of threats and displacements. Spatial distribution was linked with rank in one of the two groups: subordinate Akhals were found to stay in the periphery of the herd (rowwise matrix correlation: $rs= 0.641$, $p<0.013$, $n=14$). Frequency and distribution of sociability did not correlate significantly with rank order nor age, but in each group, strong affiliative dyads existed between horses of similar rank order. The isolation test revealed an acute increase in stress-related responses in each horse. The frequency of the stress responses was not related to rank or tendency to stay more or less spatially central but was significantly higher in horses who initiated more affiliative interactions in the herd. A separation test may illustrate the affiliative needs of horses.

#38

Born to be asocial: Newly-hatched tortoises spontaneously avoid unfamiliar individuals

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Individual recognition is important for modulating social interactions, but it is not clear to what extent this ability depends on experience gained through repeated interactions with different individuals. In wild tortoises, evidence of social interactions is limited to behaviours performed years after hatching, in the context of mating. To investigate the presence of abilities of individual recognition at the onset of life in tortoises, we used hatchlings of two species (*Testudo marginata*, *Testudo graeca*) reared with a single conspecific as unique social experience. When located in a novel environment together with the familiar conspecific, tortoises reached the average distance expected by random trajectories. On the contrary, tortoises tested with an unfamiliar conspecific first explored the mate, then actively kept a distance significantly larger than expected by chance. These results show spontaneous abilities of individual recognition in a non-social species at the onset of life, and active avoidance of unfamiliar conspecifics. We suggest that this predisposed behaviour might be adaptive for young tortoises' dispersal and that evolutionary pressures for social behaviour might be relevant for non-social species even at the onset of life.

#39

The welfare of game birds destined for release into the wild: A balance between early life care and preparation for future natural hazards

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Over 110 million game birds are reared in captivity worldwide but there is little research on their welfare during this rearing period. Game birds differ from other livestock because they experience two very distinct environments during their lives, each with its own welfare considerations. Chicks are reared in captivity under controlled and stable conditions. Afterwards they are released into the wild where they suffer high mortality due to being ill-prepared behaviourally, morphologically and cognitively. We review current rearing practices likely to affect both pre- and post-rearing welfare: 1) absence of parents; 2) unnatural rearing density; 3) spatially simple environment; 4) monotonous diet; and 5) absence of predators. In the best cases, we find a positive coincidence of interest promoting welfare pre- and post-release. However, current rearing practice, typically based on experience drawn from the poultry industry often aimed at maximising productivity may compromise post-release welfare and be the cause of the high levels of mortality observed in release populations. Manipulations to the captive environment (e.g. environmental enrichment, predator learning) have been shown to improve post-release survival of game birds, but such manipulations may compromise pre-release welfare as these experiences may invoke (short term) stress, fear and discomfort prior to release. Lack of species specific research on game bird welfare in captivity or after the birds have been released into the wild risks the possibility that the current rearing regime does not produce high standards of pre-release or post-release welfare. The management of game birds requires a delicate balance between ensuring good welfare during rearing and appropriate developmental opportunities to equip them for life in the wild.

#40

Monitoring behavioural change in elephants: development of a novel welfare assessment tool

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There has been much concern in recent years about the welfare of elephants in zoos across North America and Europe. While some previous studies have assessed zoo elephant welfare at a particular point in time, there has been little work to develop methods which could be used for regular, routine welfare assessment. Such assessment is important in order to track changes in animal welfare over time and provide early indicators of potential areas for concern. A welfare assessment tool should be rapid, reliable, and simple to complete, without requiring specialist training and facilities; welfare assessments based on behavioural observations are well suited to this purpose. This talk will describe the development of a new elephant behavioural welfare assessment tool designed for routine use by elephant keepers, with a specific focus on the identification of potential behavioural indicators of welfare and the development and testing of the tool at five UK zoological collections. This novel behavioural welfare assessment tool has been incorporated into the new SSSMZP elephant management guidelines, for use in routine elephant welfare assessment. It is currently being used by elephant-holding facilities for routine behavioural welfare monitoring, which can inform adjustments to welfare plans for individual elephants within collections, to help facilities further assess and improve zoo elephant welfare. This work provides an example of how an evidence-based behavioural welfare assessment tool for use by animal caretakers can be developed within the constraints of zoo-based research, which could be applied to a range of zoo-housed species. Use of such tools enables a more comprehensive approach to monitoring welfare over time. Determining behavioural responses to changes in management and husbandry routines enables a better understanding of the impact of management decisions on zoo animal welfare, which will help to inform policies that will support optimal welfare in zoo animals.

#41

Hot or not? Monitoring behaviour as a welfare indicator following a change in heat provision

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Ectotherm reptiles use their environment to regulate their body temperature. To optimise reptile welfare, zoos need to ensure that each animal's thermic needs are met through the provision of adequate heat sources, particularly when reptiles are housed indoors. However, it is not always easy to know when an animal's thermic needs are satisfied. This study investigated the behavioural and external carapace temperature changes in seven radiated tortoises (*Astrochelys radiata*), three adults and four juveniles, following an enclosure move to an area of increased heat provision. Behavioural data of the seven individually marked tortoises was collected using camera traps programmed to take three consecutive photos every five minutes from 8am-6pm over seven days. Two iButton data loggers were fixed to the

carapace of each tortoise, one dorsal (top) and one marginal (edge), and temperature readings were recorded once every fifteen minutes. Four data loggers also collected the ambient temperature of the enclosure, two in basking areas and two in shaded areas. Data collection was repeated for a further seven days post enclosure move. Following the enclosure move, both mean dorsal and mean marginal carapace temperature significantly increased for all individuals. There was a significant impact of the enclosure move on juvenile tortoise behaviour, with them spending significantly more time foraging and significantly less time basking. There was no significant change in behaviour of the adult animals. These results show that improving heat provision has a significant impact on radiated tortoise carapace temperature which in turn has a positive impact on behaviour, particularly for juvenile animals. This study demonstrates that monitoring behaviour of reptiles can be a good welfare indicator and highlights the importance of assessing heat provision and behaviour using available technologies to optimise animal health and wellbeing.