

## PROGRAMME FOR SPECIALE CONFERENCE DAY

Torsdag d. 27. Februar 2014

## **13.00 : Hans Malte, welcome and practical information**

### **13.05: Laia Rojano-Doñate, Where to do What? Spatial Distribution and Foraging Behaviour of Harbour seals (*Phoca vitulina*) in the Wadden Sea**

**13.20: Nicki Eriksen, Triggering factors in vertical excursions in bigeye tuna showing typical behaviour**

**13.35: Jonas Andersen, How to assess *Drosophila* cold tolerance: Chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits**

**13.50: Anders Findsen**, Why do insects enter and recover from chill coma? Low temperature and high extracellular potassium compromises muscle function in *Locusta migratoria*

## 14.05: Break

**14.15: Gitte Marie Rasmussen, The effect of temperature on glucose tolerance and insulin tolerance in mice**

**14.30: Mafalda de Freitas, Echolocation in shallow water odontocetes: characterising source parameters to help elucidate biosonar shaping pressures.**

#### **14.45: Christian Bech Christensen, *From fish to frog: New insights on the evolution of hearing in air***

**15.00: Michael Ladegaard, Harbour porpoise echolocation: Complicated clutter conditions can compromise capelin captures**

## 15.15: Break

**15.25: Mette Vesterhaab Nielsen, Can the eating behavior of land mammals be determined by head mounted accelerometers?**

**15.40:** Mette Marie Busck, *From spectroscopy to neuron number - an MR and stereology study on the Valporate-rat model of autism*

## **15.55: Renato Filogonio, Hemodynamic patterns and function of cardiac shunts in non-odilian reptiles**

## **16.10: Lauren James, Is Feeding Behaviour A Reliable Indicator Of Pain In Snakes?**

**16.25: Catherine Williams, Nociception and analgesia in *Python regius*: Do opiates work in snakes?**

**16.40: Benjamin van Soldt, Correlating structure and function of snake aortae and pulmonary arteries**

## **Where to do What? Spatial Distribution and Foraging Behaviour of Harbour seals (*Phoca vitulina*) in the Wadden Sea**

*Laia Rojano-Doñate*

**Movement and habitat use are essential population processes fundamental for the management and conservation of species. Anthropogenic activities are increasing in the Wadden Sea, becoming a potential disturbance for species present in the area.** Little is known about movement and habitat use for the Danish Wadden Sea harbour seal population. To alleviate this, 17 harbour seals were tagged in Rømø Island with Argos tracking devices that transmitted for a median of 92 days (range: 48-213). This project presents the use of State-Space models for integrating treatment of tracking system limitations and probabilistic assignment of a behavioural mode to each geolocation by means of movement metrics. The behavioural assignment was then used to infer distribution and habitat use in terms of individual variations and site fidelity. Results showed that animals travelled a median of 57 km, and up to 287 km, from initial haul-out in order to reach foraging spots, with pups or young animals travelling longer distances. Additionally, individuals tended to show haul-out and foraging site fidelity and appear to share haul-out-, but no foraging, spots. This information will allow for better identification of critical habitats and to test for effects of anthropogenic activities, such as seismic surveys and pile driving.

## **Triggering factors in vertical excursions in bigeye tuna showing typical behaviour**

*Nicki Eriksen*

Bigeye tuna are large thermoregulating predatory fish found in tropical waters around the globe. Bigeye tuna prey on the deep sound scattering layer (*dssl*) during the day; this layer is situated at several hundred meters, and is characterized by low water temperature, low ambient light levels and low concentrations of dissolved oxygen. As tunafish are active swimmers, and have high metabolisms and elevated body temperatures, this raises the question of how bigeye tuna cope with the challenges of hunting in the deep. Data tagging studies have shown that Bigeye tuna hunting in the *dssl* gradually lose heat. The fish make up for this by making regular excursions to shallower and warmer waters, where they heat up, before returning to the deep again. Furthermore, Bigeye tuna have been shown to be able to alter their conductivity by a factor of at least 10, enabling them to retain body heat well in the deep, while heating up very fast when they enter warmer waters. This study, using data from archival tags, hopes to elucidate what makes a Bigeye go up from the deep, as both body temperature and oxygen stress may be the true triggering factor.

## **How to assess *Drosophila* cold tolerance: Chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits**

*Jonas L. Andersen*

Cold tolerance is an important factor for determining the distribution of ectothermic species. Several measurements have been used to characterize insect cold tolerance, including: chill coma temperature ( $CT_{min}$ ), chill coma recovery time (CCRT), lethal temperature ( $LTe_{50}$ ), lethal time at low temperature ( $LTi_{50}$ ) and the supercooling point (SCP). Whether these measures describe the natural variance in cold tolerance that determines species distribution is, however, unclear. Similarly, it is unknown to what extend these measures are associated with common physiological mechanisms. To investigate this, we measured the 5 different cold tolerance traits in 14 *Drosophila* species with variable latitudinal distribution. Drosophilids are very often chill sensitive and accordingly, the SCP points showed poor correlations to all environmental and phenotypic variables. In contrast,  $CT_{min}$ ,  $LTe_{50}$  and  $LTi_{50}$  correlated well to environmental variables suggesting that these are superior proxies to evaluate cold adaptation in drosophilids.  $CT_{min}$  also correlated well with  $LTi_{50}$  and  $LTe_{50}$  but interestingly not to CCRT which is thought to involve a reversal of the processes leading to chill coma.

## **Why do insects enter and recover from chill coma? Low temperature and high extracellular potassium compromises muscle function in *Locusta migratoria*.**

*Anders Findsen*

When exposed to low temperatures, many insect species enter chill coma, driven by a failure of neuromuscular function. Chill coma and chill coma recovery have been associated with changes in ion-homeostasis (particularly extracellular  $[K^+]$  ( $[K^+]_{ex}$ )) and accordingly onset of chill coma has been hypothesised to result from depolarization caused by high  $[K^+]_{ex}$ . Here we examined if chill coma is associated with a disturbance in ion balance by examining the correlation between disruption of ion-homeostasis and onset of chill coma and found that marked disturbances of ion-homeostasis were not observed upon entry into coma. In a second set of experiments we used isolated tibial muscle to determine how temperature and  $[K^+]_{ex}$  affect tetanic force production. Force decreased by 80% when temperature was reduced from 23°C to 0.5°C, while an increase in  $[K^+]_{ex}$  from 10 mM to 30 mM at 23°C caused a 40% force reduction. Combining these two stressors almost abolished force production. Thus, low temperature alone may be responsible for chill coma entry, rather than a disruption of  $[K^+]_{ex}$  homeostasis. As a result of the large effect on force by  $[K^+]$  it is hypothesized that recovery of  $[K^+]_{ex}$  following chill coma could be important for the time to recover normal neuromuscular function.

## **The effect of temperature on glucose tolerance and insulin tolerance in mice.**

*Gitte Marie Rasmussen*

Mice are frequently used as a model species to study human diseases like obesity and diabetes. The glucose tolerance test (GTT) is a widely used physiological test to identify mice with impaired glucose tolerance and diabetes. Another commonly used test is the insulin tolerance test (ITT), which tests the sensitivity to insulin. Both the GTT and the ITT are highly standardized tests and are traditionally performed at room temperature (20-24°C), which is close to the human thermo neutral zone (TNZ). The TNZ of mice is around 29-31°C, and the mice are therefore commonly kept and tested at temperatures well below their lower critical temperature. The aim of my study is to test whether temperature has an impact on the outcome of the GTT and the ITT performed on mice as it is suspected that it may affect the pathophysiology of the mice. I have performed GTTs on obese mice and mice with a standard body mass, while they were subjected to one of four different temperatures, namely 15°C, 20°C, 25°C and 30°C. I will also perform insulin tolerance tests (ITTs) on mice subjected to these temperatures.

## **Echolocation in shallow water odontocetes: characterising source parameters to help elucidate biosonar shaping pressures.**

*Mafalda de Freitas*

Echolocation is a key sensory modality, required by odontocetes for successful orientation, navigation, and foraging. Click parameters provide valuable information about an animal's acoustic physiology and behavioural ecology. To date, the majority of studies stem from species which have adapted to finding prey in open water, and have thus developed a high amplitude click, with low repetition rates, better suited for long range detections. In contrast, little is known about how coastal dueling species have adapted their biosonar to suit their cluttered environments. Recordings of humpback dolphins (*Sousa chinensis*), snubfin dolphins (*Orcaella heinsohni*), tucuxi (*Sotalia fluviatilis*), and costeros (*Sotalia guanensis*) were conducted using vertical arrays in one riverine, and three costal, habitats to investigate the biosonar adaptations to shallow, cluttered environments, and help elucidate the shaping pressures of biosonar signals. Studies on harbor porpoises and bats inhabiting cluttered environments have shown them to execute low amplitude, high repetition rate clicks as a means of reducing the number of unwanted echoes, demonstrating a short-range sonar system. Thus, I predict that, similarly, the biosonar system of odontocetes in this study has been shaped by their habitats to operate analogously to that of such species, with low amplitude, high repetitions: a short-range biosonar.

## **From fish to frog: New insights on the evolution of hearing in air**

*Christian Bech Christensen*

In the transition from an aquatic to a terrestrial lifestyle during the early Carboniferous, vertebrates faced an auditory challenge due to the impedance mismatch between air and tissue. This problem was solved by the tympanic middle ear, but according to the current paleontological record this did not appear until the Triassic. Hence, tetrapods may have been deaf to aerial sounds for up to 100 million years. Here, I test this hypothesis by comparing the auditory capabilities of extant vertebrates with auditory systems resembling those of tetrapod ancestors (lungfish), early tetrapods (salamanders), and tetrapods adapted to aerial hearing (frogs). To do so, I used neuro-physiological measurements in both water and air to determine audiograms and vibrograms. I find that even though the tympanic middle ear is advantageous in aerial hearing, atympanic vertebrates are also able to detect aerial sound. Therefore, the evolutionary gap from aquatic to aerial hearing may not be as great as previously expected. Additionally, I demonstrate that lungfish are able to detect underwater sound pressure at high frequencies. This indicates that high frequency tuning of inner ear hair cells, which is considered an adaption to aerial hearing may have already been selected for in the aquatic ancestors of tetrapods.

## **Harbour porpoise echolocation: Complicated clutter conditions can compromise capelin captures**

*Michael Ladegaard*

Harbour porpoises inhabit shallow waters and must therefore cope with clutter conditions during biosonar operation. Clutter is usually defined as the presence of objects other than the object(s) of interest e.g. non-food items in vicinity of prey items. Such clutter might impair the ability of porpoises to find prey. To investigate the effects of clutter, two porpoises were fed fish under three experimental conditions: 1) clutter net (3x3 m net with 500 floats attached), 2) control net (3x3 m net without floats), 3) control without net. The porpoises were equipped with a Dtag that records both outgoing clicks and returning echoes. This allows investigation of changes in apparent source levels (SL), interclick intervals (ICI), and echo levels (EL). On-going analysis will reveal if different strategies are employed under the three conditions. I hypothesize that porpoises deal with clutter through a combination of reduced SL and varying ICI. Lower SL means a reduced acoustic window ahead of the porpoise and hence less detectable clutter echoes. Shorter ICI would be beneficial for fish tracking during approach phase through higher time resolution of the soundscape. During buzz phase a strategy of longer ICI than normal might be prudent to avoid range ambiguity of ensonified clutter elements around the fish.

## **Can the eating behavior of land mammals be determined by head mounted accelerometers?**

*Mette Vesterhaab Nielsen*

Accelerometers have become a popular tool to record activity and in some cases even behavior in domestic and in free ranging animals. The small size of accelerometers makes them ideal to attach to animals and record behavior because of little observer bias which is a problem with direct observation. A large part of the daily pattern for animals involves foraging behavior because of the influence it has on their ability to survive and thereby reproduce. In this study focus will be on the recording of behavior on terrestrial ungulate species with special attention on eating events. Attaching the accelerometer to the head of an ungulate may help in distinguishing eating bites from rumination bites/chews and thereby making it possible to quantify when ungulates eat and when they digest. In combination with GPS tracking devices we could start saying more precisely where and maybe what they eat. Accelerometer data will primarily be collected from attachment to the forehead of red deer. I hope to compare my findings on red deer with other species of ungulates to see whether there are similarities in the accelerometer signals and in the eating behavior.

## **From spectroscopy to neuron number - an MR and stereology study on the Valporate-rat model of autism**

*Mette Marie Busck*

Autism is a neurodevelopmental disorder affecting 0.5 - 1% of the population. The etiology of autism is not yet understood, but recent findings suggest that increased prenatal neurogenesis resulting in abnormally high cell numbers in the prefrontal cortex of children with autism may be an early indicator of the disorder. Many of the symptoms of autism could be explained by dysfunction in this area, placing it at the core of autism etiology. In my thesis, I will use unbiased design-based stereology to investigate whether these abnormalities are also present in the Valporate-rat (VPA) model of autism. Furthermore, I will test for correlation between the number of neurons in prefrontal cortex and the concentration of the neuronal marker N-acetyl-aspartate (NAA), as measured by MR spectroscopy. Finding increased neuron numbers in the prefrontal cortex of rats prenatally exposed to VPA will support the face validity of the model, while a correlation with NAA would open up the possibility of using MR spectroscopy as a non-invasive method for further investigation and diagnostic evaluation of autism in humans.

## **Hemodynamic patterns and function of cardiac shunts in non-crocodilian reptiles**

*Renato Filogonio*

The undivided ventricle of the reptilian heart enables blood to bypass the systemic or pulmonary circulations, resulting in left to right (L-R) or right to left (R-L) shunts, respectively, and result of two main mechanisms: “washout shunt”—referring to the residual volumes of blood within the ventricular chambers that is returned to either the systemic or pulmonary circulations—and “pressure shunt”—blood that is shunted between the intra-ventricular compartments during the cardiac cycle. I intend to describe how washout shunts are affected by the size of muscular ridge, filling pressure and contractility using *in situ* perfusion hearts of Burmese pythons and freshwater turtles. I also intend to study functionality of cardiac shunts using the South American rattlesnake, which, by means of left vagotomy, permits measurements of hemodynamic variables with and without control of pressure shunts, and will use state-of-the-art data loggers for chronic measurements of pressures and flows in undisturbed and freely behaving rattlesnakes. Finally, I wish to characterize the directional organization of the individual cardiomyocytes in both the outer compact layer of the ventricle and within the spongy inner core of the ventricle, and describe how increased filling of the heart affect the orientation of the individual fibers.

## **Is Feeding Behaviour A Reliable Indicator of Pain In Snakes?**

*Lauren James*

When measuring pain, it is pertinent to distinguish nociception from the cognitive process of pain perception; therefore the use of physiological parameters is problematic. However, the common definition of pain includes an adverse effect on normal behaviour, and while such effects have been characterised in some mammals and fish, virtually nothing is known about reptiles. This may be due to the challenges associated with assessing behaviour in these non-social and relatively inactive animals. In this study, a fixed protocol was used to examine how procedures involving either chronic or acutely painful stimuli affect the time course for the return to normal feeding in ball pythons (*Python regius*). Feeding behaviour was monitored on both a short-term (immediately after surgery) and long-term (the subsequent three weeks) basis. Preliminary results suggest that the model is potentially able to demonstrate the difference between chronic and acute painful stimuli and an effect of pre-operative analgesia on a return to normal feeding. This study aims to evaluate the use of such a model as an accurate representation of pain perception in ball pythons.

## **Nociception and analgesia in *Python regius*: Do opiates work in snakes?**

Catherine Williams

Reptiles are increasing in popularity as pets and form a large proportion of animals used in research within this department and the field of zoophysiology. Despite this, there is very limited information on analgesia suitable for invasive surgery in these animals, both in terms of post-operative anti-nociception, and effects on physiological parameters. Previous research has uncovered discrepancies in the response to opiates in snakes in comparison with other reptiles when using thermal nociceptive tests. This study identifies a chemical nociceptive test applicable to *Python regius*, and uses it to compare two commonly administered opiates with different receptor subtype bias (morphine:  $\mu$  specific agonist, and butorphanol: mixed  $\mu$  and  $\kappa$  partial agonist). Morphine and butorphanol at 10 mg/kg are found to reduce the change in heart rate caused by a subcutaneous injection of capsaicin (2mg). The effects of opiates on PO<sub>2</sub>, PCO<sub>2</sub>, heart rate and MAP are also investigated through the use of indwelling arterial catheters. Preliminary results indicate neither drug causes significant increase in PCO<sub>2</sub> as might be expected with respiratory depression commonly associated with opiates. Morphine causes increased heart rate at 5-9 hrs post administration, while butorphanol lowers it. Further investigation into these effects is warranted clinically and physiologically.

## **Correlating structure and function of snake aortae and pulmonary arteries**

*Benjamin van Soldt*

Arteries are elastic, enabling them to distend when confronted with high blood pressures. It has been shown that Pythonidae have different pressures in the aortae and pulmonary arteries; this snake group has interventricular pressure separation, while other groups typically don't. In this study we investigate the effect of interventricular pressure separation on the mechanical characteristics of the walls of various segments of the aortae and pulmonary arteries in *Python regius*. We excised proximal and distal segments from the aortae, a segment from the dorsal aorta proximal to the point of merger of the two aortae, and segments from the left and right pulmonary arteries. These were cut into rings and tested on in-house made apparatus to measure maximal artery wall distensibility and strength. We find that the mechanical characteristics of the aortae are not significantly different amongst each other, but those of the pulmonary arteries differ from those of the aortae. Histology shows that structurally the vessels appear highly similar, but collagen and elastin content measurements show different amounts of collagen and elastin between pulmonary arteries and aortae. We therefore conclude that strength differences are mediated by differences in wall thickness, which leads to different vessel wall collagen contents.