

PROGRAM FOR SPECIALEDAG

- 13.00: Poul Secher Henriksen: The influence of food composition on metabolism, heart rate and postprandial organ growth in ball python (*Python regius*)**
- 13.15: Débora Moura Claësson: Decreasing aerobic capacity with increasing temperatures in European eel: Is the heart the weak link in the mismatch between oxygen delivery and demand?**
- 13.30: Tommy Norin: Aerobic scope does not predict the responses of a tropical fish to climate warming: implications for the concept of oxygen-limited thermal tolerance**
- 13.45: William Joyce: Does cardiac shunting reduce oxidative stress in *Trachemys scripta elegans*?**
- 14.00: Break**
- 14.15: Bo Michelsen: An in vitro study of the dendritic currents in the shaping of epileptiform seizure activity in the area CA1 of the rat hippocampus**
- 14.30: Christian Damsgaard: Turtle hemoglobin function during anoxia**
- 14.45: Gitte Hovkjær Gregersen: The effect of tumor necrosis factor-alpha induced apoptosis on respiration of ISG12 expressing cells and isolated mitochondria**
- 15.00: Christian Malte: Breathing patterns and its effect on dynamics of gas exchange in the artificially ventilated Freshwater turtle (*Trachemys sp.*)**
- 15.15: Break**
- 15.30: Paul Kroer: Endosymbiotic bacteria in Priapulids**
- 15.45: Jonas Andersen: Impaired chill tolerance in digesting migratory locust (*Locusta migratoria*)**
- 16.00: Branwen Messamah. Metabolic cold adaptation in drosophilids**
- 16.15: Michael Ladegaard: Loud Escapades during Cold Nights -Vocal Courtship of the Parti-coloured Bat**
- 16: 30: Break**
- 16:45: Simone Videsen: Hunting with a harbor porpoise.**
- 17: 00 Line Hermannsen: High-frequency components of shipping noise**
- 17: 15: Kristina Ydesen: Prey engulfment in phocid seals studied with high speed cameras and accelerometry tags**
- 17:30: Jesper Madsen: Brain Shutdown for the Winter: Do Turtles Respond to Stumili during Cold Anoxia?**

ABSTRACTS

Poul Secher Henriksen: The influence of food composition on metabolism, heart rate and postprandial organ growth in ball python (*Python regius*)

Digestions in infrequent feeding snakes induce a large SDA response, elevated heart rate (HR) and growth of some visceral organs. To study the effect of different kinds of food, I took four groups of ball pythons and fed them with four types of food and then measured SDA, HR and growth of visceral organ. The four food types were, fat, glucose, protein (chicken breast) and protein + chalk. Because normal meals for snakes contains bone and therefor chalk, one objective was to see if chalk added to the protein would give an additional effect on the measured objects. The result of the study was that snakes digesting fat didn't give any effect in any of the measured objects. The snakes digesting protein showed a significant raise in SDA, HR and growth of organs. There were no additional effect in the snakes where chalk was added to the protein. However there was a significant raise in HCO_3^- when chalk was added which indicates an extra gastric acid excretion. Because there was no difference in SDA between snakes digesting protein with or without chalk, it indicates that the extra gastric acid excretion doesn't add any extra cost to the SDA response.

Déborá Moura Claësson: Decreasing aerobic capacity with increasing temperatures in European eel: Is the heart the weak link in the mismatch between oxygen delivery and demand?

Global warming results in increasing sea water temperatures, which represents a threat to marine fish. The rising temperatures affect the food web, but also exert a direct effect on the individual, by decreasing the aerobic scope with increasing temperatures above the T_{optimal} . Some of the consequences such as less energy to reproduction play a central role in the individual's fitness, diminishing it. The mechanism causing the reduced aerobic scope is still elusive, but some evidence shows that the heart is playing a central role. Research shows that limitations imposed by the heart occur before ventilatory capacity limitation in various fish species. Hence, there is reason to believe that the heart's incapacity to maintain a sufficient transport of oxygen is actually the limiting step determining thermal tolerance, by uncoupling oxygen demand and delivery. In my project, I study the influence of the heart in thermal limitation. This is done by measurements of the aerobic scope of the European eel (*Anguilla anguilla*) together with measurements of blood gases, cardiac output and manipulation of heart blood transport. All these measurements are a way of trying to point out the cause and effect mechanism of decreased aerobic capacity at increasing temperatures.

Tommy Norin: Aerobic scope does not predict the responses of a tropical fish to climate warming: implications for the concept of oxygen-limited thermal tolerance

Climate warming is predicted to impact fish populations through impairment of oxygen transport systems when temperatures exceed those which are optimal for aerobic scope (AS). This concept of oxygen-limited thermal tolerance (OLTT) is rapidly gaining popularity within climate change research and has been applied to several fish species. Here, we tested the OLTT concept by (1) measuring standard and maximum metabolic rates (SMR and MMR, respectively) and AS of juvenile barramundi (*Lates calcarifer*) at temperatures ranging from 23 to 38°C, and (2) allowing the fish to behaviourally select a preferred temperature between 29 and 38°C. A continual increase in MMR with temperature exceeded that of SMR such that AS was highest at 38°C, although the fish lost equilibrium when temperature was further increased to 41°C. In the framework of the OLTT concept, this suggests that 38°C is the preferred temperature of the fish to optimise fitness. However, when given the opportunity, the fish maintained a mean temperature of 32.2 ± 0.4 °C (mean \pm SE), a modal temperature of 31.2 ± 0.4 °C, and spent only $10 \pm 3\%$ of their time at temperatures >36 °C. Together, the results show that the thermal preference of juvenile barramundi does not coincide with the optimal temperature for AS, questioning the generality of the OLTT concept and cautioning future researchers against applying this concept without appropriate scrutiny.

William Joyce: Does cardiac shunting reduce oxidative stress in *Trachemys scripta elegans*?

All non-avian reptiles have the ability to mix oxygen-rich and oxygen-poor blood, a process known as cardiac shunting. The functional significance of cardiac shunting has been much debated in recent years. Although early opinions saw the reptilian cardiac design as 'useless' and 'imperfect', more recent hypotheses have suggested why it may be considered an adaptive trait. However, as of yet, little empirical data has supported these theories. My project will investigate a novel hypothesis that the recirculation of oxygen-poor blood in the systemic circulation reduces oxidative stress. Moreover, we suggest high atmospheric oxygen conditions in the past (i.e. $>30\%$ O₂ in the Carboniferous) may have provided a particularly strong selective pressure for the evolution of cardiac shunting. Encouragingly, recent work has suggested right-to-left shunting in American alligators (*Alligator mississippiensis*) significantly reduces oxidative stress in blood plasma (K. Felbinger, T. Owerkowicz, J. Eme, S.E. Schriener & J.W. Hicks, unpublished). Here, we seek to extend these findings to a reptile with a fundamentally different shunting mechanism, the Red-Eared Slider (*Trachemys scripta elegans*).

Bo Michelsen: An in vitro study of the dendritic currents in the shaping of epileptiform seizure activity in the area CA1 of the rat hippocampus

Epileptic seizures are caused by abnormal, hyper-synchronous discharges in populations of neurons. The behavior of neurons was originally thought to be the product of mainly a synaptic imbalance between excitation and inhibition. Through the years this view has changed, and a number of other factors are now believed to contribute to the seizure events, such as non-synaptic, electrical field interactions, and glial cells. In spite of this increasing insight into the cellular mechanisms, the role of the dendrites in relation to generation and/or maintenance of the epileptiform seizures are largely unknown. I will in my thesis attempt to uncover (i) how dendritic currents are affected during epileptic seizures; (ii) what channels are involved in dendritic currents during seizures; (iii) investigate how dendrites interact with soma during seizures. The experimental setup uses microelectrodes to measure dendritic activity in pyramidal cells from dissected rat hippocampus slices of the CA1 region, during normal conditions, and during a cesium invoked epileptic seizure.

Christian Damsgaard: Turtle hemoglobin function during anoxia

Freshwater turtles are great vertebrate models for studying hypoxia as they tolerate prolonged periods of anoxia and show extensive metabolic depression. This requires regulation of Hb-O₂ affinity to secure adequate O₂ delivery to the respiring tissues. I hypothesized that Hb-O₂ affinity would increase during anoxia to limit unloading of O₂ and tested this by drawing blood from 5 red-eared sliders, *Trachemys scripta elegans*, after a 7-day anoxic period. Anoxic turtles do not increase either hematocrit or isoHbs-ratio during anoxia, and O₂ equilibria from anoxic turtle hemolysates show no increase in O₂ affinity compared to a normoxic control in the physiological pH-range. In contrast to hibernating mammals that actively decrease the level of allosteric effectors during hibernation to increase Hb-O₂ affinity, these initial results are striking by indicating no active regulation of red cell effectors in turtles, and pH and temperature may solely be responsible for the changes in Hb-O₂ affinity during anoxia.

Gitte Hovkjær Gregersen: The effect of tumor necrosis factor-alpha induced apoptosis on respiration of ISG12 expressing cells and isolated mitochondria

Interferons (IFNs) can upregulate more than 1000 Interferon Stimulated Genes (ISGs) by binding to specific cell surface receptors and activate a cascade of intracellular signaling pathways. The antitumour activity of IFN is mediated through ISGs. One group of ISGs codes for the family of ISG12 proteins which play a role in apoptosis induction. The proteins may have a role in whether a cell should undergo apoptosis or not because some of the proteins have an anti-apoptotic effect and others a pro-apoptotic effect. When cells express the ISG12A protein they are more sensitive to tumor necrosis factor-alpha (TNF- α) induced apoptosis and the ISG12A protein has been found to localize to the mitochondria. Upregulated levels of ISG12 mRNA have been observed in cancer cells. Another finding in cancer cells is that they can reprogram their energy production by switching their energy metabolism to mainly glycolysis instead of mitochondrial oxidative phosphorylation. The aim is to investigate O₂ consumption in intact cells and isolated mitochondria during apoptosis induction. HEK293 cells will be transfected with an ISG12 protein and O₂ consumption rate will be followed as apoptosis is induced by TNF- α . Later mitochondria from transfected HEK293 cells will be isolated and corresponding measurements performed.

Christian Malte: Breathing patterns and its effect on dynamics of gas exchange in the artificially ventilated Freshwater turtle (*Trachemys sp.*)

A breathing pattern is characterised by the spacing of breaths in time, which can be done in various ways. Mammals normally space their breaths in an evenly/continuously fashion, but many ectotherms exhibit a cyclic/intermittent breathing pattern consisting of a series of consecutive ventilations interspersed by periods of apnoeas. Employing such a breathing pattern necessarily conflicts with homeostasis since the absence of a true steady state, on a breath to breath level, entails constantly fluctuating body gas stores. This study addresses the effect of different breathing patterns on gas exchange and it is hypothesised that intermittent breathing is associated with a reduced efficiency of CO₂ excretion. The investigation is carried out in anaesthetized Freshwater turtles, manipulating the breathing pattern with a respirator while keeping the overall amount of ventilation constant. Results tend to be in favour of the hypothesis, showing a decreased mean respiratory gas exchange ratio (RER), when switching from an evenly spaced breathing pattern to intermittent breathing. This indicates a transient lack of steady state since the RER is less than the respiratory quotient (RQ) indicating CO₂ retention thereby increasing bodily CO₂ stores. This effect may seem to be mitigated by potential coupling between intermittent breathing and decreasing RQ.

Paul Kroer: Endosymbiotic bacteria in Priapulids

Phylum Priapulida is a small ecdysozoan taxon of marine worms found globally. The taxon's long fossil history and basal phylogenetic position makes them interesting as model organisms for evolutionary (history) studies. Many aspects of priapulid biology are still relatively poorly known. One such aspect is the possible association with microorganisms which I have been investigating. *Priapulus caudatus* from Gullmarsfjord, Sweden were collected and DNA was extracted from the gut and an unknown 16S bacterial sequence was found. A phylogenetic analysis shows that the bacterium belongs to the Rickettsiales, an order within the Proteobacteria mostly comprising endosymbionts and some intracellular pathogens. Rickettsiales is poorly known, partly due to the difficulties in cultivating them. We suspect that the new bacterium is a specialised symbiont and our preliminary molecular phylogenetic analysis shows its closest relatives to be intra- and extracellular symbionts of arthropods. Very little is known on endosymbiosis in priapulids and this study will add new information to the known ecology of the worms and the evolution of the interactions between the symbionts and priapulid hosts. I aim to achieve this by 1) constructing phylogenies; 2) screening for bacterial proteins in the gut tissues and lumen; 3) screening the symbiont genome for known metabolic pathways; 4) obtaining similar data from other species of priapulids and their presumed associated symbionts, and 5) through comparing the phylogenies, physiological and morphological data from these investigations.

Jonas Andersen: Impaired chill tolerance in digesting migratory locust (*Locusta migratoria*)

This study explored the supposed adaptive benefit of fasting with regard to chill tolerance, since the physiological challenge of digestion and cold may interact. Earlier studies have shown that high dietary potassium loads increase hemolymph $[K^+]$, thereby causing a depolarization of muscle resting membrane potential, leading to sluggish grasshoppers¹. Similarly it has been shown that low temperature increases hemolymph $[K^+]$ due to a mismatch between active- and passive transport processes between hemolymph and intestinal tract²⁻⁴. The present study investigates cold tolerance and the regulation of ion homeostasis in fasted and fed grasshoppers in order to characterize the putative interactions of said physiological challenges. The hypothesis is that K^+ rich diets prior to a cold shock will exacerbate the subsequent K^+ imbalance, ultimately leading to increased recovery time.

Branwen Messamah. Metabolic cold adaptation in drosophilids

Metabolic cold adaptation (MCA) is the theory whereby cold climate ectotherms are expected to display an elevated metabolic rate compared to those inhabiting warmer climates at the same trial temperatures. While this idea seems logically sound as a mechanism to conserve the rate of temperature-dependent physiological processes, MCA remains controversial with numerous studies producing conflicting results. There is, however, wide-scale evidence in favour of MCA as a general trend in insects. This project aims to investigate MCA in the genus *Drosophila*, using respirometry (monitoring both oxygen consumption and carbon dioxide production) to measure and compare the metabolic rates of approximately 50 different species. All species are reared on the same medium for at least two generations and at the same temperature. Activity of flies is also measured during testing periods to control for this influence on metabolic rate. It is hoped that eliminating these variables will provide a clear picture of MCA in *Drosophila*.

Michael Ladegaard: Loud Escapades during Cold Nights -Vocal Courtship of the Parti-coloured Bat

In autumn and winter when other bats have sought refuge, the intriguing courtship of the parti-coloured bat takes place. During mating season parti-coloured bats emit a distinctive display song with peak frequency of 13.5 kHz making it audible to humans. This is much lower than regular echolocation calls which have peak frequencies around 26 kHz. What advantage is associated with lowering the voice by an octave when the purpose of vocalisation is communication? To test this, display song was recorded in the University Park of Aarhus. Transmission loss was calculated after finding range and adjusting for frequency dependant attenuation of signals. Analysis revealed source levels 10 cm from the bat of 112 dB re. 20 μ Pa (peak to peak), 99 dB re. 20 μ Pa (rms), and 82 dB re 20 μ Pa²s (energy flux density). Active space during display song behaviour was estimated to cover twice the area compared to that for echolocation calls with equivalent peak-to-peak source levels assuming detection thresholds of 0 dB re. 20 μ Pa. It is suggested that parti-coloured bats include the for bats unusually low 13.5 kHz component in their display song to maximise the chances of an encounter with a mate in the dark.

Simone Videsen: Hunting with a harbor porpoise.

Animal-borne video cameras have been used for some time to observe the behavior of animals in their own environment. That increasingly also include marine mammals, but it is unclear what can be learned from such deployments. To test this, we used a small waterproof camera with suction cups, mounted on the back of a harbor porpoise, *Phocoena phocoena* in two different locations. The porpoise was sent to catch both live and dead fish during 48 trials. During trials 3 GoPro cameras were placed in the water. From the animal-borne camera 40 prey pursuits were observed, only 3 of these proved to be unsuccessful prey captures, when comparing with footage from the GoPro cameras. Other observations during the trials such as the ability to identify prey and to see surrounding animals, suggests that the use of a video camera as a behavioral observation method has its possibilities.

Line Hermanssen: High-frequency components of shipping noise

Noise has recently been acknowledged as an important source of pollution in EU's marine strategy framework directive (MSFD descriptor 11). A main focus of the directive is low-frequency ship noise in 1/3 octave bands around 63Hz and 125Hz. However it is largely unknown if all ships radiate most of their noise in those bands, and whether marine animals are most affected at these frequencies. To test that, I recorded noise from different vessels at known distances. The noise was quantified in 1/3 octave bands and back-calculated to nominal source levels using a spherical spreading model. For all vessels recorded I found that shipping noise significantly exceeded ambient noise levels across the entire recording band (25Hz–200kHz), and reached third octave source levels up to 162 dB re μPa at 125kHz where harbor porpoises echolocate (120-150kHz). I conclude that the two low-frequency bands designated by the MSFD, as indicators of shipping noise are poor proxies for noise impacts of the vessels investigated here, at least for evaluating shipping effects on marine mammals with good ultrasonic hearing. In consequence, the higher frequencies should be included in assessment of the environmental status of marine habitats.

Kristina Ydesen: Prey engulfment in phocid seals studied with high speed cameras and accelerometry tags

Quantification of the foraging behaviour of marine mammal predators in the wild is fundamental for understanding their ecological role, but no reliable means to get information on prey capture success are available. Thus, a new technique is needed to quantify prey captures over long time series in the wild. Specific movements of the head and jaws may provide reliable feeding cues by involving fast specific changes in acceleration (jerk signatures) that might even be prey or context specific. To test this, underwater prey captures of two trained harbour seals wearing Dtag3, with triaxial accelerometers sampling at 500Hz, on their head, will be filmed with two high-speed video cameras. The hypothesis is that feeding produces prey or context specific jerk signatures that can be related to actual prey captures. If verified, the prey capture success and energetic turnover of pinnipeds in the wild can be measured over a year.

Jesper Madsen: Brain Shutdown for the Winter: Do Turtles Respond to Stimuli during Cold Anoxia?

Northern species of freshwater turtles survive months of anoxia on the bottoms of lakes to avoid freezing each winter. These animals have been reported to enter an unresponsive comatose state in cold, anoxic water, but they must on the other hand be vigilant to detect the arrival of spring. To investigate to which extent these animals enter coma during hibernation evoked potential measurements were made of responses to light and vibrations stimuli, during anoxia and at cold temperatures. I report a difference in the degree of responsiveness for light and vibrations stimuli during anoxia; the animals remain more responsive to light than vibration during anoxia. Responsiveness to both stimuli seems to decrease evenly as the animals are cooled. However, preliminary results suggest that recovery during reheating is faster for vision than vibration. The results show that these animals are not in a state of unresponsive coma during cold anoxia. Rather they display a differential shut-down of modalities as a response to anoxia. It is proposed that these animals use vision as a primary cue for detecting increasing light levels, signaling the arrival of spring.