Mini seminar for Zoophysiology students

fredag d. 6. november 2015

Abstracts and program

08:30 Mafalda de Freitas: Who's who? Attempting to distinguish small costal delphinid species based on echolocation signals.
08:45 Asbjørn G. Petersen: Aggregation of isolated hemoglobins from the turtle, *Trachemys scripta*
09:00 Lærke R. Reinholdt: How Right-to-left cardiac shunts and air convection requirements affect arterial partial pressure of oxygen and carbon dioxide in reptiles
09:15 Lauren James: An overview of currently used inhalant and injectable anaesthetic agents in reptiles and the relevance of their use in snakes
09:30 Trine Olsson: A comparative study of cold tolerance in Drosophila and its relation to hemolymph composition
09:45 Christian Damsgaard: Origin and functional diversification of the Root effect and retinal oxygen delivery in bony fishes

10:00 Coffee

10:30 Shane Gero: Social Complexity and Functional Communication in Sperm Whales
10:45 Simone K. A. Videsen: Male humpback whale escorts disrupt suckling behavior of acoustically cryptic neonate calves
11:00 Morten S. Storgaard: Toxicity of Chemical Warfare Agent (CWA) metabolites found in the Baltic Sea
11:15 Inge G. Revsbech: Comparison of circulating hydrogen sulfide and nitric oxide metabolites and their potential roles in brown bear hibernation
11:30 Mads K. Andersen: Climate change and physiology: Insight from an air-breathing fish

11:45 Lunch

12:30 Pernille M. Sørensen: Click communication in the harbor porpoise
12:40 Signe O. Jensen: Chill coma related disturbances in ion homeostasis in the Lepidoptera *Heliconius Cydno*
12:50 Amanda M. Bundgård: Nitrite-dependent modification of mitochondria and protection against oxidative stress in the red-eared slider turtle
13:05 Camilla Taulbjerg: The physiological effects of opioids on the cardiovascular system of snakes.
13:20 Christian L. Malte: Closed respirometry may underestimate tissue gas exchange and bias the respiratory exchange ratio (RER)
13:35 Laia Rojano-Doñate: Acoustics and energetics of echolocators in a noisy world

13:50 Coffee

14:15 Mai M. Madsen: The effect of feeding regime on the relationship between standard metabolic rate, specific dynamic action, maximum metabolic rate and specific growth rate in rainbow trout

14:30 Michael Ladegaard: Amazon river dolphins use a high-frequency, short-range biosonar
14:45 Simon Nørgaard: Histamine as a direct and indirect regulator of postprandial heart rate
15:00 Siri Elmegaard**:** Cognitive modulation of diving bradycardia in harbour porpoises

15:15 Cake

15:30 Catherine Williams: Drugs: what are they good for?
15:45 Salomine F. L. K. Falck: Effect of feeding regimes on behavior, swimming performance and metabolic rate in rainbow trout (*Oncorhynchus mykiss*)
16:00 Simone Strandvad: Stress response in *Scyliorhinus canicula* and *Squalus acanthias* following trawling
16:15 William Joyce: The peculiar atria of turtles

16:45 Beer

18:00 Pizza

Who's who? Attempting to distinguish small costal delphinid species based on echolocation signals.

Mafalda de Freitas

Passive acoustic monitoring (PAM) has become increasingly more common as a means to monitor marine mammals worldwide, particularly in hard to reach locations and unfavourable weather, day and night. Echolocation is a key sensory modality for all toothed whales, constituting the largest portion of their acoustic output and thus making it a potential cue for PAM. All studies to date suggest that toothed whale biosonar can be grouped into four distinct signal types: Multi-pulsed, frequency-modulated, narrowband high-frequency, and broadband transients. However, studies also suggest that echolocation source parameters are influenced by the size of the individual, which raises the questions of whether similar sized species, producing the same signal type, can be distinguished using PAM. To address this, we recorded three sympatric delphinids of similar sizes, *Tursiops aduncus*, *Sousa sahulensis*, and *Orcaella heinsohni*, using vertical 7-element and 4-element hydrophone arrays and perform discriminant analysis to elucidate potential source parameter differences

Airgun pulses in shallow water: Implications for effects on small marine mammals
Line Hermannsen

Airguns, used in oil and gas investigations, are among the most powerful anthropogenic noise sources in marine habitats. Most of the energy is at low frequencies, however part of the radiated energy may also be present at higher frequencies with potential effects on small marine mammals that have their best hearing at medium-to-high frequencies. We recorded airgun pulses in a uniform shallow water habitat using hydrophones and acoustic recorders. We show considerable energy at frequencies, where marine mammals hear well and that the spectral composition of airgun pulses is highly affected by water depth. This highlights the need for considering environmental properties in impact assessments of airguns. We conclude that the risk of hearing damage is small for both pinnipeds and porpoises. However, there is substantial potential for significant behavioral responses out to several km from an airgun, well beyond the commonly used shut down zone around airgun arrays.

Aggregation of isolated hemoglobins from the turtle, Trachemys scripta

Asbjørn Graver Petersen

In vitro hemoglobin (Hb) aggregation has been observed in all groups of the higher vertebrates. It is however relatively unknown to what extent and under what conditions this occurs in living animals.

The aims of this study were to investigate how different conditions affected the degree of aggregation and whether aggregation had implications on the O2-binding properties in Hbs from the turtle, Trachemys scripta. Using fast protein liquid chromatography we separated and isolated the two Hb isoforms, HbA and HbD. We find that only the HbA isoform aggregates and a higher heme concentration enhance the process of polymerization. The aggregation process seems independent of ATP and pH. Furthermore O2 equilibrium measurements of polymeric and tetrameric fractions obtained from gel filtration show similar O2 affinities indicating that aggregation state doesn't affect oxygenation properties of T. scripta Hb.

Future work will focus on aggregation being a marker for oxidative stress in red blood cells.

How right-to-left cardiac shunts and air convection requirements affect arterial partial pressure of oxygen and carbon dioxide in reptiles

Lærke R. Reinholdt

Multiple physiologically changes can affect the arterial partial pressure of oxygen (Pa_{O_2}) and carbon dioxide (Pa_{CO_2}). In reptiles, two of these changes are alternations in air convection requirements for CO_2 ($ACR = \dot{V}E/\dot{V}_{CO_2}$) and R-L intracardiac shunts, where a fraction of the systemic venous blood bypasses the pulmonary circulation by flowing from the right to the left side of the anatomic undivided ventricle. To analyze the degree of impact that the two variables have on the gases, two dynamic models have been developed. The models show that changes in ACR affect Pa_{CO_2} profoundly and Pa_{O_2} slightly, whereas the impact is the reverse when altering the R-L flow. When doubling the R-L flow Pa_{CO_2} increases, and to attain a similar rise in Pa_{CO_2}only a minor drop in ACR is needed. However, this change in ACR barely affects Pa_{O_2}. This asymmetry in how the two variables affect Pa_{CO_2} and Pa_{O_2} enables (at least in principle) the organism to control $Pa_{CO_2}$and Pa_{O_2} by regulating both variables.

An overview of currently used inhalant and injectable anaesthetic agents in reptiles and the relevance of their use in snakes

Lauren James

The use of appropriate anaesthesia is a priority in both research and veterinary fields, with its application to reptile surgery becoming more pertinent following an increase in exotic pet ownership and the continued use of reptile models for physiological and biomedical research. It is therefore imperative that anaesthetics and chemical methods of restraint be characterised in reptiles, with a focus on snakes, as they are neglected in the literature. The following experiments will evaluate the use of common anaesthetic agents for the induction and maintenance of surgical anaesthesia in snakes. The minimum alveolar concentrations (MAC) of isoflurane and sevoflurane will be determined, and the effects of morphine premedication investigated. The cardiovascular effects of the inhalant anaesthetics will also be described and anaesthetic induction with the injectable agent, alfaxalone, will be compared to inhalant induction with isoflurane and sevoflurane. The overall aim is to describe an appropriate anaesthetic protocol for snakes.

A comparative study of cold tolerance in Drosophila and its relation to hemolymph composition

Trine Olsson

The distribution of insects is closely linked to their ability to maintain life functions at lower temperatures, i.e. their cold tolerance, and it is therefore of great importance to understand what determines the cold tolerance of insect species. It has previously been found that cold tolerant species of e.g. *Drosophila* have low [Na^+] in their hemolymph, but the osmolality of the hemolymph does not differ significantly between species at standard conditions. The cold tolerant species must therefore have higher hemolymph concentrations of other compatible osmolytes, which is what I try to find out in my study where I use H-NMR spectroscopy to obtain quantitative measures of the organic molecules in the hemolymph in the hope of finding "the missing osmolytes".

Origin and functional diversification of the Root effect and retinal oxygen delivery in bony fishes

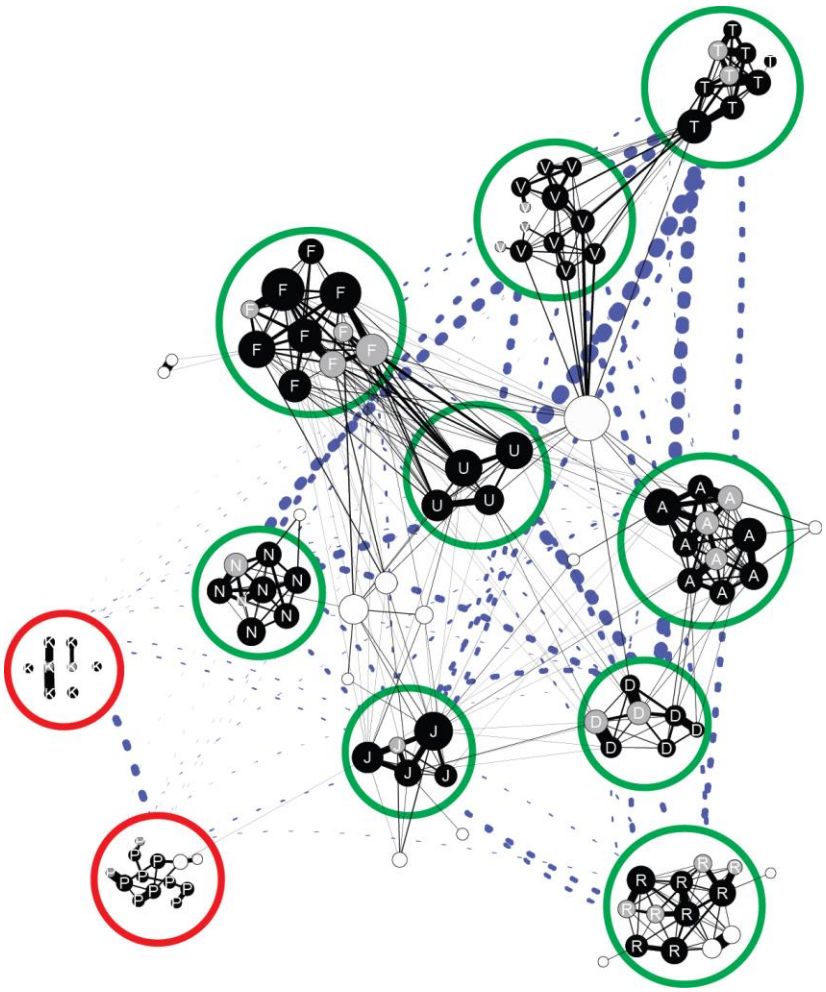
Christian Damsgaard

While retinal O_2-delivery in tetrapods is achieved through intra-retinal capillaries, advanced teleost fishes use Root effect hemoglobins and a choroid rete to secrete oxygen into their avascular retinas. To reconstruct the likely sequence in which the functional components in O_2-secretion evolved, we investigated retinal blood-flows and Root effects in 24 phylogenetically-distinct fish-species using a combination of µUS, µCT and histology. We show that the ancestral state of retinal O_2-supply is through intra-retinal capillaries, that this trait remains after the origin of the Root effect and the choroid rete, and that intra-retinal capillaries are only lost in species with well-developed Root effects. This suggests that the evolution of retinal O_2-secretion from a choroid rete depends on gradual increases in Root effect concurrent with decreased retinal capillarization. We also show that intra-retinal capillaries re-evolve and function in O_2-delivery in species that have secondary lost in any of the functional components in O_2-secretion.

Social Complexity and Functional Communication in Sperm Whales

Shane Gero

The 'social complexity hypothesis' suggests that complex social structure is a driver of diversity in animal communication systems. Sperm whales have a hierarchically-structured society in which the largest affiliative structures, the vocal clans, are marked by culturally-transmitted dialects of acoustic signals known as 'codas'. Through research completed during my postdoc, I have taken the social complexity hypothesis deeper in demonstrating not only new levels of social complexity both at temporal and social scales, but that they have the accordingly sophisticated communication to deal with the specific social interactions at various levels of their social structure. I have quantified variation in social calls between individuals, units, and clans. I connect the specific structures which create the social complexity with their expected signal diversity by demonstrating that the signals sperm whales require for social recognition do in fact exist. This is the first supporting evidence for this hypothesis in a marine species.



Three levels of sperm whale social structure (individual, unit, and clan) as defined by both social (solid black) and communication networks (dashed blue).

Male humpback whale escorts disrupt suckling behavior of acoustically cryptic neonate calves

Simone K. A. Videsen

Humpback whales annually migrate from foraging to breeding grounds to mate and calve. The dual purpose of female migrations potentially creates a conflict between choosing a mate versus maximizing the energy budget for the newborn calf by reducing exposure to male escorts. To investigate this, we deployed Dtags on eight neonate calves in Exmouth Gulf, WA. Prominent behaviors observed were travelling, resting and suckling. We show that mother-calf pairs consistently keep very close contact, and that calves suckle regularly. Conversely, the presence of antagonistic male escorts induced a disruption of suckling behavior and increased the calves' overall energy expenditure. Therefore, it is in the calf's interest to avoid attracting male escorts, which may explain why calves only produce weak sounds. We propose that this acoustic crypsis serves to maintain mother-calf contact, while reducing predation risks and attraction of male escorts that disrupt the critical transfer of milk from mothers to calves.

Toxicity of Chemical Warfare Agent (CWA) metabolites found in the Baltic Sea

Morten S. Storgaard

After 2nd World War the chemical warfare agents were prohibited by law and 11,000 tonnes of toxic agents were dumped in the Bornholm Basin east of Bornholm. The area has been screened for the presence of parent compounds and metabolites including the concentrations they are found in. The majority of the detected compounds has been found in the sediment and a minor part in the pore water. The (eco)toxicity of these compounds remain to be illuminated in which this thesis hopefully will contribute to. The toxicity of these chemicals will be described with an acute and chronic test. The acute toxicity will be described by Microtox™ with *Vibrio fischerei*, which measures inhibition in light emission as a function of different concentrations. The chronic toxicity will be described by spontaneous locomotor changes in Zebrafish (*Danio rerio*); intending to draw lines to the commercially important Cod (*Gadus morrhua*).

Comparison of circulating hydrogen sulfide and nitric oxide metabolites and their potential roles in brown bear hibernation

Inge G. Revsbech

Mammalian hibernation is a physiological wonder of temporarily downregulated metabolism, during which the animal does not eat and remains inactive for months. Understanding the underlying biochemical mechanisms may have great translational medical applications. As ubiquitous inhibitors of mitochondrial metabolism, both nitric oxide (NO) and hydrogen sulfide (H_2S) could in principle play a part in the whole body metabolic depression essential to hibernation. We investigated type and content of blood metabolites of NO and H_2S in winter hibernating and summer active, free-ranging brown bears. We found significant changes in composition of sulfide metabolites in plasma, with a decrease in plasma thiosulfate and polysulfides during hibernation, indicating that whilst hibernating, the bear may regenerate H_2S from its oxidation products, thiosulfate and polysulfides. Concurrently, high levels of free sulfide correlated with high levels of cysteine, suggesting that cysteine may be prioritized for glutathione synthesis during hibernation. Thus, this remodeling of sulfide metabolism may work to preserve plasma free cysteine for the generation of glutathione in cells, a central antioxidant also found in high levels in red blood cells during hibernation. For NO, no clear changes could be measured in circulating nitrite or in the degree of S-nitrosylation of glyceraldehyde-3-phosphante dehydrogenase, although this remains to be investigated further. Our study revealed that circulating H_2S potentially contributes to inhibition of mitochondrial respiration during hibernation.

Climate change and physiology: Insight from an air-breathing fish

Mads K. Andersen

Due to anthropogenic forcing, climate is changing at an accelerated rate, which is expected to have severe consequences for the ecology and physiology of aquatic ectotherms. We therefore investigated how increasing temperature affected swimming performance, resting and maximum oxygen uptake rates and the derived aerobic scope (AAS; maximum – resting) of the air-breathing fish *Pangasianodon hypophthalmus* using two-phase intermittent closed- and swimming respirometry. We hypothesized that *P. hypophthalmus* would maintain its AAS by increasing aerial ventilation as temperature increased, which would concomitantly lower its critical swimming speed (U_{crit}). We find that AAS was unchanged until a decrease at 39°C in both experiments. Interestingly this was achieved without increasing aerial ventilation at any temperature tested, and U_{crit} was unaffected until 39°C. These findings reveal a high resilience towards increases in temperature, and that the projected temperature increase will have little to no effect on the aerobic performance of *P. hypophthalmus*.

Click communication in the harbor porpoise

Pernille M. Sørensen

Sound plays an essential role for communication in many species of cetaceans. In some cases, assessment of acoustic communication can be complicated, as in the case of the harbor porpoise. This species only produce narrow band high frequency (NBHF) clicks. These NBHF clicks are known to play an important role in echolocation, but are now also thought to play an essential function in communication, where different types of click sequences, defined primarily by their click repetition pattern, have been shown to be correlated with specific behaviors. Initially, I wish to categorize the click communication calls used by wild harbor porpoises and to determine when, where and how often they are used, as well as putting them into behavioral context. Thereafter it could be particularly interesting to assess whether individual variation exist in the general click communication call types. Such variation would most likely play an important role in recognition of other individuals.

Chill coma related disturbances in ion homeostasis in the Lepidoptera *Heliconius Cydno*

Signe O. Jensen

At low temperatures, chill susceptible insects enter a state called chill coma, a reversible state of neuromuscular impairment. Chill coma related disturbances of ion homeostasis have been observed in different species of insects. Especially the increase in hemolymph [K^+] has been of great interest due to the effect on Nernst potential, E_K, leading to a drop in resting membrane potential. Most insects have ionic hemolymph composition of high [Na^+] and low [K^+] and [Mg^{2+}]. However this is not the case for the Lepidoptera as they have high [K^+] and [Mg^{2+}] and low [Na^+] in their hemolymph. To investigate what happens to hemolymph ion concentrations and resting membrane potential while species with this atypical ion composition are in chill coma, the lepidoptera *Heliconius Cydno* has been studied. Hemolymph and muscle samples were taken from butterflies in chill coma and concentrations of Na, K and Mg were measured using atomic absorbance spectrometry. Measurements of membrane potential are pending.

Nitrite-dependent modification of mitochondria and protection against oxidative stress in the red-eared slider turtle

Amanda M. Bundgård

Recent evidence indicates that nitrite is a cytoprotectant agent, in part because it mediates a reversible modification, S-nitrosation of complex I in the electron transport chain. This inhibits formation of toxic reactive oxygen species (ROS) from mitochondria during reoxygenation after a period of anoxia, as after stroke or heart failure, or hibernation in anoxia-tolerant animals such as the slider turtle.

During my masters project I investigated the role of nitrite as a natural cytoprotective agent in the slider turtle. I found that nitrite inhibits complex I by S-nitrosation during anoxia, reducing the level of ROS produced upon reoxygenation, which suggests that turtles naturally exploit nitrite as a strategy to prevent oxidative damage. I also found interesting changes in metabolite composition after acclimation to anoxia, such as an increase in succinate that might fuel the ROS production upon reoxygenation. This further supports the need to inhibit complex I and thereby ROS production.

The physiological effects of opioids on the cardiovascular system of snakes

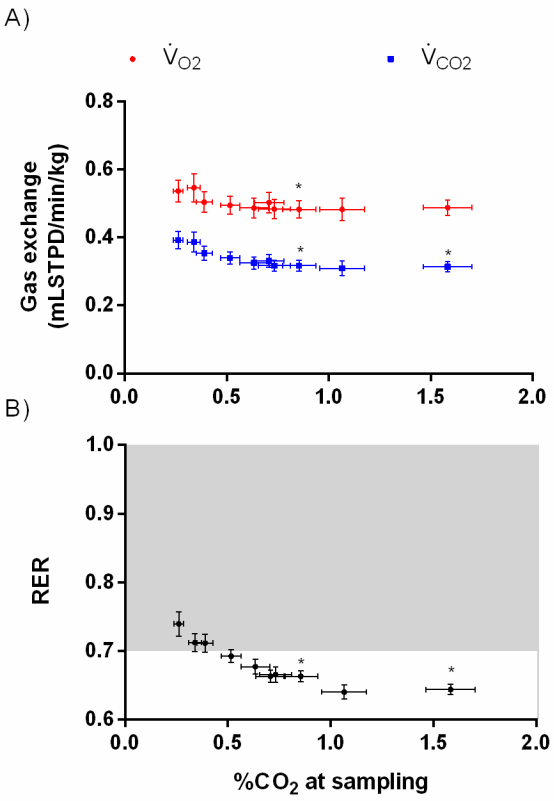
Camilla Taulbjerg

Analgesia and anesthesia is attracting more attention when working with reptiles. The following presentation will focus on opioids, especially morphine as an analgesic for snakes. Heartrate measured with ECG and behavioral analysis is used to examine the snakes' reaction when exposed to nociceptive stimuli (capsaicin) while under the influence of morphine. The results seem to implicate that when comparing heartrate, a dosage of 5 mg/kg morphine do not affect the snakes more than 1 mg/kg morphine do, but respiration rates seems to get higher when under influence of 5 mg/kg compared to the 1 mg/kg. Control studies are yet to be conducted, and behavioral analyses are not yet analysed completely.

Closed respirometry may underestimate tissue gas exchange and bias the respiratory exchange ratio (RER)

Christian L. Malte

When conducting closed respirometry, the rates of O_2 uptake and CO_2 excretion are typically assumed to be in steady-state, such that the measured rates of gas exchange equal those at tissue level. In other words, the respiratory gas exchange ratio (RER) is assumed to equal the respiratory quotient (RQ). However, because the gas concentrations change progressively during closure, the animal inspires air with a progressively increasing CO_2 concentration and decreasing O_2 concentration. These changes will eventually affect gas exchange leading to errors due to changes in the gas stores of the body and because of the higher solubility/capacitance of CO_2 in the tissues, CO_2 excretion will be more affected than O_2 uptake $\dot{V}_{O2}$. Using mathematical models and experimental data from ball pythons we show that CO_2 excretion and RER will become progressively underestimated as closure time is prolonged (*i.e.* chamber CO_2 concentration increase).



Acoustics and energetics of echolocators in a noisy world

Laia Rojano-Doñate

The growth in shipping and marine industries has raised concerns over how anthropogenic noise may affect toothed whales. Toothed whales rely on acoustic cues for critical functions such as navigation and foraging. Despite the extensive literature documenting effects of human disturbance on wildlife behaviour, there are not studies linking behavioural changes to consequences on energy budgets. In this PhD project, I hypothesize that induced behavioural changes may involve energetic costs by introducing an imbalance between energy intake and use evoked by reduced foraging success and increased movement activity. Such an imbalance could then cause a reduction in individual fitness and provoke long-term effects on population vital rates. Using porpoises as a model organism, I aim to uncover the mechanistic link between anthropogenic noise disturbance and population effects by quantifying energetic and behavioural responses to underwater noise and developing a model capable of predicting population consequences of increasing levels of noise.

The effect of feeding regime on the relationship between standard metabolic rate, specific dynamic action, maximum metabolic rate and specific growth rate in rainbow trout

Mai M. Madsen

Two groups of juvenile rainbow trout (*Oncorhynchus mykiss*) were fed different feeding regimes of either 1.05 % day^{-1} (low) or an *ad libitum* diet (high) for 7 weeks before they had their standard metabolic rate (SMR), maximal metabolic rate (MMR), specific dynamic action (SDA), and specific growth rate (SGR) measured. When comparing the two feeding regimes it was shown that the two groups had significantly different means of the SMR, absolute aerobic scope (AAS), SDA, and SGR. A significant relationship was found between the SDA and SGR and rAAS and rMMR for both feeding regimes. Furthermore, a significant relationship was found between the rMMR, SGR, and rSDA *vs.* rSMR and rMMR *vs.* rSDA for the high feeding regime, whereas this was not the case for the low feeding regime. The study showed that food ration can have a great effect on e.g. the found metabolic rates and their correlations.

Amazon river dolphins use a high-frequency, short-range biosonar

Michael Ladegaard

For the few toothed whale species fully adapted to life in river systems the task of echolocation may be more challenging than for many toothed whales at sea due to problems of clutter and reverberation in shallow waters. In general, the source parameters of echolocation clicks scale with body size, however, within comparable size ranges, toothed whales inhabit vastly different aquatic niches raising the question of whether habitat also influences biosonar parameters. To address that question we recorded wild Amazon river dolphins (*Inia geoffrensis)* in the Amazon using a vertical seven-hydrophone array to acoustically localize animals and estimate source parameters. By comparing our data with data obtained for similar-sized marine toothed whales, we show that Amazon river dolphins click at faster sampling rates, lower output levels, and higher frequency than similar-sized marine dolphins, but with equally high directionality, showing that habitat is an important co-driver of biosonar evolution in echolocating toothed whales.

Histamine as a direct and indirect regulator of postprandial heart rate

Simon Nørgaard

Ingestion of food initiates a number of processes increasing the oxygen and energetic demands of the gastrointestinal organs, which inevitably raises the demands of the cardiac tissue. The heart undergoes a postprandial tachycardia induced by a release of the cholinergic tone, an effect of one or more circulating NANC factor(s) and an increase in the histaminergic tone. Histamine induces a tachycardial effect by binding H_2 receptors directly, but observations have indicated that histamine potentially has the ability to reinforce either the cholinergic release or the adrenergic tone. By pharmacologically releasing the adrenergic innervation, we show that histamine's tachycardial mechanism works partly through a direct binding of cardiac H_2 receptors and through a strengthening of the sympathetic adrenergic signalling pathway. By stabilising histamine-containing mast cells, we extend earlier findings from *Python molurus* to *P. regius*, and show that the cardiovasoregulatory histamine is not of mast cell origin.

Cognitive modulation of diving bradycardia in harbour porpoises

Siri L. Elmegaard

All airbreathing vertebrates share an immediate response to apnoea and submergence in water. This diving reflex comprises bradycardia and peripheral vasoconstriction, which conserves blood oxygen for the brain and heart. Marine mammals benefit from modulating this response to manage oxygen levels optimally according to the duration and activity level of their dives. Here we investigate the trade-off between increasing dive time limits and risking hypoxic harmful conditions by asking: Are the harbour porpoises *(Phocoena phocoena)* able to modulate their dive response cognitively to obtain an optimal heart rate according to the planned dive duration? To address this, trained harbour porpoises were tagged with electrode-equipped DTAGs measuring heart rate during expected short and long duration dives of minimal activity. We show that porpoises do adjust their heart rates to dive duration, and we conclude that porpoises, like many seals, have conscious control of their cardiovascular responses while diving.

Drugs: what are they good for?

Catherine Williams

Providing adequate analgesia (pain relief) to animals used as scientific subjects is mandated as a refinement of experimental protocols, and can reduce the cascade of stress responses to nociception that may interfere with physiological data collection. Analgesic drugs, however, are associated with physiological effects in and of themselves, and the state of knowledge of their modulation of nociceptive and other physiological pathways in reptiles is currently poor. We review the nociceptive pathway, with recent data for the effect of morphine on cardiovascular data, in and outside of a nociceptive model in *Python regius*. Data introducing the use of non-steroidal anti-inflammatory drugs (NSAIDs) in *Crotalus durissus*, including investigation of cardiovascular, hormonal and oxidative stress parameters is presented. There is some evidence of anti-nociception, together with potentially pathological effects that may mirror those found in mammals. Further experiments, including pharmacokinetic studies, and those using alternative nociceptive models, will be outlined.

Effect of feeding regimes on behavior, swimming performance and metabolic rate in rainbow trout (*Oncorhynchus mykiss*)

Salomine F. L. K. Falck

Variation in both physiological as well as behavioral traits may, in some cases, be correlated. We investigated the link between behavior, specifically boldness, maximum aerobic metabolic rate (MMR), standard metabolic rate (SMR), critical swimming speed (U_{crit}) and anaerobic stamina in juvenile rainbow trout (*Oncorhynchus mykiss)* under two different feeding regimes. We hypothesized that individuals with higher metabolic rates are required to take more risks, and hence be bolder, to uphold their higher energy requirements. As a measure of boldness, we tested the latency to emerge from a shelter following a simulated predator attack. We found that an ample feeding regime resulted in higher SMR, higher level of boldness and higher anaerobic performance compared to a restricted feeding regime. However, it was not possible to conclude on causational relationship between feeding regime, SMR, anaerobic stamina and boldness. Thus, in we are now trying to elucidate this relationship.

Stress response in *Scyliorhinus canicula* and *Squalus acanthias* following trawling

Simone Strandvad

Survival rate of small-spotted catshark (Scyliorhinus canicula) and spiny dogfish (Squalus acanthias) after being bycatch in trawling is high, but their stress response are unknown. This was tested by constructing a trawl simulator in the lab. Reflexes and behaviour, haematocrit and blood chemistry was measured before and after simulated trawl. Results from reflexes and blood samples were compared to catsharks caught in the field in the North Sea. After trawling several reflexes did not show in the lab or in the field and routine and forage activity in juvenile catsharks decreased significantly. There was no change in behaviour in adult catsharks. Haematocrit, lactate and glucose levels increased significantly in catsharks and dogfish after trawling, signs of physical exhaustion and stress. Trawling did not affect their osmoregulation. Results from the lab was similar to results from the field, indicating that the trawl-simulator method is reliable for these species

The peculiar atria of turtles

William Joyce

In the late 1800's, unusual undulations in resting tension ('tonus waves') were reported in turtle atrial preparations. By the 1920's, these waves were married with the histological demonstration that smooth muscle coats the endocardial surface of turtle atria, unlike all other animals studied. However in the post-war era research on this smooth muscle prematurely ceased. Here, we re-visit the role and functional significance of this prominent but highly unusual feature of the turtle heart.

Pulmonary artery reactivity in South American rattlesnakes

Renato Filogonio

In non-crocodilian reptiles, the autonomic regulation of smooth muscle in the pulmonary artery walls exerts a major role in controlling both direction and magnitude of intracardiac shunts. It is well-established that the pulmonary vascular conductance is under vagal control, but the site of constriction and the influence of adrenergic, purinergic and nitric oxide regulations remain largely unknown. I measured *in vitro* vascular reactivity of four different portions of the pulmonary artery (truncus, proximal, distal and fist branch) of the South American rattlesnake (*Crotalus durissus*) to muscarinic agonist (acetylcholine), α and β-adrenergic agonists (phenylephrine and isoproterenol, respectively), purinergic agonist (adenosine) and the nitric oxide donor, sodium nitroprusside. The truncus responded to α-adrenergic stimulation with a weak vasoconstriction (65±39% of baseline), whereas the distal portion constricted more vigorously (95±41% of baseline). Isoproterenol triggered similar levels of relaxation in the branch, proximal, and distal portions up to a concentration of 10^{-5} mol l^{-1} (53±30% compared to pre-contracted values). At higher concentrations, isoproterenol lost its receptor selectivity and caused contraction in all portions, which was blocked by phentolamine (α-adrenergic antagonist). Adenosine led to weaker vasodilation (55±30% for all portions compared to pre-contracted values) than sodium nitroprusside (79±22% for all portions compared to pre-contracted values). Acetylcholine elicited very robust and similar vasoconstriction in the proximal, distal and branch portions (163±27%, 189±61, and 197±70%, respectively), but no response in the truncus. I am now checking if the pulmonary artery of South American rattlesnakes presents a diffuse vagal innervation as suggested by the reactivity experiments by investigating the distribution of vagal neuron terminations using staining with fluorogold. I am also interested in localizing the area in the brain responsible for the control of the pulmonary artery conductance by the vagus, using staining by DiI. Preliminary results indicate a diffuse vagal innervation of the pulmonary artery, corroborating the findings of the reactivity experiments.

Regulation of the Cardiorespiratory System in Air-Breathing Fish

Mikkel Thomsen

Air-breathing fish compose a diverse group possessing both gills and an air-breathing organ (ABO). This arrangement allows them to maintain a sufficiently high oxygen uptake to cover their oxidative metabolism even in aquatic hypoxia where oxygen uptake over the ABO increases. The questions I'm investigating is how ventilation and blood flow changes in response to changes in the external/internal environment, how these changes are sensed, and whether there are differences in these responses that can be ascribed to the species dependency on air-breathing in normoxic water (i.e. the degree of gill reduction). I have started out by describing *Pangasianodon hypophthalmus'* response to changes in aquatic oxygen and CO_2 and shown how it goes from behaving like a regular water-breathing fish in normoxia towards highly reduced gill ventilation in severe hypoxia (fig. 1), while CO_2 changes only affected heart rate.

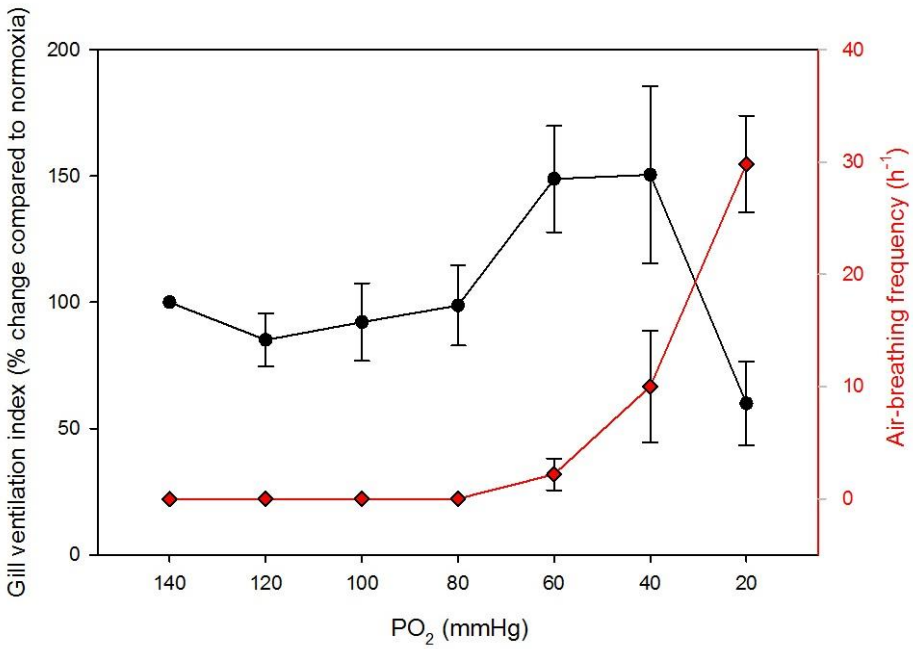


Figure 1: Changes in gill ventilation and air-breathing frequency in progressive hypoxia in *P. hypophthalmus*.

Autonomic regulation of the pulmonary circulation of reptiles

Astrid J. Hougaard

Pulmonary blood flow and cardiac shunt patterns in reptiles are controlled through autonomic innervation of smooth muscle in the pulmonary artery. Using myography, where it is possible to investigate the response of specific vessel segment, I have tried to clarify the presence of adrenergic and-, cholinergic, and a number of non-adrenergic and non-cholinergic receptors within the pulmonary arteries of turtle (*Trachemys scripta*). The results showed that the cholinergic regulation of pulmonary conductance is diffuse, rather than located in only the sphincter. Adrenergic regulation was seen, when stimulated with isoproterenol. Non-adrenergic and non-cholinergic regulation was seen, when stimulated with sodium nitroprusside, causing a nitric oxide effect. Turtle pulmonary arteries are now being collected to further investigation of the presence of the muscarinic receptors. RNA encoding M_1-M_5 is going to be purified. Human DNA sequences encoding M_1-M_5 have been blasted in the turtle genome, and specific primers have been made for qPCR analysis to support the data further.

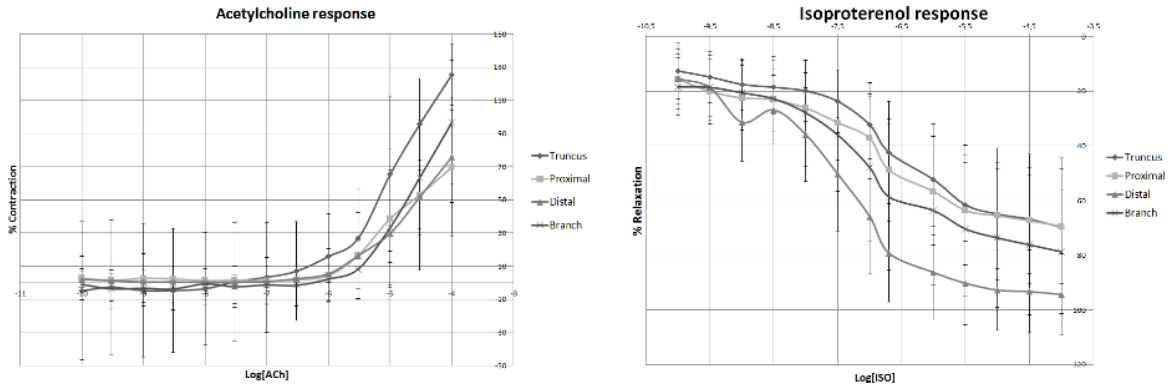


Figure 1. Autonomic regulation of segments of pulmonary artery of turtles. A) Cholinergic stimulation by acetylcholine contracts all segements of the pulmonary artery. B) Adrenergic stimulation by isoproterenol relaxes all segments of the pulmonary artery. Each point represent mean ± S.E.

Mini seminar for Zoophysiology students

Abstracts and program

08:30 Line Hermannsen: Airgun pulses in shallow water: Implications for effects on small marine mammals
08:45 Asbjørn G. Petersen: Aggregation of isolated hemoglobins from the turtle, *Trachemys scripta*
09:00 Lærke R. Reinholdt: How Right-to-left cardiac shunts and air convection requirements affect arterial partial pressure of oxygen and carbon dioxide in reptiles
09:15 Lauren James: An overview of currently used inhalant and injectable anaesthetic agents in reptiles and the relevance of their use in snakes
09:30 Trine Olsson: A comparative study of cold tolerance in Drosophila and its relation to hemolymph composition
09:45 Christian Damsgaard: Origin and functional diversification of the Root effect and retinal oxygen delivery in bony fishes

10:00 Coffee

10:30 Shane Gero: Social Complexity and Functional Communication in Sperm Whales
10:45 Simone K. A. Videsen: Male humpback whale escorts disrupt suckling behavior of acoustically cryptic neonate calves
11:00 Morten S. Storgaard: Toxicity of Chemical Warfare Agent (CWA) metabolites found in the Baltic Sea
11:15 Inge G. Revsbech: Comparison of circulating hydrogen sulfide and nitric oxide metabolites and their potential roles in brown bear hibernation
11:30 Mads K. Andersen: Climate change and physiology: Insight from an air-breathing fish

11:45 Lunch

12:30 Pernille M. Sørensen: Click communication in the harbor porpoise
12:40 Signe O. Jensen: Chill coma related disturbances in ion homeostasis in the Lepidoptera *Heliconius Cydno*

12:50 Amanda M. Bundgård: Nitrite-dependent modification of mitochondria and protection against oxidative stress in the red-eared slider turtle
13:05 Camilla Taulbjerg: The physiological effects of opioids on the cardiovascular system of snakes.
13:20 Christian L. Malte: Closed respirometry may underestimate tissue gas exchange and bias the respiratory exchange ratio (RER)
13:35 Laia Rojano-Doñate: Acoustics and energetics of echolocators in a noisy world

13:50 Coffee

14:15 Mai M. Madsen: The effect of feeding regime on the relationship between standard metabolic rate, specific dynamic action, maximum metabolic rate and specific growth rate in rainbow trout
14:30 Michael Ladegaard: Amazon river dolphins use a high-frequency, short-range biosonar
14:45 Simon Nørgaard: Histamine as a direct and indirect regulator of postprandial heart rate
15:00 Siri Elmegaard**:** Cognitive modulation of diving bradycardia in harbour porpoises

15:15 Cake

15:30 Catherine Williams: Drugs: what are they good for?
15:45 Salomine F. L. K. Falck: Effect of feeding regimes on behavior, swimming performance and metabolic rate in rainbow trout (*Oncorhynchus mykiss*)
16:00 Simone Strandvad: Stress response in *Scyliorhinus canicula* and *Squalus acanthias* following trawling
16:15 William Joyce: The peculiar atria of turtles

16:30 Beer

18:00 Pizza

Airgun pulses in shallow water: Implications for effects on small marine mammals

Line Hermannsen

Airguns, used in oil and gas investigations, are among the most powerful anthropogenic noise sources in marine habitats. Most of the energy is at low frequencies, however part of the radiated energy may also be present at higher frequencies with potential effects on small marine mammals that have their best hearing at medium-to-high frequencies. We recorded airgun pulses in a uniform shallow water habitat using hydrophones and acoustic recorders. We show considerable energy at frequencies, where marine mammals hear well and that the spectral composition of airgun pulses is highly affected by water depth. This highlights the need for considering environmental properties in impact assessments of airguns. We conclude that the risk of hearing damage is small for both pinnipeds and porpoises. However, there is substantial potential for significant behavioral responses out to several km from an airgun, well beyond the commonly used shut down zone around airgun arrays.

Aggregation of isolated hemoglobins from the turtle, Trachemys scripta

Asbjørn Graver Petersen

In vitro hemoglobin (Hb) aggregation has been observed in all groups of the higher vertebrates. It is however relatively unknown to what extent and under what conditions this occurs in living animals.

The aims of this study were to investigate how different conditions affected the degree of aggregation and whether aggregation had implications on the O2-binding properties in Hbs from the turtle, Trachemys scripta. Using fast protein liquid chromatography we separated and isolated the two Hb isoforms, HbA and HbD. We find that only the HbA isoform aggregates and a higher heme concentration enhance the process of polymerization. The aggregation process seems independent of ATP and pH. Furthermore O2 equilibrium measurements of polymeric and tetrameric fractions obtained from gel filtration show similar O2 affinities indicating that aggregation state doesn't affect oxygenation properties of T. scripta Hb.

Future work will focus on aggregation being a marker for oxidative stress in red blood cells.

How right-to-left cardiac shunts and air convection requirements affect arterial partial pressure of oxygen and carbon dioxide in reptiles

Lærke R. Reinholdt

Multiple physiologically changes can affect the arterial partial pressure of oxygen (Pa_{O_2}) and carbon dioxide (Pa_{CO_2}). In reptiles, two of these changes are alternations in air convection requirements for CO_2 ($ACR = \dot{V}E/\dot{V}_{CO_2}$) and R-L intracardiac shunts, where a fraction of the systemic venous blood bypasses the pulmonary circulation by flowing from the right to the left side of the anatomic undivided ventricle. To analyze the degree of impact that the two variables have on the gases, two dynamic models have been developed. The models show that changes in ACR affect Pa_{CO_2} profoundly and Pa_{O_2} slightly, whereas the impact is the reverse when altering the R-L flow. When doubling the R-L flow Pa_{CO_2} increases, and to attain a similar rise in Pa_{CO_2} only a minor drop in ACR is needed. However, this change in ACR barely affects Pa_{O_2}. This asymmetry in how the two variables affect Pa_{CO_2} and Pa_{O_2} enables (at least in principle) the organism to control Pa_{CO_2} and Pa_{O_2} by regulating both variables.

An overview of currently used inhalant and injectable anaesthetic agents in reptiles and the relevance of their use in snakes

Lauren James

The use of appropriate anaesthesia is a priority in both research and veterinary fields, with its application to reptile surgery becoming more pertinent following an increase in exotic pet ownership and the continued use of reptile models for physiological and biomedical research. It is therefore imperative that anaesthetics and chemical methods of restraint be characterised in reptiles, with a focus on snakes, as they are neglected in the literature. The following experiments will evaluate the use of common anaesthetic agents for the induction and maintenance of surgical anaesthesia in snakes. The minimum alveolar concentrations (MAC) of isoflurane and sevoflurane will be determined, and the effects of morphine pre-medication investigated. The cardiovascular effects of the inhalant anaesthetics will also be described and anaesthetic induction with the injectable agent, alfaxalone, will be compared to inhalant induction with isoflurane and sevoflurane. The overall aim is to describe an appropriate anaesthetic protocol for snakes.

A comparative study of cold tolerance in Drosophila and its relation to hemolymph composition

Trine Olsson

The distribution of insects is closely linked to their ability to maintain life functions at lower temperatures, i.e. their cold tolerance, and it is therefore of great importance to understand what determines the cold tolerance of insect species. It has previously been found that cold tolerant species of e.g. *Drosophila* have low [Na^+] in their hemolymph, but the osmolality of the hemolymph does not differ significantly between species at standard conditions. The cold tolerant species must therefore have higher hemolymph concentrations of other compatible osmolytes, which is what I try to find out in my study where I use H-NMR spectroscopy to obtain quantitative measures of the organic molecules in the hemolymph in the hope of finding "the missing osmolytes".

Origin and functional diversification of the Root effect and retinal oxygen delivery in bony fishes

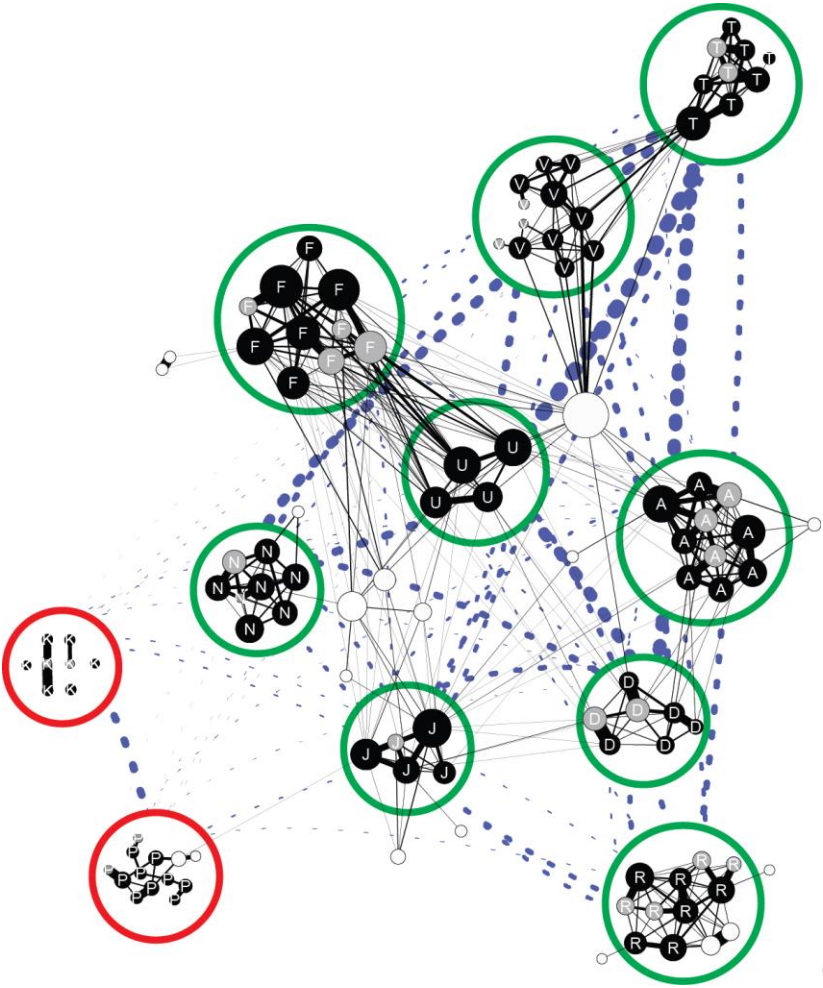
Christian Damsgaard

While retinal O_2-delivery in tetrapods is achieved through intra-retinal capillaries, advanced teleost fishes use Root effect hemoglobins and a choroid rete to secrete oxygen into their avascular retinas. To reconstruct the likely sequence in which the functional components in O_2-secretion evolved, we investigated retinal blood-flows and Root effects in 24 phylogenetically-distinct fish-species using a combination of µUS, µCT and histology. We show that the ancestral state of retinal O_2-supply is through intra-retinal capillaries, that this trait remains after the origin of the Root effect and the choroid rete, and that intra-retinal capillaries are only lost in species with well-developed Root effects. This suggests that the evolution of retinal O_2-secretion from a choroid rete depends on gradual increases in Root effect concurrent with decreased retinal capillarization. We also show that intra-retinal capillaries re-evolve and function in O_2-delivery in species that have secondary lost in any of the functional components in O_2-secretion.

Social Complexity and Functional Communication in Sperm Whales

Shane Gero

Multidimensional network depicting patterns of social interactions and acoustic similarity across three levels of sperm whale social structure in the Eastern Caribbean. Individuals (small nodes) within units (large nodes) within vocal clans (colour of large nodes). In the social network, individuals are connected by social relationships (black solid lines) weighted based on the Half-Weight Index of associations. Individual nodes are sized relative to their measure of degree (number of connected individuals) and coloured based on class (black – adult females, grey – dependent calves, white – mature male escorts). The only social connection between clans is a mature male escort who associated with both Unit P and Unit J and there are no direct associations between females in differing clans. In the overlapped acoustic network, units (large nodes) are connected by coda repertoire similarity (dashed blue lines) weighted based multivariate similarity and coloured based on clan (Green - Eastern Caribbean Clan, Red – two rare units). Note that the weighting of the edges for acoustic similarity and social association differ and the relative thickness of lines between social and acoustic networks are not related.



Three levels of sperm whale social structure (individual, unit, and clan) as defined by both social (solid black) and communication networks (dashed blue).

Male humpback whale escorts disrupt suckling behavior of acoustically cryptic neonate calves

Simone K. A. Videsen

Humpback whales annually migrate from foraging to breeding grounds to mate and calve. The dual purpose of female migrations potentially creates a conflict between choosing a mate versus maximizing the energy budget for the newborn calf by reducing exposure to male escorts. To investigate this, we deployed Dtags on eight neonate calves in Exmouth Gulf, WA. Prominent behaviors observed were travelling, resting and suckling. We show that mother-calf pairs consistently keep very close contact, and that calves suckle regularly. Conversely, the presence of antagonistic male escorts induced a disruption of suckling behavior and increased the calves' overall energy expenditure. Therefore, it is in the calf's interest to avoid attracting male escorts, which may explain why calves only produce weak sounds. We propose that this acoustic crypsis serves to maintain mother-calf contact, while reducing predation risks and attraction of male escorts that disrupt the critical transfer of milk from mothers to calves.

Toxicity of Chemical Warfare Agent (CWA) metabolites found in the Baltic Sea

Morten S. Storgaard

After 2nd World War the chemical warfare agents were prohibited by law and 11,000 tonnes of toxic agents were dumped in the Bornholm Basin east of Bornholm. The area has been screened for the presence of parent compounds and metabolites including the concentrations they are found in. The majority of the detected compounds has been found in the sediment and a minor part in the pore water. The (eco)toxicity of these compounds remain to be illuminated in which this thesis hopefully will contribute to. The toxicity of these chemicals will be described with an acute and chronic test. The acute toxicity will be described by Microtox™ with *Vibrio fischerei*, which measures inhibition in light emission as a function of different concentrations. The chronic toxicity will be described by spontaneous locomotor changes in Zebrafish (*Danio rerio*); intending to draw lines to the commercially important Cod (*Gadus morrhua*).

Comparison of circulating hydrogen sulfide and nitric oxide metabolites and their potential roles in brown bear hibernation

Inge G. Revsbech

Mammalian hibernation is a physiological wonder of temporarily down-regulated metabolism, during which the animal does not eat and remains inactive for months. Understanding the underlying biochemical mechanisms may have great translational medical applications. As ubiquitous inhibitors of mitochondrial metabolism, both nitric oxide (NO) and hydrogen sulfide (H_2S) could in principle play a part in the whole body metabolic depression essential to hibernation. We investigated type and content of blood metabolites of NO and H_2S in winter hibernating and summer active, free-ranging brown bears. We found significant changes in composition of sulfide metabolites in plasma, with a decrease in plasma thiosulfate and polysulfides during hibernation, indicating that whilst hibernating, the bear may regenerate H_2S from its oxidation products, thiosulfate and polysulfides. Concurrently, high levels of free sulfide correlated with high levels of cysteine, suggesting that cysteine may be prioritized for glutathione synthesis during hibernation. Thus, this remodeling of sulfide metabolism may work to preserve plasma free cysteine for the generation of glutathione in cells, a central antioxidant also found in high levels in red blood cells during hibernation. For NO, no clear changes could be measured in circulating nitrite or in the degree of S-nitrosylation of glyceraldehyde-3-phosphante dehydrogenase, although this remains to be investigated further. Our study revealed that circulating H_2S potentially contributes to inhibition of mitochondrial respiration during hibernation.

Climate change and physiology: Insight from an air-breathing fish

Mads K. Andersen

Due to anthropogenic forcing, climate is changing at an accelerated rate, which is expected to have severe consequences for the ecology and physiology of aquatic ectotherms. We therefore investigated how increasing temperature affected swimming performance, resting and maximum oxygen uptake rates and the derived aerobic scope (AAS; maximum – resting) of the air-breathing fish *Pangasianodon hypophthalmus* using two-phase intermittent closed- and swimming respirometry. We hypothesized that *P. hypophthalmus* would maintain its AAS by increasing aerial ventilation as temperature increased, which would concomitantly lower its critical swimming speed (U_{crit}). We find that AAS was unchanged until a decrease at 39°C in both experiments. Interestingly this was achieved without increasing aerial ventilation at any temperature tested, and U_{crit} was unaffected until 39°C. These findings reveal a high resilience towards increases in temperature, and that the projected temperature increase will have little to no effect on the aerobic performance of *P. hypophthalmus*.

Click communication in the harbor porpoise

Pernille M. Sørensen

Sound plays an essential role for communication in many species of cetaceans. In some cases, assessment of acoustic communication can be complicated, as in the case of the harbor porpoise. This species only produce narrow band high frequency (NBHF) clicks. These NBHF clicks are known to play an important role in echolocation, but are now also thought to play an essential function in communication, where different types of click sequences, defined primarily by their click repetition pattern, have been shown to be correlated with specific behaviors. Initially, I wish to categorize the click communication calls used by wild harbor porpoises and to determine when, where and how often they are used, as well as putting them into behavioral context. Thereafter it could be particularly interesting to assess whether individual variation exist in the general click communication call types. Such variation would most likely play an important role in recognition of other individuals.

Chill coma related disturbances in ion homeostasis in the Lepidoptera *Heliconius Cydno*

Signe O. Jensen

At low temperatures, chill susceptible insects enter a state called chill coma, a reversible state of neuromuscular impairment. Chill coma related disturbances of ion homeostasis have been observed in different species of insects. Especially the increase in hemolymph [K^+] has been of great interest due to the effect on Nernst potential, E_K, leading to a drop in resting membrane potential. Most insects have ionic hemolymph composition of high [Na^+] and low [K^+] and [Mg^{2+}]. However this is not the case for the Lepidoptera as they have high [K^+] and [Mg^{2+}] and low [Na^+] in their hemolymph. To investigate what happens to hemolymph ion concentrations and resting membrane potential while species with this atypical ion composition are in chill coma, the lepidoptera *Heliconius Cydno* has been studied. Hemolymph and muscle samples were taken from butterflies in chill coma and concentrations of Na, K and Mg were measured using atomic absorbance spectrometry. Measurements of membrane potential are pending.

Nitrite-dependent modification of mitochondria and protection against oxidative stress in the red-eared slider turtle

Amanda M. Bundgård

Recent evidence indicates that nitrite is a cytoprotectant agent, in part because it mediates a reversible modification, S-nitrosation of complex I in the electron transport chain. This inhibits formation of toxic reactive oxygen species (ROS) from mitochondria during reoxygenation after a period of anoxia, as after stroke or heart failure, or hibernation in anoxia-tolerant animals such as the slider turtle.

During my masters project I investigated the role of nitrite as a natural cytoprotective agent in the slider turtle. I found that nitrite inhibits complex I by S-nitrosation during anoxia, reducing the level of ROS produced upon reoxygenation, which suggests that turtles naturally exploit nitrite as a strategy to prevent oxidative damage. I also found interesting changes in metabolite composition after acclimation to anoxia, such as an increase in succinate that might fuel the ROS production upon reoxygenation. This further supports the need to inhibit complex I and thereby ROS production.

The physiological effects of opioids on the cardiovascular system of snakes

Camilla Taulbjerg

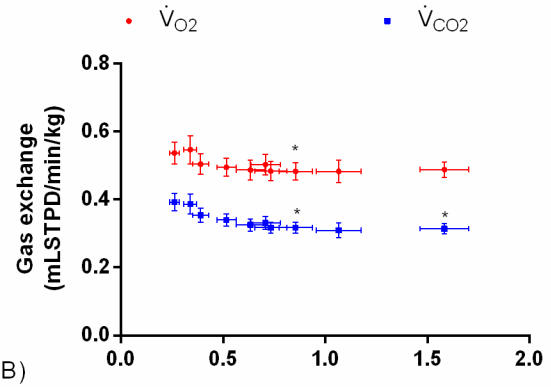
Analgesia and anesthesia is attracting more attention when working with reptiles. The following presentation will focus on opioids, especially morphine as an analgesic for snakes. Heartrate measured with ECG and behavioral analysis is used to examine the snakes' reaction when exposed to nociceptive stimuli (capsaicin) while under the influence of morphine. The results seem to implicate that when comparing heartrate, a dosage of 5 mg/kg morphine do not affect the snakes more than 1 mg/kg morphine do, but respiration rates seems to get higher when under influence of 5 mg/kg compared to the 1 mg/kg. Control studies are yet to be conducted, and behavioral analyses are not yet analysed completely.

Closed respirometry may underestimate tissue gas exchange and bias the respiratory exchange ratio (RER)

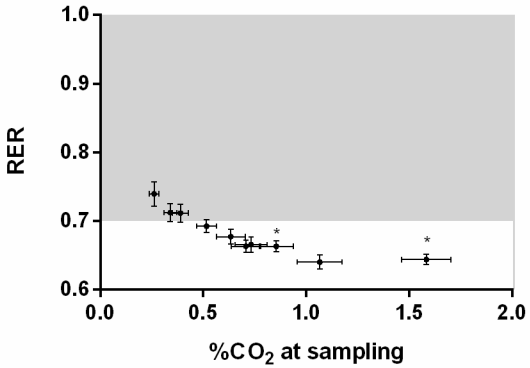
Christian L. Malte

When conducting closed respirometry, the rates of O_2 uptake and CO_2 excretion are typically assumed to be in steady-state, such that the measured rates of gas exchange equal those at tissue level. In other words, the respiratory gas exchange ratio (RER) is assumed to equal the respiratory quotient (RQ). However, because the gas concentrations change progressively during closure, the animal inspires air with a progressively increasing CO_2 concentration and decreasing O_2 concentration. These changes will eventually affect gas exchange leading to errors due to changes in the gas stores of the body and because of the higher solubility/capacitance of CO_2 in the tissues, CO_2 excretion will be more affected than O_2 uptake $\dot{V}_{O2}$. Using mathematical models and experimental data from ball pythons we show that CO_2 excretion and RER will become progressively underestimated as closure time is prolonged (*i.e.* chamber CO_2 concentration increase).

A)


$\dot{V}_{O2}$
$\dot{V}_{CO2}$
Gas exchange (mLSTPD/min/kg)
0.8
0.6
0.4
0.2
0.0
0.0
0.5
1.0
1.5
2.0
*
*
*

B)


RER
1.0
0.9
0.8
0.7
0.6
0.0
0.5
1.0
1.5
2.0
%CO_2 at sampling
*
*

Acoustics and energetics of echolocators in a noisy world

Laia Rojano-Doñate

The growth in shipping and marine industries has raised concerns over how anthropogenic noise may affect toothed whales. Toothed whales rely on acoustic cues for critical functions such as navigation and foraging. Despite the extensive literature documenting effects of human disturbance on wildlife behaviour, there are not studies linking behavioural changes to consequences on energy budgets. In this PhD project, I hypothesize that induced behavioural changes may involve energetic costs by introducing an imbalance between energy intake and use evoked by reduced foraging success and increased movement activity. Such an imbalance could then cause a reduction in individual fitness and provoke long-term effects on population vital rates. Using porpoises as a model organism, I aim to uncover the mechanistic link between anthropogenic noise disturbance and population effects by quantifying energetic and behavioural responses to underwater noise and developing a model capable of predicting population consequences of increasing levels of noise.

The effect of feeding regime on the relationship between standard metabolic rate, specific dynamic action, maximum metabolic rate and specific growth rate in rainbow trout

Mai M. Madsen

Two groups of juvenile rainbow trout (*Oncorhynchus mykiss*) were fed different feeding regimes of either 1.05 % day^{-1} (low) or an *ad libitum* diet (high) for 7 weeks before they had their standard metabolic rate (SMR), maximal metabolic rate (MMR), specific dynamic action (SDA), and specific growth rate (SGR) measured. When comparing the two feeding regimes it was shown that the two groups had significantly different means of the SMR, absolute aerobic scope (AAS), SDA, and SGR. A significant relationship was found between the SDA and SGR and rAAS and rMMR for both feeding regimes. Furthermore, a significant relationship was found between the rMMR, SGR, and rSDA *vs.* rSMR and rMMR *vs.* rSDA for the high feeding regime, whereas this was not the case for the low feeding regime. The study showed that food ration can have a great effect on e.g. the found metabolic rates and their correlations.

Amazon river dolphins use a high-frequency, short-range biosonar
Michael Ladegaard

For the few toothed whale species fully adapted to life in river systems the task of echolocation may be more challenging than for many toothed whales at sea due to problems of clutter and reverberation in shallow waters. In general, the source parameters of echolocation clicks scale with body size, however, within comparable size ranges, toothed whales inhabit vastly different aquatic niches raising the question of whether habitat also influences biosonar parameters. To address that question we recorded wild Amazon river dolphins (*Inia geoffrensis)* in the Amazon using a vertical seven-hydrophone array to acoustically localize animals and estimate source parameters. By comparing our data with data obtained for similar-sized marine toothed whales, we show that Amazon river dolphins click at faster sampling rates, lower output levels, and higher frequency than similar-sized marine dolphins, but with equally high directionality, showing that habitat is an important co-driver of biosonar evolution in echolocating toothed whales.

Histamine as a direct and indirect regulator of postprandial heart rate

Simon Nørgaard

Ingestion of food initiates a number of processes increasing the oxygen and energetic demands of the gastrointestinal organs, which inevitably raises the demands of the cardiac tissue. The heart undergoes a postprandial tachycardia induced by a release of the cholinergic tone, an effect of one or more circulating NANC factor(s) and an increase in the histaminergic tone. Histamine induces a tachycardial effect by binding H_2 receptors directly, but observations have indicated that histamine potentially has the ability to reinforce either the cholinergic release or the adrenergic tone. By pharmacologically releasing the adrenergic innervation, we show that histamine's tachycardial mechanism works partly through a direct binding of cardiac H_2 receptors and through a strengthening of the sympathetic adrenergic signalling pathway. By stabilising histamine-containing mast cells, we extend earlier findings from *Python molurus* to *P. regius,* and show that the cardiovasoregulatory histamine is not of mast cell origin.

Cognitive modulation of diving bradycardia in harbour porpoises
Siri L. Elmegaard

All airbreathing vertebrates share an immediate response to apnoea and submergence in water. This diving reflex comprises bradycardia and peripheral vasoconstriction, which conserves blood oxygen for the brain and heart. Marine mammals benefit from modulating this response to manage oxygen levels optimally according to the duration and activity level of their dives. Here we investigate the trade-off between increasing dive time limits and risking hypoxic harmful conditions by asking: Are the harbour porpoises *(Phocoena phocoena)* able to modulate their dive response cognitively to obtain an optimal heart rate according to the planned dive duration? To address this, trained harbour porpoises were tagged with electrode-equipped DTAGs measuring heart rate during expected short and long duration dives of minimal activity. We show that porpoises do adjust their heart rates to dive duration, and we conclude that porpoises, like many seals, have conscious control of their cardiovascular responses while diving.

Drugs: what are they good for?
Catherine Williams

Providing adequate analgesia (pain relief) to animals used as scientific subjects is mandated as a refinement of experimental protocols, and can reduce the cascade of stress responses to nociception that may interfere with physiological data collection. Analgesic drugs, however, are associated with physiological effects in and of themselves, and the state of knowledge of their modulation of nociceptive and other physiological pathways in reptiles is currently poor. We review the nociceptive pathway, with recent data for the effect of morphine on cardiovascular data, in and outside of a nociceptive model in *Python regius*. Data introducing the use of non-steroidal anti-inflammatory drugs (NSAIDs) in *Crotalus durissus*, including investigation of cardiovascular, hormonal and oxidative stress parameters is presented. There is some evidence of anti-nociception, together with potentially pathological effects that may mirror those found in mammals. Further experiments, including pharmaco-kinetic studies, and those using alternative nociceptive models, will be outlined.

Effect of feeding regimes on behavior, swimming performance and metabolic rate in rainbow trout (*Oncorhynchus mykiss*)

Salomine F. L. K. Falck

Variation in both physiological as well as behavioral traits may, in some cases, be correlated. We investigated the link between behavior, specifically boldness, maximum aerobic metabolic rate (MMR), standard metabolic rate (SMR), critical swimming speed (U_{crit}) and anaerobic stamina in juvenile rainbow trout (*Oncorhynchus mykiss)* under two different feeding regimes. We hypothesized that individuals with higher metabolic rates are required to take more risks, and hence be bolder, to uphold their higher energy requirements. As a measure of boldness, we tested the latency to emerge from a shelter following a simulated predator attack. We found that an ample feeding regime resulted in higher SMR, higher level of boldness and higher anaerobic performance compared to a restricted feeding regime. However, it was not possible to conclude on causational relationship between feeding regime, SMR, anaerobic stamina and boldness. Thus, in we are now trying to elucidate this relationship.

Stress response in *Scyliorhinus canicula* and *Squalus acanthias* following trawling

Simone Strandvad

Survival rate of small-spotted catshark (Scyliorhinus canicula) and spiny dogfish (Squalus acanthias) after being bycatch in trawling is high, but their stress response are unknown. This was tested by constructing a trawl simulator in the lab. Reflexes and behaviour, haematocrit and blood chemistry was measured before and after simulated trawl. Results from reflexes and blood samples were compared to catsharks caught in the field in the North Sea. After trawling several reflexes did not show in the lab or in the field and routine and forage activity in juvenile catsharks decreased significantly. There was no change in behaviour in adult catsharks. Haematocrit, lactate and glucose levels increased significantly in catsharks and dogfish after trawling, signs of physical exhaustion and stress. Trawling did not affect their osmoregulation. Results from the lab was similar to results from the field, indicating that the trawl-simulator method is reliable for these species

The peculiar atria of turtles

William Joyce

In the late 1800's, unusual undulations in resting tension ('tonus waves') were reported in turtle atrial preparations. By the 1920's, these waves were married with the histological demonstration that smooth muscle coats the endocardial surface of turtle atria, unlike all other animals studied. However in the post-war era research on this smooth muscle prematurely ceased. Here, we revisit the role and functional significance of this prominent but highly unusual feature of the turtle heart.

Pulmonary artery reactivity in South American rattlesnakes

Renato Filogonio

In non-crocodilian reptiles, the autonomic regulation of smooth muscle in the pulmonary artery walls exerts a major role in controlling both direction and magnitude of intracardiac shunts. It is well-established that the pulmonary vascular conductance is under vagal control, but the site of constriction and the influence of adrenergic, purinergic and nitric oxide regulations remain largely unknown. I measured *in vitro* vascular reactivity of four different portions of the pulmonary artery (truncus, proximal, distal and fist branch) of the South American rattlesnake (*Crotalus durissus*) to muscarinic agonist (acetylcholine), α and β-adrenergic agonists (phenylephrine and isoproterenol, respectively), purinergic agonist (adenosine) and the nitric oxide donor, sodium nitroprusside. The truncus responded to α-adrenergic stimulation with a weak vasoconstriction (65±39% of baseline), whereas the distal portion constricted more vigorously (95±41% of baseline). Isoproterenol triggered similar levels of relaxation in the branch, proximal, and distal portions up to a concentration of 10^{-5} mol l^{-1} (53±30% compared to pre-contracted values). At higher concentrations, isoproterenol lost its receptor selectivity and caused contraction in all portions, which was blocked by phentolamine (α-adrenergic antagonist). Adenosine led to weaker vasodilation (55±30% for all portions compared to pre-contracted values) than sodium nitroprusside (79±22% for all portions compared to pre-contracted values). Acetylcholine elicited very robust and similar vasoconstriction in the proximal, distal and branch portions (163±27%, 189±61, and 197±70%, respectively), but no response in the truncus. I am now checking if the pulmonary artery of South American rattlesnakes presents a diffuse vagal innervation as suggested by the reactivity experiments by investigating the distribution of vagal neuron terminations using staining with fluorogold. I am also interested in localizing the area in the brain responsible for the control of the pulmonary artery conductance by the vagus, using staining by DiI. Preliminary results indicate a diffuse vagal innervation of the pulmonary artery, corroborating the findings of the reactivity experiments.

Regulation of the Cardiorespiratory System in Air-Breathing Fish

Mikkel Thomsen

Air-breathing fish compose a diverse group possessing both gills and an air-breathing organ (ABO). This arrangement allows them to maintain a sufficiently high oxygen uptake to cover their oxidative metabolism even in aquatic hypoxia where oxygen uptake over the ABO increases. The questions I'm investigating is how ventilation and blood flow changes in response to changes in the external/internal environment, how these changes are sensed, and whether there are differences in these responses that can be ascribed to the species dependency on air-breathing in normoxic water (i.e. the degree of gill reduction). I have started out by describing *Pangasianodon hypophthalmus'* response to changes in aquatic oxygen and CO_2 and shown how it goes from behaving like a regular water-breathing fish in normoxia towards highly reduced gill ventilation in severe hypoxia (fig. 1), while CO_2 changes only affected heart rate.

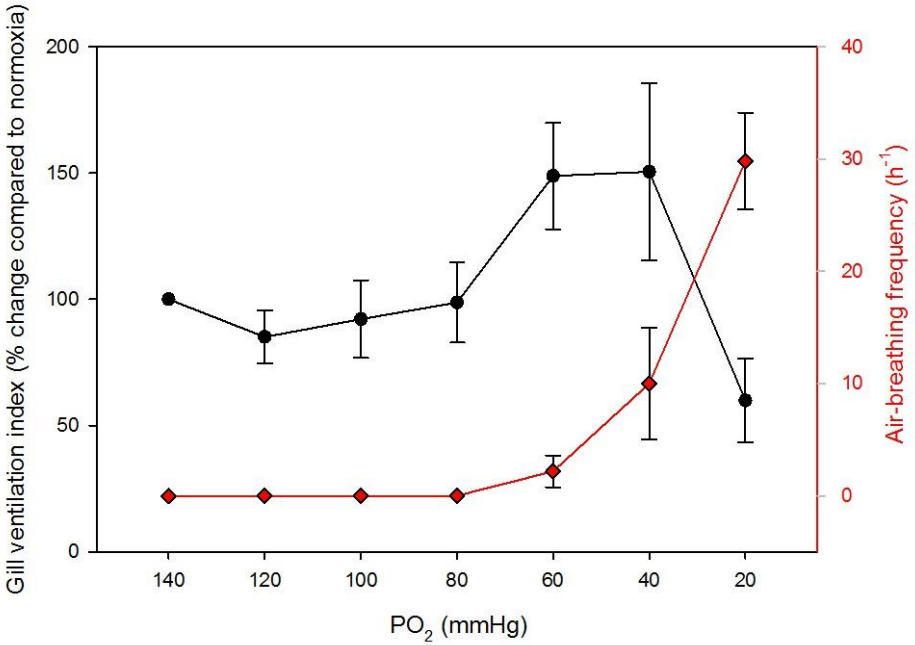


Figure 1: Changes in gill ventilation and air-breathing frequency in progressive hypoxia in *P. hypophthalmus*.

Autonomic regulation of the pulmonary circulation of reptiles

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Pulmonary blood flow and cardiac shunt patterns in reptiles are controlled through autonomic innervation of smooth muscle in the pulmonary artery. Using myography, where it is possible to investigate the response of specific vessel segment, I have tried to clarify the presence of adrenergic and-, cholinergic, and a number of non-adrenergic and non-cholinergic receptors within the pulmonary arteries of turtle (*Trachemys scripta*). The results showed that the cholinergic regulation of pulmonary conductance is diffuse, rather than located in only the sphincter. Adrenergic regulation was seen, when stimulated with isoproterenol. Non-adrenergic and non-cholinergic regulation was seen, when stimulated with sodium nitroprusside, causing a nitric oxide effect. Turtle pulmonary arteries are now being collected to further investigation of the presence of the muscarinic receptors. RNA encoding M_1-M_5 is going to be purified. Human DNA sequences encoding M_1-M_5 have been blasted in the turtle genome, and specific primers have been made for qPCR analysis to support the data further.

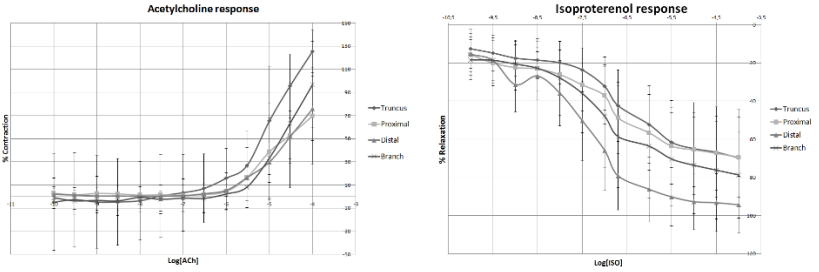

Figure 1. Autonomic regulation of segments of pulmonary artery of turtles. A) Cholinergic stimulation by acetylcholine contracts all segements of the pulmonary artery. B) Adrenergic stimulation by isoproterenol relaxes all segments of the pulmonary artery. Each point represent mean ± S.E.