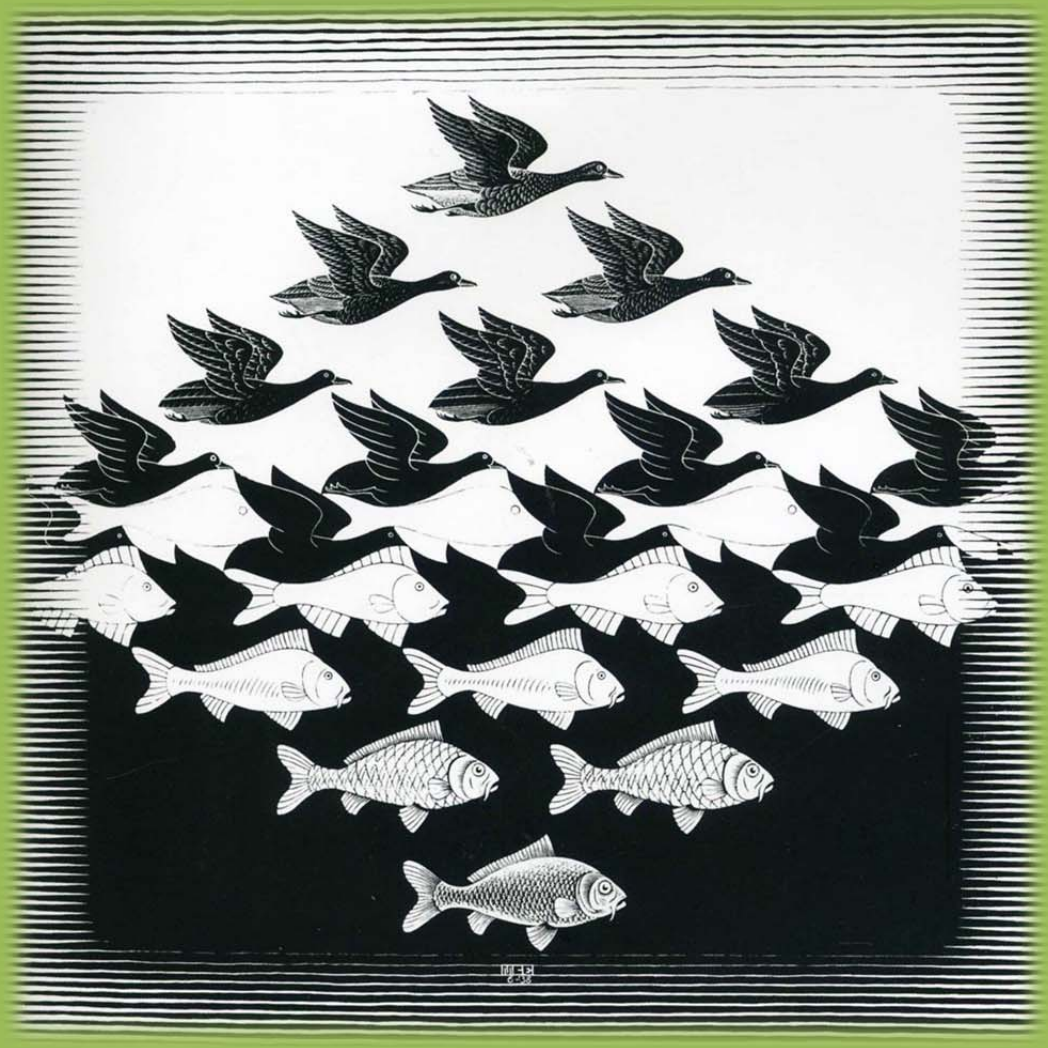
12-04-2018

# ZOOPHYSIOLOGY SPECIALEDAG



Zoophysiology | Aarhus University

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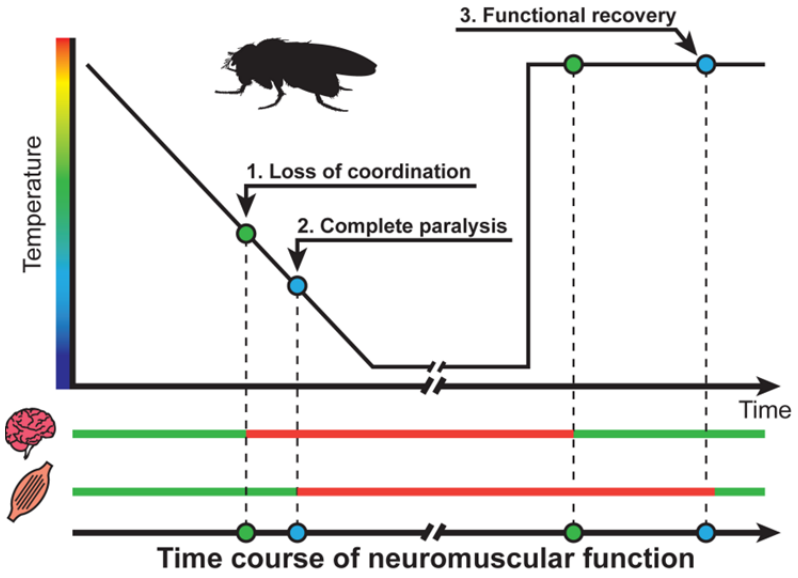
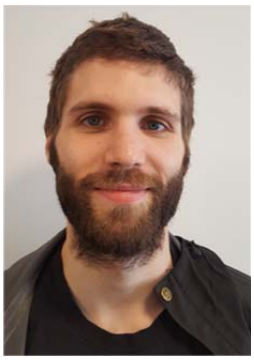
# Abstracts

**Mads Kuhlmann Andersen**
Supervisor(s): Johannes Overgaard

## Chill coma onset and recovery: differing roles of the central nervous system and muscular function

When insects are cooled, they first lose their ability to perform coordinated movements at a critical temperature ($CT_{min}$) and later enter a state of complete neuromuscular paralysis at the chill coma onset temperature (CCO). To regain fitness, function must be recovered when temperature becomes favourable, and the time it takes is referred to as the chill coma recovery time (CCRT). These three phenotypes ($CT_{min}$, CCO, and CCRT) are popular measures of insect cold tolerance, but despite this, relatively little is known about their underlying mechanisms. Here, we used a model system of three *Drosophila* species with varying cold tolerances to investigate the functional roles of the central nervous system (CNS) and muscle membrane polarization ($V_m$) in setting the lower thermal limits ($CT_{min}$ and CCO) and in delaying chill coma recovery. This was done by performing electrophysiological measurements of extracellular field potentials in the CNS and muscle $V_m$ using glass microelectrodes. Our results show that the entry into chill coma, initiated by a loss of coordination, is caused by the onset of a spreading depolarization in the CNS which is followed shortly by a shutdown of muscle function. After reheating, the CNS function recover rapidly, and functional recovery was limited by the return of muscle function – except in the most cold-tolerant species where muscle function was seemingly never lost and functional recovery is dictated by CNS recovery. Thus, we demonstrate the primacy of different physiological systems in setting the most commonly used cold tolerance measures for insects.



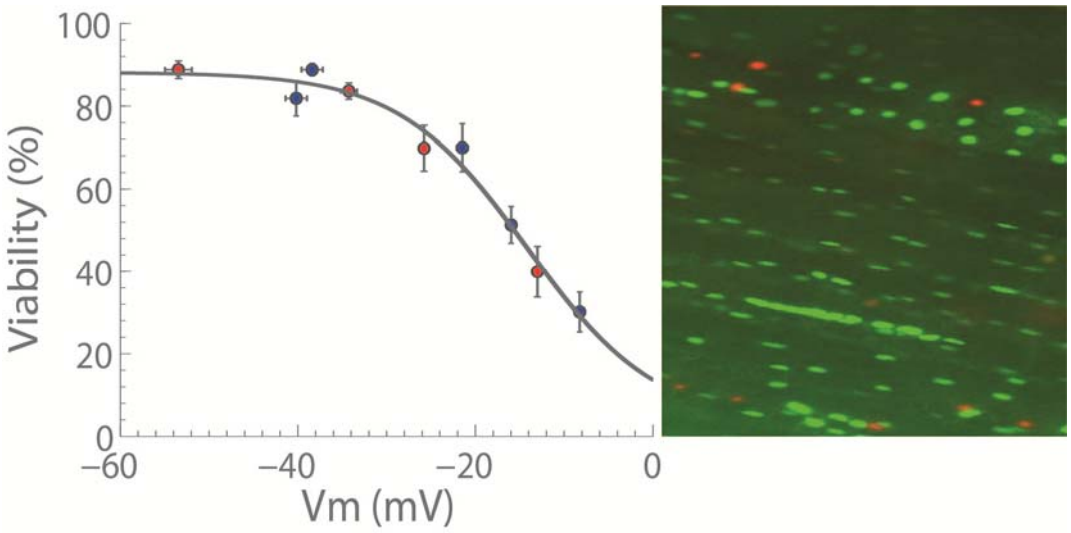


**Jeppe Bayley**
Supervisor(s): Johannes Overgaard

## Why do insects die at low temperature?

### Understanding the physiological dysfunctions during cold-stress

Cold tolerance of insects is known to be critically important for their distribution. Acute cold stress has been observed to coincide with an initial depolarization of the excitable tissue and a reversible loss of neuromuscular coordination in many insects. If the cold exposure is increased (duration or intensity), insects lose transmembranal ionic homeostasis which further depolarizes the cell and non-reversible injury starts to develop. Using a fluorescent dual DNA staining method to estimate cell viability in muscle fibres, extent of cell injury under chilling/depolarizing conditions was measured. Furthermore, to look for possible mechanisms behind chill induced cell injury, both the extracellular and intracellular environments were manipulated. Using this approach, we discovered that the accumulation of intracellular $Ca^{2+}$ was essential for the development of chill induced cell injury. Furthermore, blocking the voltage-gated $Ca^{2+}$ channel protected fibre viability. Therefore, the $Ca^{2+}$ current of muscles from warm- and cold acclimated locusts was measured using a three-electrode voltage clamp. Fibre excitability was also assessed using an intracellular electrode to inject a constant current. Surprisingly cold acclimation resulted in increased peak $Ca^{2+}$ currents and increased fibre excitability at intermediate chamber temperatures and unchanged $Ca^{2+}$ currents at low temperatures. Thus, the conclusions of my studies so far are 1) accumulation of intracellular $Ca^{2+}$ ions is essential for development of chill induced cell injury, 2) intracellular $Ca^{2+}$ accumulation appears to be mediated by the voltage gated $Ca^{2+}$ channel, and 3) cold acclimation results in increased excitability and $Ca^{2+}$ currents at room temperature.

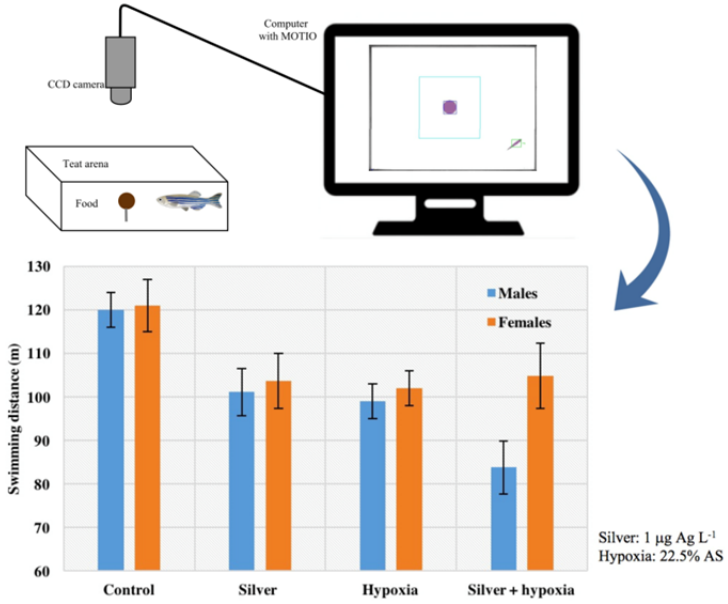


**Mie Cederdorff**
Supervisor(s): Erik Baatrup

# The effects of hypoxia and silver on feeding behaviour and spontaneous swimming activity of zebrafish (*Danio rerio*)

Hypoxia occurs both naturally and as a result of eutrophication in aquatic systems and is a global problem to aquatic life. Additionally, hypoxia often co-occurs with the presence of pollutants. Despite of this, little is known about the combined effects of hypoxia and contaminants on aquatic organisms. The objective of this study is to investigate the combined effects of hypoxia and silver on spontaneous swimming activity and feeding behaviour of zebrafish (*Danio rerio*). Silver is discharged to the aquatic environment from leaching, mining and other anthropogenic sources. It is traditionally incorporated in e.g. jewellery, coins, electronics, and photographic manufacturing. Furthermore, the antibacterial properties of both ionic silver and nanosilver has expanded the use of silver to being incorporated in products including clothing, plastic, paints, food packaging and household appliances such as refrigerators and washing machines. Silver is known to disturb the osmoregulation of the gills and cause respiratory stress.

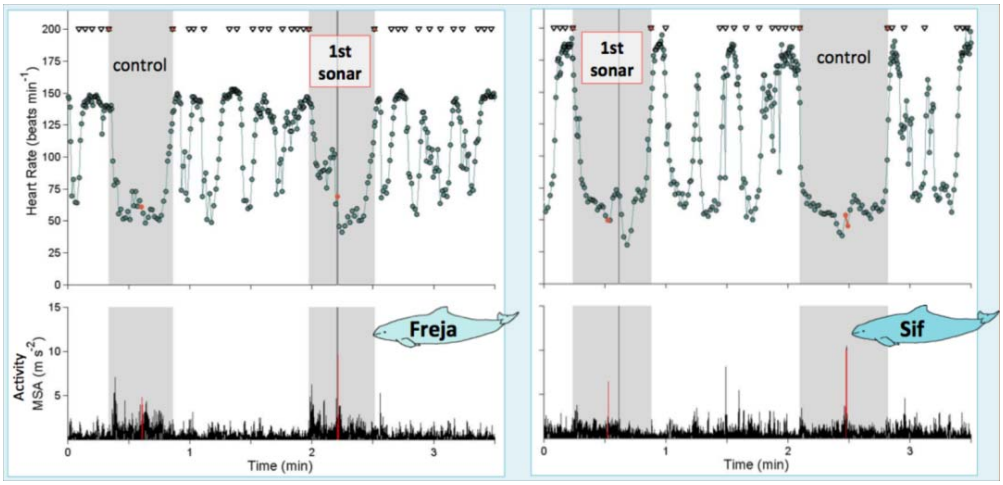
Behavioural effects are important, from an ecological point of view, because they have the potential to alter factors that are important for both short term survival of the individual and long term stability of the population. In this study, male and female zebrafish are exposed to either silver (nominal concentrations of 0.1, 1, or 10 µg $L^{-1}$) for two weeks, hypoxia (50.7 ± 0.5% or 22.5 or 0.3% air saturation, AS) for 20 hours, or the combination of silver and hypoxia. Subsequently, different behavioural parameters are quantified for 30 min using an automated tracking system.



**Siri L. Elmegaard**
Supervisor(s): Peter T. Madsen

## Cardiac responses of captive harbour porpoises to anthropogenic noise

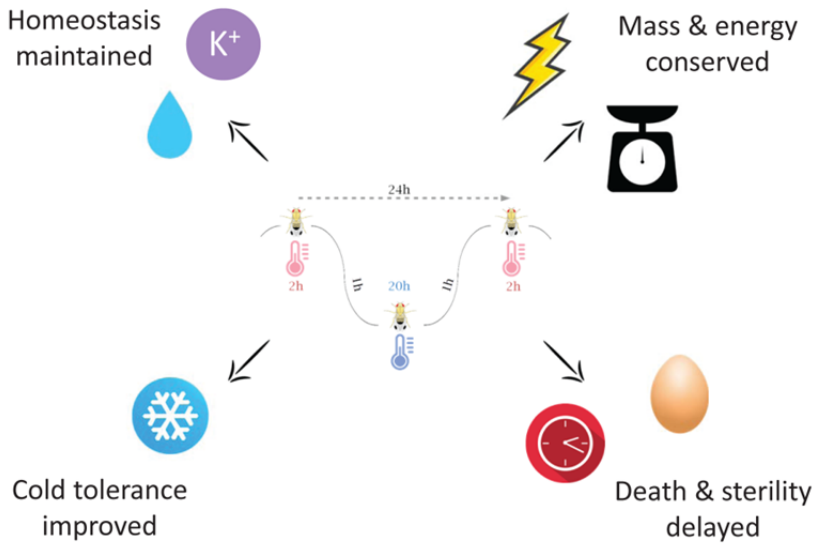
Increasing levels of anthropogenic noise in marine ecosystems may have negative effects on marine mammals, including behavioural or physiological responses impairing individual and population fitness. Mass strandings of beaked whales and porpoises have coincided with naval sonar exercises, and beaked whales display clear avoidance and alteration of diving behaviour caused by mid-frequency naval sonar. Unfavourable cardiovascular responses to such noise sources could impair gas management and recovery time at the surface of breath-hold divers; however, little is known about cetacean heart rate responses to acoustic disturbances. Many terrestrial animals increase heart rate and muscle blood flow when startled, but pinnipeds decrease heart rate to levels resembling their dive response. This decrease enables longer dives by conserving blood oxygen, which is critical if a sudden danger is perceived at depth. In this study we assessed the heart rate response to sonar and a sound designed to create a startle response in two captive harbour porpoises (*Phocoena phocoena*) during playback experiments. We measured heart activity (electrocardiogram) and fine scale behaviour (depth, acceleration, sound) using a small digital datalogger (ecg-dtag3) attached with suction cups. The non-invasive, quick attachment and small size of the tag allows the animals to move freely and conduct their normal or experimentally induced behaviour immediately after deployment. We found that the first sonar exposure (received level 155 dB re 1µPa (rms)) to the naïve animals caused a pronounced drop in heart rate (24 beats $min^{-1}$ and 38 beats $min^{-1}$). Neither subsequent sonar exposures nor startling sounds caused acute heart rate changes. Although our sample size is small, these results suggest that porpoises can respond physiologically to noise exposures, but that they may habituate quickly. The initial sonar-induced heart rate reduction suggests that these small odontocetes have adapted to improve stress handling time when submerged.



**Clayre Grumiaux**
Supervisor(s): Johannes Overgaard, Hervé Colinet

## Storing insects for biological control: fluctuating thermal regime ensures maintenance of physiological homeostasis and reproductive capacity in *Drosophila suzukii*

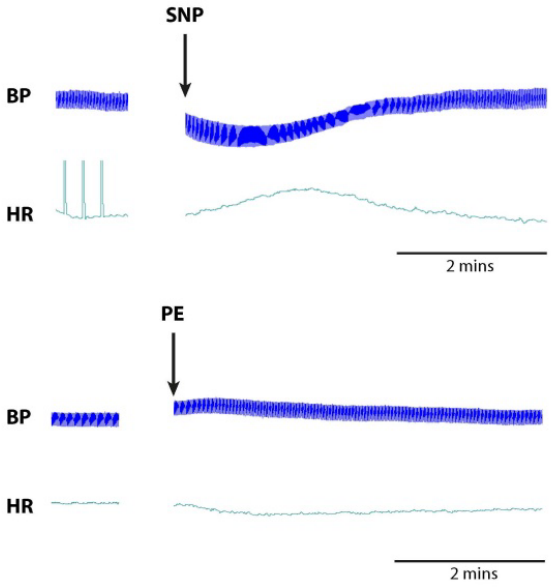
*Drosophila suzukii*, an invasive species recently introduced in Europe, lay their eggs in thin-skinned fruits and cause huge financial losses to fruit growers. One way to control this pest is the Sterile Insect Technique (SIT) where millions of sterile males are released to outcompete the natural reproductive males. Production of such quantities demands a huge stock of reproductive females available on demand. Unfortunately, *Drosophila* stocks die quickly of old age and loose fecundity when maintained at warm temperatures. On the other hand, they die from chill injury if maintained at constant low temperature. Here we investigate the potential of a new storage method using Fluctuating Thermal Regime (FTR) that harnesses the benefits of both warm and cold storage. Using a FTR regime with a daily warm period (3 hours at 25°C) and cold period (21 hours at 3°C) we compare ion, water and energy balances between FTR females and females exposed to constant 25°C and 3°C. Further we assessed chill-tolerance, as survival and fecundity rates to see if FTR was inducing chilling injuries or compromised reproduction. Flies maintained at 3°C quickly died from chill injuries as manifested from the loss of ion and water balance. In contrast, FTR flies maintained stable ion and water balance (similar to with 25°C flies) and also maintained mass and energy storage (lipid and glycogen content). When compared to 25°C flies, FTR flies had increased cold tolerance (faster chill coma recovery) and experienced much slower senescence as observed from tripling in lifespan and preserving fecundity.



**Lauren James**
Supervisor(s): Tobias Wang, Mads F. Bertelsen

## Cardiovascular control under anaesthesia in the ball python (*Python regius*)

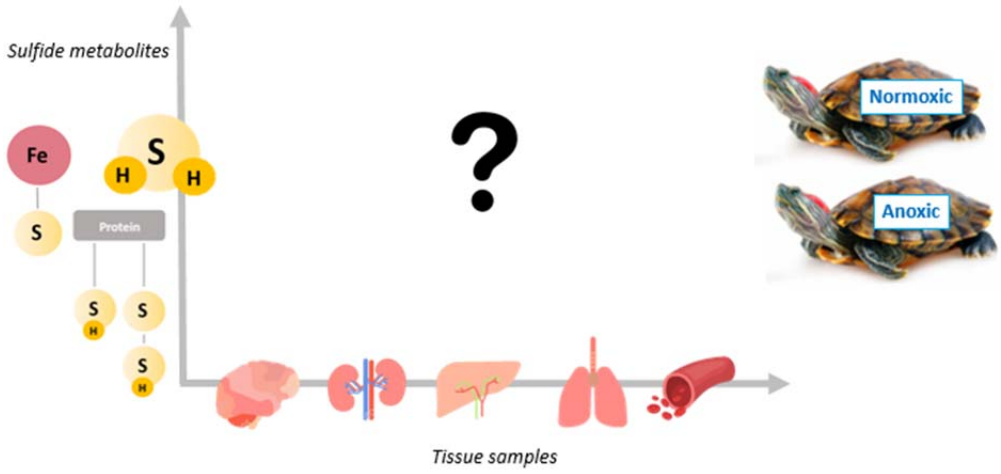
Vertebrates regulate arterial blood pressure, and exhibit reciprocal heart rate responses when blood pressure changes. This cardiac arm of the barostatic reflex depends on integration of various afferent inputs within the central nervous system and is therefore studied best in recovered animals. However, due to the invasive nature of some cardiovascular studies, there is considerable desire to develop protocols that allow this important reflex to be studied under anaesthesia. In addition, the ability to maintain an animal at baseline is highly desirable from a clinical perspective. In the present study, we characterised the heart rate responses to pharmacological manipulations of blood pressure using sodium nitroprusside and phenylephrine (the "Oxford Method") in ball pythons *(Python regius)* under several types of anaesthesia: pentobarbital, propofol, alfaxalone and inhaled isoflurane and compared them to the resting situation. Alfaxalone was the only anaesthetic that did not dampen or completely abolish the barostatic response, corroborating previous findings that minimal changes in heart rate and blood pressure occur that are not related to handling for injection. Propofol considerably dampened the response, with some animals exhibiting no baroreflex at al. Preliminary results suggest that pentobarbital abolishes the barostatic response and isoflurane partially dampens the response to phenylephrine, but not sodium nitroprusside. Our findings highlight the importance of carefully considering the anaesthetic protocols used when conducting physiological experiments; and suggest that alfaxalone may serve as a useful alternative to classically used agents, such as pentobarbital and isoflurane.




**Birgitte Jensen**
Supervisor(s): Angela Fago

## Hydrogen sulfide metabolites in tissue samples of anoxia tolerant freshwater turtles (*Trachemys scripta*)

Hydrogen sulfide ($H_2S$) is a newly discovered signaling molecule with profound physiological effects. Low concentrations of exogenous free $H_2S$ can reversibly reduce metabolic rate. In hibernating bears where metabolism is drastically reduced, endogenous changes in $H_2S$ metabolites have been observed, suggesting that sulfide signaling is part of a hypometabolic state. A hypometabolic state is pivotal for hibernating animals, yet the freshwater turtles are unique, as the metabolic rate of a hibernating turtle at its usual hibernating temperature is over 10 000 times lower than that of a similarly sized mammal resting at its normal body temperature. Furthermore, these turtles can survive for several months at the bottom of a frozen lake in complete absence of oxygen without sustaining any tissue damage. We hypothesize that sulfide remodeling must play a profound role in these turtles, contributing to molecular adaptions during winter hibernation. Red-eared sliders (*Trachemys scripta*) were acclimated to low temperature and to either normoxic or anoxic conditions. Tissue samples, including liver, brain, lung, kidney, and blood, were harvested and sulfide metabolites (free sulfide, bound sulfane sulfur and acid-labile sulfide) were measured with a fluorescent monobromobimane assay coupled with reverse-phase high-performance liquid chromatography (RP-HPLC). To encounter for enzymatically produced sulfide, an enzyme assay will be performed to compare the activity of the three main sulfide-producing enzymes. Preliminary results of the non-acclimated turtles showed high concentrations of free sulfide in the plasma corresponding to 0.9±0.1 µM (n = 4), whereas acclimated samples are yet to be finalized. We expect the metabolites of sulfide to change during anoxia acclimation reflecting the change in metabolic rate. Beside metabolic depression, sulfide contributes to an array of physiological functions ranging from neuroprotection to vasoconstriction, implying that sulfide concentrations between different tissues will vary as well.



**Christian Jensen**
Supervisor(s): Mark Bayley

## Vasomotor responses in the branchial and systemic vasculature by treatment with serotonin in the iridescent shark catfish, *Pangasianodon hypophthalmus*

Vasomotor responses mediated by serotonin have been described in several teleosts. One of these is the hypoxia mediated granulation of serotonin-containing neuro-epithelial cells in the gill filaments. This is thought to decrease functional surface area by stopping the distal filamental artery blood flow in concert with a vasoconstriction of the filamental arteries. The decrease in functional surface area is advantageous during severe hypoxia as loss of oxygen from the blood to the surrounding water is minimized. Furthermore, the reduced functional surface area also serves to maintain water and ion balance.

As the facultative air-breather, *P. hypophthalmus*, often experiences hypoxia and is able to extract oxygen from the air, an ability to decrease of functional surface area would be beneficial. I will investigate the role of serotonin in the branchial vasculature through isolated perfused gill arch preparations. As the 5-HT receptors responsible for the vasomotor responses in other teleosts have proved to be 5-$HT_2$ receptors, I will use the specific 5-$HT_2$-antagonist Ritanserine to verify 5-$HT_2$ mediated responses in *P. hypophthalmus*. Furthermore, I will attempt to visualize the change in blood flow by perfusion with a dye which does not exit the circulation or by using an epi-illumination microscope as described by Sundin et al., 1995. Additionally, the branchial arches drain into two different arteries in *P. hypophthalmus*. The 1$^{st}$ and 2$^{nd}$ arches drain into the dorsal aorta (DA), while the 3$^{rd}$ and 4$^{th}$ drain into the coeliaco-mesenteric artery (CMA). The DA supplies the systemic tissues while the CMA supplies the air-breathing organ. A shunt between the DA and CMA has previously been observed. Based on these observations, the serotonin-mediated vasomotor responses of several parts of the circulation will be investigated through myography.

Aarhus University
April 2018

**Malene Brøgger Jensen**
Supervisor(s): Tobias Wang

## Effect of hypothyroidism on glucose tolerance, heart rate and arterial blood pressure in *Python molurus*

Thyroid hormones (THs) are essential for development, growth and serves as the main regulators of the metabolic rate in vertebrates. Thyroid deficiency (hypothyroidism) is known to cause a decrease in body temperature, heart rate and metabolism in mammals. However, while the effect and importance of THs are well established for mammals, the regulatory effects of the of THs in reptiles are poorly investigated. A previous study has shown that thyroid deficiency in *Python regius* caused an elevated metabolic rate, which resulted in a prolonged specific dynamic action. To further investigate the possible effects of THs, this study examines heart rate, arterial blood pressure and glucose tolerance in euthyroid- and hypothyroid snakes (*Python molurus*) during fasting and digestion. A hypothyroid state was achieved by thyroidectomy, which led to a decrease in circulating THs. Snakes were equipped with an arterial catheter for the collection of blood samples for the glucose tolerance test which simultaneously allowed measurements of heart rate and blood pressure. For the glucose tolerance test, snakes were administered a bout of glucose (1g/kg) through the catheter which caused an immediate rise in plasma glucose concentration. The ability to clear of glucose from the bloodstream was then measured over a period of 24h. Initial results from this study shows no significant change in heart rate and arterial pressure between the hypothyroid and control group during digestion but fasting hypothyroid snakes shows a tendency to have a lowered heart rate compared to the euthyroid group. Furthermore, THs seem to have a small effect on the glucose clearance rate, as the plasma glucose concentration of hypothyroid snakes have slower clearance rate after administration of glucose compared with the control group.

## Sofie Skafsgård Jeppesen
Supervisor(s): Mark Bayley

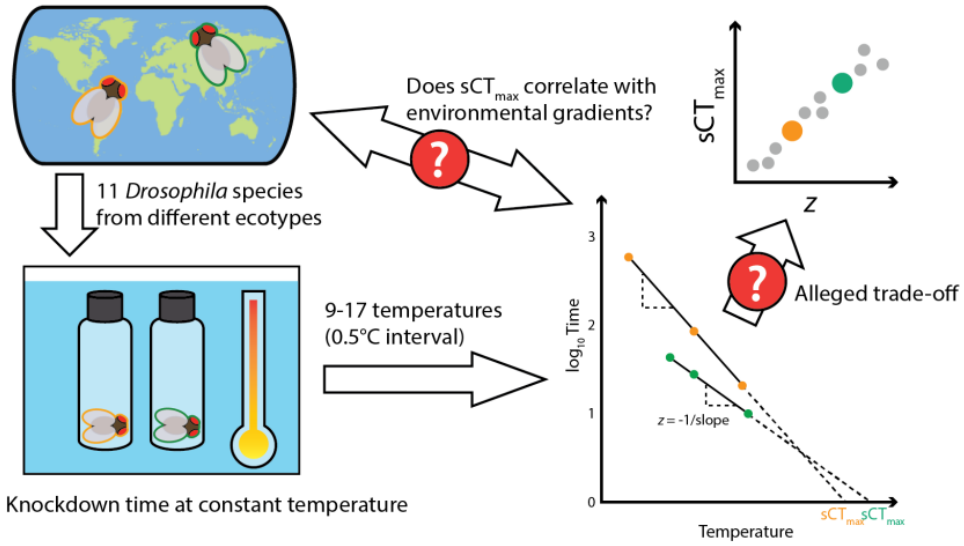
### Stress behaviour in pangasius (*Pangasianodon hypophthalmus*) during perceived risk of predation: the influence of catecholamines on behaviour

It has been shown that the air-breathing *Pangasianodon hypophthalmus* can suppress its ventilatory reflex to air-breathe during a perceived risk of predation. Thus, the appearance of a stuffed heron caused pangasius to double its air-breathing interval from 5.25 minutes to 10.09 minutes, in hypoxic water. Myography studies have shown that the branch of the celiac mesenteric artery supplying the air-breathing organ contracts strongly under elevated catecholamines. The studies in my master's thesis will examine the connection between perceived risk of predation and the physiological aspects of the stress response by performing the simulated predation exposure on animals with in-dwelling catheters to allow measurement of circulating catecholamines, glucose and cortisol. In addition, these catheters will allow the reverse causality to be addressed by examining the behavioural response following injection of a catechol bolus into the dorsal aorta. The perceive risk of predation is being illustrated by an observer with a fishing net trying to catch and irritate the fish without conflicting with the catheter. Air-breathing and behaviour will be recorded by a camera and blood samples will be taken before, during and after the disturbance.

**Lisa Bjerregaard Jørgensen**
Supervisor(s): Johannes Overgaard

## How to measure insect heat tolerance: unifying static and dynamic assays

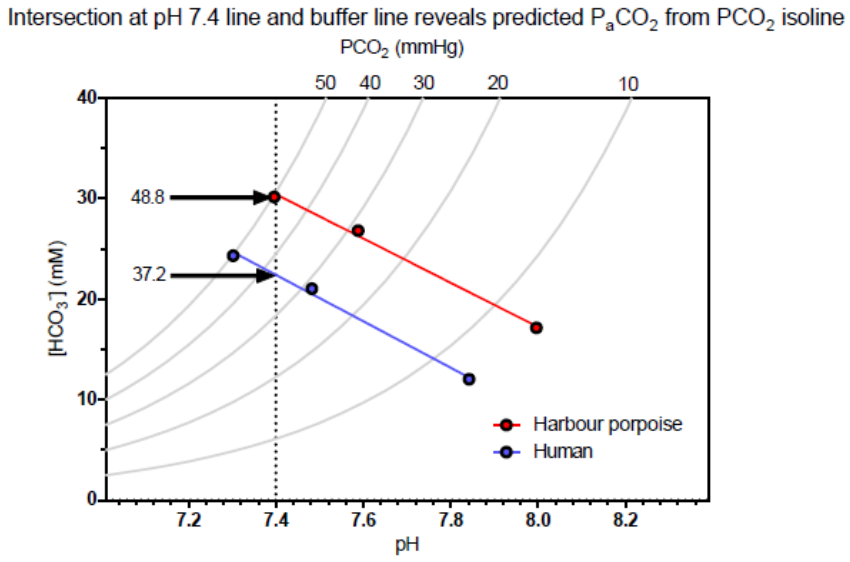
Temperature is one of the most important determinants of species distribution and climate change will likely affect future distributions of many species. Prediction of such distributional changes calls for simple and comparable measures of heat tolerance that correlate with species performance in their natural environments. A recent model (thermal tolerance landscapes – TTLs) uses the exponential relation between temperature and knockdown time to describe the thermal tolerance of ectotherms in different time/temperature intervals. TTLs therefore allow for parametrisation of the complex interaction between absolute temperature (i.e. heat stress intensity) and duration of heat exposure across a range of stressful temperatures. Previous analyses of TTLs have reported an apparent trade-off between tolerance to acute and chronic heat stress in ectotherms. However, this trade-off may represent an inherent property of the model, rather than a true biological phenomenon. To test the "ecological applicability" of TTLs and examine the apparent trade-off, we measured knockdown time at 9-17 static temperatures (0.5°C intervals) to establish TTLs for 11 species of *Drosophila* representing different thermal ecotypes. Additionally, we measured knockdown temperature during three dynamic assays (heating flies with different ramp rates). With these data we show that static and dynamic assays give comparable information on heat tolerance. We also show that both dynamic and static measures of heat tolerance correlate tightly with the environmental characteristics encountered by the 11 species. Finally, our data clearly demonstrate that trade-offs between chronic and acute tolerance are absent within and between species when the data is analysed using curve interpolation.



**Niels Kristensen**
Supervisor(s): Hans Malte, Peter T. Madsen, Tobias Wang

## The acid-base physiology of marine mammals

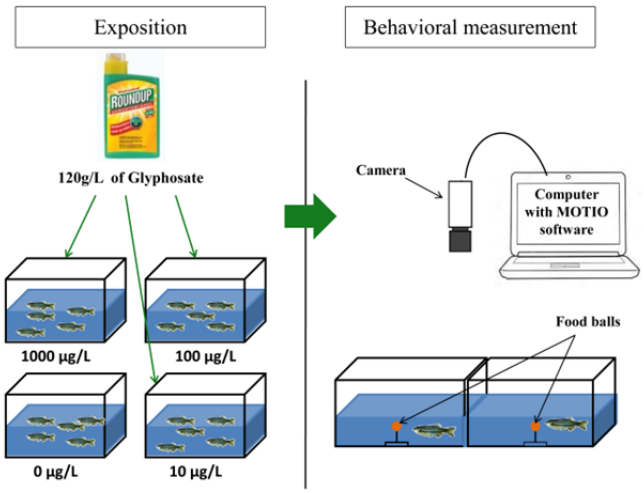
The diving lifestyles of marine mammals complicate the regulation of their arterial partial pressure of $CO_2$ ($P_aCO_2$), which in turn could affect the acid-base status of these animals. Previous studies have estimated the $P_aCO_2$ primarily using the End-Tidal $PCO_2$ as an end-point, but the results do not agree whether this group of mammals displays similar or higher values of $P_aCO_2$ compared to terrestrial mammals. The current study uses another approach in which the relation of the three acid-base variables pH, $[HCO_3^-]$ and $PCO_2$ of the blood is determined. This allows for the calculation of the $P_aCO_2$ under the assumption of an arterial pH value of 7.4 and enables drawing of the blood $CO_2$ dissociation curve and calculation of the blood buffering capacity. Animals to be included are three cetacean species, harbour porpoise (*Phocoena phocoena*), beluga whale (*Delphinapterus leucas*), Bottlenose dolphin (*Tursiops truncatus*) and two pinnipeds species, south American sea lion (*Otaria flavescens*) and Walrus (*Odobenus rosmarus*). Preliminary results from a harbour porpoise revealed an increased $P_aCO_2$ of (48.8 mmHg) compared to human control (37.2 mmHg). To avoid low blood pH values the higher $P_aCO_2$ was shown to be compensated by an increased bicarbonate concentration $[HCO_3^-]$. Calculation of the true plasma non-bicarbonate buffer value ($\beta_{NB}$) showed how the buffering capacity of the harbour porpoise ($\beta_{NB}$=22.0 slykes) was similar to that of a human ($\beta_{NB}$=23.3), suggesting that the metabolically compensated increase in blood $[HCO_3^-]$ is the primary strategy of avoiding low blood pH. Thus preliminary results of this study support the findings of previous studies showing an increase in the $P_aCO_2$ of marine mammals compared to terrestrial mammals. These findings could be of great use to strategies used in the calculation of the metabolic rate of marine mammals, since these uses the $P_aCO_2$ to calculate the lung $O_2$ extraction efficiency.



**Mathilde Liégeois**
Supervisor(s): Erik Baatrup

## Effect of Round-up on the swimming and foraging behaviors of zebrafish

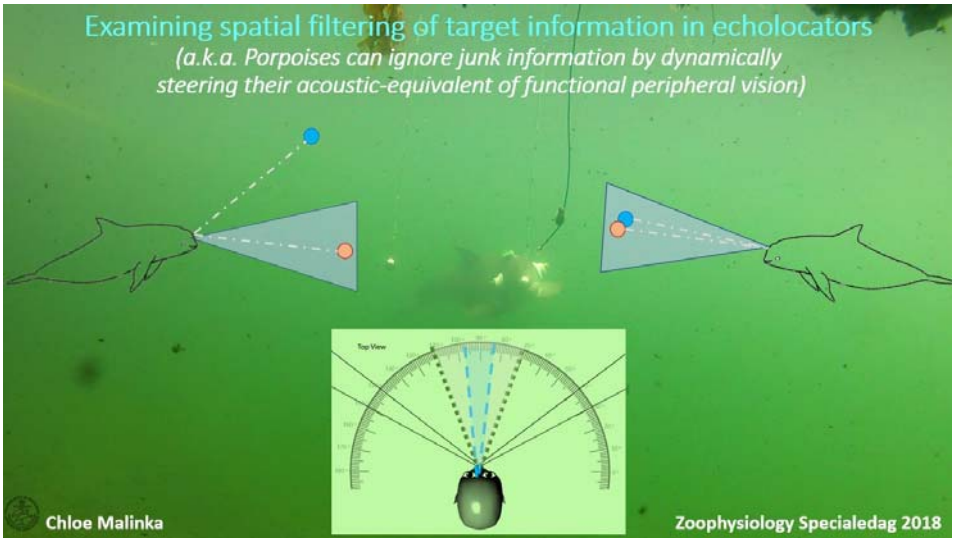
Glyphosate has become the most widely used herbicide in the world, due to the increase of glyphosate resistant crops in agricultural areas after its introduction in 1996. Whilst Glyphosate can be used alone, this compound is commonly applied as an active ingredient of the herbicide Roundup®. This pesticide contains several adjuvants, such as the isopropylamine salt, which potentially promote an unknown toxicity. Its application poses numerous problems, both for the health of the applicators and food consumers as well as for the environment by contaminating the soil, water and leading to the death of plants and animals. Therefore, Round-Up exhibits greater side effects than glyphosate alone and its wide application use makes necessary to assess its impacts on non-target organisms. It is important to understand not only the lethal but also the sublethal effects of xenobiotics like the behavioral effects. Studies with other pesticides have already highlighted that behavioral changes in zebrafish are suitable markers in order to evaluate toxicological mechanisms triggered by exposure to these agents. Thus, the aim of this study is to evaluate the effects of exposure to Roundup® on behavioral parameters on adult zebrafish. To this end, Zebrafish adults were exposed for 7 days to Roundup® to obtain glyphosate concentrations of 0, 10, 100 and 1000 µg $L^{-1}$ in the exposure tanks. Immediately after the exposure, the foraging behavior experiments were performed on one female and one male simultaneously with a camera recording during 30 minutes. The camera was interfaced with a computer with the MOTIO vision system software which was controlling the measurements. The male and female swimming and foraging behaviors were evaluated on the basis of different parameters. Further experiments will be done with other concentrations to target more accurately the effective concentration.



**Chloe E. Malinka**
Supervisor(s): Peter T. Madsen

## Using acoustic recording tags to examine spatial filtering of target information in echolocators

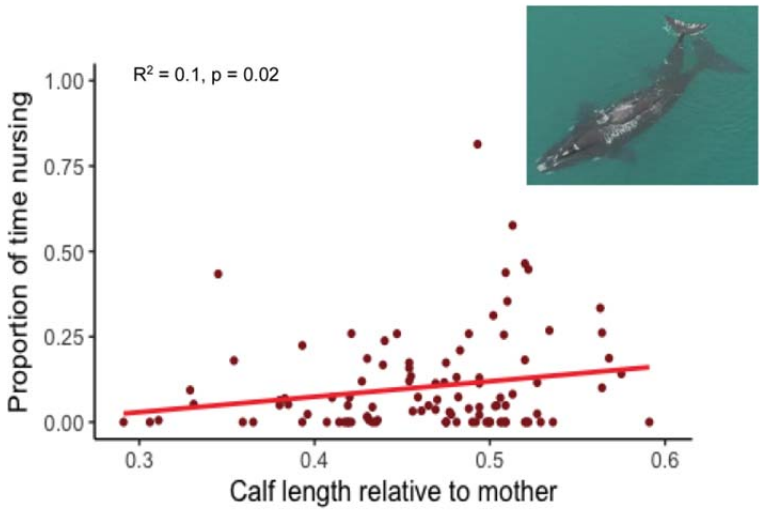
Toothed whales produce narrow forward-directed biosonar beams and appear to be able to dynamically adjust beamwidth as they home in on a target. High beam directionality during search-phase echolocation reduces acoustic clutter, and the widening of the beam during final phases of prey capture allows for keeping fast-moving, evasive prey within the field of view. Biosonar beamwidths are conventionally quantified by their half-power width but this arbitrary bound may not be biologically relevant: Toothed whales steer their acoustic gaze to include and exclude information, but it is unknown what the functional beamwidth is, i.e., how far off-axis can information still be collected. Additionally, we do not know if echolocators modify their beamwidth when multiple targets are present. This study sought to understand how echolocators spatially filter their echoic scene to modulate information flow. Harbour porpoises (*Phocoena phocoena*) at Fjord&Bælt were trained to close in on simultaneously presented spherical targets while performing a two-alternative forced-choice task. Distances between the targets varied (13.5-108 cm) and were presented at varying orientation orders. The free-swimming porpoises wore eyecups and were tagged with a sound and movement tag (dtag4) to record their echoic scene. The known ranges between targets and the porpoise, combined with the sound levels received on the target-mounted hydrophones revealed how the porpoises controlled their acoustic field of view. When targets were close together and the discrimination task was more difficult since the echo ratio between targets was low, buzzes were longer and started from farther away, and source levels were greater. Porpoises were found to maintain the buzz phase when switching attention to the other target. Here we show that the animals need to be closer to targets to discriminate between them when the targets are spaced closely together, revealing that the narrow beam of echolocators serve as a spatial filter.



**Mia L. K. Nielsen**
Supervisor(s): Peter T. Madsen

## Nursing behaviour of young southern right whale calves

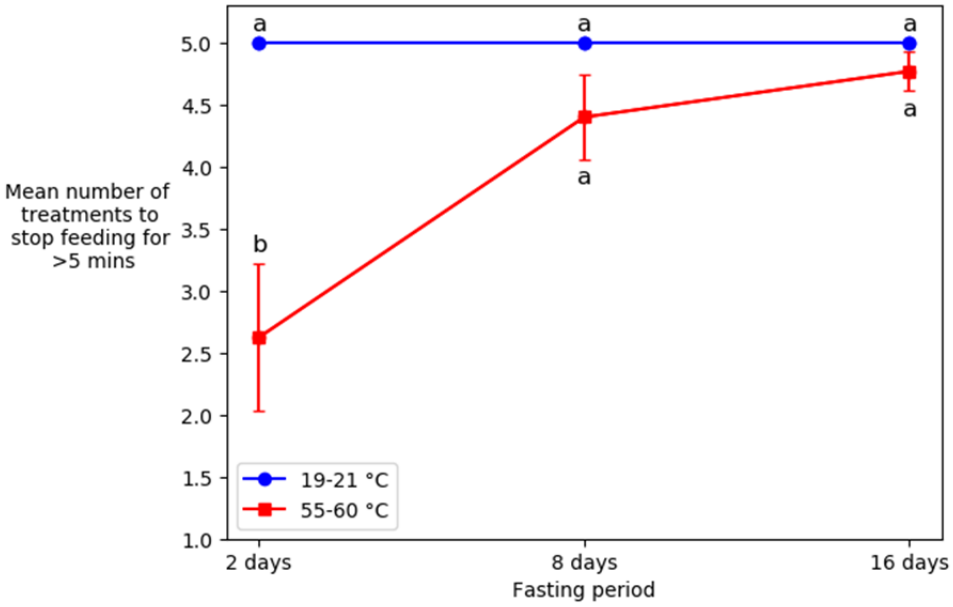
During the early stages of development, mammals are reliant on their mothers, and the maternal care provided during this period is critical for offspring survival. For baleen whales, that generally reside in shallow, coastal waters during the first months of nursing their calves, it is largely unknown how important behaviours associated with maternal care are allocated at these nursing grounds. Here, we test if mother-calf pairs with neonate calves spend more time engaged in nursing and back-riding compared to mother-calf pairs with older calves, as these behaviours have been described as having the greatest energetic costs for the fasting mother. To test this, we compared the proportion of time observed nursing and back-riding of 51 southern right whale mother-calf pairs during the breeding season at a sheltered nursing ground in South Australia. Behavioural observations from the air were performed using a DJI phantom 3 Pro. Body morphometric measurements of the 51 mother-calf pairs were obtained using an Inspire 1, which was used to categorize the age classes of the calves. We show that back-riding was a more pronounced activity for the smallest compared to the biggest calves, while nursing was more pronounced for the biggest calves, suggesting that the biggest calves utilize their increased breath-hold ability to maximize their lipid stores before the migration to the feeding grounds. Further, we provide estimates of frequency and occurrence of these crucial behaviours related to maternal care in a healthy, undisturbed population that is showing strong recovery from whaling. These baseline estimates are important to identify possible disruptions of crucial nursing behaviours, and in the management of human activities in known nursing areas of this and related species.



**Jon Poulsen**
Supervisor(s): Tobias Wang

## Do crabs feel pain? Shore crabs display a motivational trade-off in response to noxious stimulation, but poor learning ability

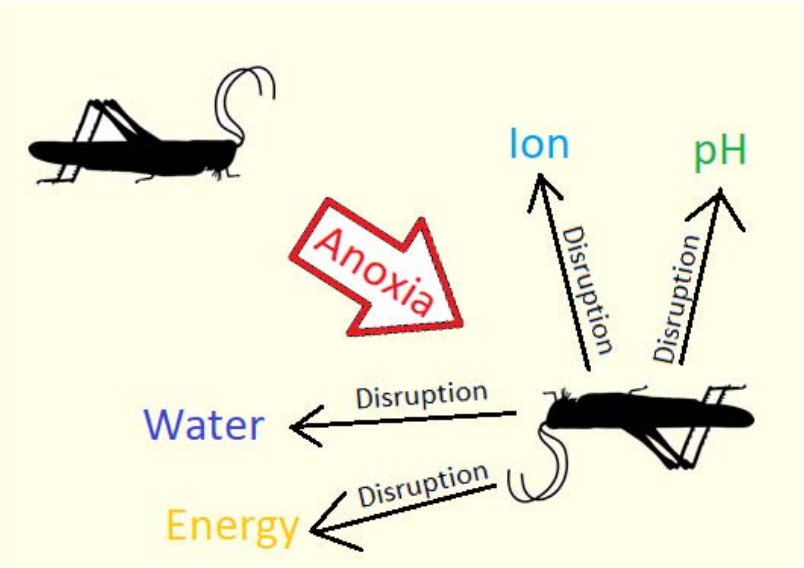
The capacity for non-human animals to experience pain is a controversial topic of critical importance with respect to animal welfare and the interpretation of behaviours such as defensive responses and risk-taking. Published studies indicate that decapod crustaceans are capable of motivational trade-off decisions and learning in response to noxious stimuli. However, both abilities also appear quite limited which makes it hard to draw a firm conclusion on the involvement of pain. I have conducted behavioural experiments on shore crabs (*Carcinus maenas*) to better assess the extent of these abilities. In one experiment, crabs were fasted for 2, 8 or 16 days before being offered a meal of blue mussel. The crabs received a spray of hot water (1 mL, 55-60 ℃) on the claw every time they attempted to feed. There was a clear influence of the previous fasting period, such that crabs having fasted for 2 days were easily deterred from feeding, while those that had fasted for 8 or 16 days required more heat applications before abandoning the meal. Learning ability was tested in a different experiment in which crabs were punished for moving into either a decorated or barren corner of the test area. The crabs failed to make an association after 6 trials in rapid succession. In conclusion, noxious heat influenced motivation to feed in the crabs but did not lead to associative learning.

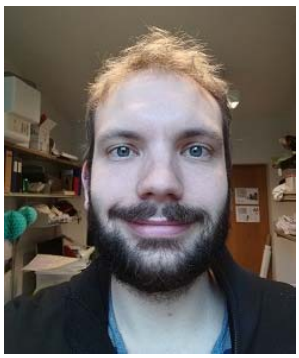


**Mathias Ravn**
Supervisor(s): Johannes Overgaard

## Why do insects die from hypoxia?

Most animals rely on aerobic ATP production and thus depend on oxygen availability to survive, but insects generally have considerable anoxic tolerances. When exposed to anoxia, most insects rapidly go into a defensive hypometabolic coma from which they can recover when exposed to normoxia again. However, prolonged anoxic bouts result increased recovery times and in cell damage and death, and any insect, like most animals, will eventually face death. During anoxic coma in insects, body homeostasis is lost including energy and ion homeostasis and possibly water and acid-base balance as well. However, it is not known how fast and to what degree each of the factors are disrupted compared to each other or how quickly they are recover, and it has not been investigated which disruptions are the primary source of tissue damage. Here we show in the migratory locust (*Locusta migratoria*) that prolonged anoxic exposures are associated with increased recovery time, decreased survival, and rapidly disrupted ion, energy, acid-base, and water homeostasis, and we show which factors are the primary cause of anoxic cell injury. Locusts could not fully recover after 4 hours of anoxia at 30 °C, and at this point hemolymph [$K^+$] and [$Na^+$] had elevated 5-fold and 2-fold respectively, muscle tissue [ATP] had decreased to ≤1% of normoxic values, hemolymph pH had dropped 0.8 units from 7.3 to 6.5, and hemolymph water content was halved. Furthermore, the isolated and combined effects of anoxia, high [$K^+$] and lowered pH on cell injury *in vitro* is discussed. These results suggest what the most damaging physiological factors are for insects during anoxia, and thus why insects ultimately die from anoxia.

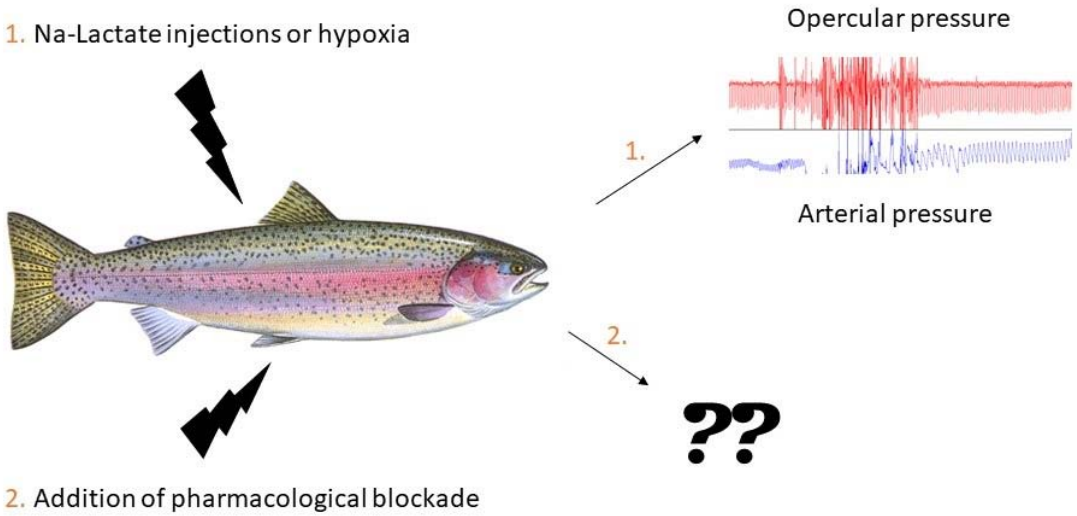




**Mikkel T. Thomsen**
Supervisor(s): Mark Bayley

## The transduction of the lactate ventilator response

Lactate – the metabolic end product of anaerobic metabolism – is currently emerging as an important signalling molecule in multiple biochemical and physiological processes. Several functions for the lactate ion have now been documented, including a stimulation of ventilation in mammals by direct interaction with a carotid body receptor. Following this discovery, we demonstrated the presence of a similar response in two teleosts (*Pangasianodon hypophthalmus* and *Oncorhynchus mykiss*) by intra-arterial injections of Na-Lactate under constant arterial $PO_2$ and pH. Further, we showed an attenuation of the ventilatory response by removal of afferent input from the first gill arch, one of the main sites of hypoxia sensing and we identified the expression of a putative lactate receptor in gill tissue, likely homologous to the mammalian lactate receptor. Collectively, the current data suggest that the lactate ion serves as an important modulator of ventilation and that the mechanism is conserved within vertebrates. Currently, I am conduction a pharmacological investigation of the lactate response. By blocking of specific receptors and comparing the ventilatory responses to hypoxia and lactate, the aim is to determine whether the same sensory pathway is used, which will aid in establishing the transduction mechanism underlying the response. Preliminary data from this study suggest that the lactate mediated ventilatory response can be eliminated by blocking of a serotonin receptor. This provides indirect evidence of the likely connection to the serotonergic neuroepithelial cells (NEC), which are the putative chemosensory cells for sensing of respiratory gases in the gills.



**Christian Ørskov**
Supervisor(s): Johannes Overgaard

## A new method to study behavioral thermoregulation in locusts

Locusts, like *Locusta migratoria* are capable of regulating their body temperature when necessary by seeking microclimates with temperatures that suit their needs. This behavioural thermoregulation is the first line of defence against temperature changes is the immediate surroundings of the insects, and has been extensively studied in different locust species. However, most studies have been conducted during the daytime hours using thermocouples, a very invasive method, that limits the possibility of continuous data-logging. Here, the advancing field of insect telemetry is considered. The aim of this study was to establish a linear correlation between the method of thermocouples and the, scarcely used, telemetry system in a thermal gradient, and, using telemetry, replicate existing data obtained with thermocouples This would present a viable alternative to thermocouples in field-studies of thermoregulatory behaviour in large insects, one that is less invasive and better able to log data continuously, even during the night where the tracking of minute insects historically has been difficult.