Suggested project for the home exam in MBV4330/9330

Project in Field work:

Climate changes affect the marine ecosystem and can lead to local changes in the composition and distribution of species in the food chain. Seabirds are top consumers in marine foodwebs and are good indicators of changes in physical parameters of the marine ecosystem. Their breeding success is ofter well correlated with factors such as food availability and climate. Seabirds breeding in the arctic often raise fewer chicks than birds at more southern breeding sites indicating aditional challenges for birds in the arctic. In the present study we want to address whether seabirds are working on the edge of their metabolic capacity to raise chicks. If so, climate changes that decrease food availability may have detrimental effects on the breeding success of birds breeding in the arctic. We wil use black-legged kittiwakes (Rissa tridactyla) as our research animal. The kittiwake is a sea-gull with a circumpolar distribution ranging from breeding colonies in southern Europe to Northern Norway and Sptisbergen. At their northermost breeding sites at Sptisbergen, the number of chicks is less (1-2 chick) on average compared to breeding colonies in Northern Norway (2-3 chick) indicating that birds at Spitsbergen may be vulnarable to negative changes in local food availability. We will measure energy expenditure among breeding kittiwakes at two breeding sites Kongsfjorden (Spitsbergen) and Hornøya (Northern Norway) when their chicks are 10-20 days old. At this time the parents need to cover their own as well as the increasing energy needs of the chicks in the nest. We will use the doubly-labelled water technique which is an effiecient and precise way of measuring the animal's energy expenditure during 24 hours. The birds will be captured on their nest using a rod with a snare at the end. The birds will be weighed, ring marked (metal rings from Stavanger Museum) and beak and tarsus measured. One ml of doubly-labelled water ($^{3}H_{2}{}^{18}O$) will be injected into the bird's pectoralis. A blood sample (using a syringe needle) will be taken from the foot vein before it is released. After 24 hours the bird will be re-captured on the nest and a new blood sample will be taken. The difference in the amount of doubly-labelled water between the initial and last blood sample is a measure of energy expenditure. In order to limit the stress on the breeding pair only one of the parents will be used in the procedure. The behaviour of both parents and birds in neirghbouring nests (control nests) will be monitored before, during and after the experiment. The chicks in the nests will be weighed at regular intervals to estimate growth curves from experimental nests and control nests. The behaviour of the parents, weight development and growth curves of the chicks are measures of effects of the procedure on the birds. The procedure will be performed at the two breeding locations. The data from this study will be of great importance for estimating the possible effects of climate changes on marine food chains and fisheries.

2. Project in fish:

Does hypoxia induce adaptational changes in crucian carp gills in response to sesonal variations of oxygen levels?

In vertebrates, the need for gas exchange forces the animal to expose its interior milieu to the external world. For animals breathing with lungs, the large respiratory surface area can be problematic with respect to infection, heat and water loss. Fish respirating in seawater lose water over the gills to the hyperosmotic environment, while freshwater fish accumulate water and lose salts owing to the gradients between the animal and the environment. Large water uptake results in high rates of dilute urine excretion, whereas ion losses must be compensated for by energetically costly ion pumping. The structure of the respiratory organ is therefore likely to be a compromise between the need for gas exchange and a desire to minimise ion and water fluxes. The crucian carp (*Carassius carassius*) is a North European freshwater fish that often inhabits small ponds that, due to ice coverage, become hypoxic and finally anoxic for several months every winter. Its exceptional hypoxia and anoxia tolerance make it the sole fish species in this habitat. In both Norwegian and Swedish populations of this carp, we have observed a lack of protruding lamellae, the respiratory units in fish (sometimes denoted secondary lamellae). This is a highly exceptional feature in fish since the lamellae are the primary site for gas exchange, making up most of the respiratory surface area of fish gills. However, a favourable aspect of a small gill area would be reduced water influx and ion losses.
The hemoglobin of *Carassius* has an extremely high affinity for O_2; a P_{50} for O_2 of 0.347 kPa has been measured in goldfish (*Carassius auratus*; Burggren, 1982). This may explain why, under normoxic conditions, crucian carp do not need a large respiratory surface area. Thus, the lack of protruding lamellae could be a morphological adaptation for reducing water and ion fluxes. However, such an unusual trait should limit the ability of the crucian carp to cope with falling environmental O_2 conditions, unless relatively rapid morphological changes can take place.

Here we ask whether the gills of the crucian carp undergo morphological changes in resonse to severe hypoxia. Crucian carp will be caught in June in the Tjernsrud pond, Oslo and kept in tanks at Department of Molecular Biosciences. The fish will be fed daily and continuosly supplied with aerated water. During hypoxia exposure, the fish will be kept in dark 25-litre plastic tanks continuously supplied with N_2-bubbled dechlorinated Oslo tap water. The oxygen level will be monitored with an oxygen electrode (WTW, Weilheim, Germany). The water temperature will be kept at 8°C. The fish will be exposed to hypoxia for up to 14 days. Fish will be taken out for sampling at 0, 1, 3, 7 and 14 days of exposure. The O_2 level will be kept at 0.75±0.15 mg l^{-1} (6-8% of air saturation). This is slightly below their critical O_2 concentration ($[O_2]_{crit}$), which is the level of O_2 where the fish can no longer meet their energy requirements through aerobic metabolism alone. A group of fish will be put back into normoxia for 7 days after 14 days of hypoxia exposure and will then be sampled. Control fish (and reoxygenated fish) will be kept in an identical tank, which will be supplied with aerated water rather than N_2-bubbled water. The hypoxia experiments will be done from March to October. Gills will be fixed in 3% glutaraldehyde in 0.1 mol l^{-1} Nacacodylate buffer. The lamellae of the gills sampled at different time points from the respective groups will be measured using a light microscope. Metabolic rate will be measured as rate of oxygen consumption using closed respirometry. Groups of fish will be kept in normoxia (with embedded lamellae) and compared with fish exposed to hypoxia (with protruding lamellae). The latter group will be exposed to hypoxia for 7 days and then kept in normoxic water for 24 h to minimise the possibility that the fish suffer from hypoxia-induced energy deficiency,

even if this is unlikely for crucian carp. The rate of O_2 consumption ($\dot{V}_{O2}$) during falling water [O_2] will be determined.

The outcome of these experiments will reveal whether an increased respiratory surface area due to lamellae has a significant effect on water and ion fluxes and may reveal increased costs of the ion pumping needed to maintain homeostasis.

3. Project in mammals:

The aim is to research the bacterial toxin lipopolysaccharide (LPS) in a rodent model of sepsis. LPS will concentration-dependently lead to organ failure and if given in a large enough dose, death.

Your research target is to study the downstream signalling of organ injury, with the perspective of developing a therapy in the treatment of sepsis.

For you to decide: What are the end-points; organ function, death of animals, a serum marker of inflammation, activation of inflammatory cells? Any other? You will want to do tissue analysis to study signalling downstream to LPS. Which tissues will you collect, how will you analyse them? Do you want to do gain-of function or loss-of function studies; if so, pharmacology or genetically engineered mice? Which mice? Has a GMO been sent to the Norwegian authorities on the mice? Which positive and negative controls are needed? Do you plan a dose-response study?