Experiment with round gobies

Student learning outcome

To understand how scientists use knowledge about cell respiration, membrane transport and physiology to construct experiments, that will provide information needed in nature management. The document can work as a report, where you fill in the results and write the discussion.

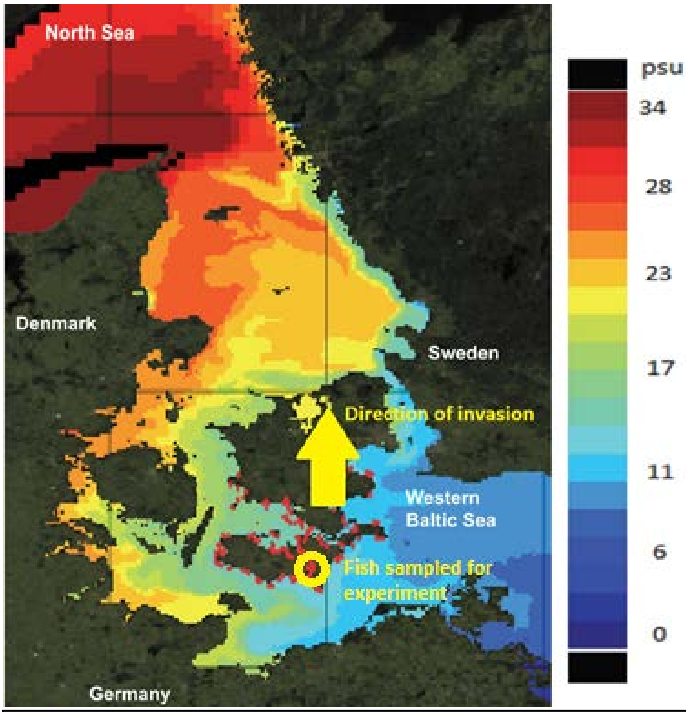
Purpose of the experiment

To evaluate the dispersal potential of an invasive fish.

Introduction

Non-indigenous species are defined as invasive when they cause adverse effects on more than one of the following three parameters, which include 1) the local environment, 2) local economies, or 3) human health. Predicting the dispersal potential of an invasive species is therefore a key tool for managers when they evaluate what would be the appropriate management action.

In this experiment the dispersal potential for the round goby was evaluated. The round goby is invasive in the Western Baltic Sea, where it is changing the structure of the ecosystem with severe impact for particular fisheries. As it appears to be dispersing rapidly, it is crucial for managers to understand when and where this invasion is going to stop. The latest published map showing the extent of the "invasion" is from 2013 and shown to the right. After the publication of the map round gobies have been found even further north. The round goby thrives in fresh and brackish water, but so far no round gobies have been observed in high-saline oceanic waters. It is there for hypothesized, that its dispersal will be limited by the higher salinities in the North-Western part of the Baltic Sea in the area around Denmark (see map). To test this hypothesis an experiment was carried out which focused on the round goby's tolerance for water of different salinities. The tolerance was evaluated by measuring 1) the percentage of gobies able to survive at different salinities, and



Map showing the Western Baltic Sea and the waters around Denmark. Red dots indicate places where round gobies had been identified in 2013. The round goby has is currently expanding into areas of still higher salinities; from the most western part of the Baltic Sea northward along the coastline at a rate of approximate 30 km year^{-1} .

The colors in the sea indicate the different salinities in Danish waters between 0-5m depth. PSU stands for Practical Salinity Units where the listed numbers translate into "per thousandths". The color yellow therefore represent a salinity of approximately 20, meaning that 20/1000 or 2% of a water sample here will be salt, and most of it sodium chloride. (Modified map showing direction and sample area from Behrens et al. 2017)

2) changes in oxygen consumption, which is an indicator of the costs associated with physiological adaption to different salinities.

Theory 1 – the need for oxygen

Fish are vertebrates and thus eukaryotes, which have **cells** containing the same organelles as humans. This means that they for example carry out aerobic respiration in their mitochondria in order to produce energy in the form of the energy storing molecule adenosine triphosphate (**ATP**). Given that this is an **aerobic process**, fish like humans, must use oxygen (**O_2**) for this process, which must be obtained from their surroundings. Additionally fish must (obviously) also get rid of carbon dioxide (CO_2) which is the byproduct of aerobic respiration. Although we live in different environments (i.e. water versus air), the cellular processes, which support the metabolism in fish and thus maintain their **physiological** demands, are therefore in many ways identical to those of humans.

Like humans, fish **must increase their oxygen consumption** in order **to increase energy production** to support activities related to e.g. catch of prey, predator avoidance or growth. It is therefore not surprising that many studies has linked the individual fitness and growth potential of fish populations to their oxygen transport. The degree to which a fish is able to increase its oxygen consumption is thus a good indicator for its ability to support energy demanding processes and hence cope with any internal or external factor affecting its energy demands. How well a fish does this is reflected in its so-called "aerobic scope" (AS). To find the aerobic scope of a fish two measurements of oxygen consumption must be carried out 1) where the fish is at rest and not digesting, and 2) where the fish is as active as possible. The difference in the rate of oxygen consumption between these two activity states is the aerobic scope (AS). In fish research these measurements are called:

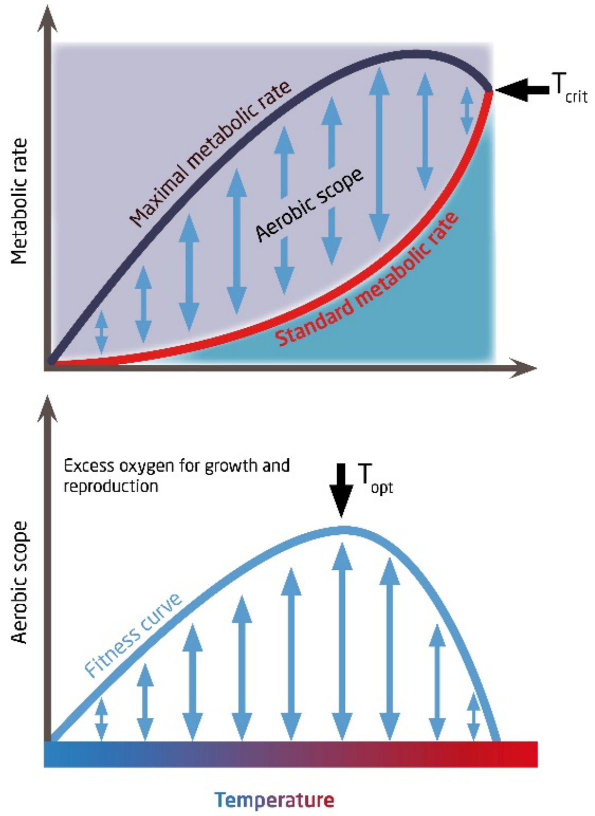
1) The Standard Metabolic Rate (SMR) = the oxygen consumption where the fish is resting and not digesting
2) The Maximum Metabolic Rate (MMR) = the rate of oxygen consumption where the fish is as active as possible

The relation between these measurements can be described by the equation:

$$\text{Aerobic scope (AS)} = \text{MMR} - \text{SMR}$$

In fish, both SMR and MMR – and hence AS - are affected by temperature and salinity (an example with effects of increasing temperature on AS can be seen in Figure 1). Notably, with increasing temperatures MMR increases at a faster rate compared to SMR until a maximum level is reached for MMR where it starts to decrease. MMR drops in the end because the cardio-respiratory system starts to fails at these high temperatures. In figure 1 the temperature shown as "T_{crit}", hereby indicates that the fish is reaching a critical temperature above which, it will not be able to catch prey, grow or avoid predators. Additionally the temperature shown as "T_{opt}" indicates the temperature at which, the fish has the highest levels of oxygen available for the same things.

Figure 1.Aerobic scope and metabolic rate in relation to temperature. Modified from Verbek et al, 2016.



Question to ensure understanding

<u>Question A</u>

Explain what the aerobic scope is, and how it can be calculated for a fish.

In the figure above the lines meet in the right corner. This indicates that the aerobic scope reaches 0 for this species of fish at this specific temperature (what the actual temperature is, is not important).

Imagine two fish in an experiment.

1) Fish 1 is in large aquarium where the temperature is very high, resulting in a substantially reduced AS for this fish
2) Fish 2 is in a large aquarium where the temperature is lower, and closer to the natural and ideal temperature for this species.

<u>Question B</u>

The two fish are kept in both aquariums for two years, where they are being fed the exact same types and amounts of food. Which of the two fish do you think will have gained the most weight? Support your answer.

<u>Question C</u>

A larger predatory fish is released in each of the aquariums. Which of the two fish do you think have the best chance of escaping the predator, and why?

Theory 2 – coping with salinity

Fish like humans have tissue and cells which need to have the right concentrations of different ions and water for them to function properly. In other words, the fish need to maintain internal homeostasis. Through evolution all organisms (including fish) have been adapted to cope with particular environments. In aquatic environments, organisms must cope with different levels of salts, and their physiology is therefore fine-tuned to the ranges of salinity, which they normally encounter.

Many organisms including fish are however faced with the need to have a slightly different concentration of ions in e.g. their blood and tissue than the surrounding environment. Due to osmosis, such ion gradients will only be maintained if an active regulation takes place in the exposed tissue's permeable cells. In fish this tissue is mainly the gills and the gut. Especially the gills are so permeable that not only oxygen and carbon dioxide can across its thin membrane but also sodium and chloride. To keep the internal concentrations under control **osmoregulation** is therefore required.

Osmoregulation is the **active process** to control differences of osmotic pressure between internal and external fluids by keeping, obtaining or releasing water, ions and other solutes. The process is carried out by specific pumps in the cell membrane. Marine fish are **hypotonic** to salt water, causing them to passively loose water and gain ions over their gills and gut, while freshwater fish, being **hypertonic** to their environment, gain water and loose ions. To counteract this, fish must use energy in the form of ATP to counteract the passive osmosis. So, in theory, this indicates that fish inhabiting salinity levels either above or below their internal salt concentrations will have to spend more ATP to maintain the internal homeostasis, than a fish living in water where the ambient salinity is closer to the internal concentrations of the fish's blood and tissue (table 1).

The link between fish, salinity and energy requirements

Fish	Salinity in water	Effect on exposed tissue	Cellular requirement	ATP demand	Oxygen demand	SMR	AS
A	Isoosmotic* to the internal environment of the fish	Constant water content in tissue	A bit of osmoregulation	Low ATP use	Low	Low	Unaffected
B	Much lower than the fish's internal concentrations	Absorb water from surrounding water	Substantial osmoregulation	High ATP use	High	High	Reduced
C	Much higher than the fish's internal concentrations	Loose water to surrounding	Substantial osmoregulation	High ATP use	High	High	Reduced

*Isoosmotic means that the salinity inside the fish is the same as the water around it.

Table 1: The effect of water of various salinities on fish. Each column demonstrates how e.g. a high salinity will increase the loss of water over the gills due to osmosis. To counteract this passive loss of water, substantial osmoregulation is required by the cells. This osmoregulation is an active process, and will thus demand a high use of ATP which again will only be available if the fish increase its aerobic metabolism, where oxygen is demanded. Finally as a consequence, the standard metabolic rate (SMR) will increase and thus reduce the aerobic scope (AS).

Most fish are adapted to cope with a narrow range of salinities, and they will die if exposed to salinities deviating much from their optimal salinity. Some species however very good at osmoregulating and can hence tolerate a wide range of salinities. One way to test the osmoregulating ability of fish, is therefore simple to perform an experiment with different salinities.

Methods

As the aerobic scope is a key indicator for fish' ability to thrive in an ecosystem (reproduce, grow, avoid predators, move to important habitats etc.), experiments can be made to assess potential impact of environmental factors like salinity on the aerobic scope of the individual, and from this it can be evaluated how likely it is that an invasive fish like the round goby will disperse into waters of different salinities.

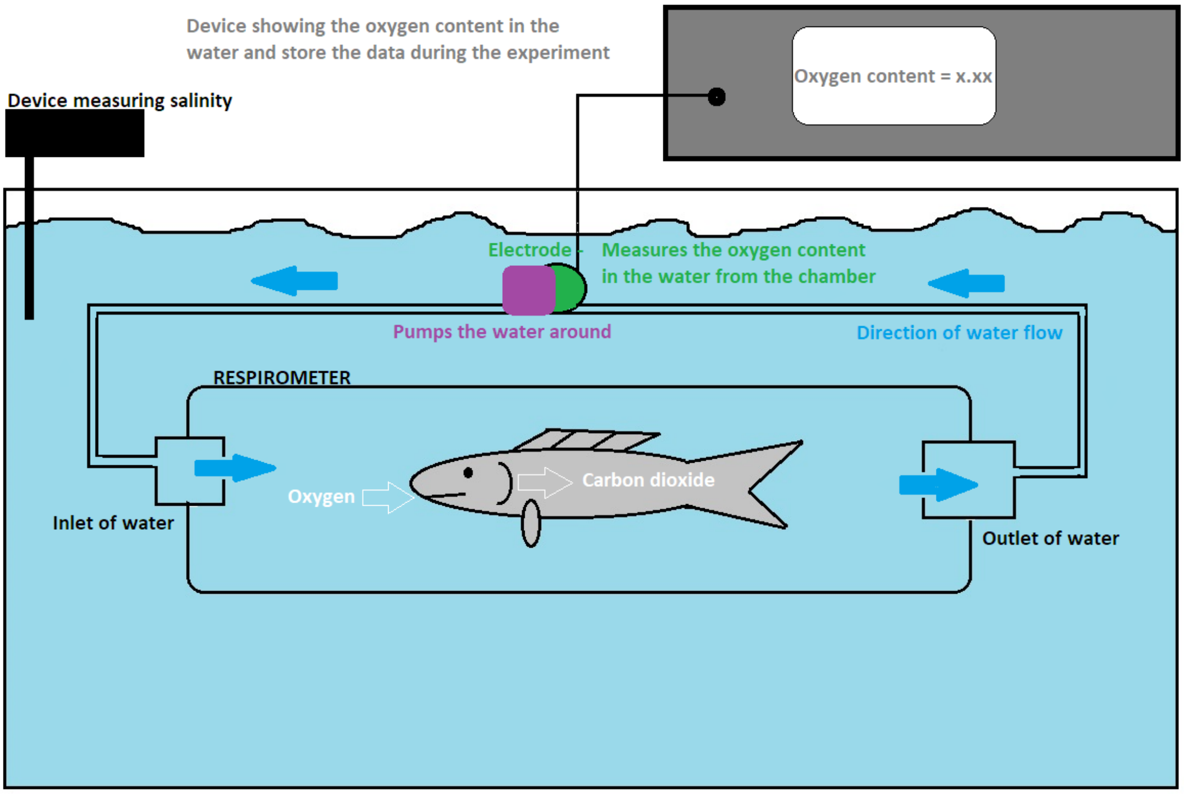
The experiment was carried out in the following order. Ethical permit 2015-15-0201-00546 from the Danish Animal Ethics Committee covered all experiments reported here.

1) Round gobies were caught in fyke nets in brackish water (salinity 10 PSU) in the Western part of the Baltic, in the area called Guldborgsund. From there the fish were transported to the National Aquarium of Denmark.
2) At the aquarium the fish were held in freshwater (0 PSU) for 3 months until the acclimation to higher salinities began. Fish were fed three times a week with fish pellets. Fish were observed twice daily, by animal caretakers. Fish demonstrating signs of suffering or distress were euthanized.
3) Twenty round goby were kept in freshwater, and 100 additional fish were ivided into five new experimental aquariums with brackish water (10 PSU) and kept for a week. Twenty fish stayed at 10 PSU, whereas the remaining fish were slowly ((with an increase of 5 PSU per week) acclimated to 15, 20, 25 and 30 PSU, respectively. When the batch of fish reached their treatment salinity, they were kept in that salinity for approx. three weeks, before initiating measurements of SMR and MMR.
   The length and weight of the fish used in the experiment, and the number (N) used for experiments, are shown in the table below.

Treatment salinity (PSU)	**0**	**10**	**15**	**20**	**25**	**30**
Number of fish used (N)	8	8	8	8	8	8
Mean length (cm)	15.2	15.4	15.4	15.7	14.9	14.8
Mean weight (g)	53	53	56	54	45	45

4) To measure the MMR of the fish, individual fish were manually exercised to exhaustion (between 2 and 5 min) in a circular tank (40 cm diameter, water depth 10 cm) and subsequently placed in the respirometer. Here an electrode measured the decrease in oxygen within the respirometer. Knowing this decrease of oxygen, the weight of the fish, the volume of the respirometer and the oxygen solubility (which varies with temperature and salinity), one can estimate how much oxygen the fish consumes (i.e. how much energy the fish uses).
5) After the MMR measurements, the fish would stay in the respirometer overnight, allowing it to calm down and rest, where after measurements of the individuals SMR could be made.

Figure demonstrating a respirometer



6) Because larger fish (not surprisingly) consumes more oxygen as compared to smaller fish, the SMR and MMR (and hence AS) is always standardized to oxygen consumption per kilogram of fish (per unit time). In this way, to oxygen consumption is directly comparable, irrespectively of the size of the fish. The data in the excel sheet therefore has the following units.

   SMR = standard metabolic rate (mg 0_2 kg^{-1} h^{-1})
   MMR = maximum metabolic rate (mg 0_2 kg^{-1} h^{-1})
   AS = Aerobic Scope (mg 0_2 kg^{-1} h^{-1})

7) The number of dead fish during the experimental period was noted down for each salinity treatment, and from this a survival percentage could be calculated. Results are presented in the data sheet.

8) At the end of the experiment all fish were euthanized.

Data analysis

In order to understand the effect of different salinities on the round gobies aerobic scope (AS) we must compile the data on MMR and SMR and calculate AS.

Aerobic scope (AS) = MMR – SMR

Important data considerations

Data, (unfortunately) do not speak for itself. In order to move from data points to meaningful information, data must be presented in an intuitive way and interpreted by scientists in light of our theoretical understanding. To guide your presentation and interpretation of the data, it might be worth considering the following things.

a) *As you will see in the Excel data file the measurements of oxygen consumption for different fish was not identical at the same salinities. Does this mean that there was a mistake in in the experiment?*
   The authors of the study did not think so, especially as individual differences is always expected assuming that biological factors such as size, age or gender could affect the oxygen consumption slightly. You're not exactly performing equally to your friend, brother or sister or neighbough – right? The same inter-individual difference is expected between fish.

b) *Given that the measured values are not the same, you must decide on how to present the data. One way to do it, could be to take the mean value of SMR and MMR for the 8 fish used at the 6 different salinities, and then calculate the AS for each salinity.*
   a. *If you are more advanced you could also calculate the standard deviation for each of the salinities.*

c) *When you have calculated the AS at the different salinities, you must present the results in a meaningful way. The scientists in this study did this, by making a plot with AS (Y-axis) as a function of salinity (X-axis)*

d) *Finally you must also decide how to present the development in survival for the fish at the different salinities*

Results

Place your presentation of the data here – this way the document will work as a general scientific report. Remember to add comments, where you interpret what the data is showing.

Discussion

As the round goby is generally found in fresh and brackish water, it was the hypothesis, that the round goby's dispersal will be limited by the higher salinities in the North-Western part of the Baltic Sea in the areas around Denmark.

1) Discuss to what degree the results have provided evidence for this hypothesis.
2) If possible, try to take the map from the introduction and show at where you believe that the round gobies will start to experience significantly reduced performance, i.e. low AS.
3) Provide three recommendations for the Nature Agencies in Denmark, Sweden and Germany, which will help them decide how to manage the round goby invasion in the coming years.

Conclusion

1) Conclude whether it is likely that the round gobies dispersal will be limited by the higher salinities in the North-Western part of the Baltic Sea.

References

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