Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Marie Reiersen

The effects of oxygen availability and acclimation temperature on upper thermal tolerance in zebrafish (*Danio rerio*)

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Supervisor: Fredrik Jutfelt
Co-supervisor: Anna H. Andreassen
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NTNU
Kunnskap for en bedre verden

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Abstract

Global temperatures are increasing, and heat waves are becoming more frequent. Therefore, it is increasingly important to understand how different organisms respond to changes in temperature. It has been suggested that a constraint in capacity to supply tissues with oxygen causes a decline in animal performance when temperatures approach limiting values. Results from previous studies are however contradicting, and it seems like the effect of oxygen availability on upper thermal tolerance is dependent on species and context. We investigated whether ambient oxygen level (30 %, 100 % and 200 % of air saturation) is a limiting factor for upper thermal tolerance measured as CT_{max} in zebrafish acclimated to 20 ℃, 28 ℃ and 34 ℃. In a second experiment the effect of oxygen (50 %, 100 %, 150 % and 250 % of air saturation) on upper thermal tolerance and aerobic scope was further investigated for cold acclimated fish. We hypothesised that fish acclimated to different temperatures might respond differently to oxygen levels above or below usual levels because the physiology of the fish change with acclimation temperature. Fish exposed to 50 % air saturated water was able to maintain a relatively high aerobic scope when acclimated to 20 ℃, and aerobic scope increased with level of oxygen saturation in hyperoxia. Moreover, CT_{max} was considerably reduced when fish were exposed to 30 % air saturated water for all acclimation temperatures. Hyperoxia seemed to only affect CT_{max} in cold acclimated fish, where hyperoxia increased CT_{max} up to a level before declining. Thus, the effect of oxygen was dependent on acclimation temperature. As aerobic scope was highest at the highest levels of ambient oxygen, upper thermal limits might be dependent on oxygen only until aerobic scope reach a certain magnitude, after which the benefit of hyperoxia was saturated and other physiological functions became limiting for thermal tolerance.

Sammendrag

Globale temperaturer øker, og hetebølger forekommer oftere. Det blir derfor stadig mer viktig å forstå hvordan ulike organismer responderer til temperaturendringer. Det har blitt foreslått at en begrensning i dyrs kapasitet til å forsyne vevene med oksygen fører til en nedgang i ytelse når temperaturer nærmer seg begrensende verdier. Resultater fra tidligere studer er derimot motstridende, og det ser ut til at effekten av oksygentilgjengelighet på øvre temperaturtoleranse er avhengig av art og sammenheng. I denne studien undersøkte vi om oksygennivå i omgivelsene (30 %, 100 % og 200 % luftmetning) er en begrensende faktor for øvre temperaturtoleranse målt som CT_{max} hos sebrafisker akklimert til 20 ℃, 28 ℃ og 34 ℃. I et nytt eksperiment ble effekten av oksygen (50 %, 100 %, 150 % og 250 % luftmetning) på øvre temperaturtoleranse og aerob kapasitet videre undersøkt for kuldeakklimerte fisk. Vår hypotese var at fisker som har blitt akklimert til ulike temperaturer kan respondere ulikt på oksygennivåer som er høyere eller lavere enn vanlige nivåer. Dette fordi fiskers fysiologi endres med akklimeringstemperatur. Vi fant at fisk eksponert for 50 % luftmettet vann klarte å opprettholde en relativt høy aerob kapasitet når de hadde blitt akklimert til 20 ℃, og at den aerobe kapasiteten økte med nivå av luftmetning i hyperoksi. Videre ble CT_{max} betraktelig redusert ved eksponering for 30 % luftmettet vann for alle akklimeringstemperaturer. Hyperoksi så bare ut til å påvirke CT_{max} for kuldeakklimerte fisker, hvor hyperoksi økte varmetoleranse opp til et nivå før den avtok. Dermed var effekten av oksygen på øvre temperaturtoleranse avhengig av akklimeringstemperatur. Siden den aerobe kapasiteten var høyest ved de høyeste oksygennivåene er det mulig at øvre temperaturtoleranse bare er avhengig av oksygen frem til den aerobe kapasiteten når et visst omfang der fordelen av hyperoksi er fullt utnyttet og andre fysiologiske funksjoner blir begrensende for temperaturtoleranse.

Acknowledgements

First and foremost, I would like to thank Associate Professor Fredrik Jutfelt and Ph.D. candidate Anna H. Andreassen for fantastic supervision. Your guidance, support, and encouragement have been extremely valuable in this process. I would also like to thank fellow master students Anders Jorud Meyer and Sondre Kristenstuen for practical help in the lab. This project would not have been possible to conduct without the two of you. Moreover, I would like to thank Ph.D. candidate Mette Finnøen, who provided valuable advice concerning the statistical work, and the rest of the zebrafish group for the inclusive culture and educational discussions. The weekly group meetings have been something to look forward to in a hectical year influenced by the coronavirus. Lastly, I would like to thank friends and family for all love and support throughout my years in Trondheim.

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Abbreviations

CTM	Critical thermal method
CT_{max}	Critical thermal maximum.
$\dot{M}O_{2,max}$	Maximum oxygen consumption rate
$\dot{M}O_{2,min}$	Minimum oxygen consumption rate
$\dot{M}O_{2,routine}$	The lowest oxygen consumption rate of fish during the first 30 minutes after transfer to a respirometer
OCLTT	Oxygen and capacity limited thermal tolerance

1 Introduction

1.1 Ectotherm thermal tolerance

The body temperature of ectotherms is generally following the ambient temperature (Schulte, 2015). Animals are generally adapted to a limited temperature range and temperatures above or below this range will have negative consequences for physiological functions and processes (Fangue, Todgham, & Schulte, 2021). Both upper and lower limits of temperatures tolerated by fish is strongly dependent on genetics, temperature during development and acclimation temperature (López-Olmeda & Sánchez-Vázquez, 2011). Global temperatures are increasing and heat waves are becoming more frequent (IPCC, 2007) and it is therefore especially important to understand the upper thermal tolerance of animals and how different organisms respond to changes in temperature.

1.1.1 Acclimation

Because biochemical rates are temperature sensitive, they are also partly controlled by the environment (Schulte, 2015). To reduce the extent to which biological rates change in response to long-term changes in temperature ectotherms can acclimate by remodelling their physiology through different processes (Jayasundara & Somero, 2013; Seebacher et al., 2010). The term acclimation is commonly used to describe a, usually reversible, response to a single environmental factor, for example temperature (Lagerspetz, 2006). These processes can involve changes in protein isoforms (Crockford & Johnston, 1990), mitochondrial density (O'Brien, 2011) and membrane lipid composition (Hazel, 1995). Moreover, there is evidence that thermal acclimation can affect the cardiorespiratory system through for example changes in cardiac morphology (Klaiman, Fenna, Shiels, Macri, & Gillis, 2011) and in the molecular machinery of cardiac excitation-contraction coupling (Vornanen, Shiels, & Farrell, 2002). The temperature an animal is acclimated to is among the most critical factors influencing thermal tolerance of fishes. As acclimation temperature increases, maximum temperatures tolerated generally increase while tolerance to cold is reduced (Beitinger, Bennett, & McCauley, 2000). Correspondingly, acclimation to cold overall enhance tolerance to cold, while upper thermal tolerance is reduced.

1.1.2 Effects of oxygen

Temperature affects both minimum aerobic metabolic rate ($\dot{M}O_{2,min}$) and maximum aerobic metabolic rate ($\dot{M}O_{2,max}$) in fishes, but the shape of this relationship between temperature and metabolic rate is not necessarily the same (Figure 1.1A) (Fangue et al., 2021; Verberk et al., 2016). An exponential rise in $\dot{M}O_{2,min}$ until lethal temperatures has often been observed, while $\dot{M}O_{2,max}$ often plateau or even decrease before the whole animal critical thermal maximum (Fry & Hart, 1948; Norin & Clark, 2016). Consequently, temperature can have strong effects on aerobic scope, defined as the difference between $\dot{M}O_{2,min}$ and $\dot{M}O_{2,max}$. It has been suggested that a constraint in the animals capacity to supply tissues with oxygen causes a decline in their performance when temperatures approach limiting values (Pörtner, Bock, & Mark, 2017). This concept is referred to as "oxygen- and capacity limited thermal tolerance" (OCLTT) and suggests that biochemical and physiological capacities of aquatic ectotherms have evolved so that aerobic scope is maximized to optimize fitness related performance in a specific temperature range. This would imply that traits like growth rate, locomotion and reproduction is connected to aerobic scope, and animals should have optimal fitness when living at these temperatures (Clark, Sandblom, & Jutfelt, 2013). Studies on several fish species shows that acute heat tolerance is reduced when the level of oxygen in the water drops below normal levels (Healy & Schulte, 2012; Islam et al., 2020; Rutledge & Beitinger, 1989). These results support the idea of oxygen supply as the limiting factor for heat tolerance (Brijs et al., 2015).

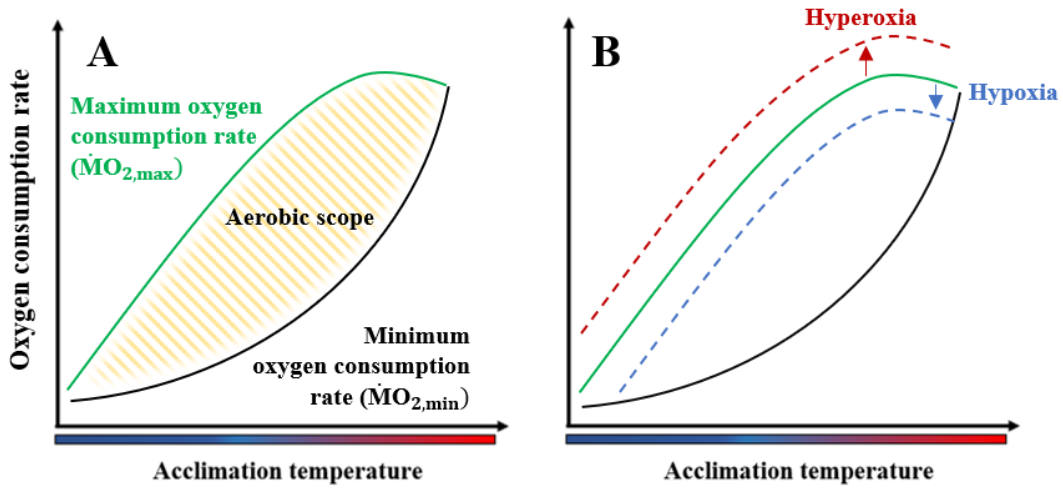


Figure 1.1 A Thermal dependency of maximum ($\dot{M}O_{2,max}$) and minimum ($\dot{M}O_{2,min}$) oxygen consumption rate , and aerobic scope defined as the $\dot{M}O_{2,max}$- $\dot{M}O_{2,min}$. (Verberk et al., 2016). **B** Ambient hypoxia can be expected to decrease aerobic scope (Portner & Farrell, 2008). Several studies have also found elevated aerobic scope in fish exposed to hyperoxia (McArley, Sandblom, & Herbert, 2021).

Even though there is empirical support for the OCLTT hypothesis in certain contexts, results from other studies are contradicting the hypothesis (Jutfelt et al., 2018). Brijs et al. (2015) found that fish exposed to environmental hyperoxia did not have a higher CT_{max}, nor did fish exposed to anaemia (experimentally induced hypoxia) have lower CT_{max}. Moreover, aerobic scope has been found to keep increasing above the animals preferred temperature and until the temperature approaches lethal levels before rapidly declining (Clark et al., 2013; Norin, Malte, & Clark, 2014). In addition, some studies have also reported that growth rate is highest at temperatures lower than when the aerobic scope is maximized (Brijs et al., 2015; Grans et al., 2014; Schulte, 2015). It has been suggested that these results could be explained by different physiological processes being optimized at different temperatures, a "multiple performances – multiple optima", MPMO, hypothesis (Clark et al., 2013). This hypothesis implies that thermal tolerance is controlled by multiple parameters, and the importance of these parameters could vary between species, life stages and the nature of the thermal challenge.

1.1.3 Measuring thermal tolerance: CTM

Thermal tolerance of ectothermic animals are often assessed by the critical thermal methodology (Cowles & Bogert, 1944). Beitinger et al. (2000) defined the terms CT_{max} and CT_{min} as a measure for upper and lower thermal tolerance with the CTM, respectively. Upper thermal limits of ectothermic vertebrates are commonly quantified as the temperature where locomotion is disorganised, and thus fish are unable to escape from deadly conditions (Beitinger et al., 2000). The CTM require a constant linear change in temperature from acclimation temperature until a sublethal physical disorganised response occurs, usually loss off equilibrium, but other criteria can also be used (Becker & Genoway, 1979; Beitinger et al., 2000). The rate of temperature change is a critical factor when performing CTM tests. CTM values has shown to vary considerably with different rates of heating (Becker & Genoway, 1979). Slow heating rates can allow the animal to acclimate to the thermal challenge, while fast heating rates can lead to a temperature shock or a lag in deep body temperature (Becker & Genoway, 1979; Beitinger et al., 2000). As a compromise Becker and Genoway (1979) recommend a rate of 0.3 °C/min as a standard for CTM determinations with freshwater fish. Beitinger et al. (2000) argues that CTM are best used as a relative and not absolute measure of temperature tolerance because of the effect of heating rate and differences in end point criteria being used.

1.1.4 The study species: zebrafish (*Danio rerio*)

Zebrafish (*Danio rerio*) belongs to the Cyprinidae family of freshwater fishes, and has become an important model organisms in many fields of research (López-Olmeda & Sánchez-Vázquez, 2011). It is a small and robust fish, with short generation time and large numbers of specimens can easily and cheaply be kept in the laboratory (Spence, Gerlach, Lawrence, & Smith, 2008). In the wild they are mainly located around the Ganges and Brahmaputra basins in north-eastern India, Bangladesh and Nepal (López-Olmeda & Sánchez-Vázquez, 2011). Zebrafish are usually found in slow-moving or standing shallow waters (Spence et al., 2006). Sundin et al. (2019) found zebrafish in temperatures ranging from 24.5 ℃ to 35.3 ℃ in India. Other studies have reported that and they have been found in temperatures as low as 16.5 ℃ and as high as 38.6 ℃ (López-Olmeda & Sánchez-Vázquez, 2011; Spence et al., 2006). In the wild they can experience great variations in temperature as daily temperature fluctuations up to 5.6 ℃ has been recorded in the Ganges basin (López-Olmeda & Sánchez-Vázquez, 2011). Several studies have focused on the thermal tolerance of zebrafish. For example, the effect of acclimation temperature (Cortemeglia & Beitinger, 2005; Morgan et al., 2019), developmental temperature (Schaefer & Ryan, 2006) and evolution of CT_{max} performance (Morgan, Finnøen, Jensen, Pélabon, & Jutfelt, 2020) have been investigated. To my knowledge, no previous study has investigated the effect of hypoxia or hyperoxia on CT_{max} in adult zebrafish.

Oxygen limitation does not seem to be a universal mechanism constraining thermal tolerance across all contexts and species (Andreassen, Hall, Khatibzadeh, Jutfelt, & Kermen, 2020; Brijs et al., 2015; Ern, Norin, Gamperl, & Esbaugh, 2016). In accordance with OCLTT the CT_{max} of zebrafish larvae have been found to be sensitive to oxygen, but it is unclear whether this sensitivity is limited to early life stages, or also apply to adult zebrafish (Andreassen et al., 2020). Moreover, it is unknown how acclimation temperature affects the effect of oxygen. Brijs et al. (2015) found no effect of hyperoxia for European perch (*Perca fluviatilis*) acclimated to 23 ℃, while another study on the same species found an increase in CT_{max} for fish acclimated to 18 ℃ when exposed to hyperoxia (Ekström et al., 2016). Together with the physiological changes that might occur through acclimation, this leaves an interesting area for more investigation.

1.2 Study aim

The aim for this study was to investigate whether oxygen is a limiting factor for heat tolerance in zebrafish. CT_{max} was generally expected to increase with acclimation temperature. According to the OCLTT-hypothesis ambient hypoxia is predicted to lead to a lower thermal tolerance and hyperoxia is predicted to lead to an increased thermal tolerance if ambient oxygen levels translate into increased oxygen supply to the tissues (Figure 1.1A,B). It could also be predicted that fish acclimated to different temperatures might respond differently to oxygen levels above or below usual levels (Figure 1.2C) since the physiology of the fish change with acclimation temperature (Jayasundara & Somero, 2013).

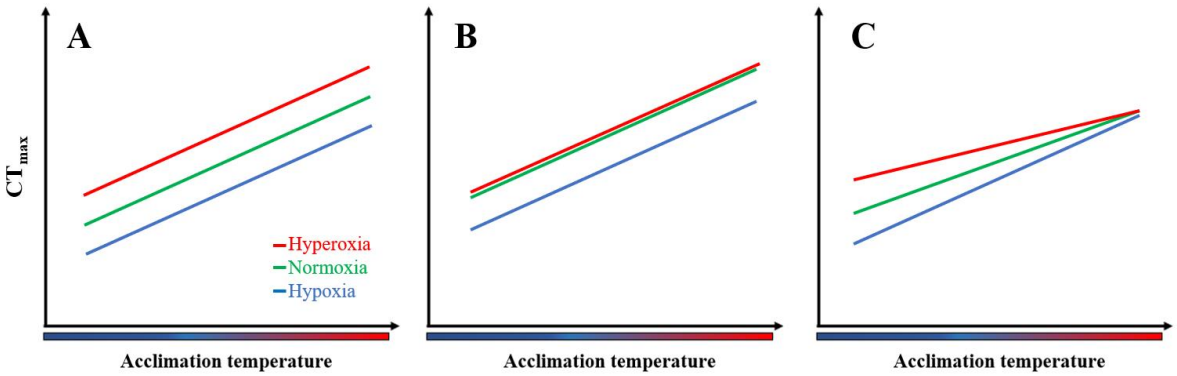


Figure 1.2 Three possible outcomes from the experiment. Blue, red, and green lines represent hypoxia, normoxia and hyperoxia treatments, respectively. From OCLTT CT_{max} can be expected to increase with oxygen availability (A). There is also a possibility that increased water air saturation does not translate into increased oxygen supply to the tissues, and therefore not affect upper thermal tolerance (B). The effect of oxygen on CT_{max} might also be dependent on acclimation temperature (C).

To test these predictions, we first performed an experiment (Experiment 1) where individuals were acclimated to either 20 °C, 28 °C or 34 °C for 14-17 days before acute heat tolerance was measured as CT_{max} at three different oxygen levels: hypoxia (30 %), normoxia (100 %) and hyperoxia (200 %), reported in percentage of air saturation. In a second experiment (Experiment 2) we further investigated the effect of oxygen saturation with a focus on environmental hyperoxia in cold acclimated fish (20 °C). The oxygen levels used for this additional experiment were 50 %, 100 %, 150 % and 250 % of air saturation. Furthermore, according to OCLTT increased performance due to a higher availability of oxygen also requires that the animal have the capacity to consume and transport this oxygen, that is increase aerobic scope (Figure 1.1B). To test whether environmental oxygen levels affected aerobic scope routine oxygen consumption rate ($\dot{M}O_{2,routine}$) and maximum oxygen consumption rate ($\dot{M}O_{2,max}$) were measured.

2 Methodology

The fish used for the experiments were from a line originating from a wild caught zebrafish (*Danio rerio*) population and kept in the animal facility at the Norwegian University of Science and Technology (NTNU), Trondheim, Norway. All fish were kept in glass aquaria (30 x 30 x 50 cm) containing air supply, an ornamental plastic plant, titanium heaters (TH-100, Aqua Medic, Bissendorf, Germany) controlled by thermostats (ITC-306T, Inkbird, Shenzhen, China), and temperature sensors (type K thermocouple) connected to a data logger (Picotech TC-08, Cambridgeshire, UK) were set up on three shelves in a climate-controlled room. Water was carbon filtered and added 0.25 ml/L salt and 0.25 ml/L Aquasafe (Aquasafe, Tetra Sales, Blacksburg, VA, USA) for all experiments and the acclimation. Before the experiments all aquaria held a water temperature of 28 ℃.

2.1 Experiment 1

The purpose of this experiment was to investigate the effect of oxygen on upper thermal tolerance, measured as CT_{max}. To investigate whether the effect of oxygen was dependent on acclimation temperature, fish were acclimated to different temperatures. A total of 207 fish (n=23 per treatment), including two spare fish, were kept in nine aquaria. Eleven additional fish were kept in each aquarium for another experiment (n=34 per aquarium). As the experiment commenced each aquarium was assigned one of three acclimation treatments (20 ℃, 28 ℃ or 34 ℃) leaving three replicate tanks per temperature. To control for differences in for example light conditions, temperature, feeding, movements, or other disturbances every shelf contained one aquarium of each temperature and the different temperatures had different positions on the three shelves (Figure 2.1). The aquaria were marked with numbers and color-coded tape according to the assigned temperature.

2.1.1 Acclimation

All aquaria were kept at normoxia. The temperature was changed with 2 ℃ each day until the assigned acclimation temperature was reached. Subsequently, the fish were left to acclimate for 14-17 more days. During this period, the fish were fed twice a day on weekdays and once a day in weekends with dry fish flakes (TetraPro, Tetra Sales, Blacksburg, VA, USA) controlled for signs of sickness, mortality (n=1) was recorded, dead fish were removed, and water temperature was measured. In addition, water temperature was continuously monitored using temperature loggers.

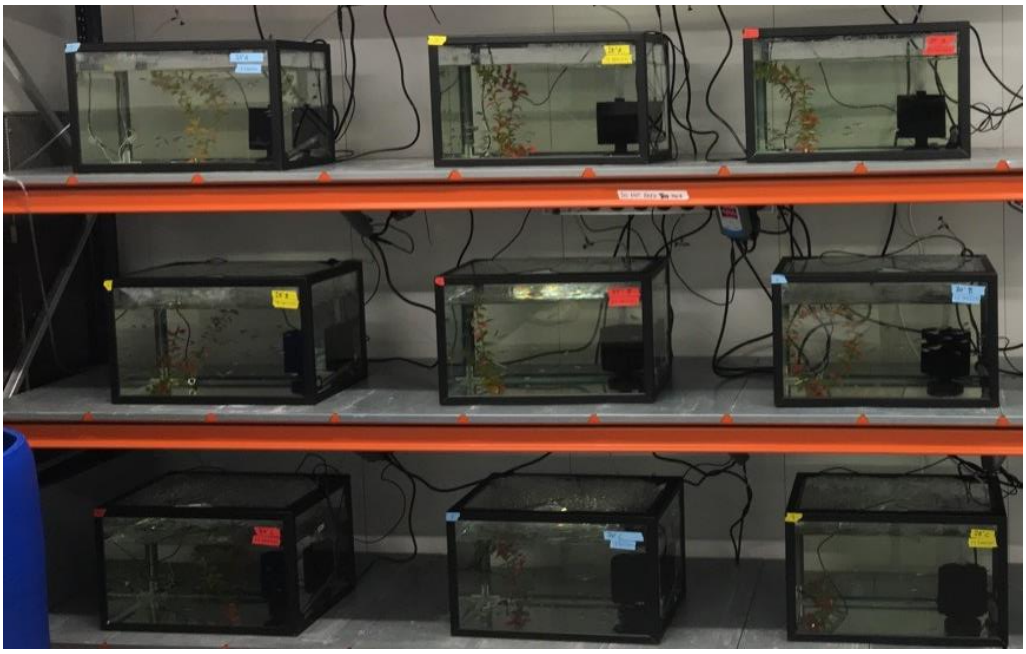


Figure 2.1 Setup of aquaria. Blue, yellow and red tapes represent acclimation temperatures of 20, 28 and 34 ℃ respectively.

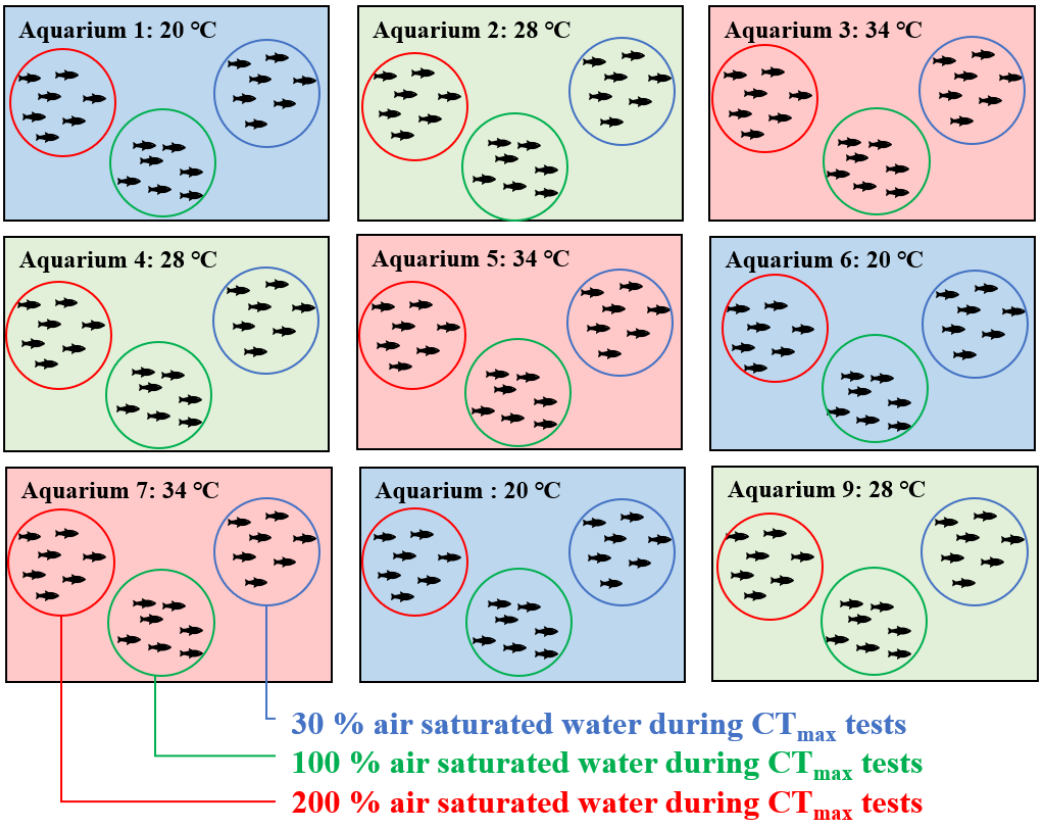


Figure 2.2 Experimental design for Experiment 1. Nine aquariums with 21 fish acclimated to either 20 ℃, 28 ℃ and 34 ℃ were used for this experiment. Fish were tested for CT_{max} in batches of 7 fish (=3 batches per aquarium). Fish in the same batch was tested at a common level of oxygen. Each batch from the same aquarium were tested at different levels of oxygen.

2.1.2 Testing upper thermal tolerance: CT_{max} tests

After the acclimation period the CT_{max} tests were conducted over a period of three days. The remaining untested fish were fed once at the end of the day during this period. Fish would then be fasted for >16 h prior to the tests. This way, fish were in approximately the same state of hunger while conducting the CT_{max} measurements. The fish in each aquarium were divided into three groups of seven fish (Figure 2.2). Each group from the same aquarium was assigned a different oxygen treatment so all combinations of tank and oxygen treatments and were tested. Nine trials were conducted each day and a total of 27 trials were performed. The trials were ordered so that the same tanks were not tested at the same time each day. For practical reasons hypoxia trials were performed either before or after lunch, and the opposite for the hyperoxia trials. The order was changed each day. Normoxia trials could be performed simultaneously as other trials.

The setup and equipment used for CT_{max} tests was the same as used by Morgan, Finnøen, and Jutfelt (2018) (Figure 2.3). A heating tank (25 x 22 x 18 cm) was set up with a 300 W coil heater placed in a cylindrical custom-made steel heating case attached to a water pump (Eheim Universal 300, Deizisau, Germany) and filled with 9 L of water holding the respective acclimation temperature. This resulted in a heating rate of 0.34 ± 0.02 °C per minute and continuous mixing. In addition, the heating tank was set up with air supply, oxygen or nitrogen supply, a temperature sensor and two robust fiber-optic oxygen sensors (Firesting O2, PyroScience, Aachen, Germany). The heater, pump and oxygen/nitrogen supply were separated from the fish with a mesh, whereas the temperature sensor and an oxygen probe were kept in the same compartment as the fish. To achieve the respective oxygen levels either nitrogen (for hypoxia treatments), regular air (control treatments), or oxygen (hyperoxia treatments) was added to the water through an air stone. The oxygen level was continuously monitored and manually adjusted throughout the test. Deviations in level of air saturation of ± 5 % and ± 10 % from the assigned oxygen treatments were considered acceptable for the hypoxia and hyperoxia treatment, respectively. The heating tanks were covered with a transparent plastic plate for most part of the tests, to easier maintain the desired level of oxygen in the water. Water was changed between each trial.

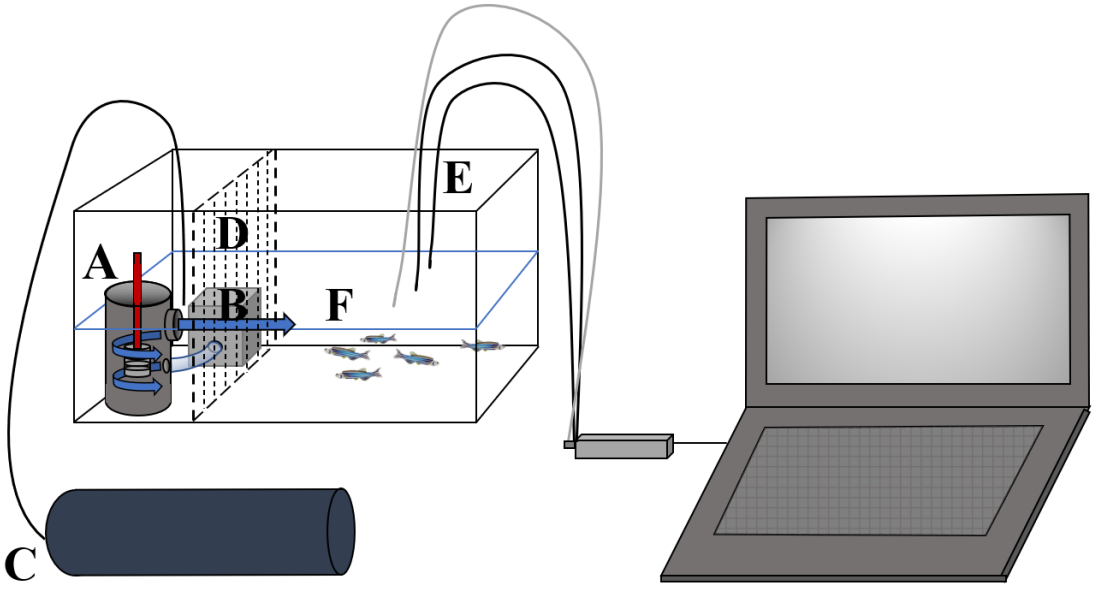


Figure 2.3 Experimental setup for CT_{max} tests, ensuring homogenous water temperature and level of air saturation, and a constant heating rate. (A) Cylindrical heating case with a 300 W coil heater; (B) water pump; (C) oxygen/nitrogen supply; (D) mesh; (E) temperature sensor fibre-optic oxygen sensor; (F) fish compartment. Adapted from Morgan et al. (2018).

Fish were added to the heating tanks in batches of seven fish per trial. Loss of equilibrium, defined by Becker and Genoway (1979) as the point where fish lost the ability to remain dorsoventrally upright for three seconds and this ability was not regained, was used as the endpoint criteria. When individual fish lost equilibrium, an observer immediately transferred the fish to a numbered container filled with ice water, and temperature and time when the fish lost equilibrium was noted by a second person. To minimize bias the observer did not know the water temperature. As each fish was transferred to a separate container individual fish was weighted to nearest 0.01 g. Additionally, length of each fish from trials performed the two last days as also recorded with a precision of 0.1 mm using a digital calliper.

2.2 Experiment 2

The purpose of the second experiment was to further investigate the effect of oxygen, mainly hyperoxia, on upper thermal tolerance, and investigate links to aerobic scope. Three aquaria were set up and 135 fish distributed randomly between them (n=45 per aquarium). The fish used for this experiment were from the same line as the Experiment 1 and kept the same way. A mortality of n=1 was recorded. Like Experiment 1, temperature was lowered 2 ℃ each day starting at 28 ℃ until the desired temperature of 20 ℃ was achieved, and all aquaria were kept

at normoxia. The fish were then left to acclimate for 13-17 days, before 15-18 fish from each aquarium went through $\dot{M}O_2$ measurements (Figure 2.4). $\dot{M}O_2$ measurements were conducted over a period of five days, and CT_{max} tests over two days. In this period, the fish were fed at the end of the day to ensure similar state of hunger and minimize potential effects of digestion. The fish would then been fasted for >16 h prior to the tests. After metabolic rate measurements the fish were kept in four separate tanks according to oxygen exposure during the metabolic rate measurements. CT_{max} test were performed 3-4 days after the last $\dot{M}O_2$ measurements.

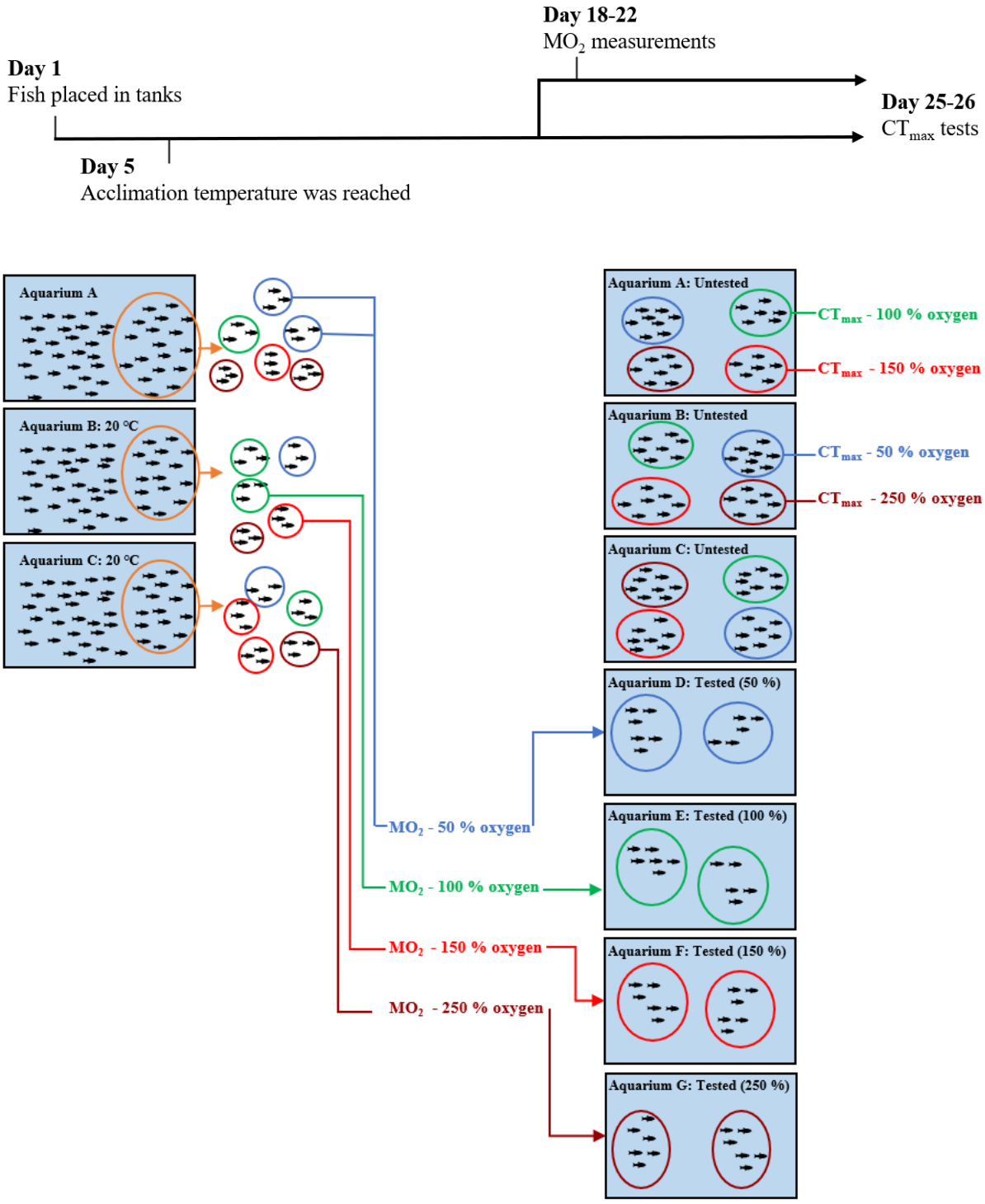


Figure 2.4 Timeline and experimental design for Experiment 2. A total of 135 fish were acclimated to 20 ℃ for 18-22 days (including a stepwise lowering of temperature by 2 ℃ per day from 28 ℃) before 48 fish went through $\dot{M}O_{2,max}$ and $\dot{M}O_{2,routine}$ measurements at different levels of oxygen. Fish that had been through $\dot{M}O_2$ measurements were placed in new aquaria according to oxygen treatment. At the end of the experiment, 25-26 days after fish had been placed in the aquaria all fish were tested for CT_{max} in batches of 5-8 fish. Fish that had been tested for $\dot{M}O_2$ were tested with the same oxygen treatment. One batch from each of the aquaria with untested fish were tested with each oxygen treatment.

2.2.1 $\dot{M}O_2$ measurements and calculations

For the measurements of aerobic scope, a large aquarium (60 x 35 x 35 cm) was set up as a water bath with air supply, oxygen or nitrogen supply, a temperature sensor, and a robust fiber-optic sensor (Firesting O2, PyroScience, Aachen, Germany), in addition to a small water pump (Eheim CompactON 300, Deizisau, Germany) to ensure mixing and a homogenous oxygen concentration (Figure 2.5). This way, oxygen concentration in the aquarium could be controlled and adjusted when needed. Later in the experiment two additional oxygen sensors were placed in the aquarium. To be able to control temperature, a titanium heater (TH-100, Aqua Medic, Bissendorf, Germany) was also placed in the aquarium.

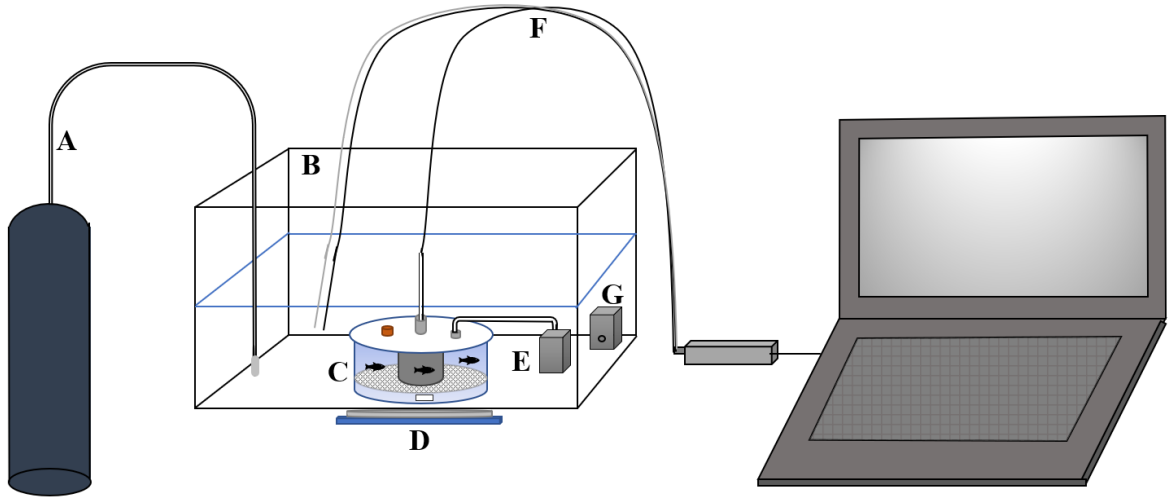


Figure 2.5 Experimental setup for oxygen consumption measurements: (A) Oxygen/nitrogen supply; (B) large aquarium; (C) respirometer, (D) magnet stirrer; (E) flush pump; (F) temperature sensor and fibre-optic sensors; (G) pump to ensure mixing of water in the large aquarium.

A customised IKEA 0.4 L circular glass food container (6 x 14 cm, 331.4 mL) with a plastic lid was used as respirometer and placed in the large aquarium (Figure 2.5C). Three holes were drilled into the lid. A flush pump (Eheim CompactON 300, Deizisau, Germany) was attached with a silicone tube to one hole, and a glass water outlet tube to the second hole. The last hole was so the fish could be transferred to the respirometer using a funnel. When fish were placed in the respirometer the hole was covered with a rubber stopper. A robust fiber-optic sensor (Firesting O2, PyroScience, Aachen, Germany), was placed in the respirometer through the water outlet tube to measure the oxygen concentration inside the respirometer. To keep the fish swimming in the outer circumference of the chamber a piece of 4 cm diameter plastic pipe was added to the respirometer. A magnet stir bar was placed in the bottom of the respirometer and

separated from fish with a mesh. A magnet drive plate was placed under the large aquarium to create an even current for the fish to swim in, and so water flow speed, and thus swimming speed, could be increased by adjusting the magnetic stirrer.

Measurements of $\dot{M}O_{2,routine}$ and $\dot{M}O_{2,max}$ were done using intermittent-flow respirometry. $\dot{M}O_{2,routine}$, was measured as an approximation of $\dot{M}O_{2,min}$. $\dot{M}O_{2,routine}$ refer to the rate of oxygen consumption when fish is undergoing normal or another predefined activity (Metcalfe, Van Leeuwen, & Killen, 2016). In contrast, $\dot{M}O_{2,min}$ ideally involves for instance a fasting period of 36-48 h prior to the measurements and leaving fish in the respirometer for 24-26 h to ensure that $\dot{M}O_2$ are close to minimal levels required to sustain life (Clark et al., 2013). Here, $\dot{M}O_{2,routine}$ was defined as the lowest rate of oxygen consumption during the first 30 minutes after the fish were transferred in the respirometer. $\dot{M}O_{2,max}$ was measured directly after $\dot{M}O_{2,routine}$. All rounds of measurements were conducted over a period of 5 days, each round lasting less than an hour. Level of air saturation in the respirometer was logged with a sampling frequency of 0.5 Hz for all measurements, and sensors calibrated to 100% dissolved oxygen between each round. For each round of $\dot{M}O_2$ measurements three fish randomly picked from an aquarium were placed in the respirometer (431.4 mL). The fish were not separated in any way and could interact freely. Four rounds with $\dot{M}O_2$ measurements were performed for each treatment (a total of 16 rounds and 48 individual fish). The order was allocated so all measurements with the same treatment were not performed at the same day or time of day. The water used in the respirometer held a temperature of 20.0 ± 0.6℃, the same as water temperature in holding aquaria, and was changed between every or every other round. To be able to verify that the fish were calm during $\dot{M}O_{2,routine}$ measurements, and to be able to monitor the fish without disturbing them, the fish were filmed during measurements. For the same reasons, the aquarium was covered, and plants were placed in the aquarium.

After the fish were placed in the respirometer the magnet drive plate was turned on the slowest speed for measurements of $\dot{M}O_{2,routine}$. Oxygen recordings were started immediately after the fish were transferred to the respirometer. The flush pump was turned off for approximately 10 minutes before it was turned on for approximately 2.5 minutes to restore the oxygen level. This was repeated two more times to get three periods for $\dot{M}O_{2,routine}$ slopes. To obtain $\dot{M}O_{2,max}$ the speed of the water was increased in a stepwise manner until the maximum speed was reached. The speed was adjusted by fixed steps on the spinner and later converted based on previous

measurements of water speed in the chamber at the different settings (Morgan et al., Unpublished manuscript). The fish were first allowed to swim at a speed of 3.4 cm/s, 6.1 cm/s and 13.1 cm/s for five minutes each before increasing the speed to the maximum speed that the fish could sustain for 1-2 minutes. After about 7.5 minutes, when water speed was approximately 6.1 cm/s, the flush pump was turned on to restore the oxygen level, so the fish had the proper oxygen treatment before the measurement at maximum swim speed. The maximum speed was obtained by increasing the speed until fish could not swim any more, and then reducing it slightly. During both routine and $\dot{M}O_{2,max}$ measurements, the level of air saturation in the large aquarium was monitored and adjusted continuously to ensure correct saturation when flushing.

Afterwards fish were transferred to a separate container, before they, one at the time, were anesthetized using buffered tricaine methanesulfonate (MS-222) in water (3 ml: 90 ml) before weighing and measuring length to the nearest 0.01 g and 0.1 mm, respectively. The fish were then placed in new aquaria according to oxygen treatments (11-12 fish in each aquarium, 2 fish died during the anesthesia). This was done to be able to account for any effects of the metabolism measurements on the later CT_{max} tests.

Oxygen consumption was calculated using the following formula (Clark et al., 2013):

$$MO_2 = \frac{(V_r - V_f) \times \Delta C_{wO_2}}{\Delta t \times M_f},$$

where V_r and V_f are respirometer and fish volume (1 g fish assumed to be equivalent to 1 ml fish), respectively, ΔC_{wO2} the change in oxygen concentration in the respirometer water, Δt the change in time over which ΔC_{wO2} was measured, and M_f the total weight of fish in g.

Oxygen concentration in the respirometer water was measured as % of air saturation with a sampling frequency of 0.5 Hz. The analysis software LabChart Reader (LabChart v.8.1.13, ADInstruments, NSW, Australia) was used to get the percentage decline in saturation per second. Intervals of approximately one minute were used to calculate the slopes. For $\dot{M}O_{2,routine}$ calculations, the lowest rate of oxygen consumption from the three first measurement intervals was used. For $\dot{M}O_{2,max}$ measurements the highest rate was used (from the last 2-3 minutes). An online oceanographic calculator and unit conversion tool developed by the International Council of the Exploration of the Sea was used to convert the change in oxygen concentration from percent change in level of air saturation per second to mg change per hour (ICES, 2021).

2.2.2 CT_{max} tests

CT_{max} tests for the second experiment were conducted the same way as in Experiment 1, but with 50 %, 100 %, 150 % and 250 % of air saturated water, respectively. The heating rate was eating rate of 0.36 ± 0.01°C per minute. Tests were performed over a period of two days, 3-4 days after the last $\dot{M}O_2$ measurements. Due to previously conducted $\dot{M}O_2$measurements there was a variable number of fish in each aquarium, and thus the group size of each trial varied between 5 and 8 fish. The fish that had been through $\dot{M}O_2$ were tested with the same oxygen treatments as the metabolism measurements. For practical reasons hypoxia and normoxia trials were conducted in the same heating tank and another tank were used for hyperoxia treatments. That way, hyperoxia trials could be performed simultaneously as either hypoxia or normoxia trials. Heating tanks were switched between days to correct for any effect of tank.

After CT_{max} fish were transferred to containers with 100% air saturated water. Two fish died from CT_{max}, and survival was considered a criterium for including fish in later analyses. Fish were anesthetized using buffered MS-222 in water (3 ml:90 ml) before measuring length and weight to the nearest 10-2 g and 10-1 mm, respectively. Afterwards, the fish was euthanized.

2.3 Statistical analyses

All statistical analyses were conducted in R 3.6.1 (Team, 2013). Linear mixed-effects models were fitted using the "lmer()" function in the lmerTest package (Kuznetsova, Brockhoff, & Christensen, 2015). Linear models were fitted for models where no random effects were included using the "lm()" function in the stats package (Team, 2013). P-values lower than 0.05 were considered statistically significant for the effect factors, and P-values lower than 0.15 were considered significant for interactions. Model selection was performed by fitting models with maximum likelihood (ML) and ranking them according to Aikaike Information Criterion adjusted for sample size (AICc) using the "model.sel()" function in the MuMIn package (Barton & Barton, 2015). In cases where models were ranked equally high (ΔAICc < 2), models were refitted with REML and the simplest model was chosen unless additional terms had a significant effect or notably improved the amount of variation explained by the model (R^2, "r.squaredGLMM()" function in the MuMIn package)(Barton & Barton, 2015). Residuals were plotted and visually examined using the "plot_model()" function from the sjPlot package with "type="diag"" (Lüdecke & Lüdecke, 2015).

All plots were created using the "ggplot()" function in the ggplot2 package (Wickham, 2011) with estimates predicted from the respective models using the "ggpredict()" function in the ggeffects package (Lüdecke, Aust, Crawley, & Ben-Shachar, 2020). All values are reported as mean ± SE.

2.3.1 Experiment 1

In the first experiment data on CT_{max}, acclimation temperature, level of oxygen, length and weight were recorded for individual fish, in addition to which day the fish were tested and the tank (aquarium the fish came from) and batch (group of fish tested together). Heating rate was also measured for each trial. To test whether CT_{max} were affected by acclimation temperature and level of oxygen, data was analysed using a linear mixed-effects model, considering CT_{max} as a response to the fixed effects level of oxygen and acclimation temperature. To investigate whether the effect of oxygen was dependent on acclimation temperature an interaction term was also included. Both tank and batch are factors that might cause dependency in the data. To account for these effects a nested tank/batch structure was included as random effects. A Pearson correlation test showed that length and weight were highly correlated (r=0.84). Because length was only recorded for 2/3 of the fish, whereas weight was recorded for all fish, weight was used in the analyses. Possible effects of fish size and heating rate were accounted for by comparing models with and without these variables as fixed factors.

2.3.2 Experiment 2

For the oxygen consumption measurements of $\dot{M}O_{2,routine}$ and $\dot{M}O_{2,max}$, oxygen treatment, individual length and weight, total weight for each batch, tank, date and daily order of test were recorded. In addition, mean temperature in the holding aquarium was calculated, and aerobic scope (AS) was calculated as the difference between maximum and $\dot{M}O_{2,routine}$. To test whether environmental hypoxia and hyperoxia treatments could affect AS, that is, if $\dot{M}O_{2,max}$ and/or $\dot{M}O_{2,routine}$ differed between oxygen treatments, data were analysed using linear models. Effects of weight, temperature differences in the respirometer during the tests, holding tank, order and interactions between oxygen and temperature, and weight and temperature were controlled for by comparing models with different fixed factors and interactions included.

For CT_{max} the same variables as in Experiment 1 were recorded. It was also recorded whether fish had been through metabolism measurements prior to the CT_{max} test. To test the effect of the different levels of oxygen on CT_{max}, data was analysed using linear mixed-effects models,

considering CT_{max} as a response to oxygen and a nested tank/batch random effect structure. To account for any effects of previously conducted metabolism measurements, length or weight, models with different fixed factors, fitted with ML, were compared based on AICc.

2.3.3 Combined results of CT_{max} of fish acclimated to 20 °C

Data on CT_{max} from Experiment 1 and 2 on fish acclimated to 20 °C were combined an analysed in a separate linear mixed-effects model with a nested tank/batch random effect structure. For this analysis oxygen was considered a continuous variable. Any differences between the two experiments, differences in heating rates and effects of being tested for $\dot{M}O_2$ was accounted for by comparing models with different fixed factor based on AICc. Because there seemed to be a curved relationship between oxygen and CT_{max}, models with and without a quadratic oxygen term were also compared.

3 Results

3.1 Experiment 1

Acclimation temperature had a significant effect on CT_{max}, with fish generally having a 2.8 ± 0.3 °C (mean±S.E.) lower CT_{max} when acclimated to 20 °C (t= -9.27, p<0.001), and a 1.5 ± 0.3 °C higher CT_{max} when acclimated to 34 °C (t = 5.07, p<0.001) compared to fish acclimated to 28 °C (Figure 3.1). Moreover, fish exposed to hypoxia during CT_{max} tests generally had a lower CT_{max} (1.3 ± 0.3 °C, t = -7.55, p<0.001). On the contrary, hyperoxia did not have an effect (0.17 ± 0.3 °C, t =1.0, p=0.57). No significant interaction between acclimation temperature and oxygen was found (t ϵ [-0.53, 0.98], p>0.34 for all possible interactions). Neither weight nor heating rate seemed to influence CT_{max} in this experiment (ΔAIC<2, $\Delta R^2 \approx 0$, Appendix Table C.1).

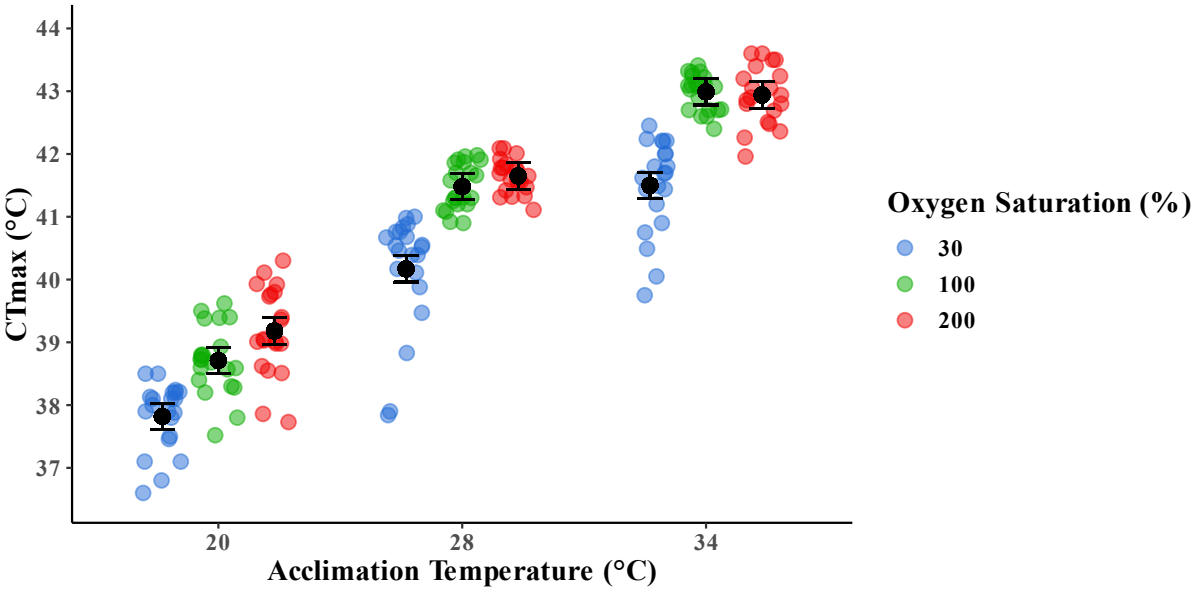


Figure 3.1 CT_{max} of Zebrafish (*Danio rerio*) acclimated to three different temperatures (20, 28 and 34 °C) tested at three levels of oxygen. Blue: 30 % oxygen of air saturated water, green:100 % oxygen and red:200 % oxygen. Black dots are predicted CT_{max} at the respective acclimation temperatures and oxygen levels. Bars are standard errors.

3.2 Experiment 2

In the second experiment effect of oxygen, mainly hyperoxia, on upper thermal tolerance, and links to aerobic scope were investigated. To test the effect of oxygen availability on metabolic rates, fish acclimated to 20 °C were acutely exposed to either 50, 100, 150 or 250 % of air saturated water. $\dot{M}O_{2,routine}$ for fish exposed to 100 % air saturated water was 0.44 ± 0.05 mg

O_2 h^{-1} g^{-1}, and was not significantly different from the other oxygen treatments, although fish exposed to 250 % of air saturated water had slightly higher $\dot{M}O_{2,routine}$ (t = 1.8, p = 0.10, Table 3.1, Figure 3.2A). $\dot{M}O_{2,max}$ for fish exposed to 100 % air saturated water was 1.39 ± 0.08 mg O_2 h^{-1} g^{-1}, $\dot{M}O_{2,max}$ of fish exposed to 50 % oxygen saturated water was not significantly lower (t=-0.87, p=0.40). The hyperoxia treatments significantly increased $\dot{M}O_{2,max}$ with 39 % (t = 4.67, p<0.001) and 63 % (t = 6.58, p<0.001) for 150 % and 250 % air saturated water respectively. As a result of this, aerobic scope increased with level of oxygen saturation. Aerobic scope was 0.94 ± 0.07 mg O_2 h^{-1} g^{-1} for fish exposed to 100 % air saturated water, 21 % lower for fish exposed to hypoxia even though the difference was not significant (t=-1.98, p=0.08), and 68 % (t=5.61, p<0.001) and 80 % (t=6.21, p<0.001) higher for fish exposed to 150 % and 250 % air saturated water, respectively. Small temperature differences in the respirometer during measurements seemed to significantly affect $\dot{M}O_{2,routine}$ and aerobic scope, and total weight seemed to affect $\dot{M}O_{2,max}$ and aerobic scope. Neither holding tank, day nor order seemed to have any effect on $\dot{M}O_2$ (ΔAICc > 2, Appendix Table C.2, Table C.4)

Table 3.1 Parameter estimates with standard errors from linear and linear mixed effect models explaining variation in $\dot{M}O_{2,routine}$, $\dot{M}O_{2,max}$, aerobic scope and CT_{max}. Estimates significantly different from 100 % with a significance level of p< 0.05 are indicated with a *.

Oxygen treatment	$\dot{M}O_{2,routine}$	$\dot{M}O_{2,max}$	Aerobic scope	CT_{max}
50 %	0.51 ± 0.05	1.29 ± 0.08	0.74 ± 0.08	38.84 ± 0.15
100 %	0.44 ± 0.05	1.39 ± 0.08	0.94 ± 0.07	38.86 ± 0.14
150 %	0.41 ± 0.05	1.93 ± 0.09 *	1.58 ± 0.09 *	39.77 ± 0.15 *
250 %	0.57 ± 0.05	2.26 ± 0.10 *	1.69 ± 0.09 *	39.28 ± 0.14

As in the first experiment CT_{max} seemed to be affected by the water oxygen level in the second experiment (Table 3.1, Figure 3.2B). However, the effect of 50 % oxygen was not significant (t=1.65, p=0.12). Fish exposed to 150 % saturation had a 0.6 ± 0.2 ℃ higher CT_{max} compared to fish exposed to 100 % air saturated water (t=3.21, p= 0.006). The effect of 250 % oxygen was on the other hand smaller and not significant (0.22 ± 0.2 ℃, t=1.12, p=0.29). Weight did not seem to affect CT_{max}. A model with heating rate was preferred (ΔAICc>2, Appendix Table C.5), and there were indications of heating rate being an important factor (-17.2 ± 8.5 ℃ per 1 ℃ increase in heating rate, t = -2.03, p = 0.06).

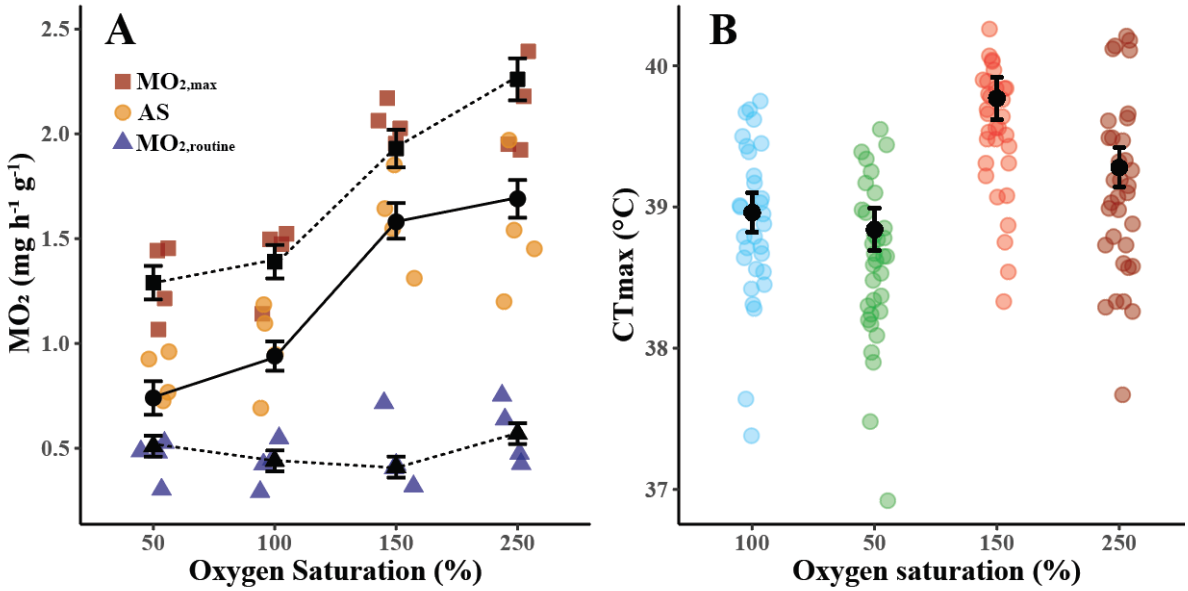


Figure 3.2 Results from Experiment 2 on zebrafish (*Danio rerio*) acclimated to 20 °C tested at four different oxygen saturations (50,100…). **A** Each datapoint is mean mass specific oxygen consumption rates ($\dot{M}O_2$) for a group of fish (n=3). Red squares are maximum consumption rates ($\dot{M}O_{2,max}$); blue triangles are routine oxygen consumption rates ($\dot{M}O_{2,routine}$); and yellow dots are aerobic scope (AS). $\dot{M}O_2$ estimates are adjusted for a mean group weight of 0.90 g. **B** Each dot represents CT_{max} of individual fish. Black symbols are predicted values at de respective oxygen saturations from the models and bars are standard error.

3.3 Combined results for the two experiments

Lastly, data on CT_{max} from Experiment 1 and 2 on fish acclimated to 20 ℃ were combined. A model with a quadratic oxygen term was preferred over a model without a quadratic term (ΔAICc = 19.5, Appendix Table C.6). CTmax increased with level of water oxygen saturation until water oxygen saturation was approximately 150 – 200 % before it declined (Figure3.3). Generally, fish had a 0.44 ± 0.15 ℃ higher CTmax in the second experiment (t = 2.93, p = 0.007) and being previously tested for $\dot{M}O_2$ decreased CTmax with 0.34 ± 0.16 ℃ (t = -2.18, p = 0.04). Weight and heating rate did not seem to have any effect on CTmax (ΔAICc > 2, Appendix Table C.6).

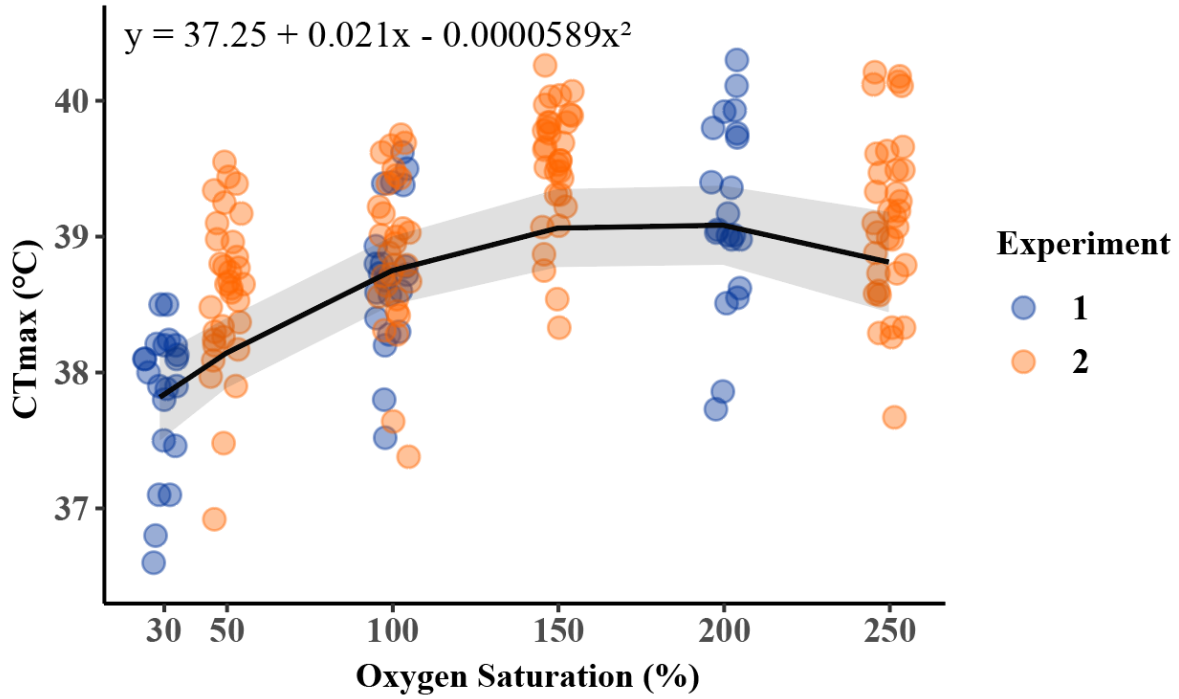


Figure 3.3 CT_{max} of zebrafish (*Danio rerio*) acclimated to 20 ℃ for both experiments combined (Experiment 1: blue, Experiment 2: orange). The black line is predicted values from a linear mixed-effects model and the grey area is 95 % confidence intervals. Estimates are adjusted for Experiment 1 and fish not tested for $\dot{M}O_2$. Datapoints represent individual fish.

4 Discussion

4.1 Effect of ambient oxygen levels on aerobic Scope

In this study, no significant effect of hyperoxia on $\dot{M}O_{2,routine}$ was found. This is consistent with the findings of McArley et al. (2021) who found that out of 22 studies examining the effect of hyperoxia on either $\dot{M}O_{2,min}$ or $\dot{M}O_{2,routine}$ all found either no change or an increase in $\dot{M}O_{2,routine}$ during exposure to hyperoxia. Although not significant, the data indicated a slightly higher $\dot{M}O_{2,routine}$in fish exposed to 250 % air sat. Similarly fish exposed to hypoxia in this experiment seemed to have an unchanged or slightly, non-significantly increased $\dot{M}O_{2,routine}$. Generally, hypoxia was expected to either not change or decrease $\dot{M}O_{2,routine}$ (McBryan, Anttila, Healy, & Schulte, 2013; Norin & Clark, 2016; Wang, Lefevre, van Cong, & Bayley, 2009). As $\dot{M}O_{2,routine}$ was measured the first 30 minutes after transfer to the respirometer from 100 % air saturated water the observed differences might be due to stress caused by the change in environment, and the transfer to 50 % and 250 % of air saturated water being more stressful to the fish. However as the potential differences were small, the fish was most like not highly stressed during measurements.

Contrary to resting values of $\dot{M}O_{2,routine}$, $\dot{M}O_{2,max}$ values are expected to be sensitive to reduction in oxygen availability (Wang et al., 2009), and can decrease in parallel with oxygen availability such that increasing levels of hypoxia gradually reduce $\dot{M}O_{2,max}$ (Ern et al., 2016; Norin & Clark, 2016). Although a slightly lower $\dot{M}O_{2,max}$ was observed for fish exposed to hypoxia, the effect was not significant. However, zebrafish are likely to experience low oxygen levels in their natural warm and tropical environmentsand are hypoxia tolerant (Roesner, Hankeln, & Burmester, 2006; Wells, 2009), and acute hypoxia (approximately 1 h exposure) have been shown to increase haemoglobin O_2 affinity (Cadiz, Bundgaard, Malte, & Fago, 2019). Therefore, it appears zebrafish can sustain a high $\dot{M}O_{2,max}$ when exposed to 50 % air saturated water.

In normoxia, the haemoglobins in the blood flowing through the gills are generally assumed to be fully saturated (Wells, 2009). As the majority of oxygen transported in fish blood is bound by haemoglobin, it may seem counterintuitive that $\dot{M}O_{2,max}$ and thereby aerobic scope can increase when exposed to hyperoxia (Brijs et al., 2015). However, consistent with my findings, three studies out of out of six studies reviewed by McArley et al. (2021) found an increase in

$\dot{M}O_{2,max}$ when fish were exposed to hyperoxia, and the remaining studies found no effect. The largest effect was found in European perch where fish exposed to 200 % of air saturated water exhibited an aerobic scope that was 90 % higher than control group, and the difference in aerobic scope was caused by an increase in $\dot{M}O_{2,max}$ alone (Brijs et al., 2015). Thus, an aerobic scope that is 64 % and 80 % higher compared to fish exposed to normoxia for fish exposed to 150 % and 250 % of air saturated water seems in line with these previous reports.

4.2 The effect of oxygen on CT_{max}

Compatible with the OCLTT-hypothesis, an effect of hypoxia (30 % air saturated water) on thermal tolerance was observed in Experiment 1. This is in accordance with several previous studies (Healy & Schulte, 2012; Islam et al., 2020; Rutledge & Beitinger, 1989). However, no effect of 50 % air saturation on thermal tolerance was found in Experiment 2. Ern et al. (2016) showed that despite of substantial reductions in aerobic scope, upper thermal tolerance was independent of oxygen availability over a wide range of oxygen levels before declining sharply with water oxygen tension for red drum (*Sciaenops ocellatus*) and lumpfish (*Cyclopterus lumpus*). The same pattern was found in an additional experiment on three stenothermal species (Ern, Johansen, Rummer, & Esbaugh, 2017). Because aerobic scope was considerably reduced before CT_{max} was affected in these studies, there is a possibility that the thermal tolerance of water breathing ectotherms generally is dependent on oxygen only until aerobic scope reach a certain magnitude, where other physiological ceilings might limit CT_{max} (Sandblom et al., 2016). Oxygen independence of CT_{max} is however most likely not the explanation behind the lack of effect of hypoxia in this experiment, as CT_{max} increased in hyperoxia at the same temperature. The high CT_{max} at 50 % saturation is therefore probably rather explained by the relatively high aerobic scope observed at this oxygen level.

It seems that hyperoxia can improve heat tolerance for some species, but not others (McArley et al., 2021). For example, no effect of hyperoxia on thermal tolerance have been found in red shiner (*Notropis lutrensis*), blackstripe topminnow (*Fundulus notatus*)(Rutledge & Beitinger, 1989), white- or red-blooded Antarctic Notothenioid fishes (Devor, Kuhn, O'Brien, & Crockett, 2016). In European perch (*Perca fluviatilis)* and in a triplefin (*Forsterygion lapillum*) CT_{max} increased with 1.1 ℃ and 0.43 ℃ respectively, when fish were exposed to 200 % air saturated water (Ekström et al., 2016; McArley, Hickey, & Herbert, 2018). However, hyperoxia seemed to have no effect on CT_{max} in a more thermally tolerant population of European perch exposed to decades of environmental warming (Brijs et al., 2015). In zebrafish larvae a lower CT_{max}

have been found for larvae exposed to hypoxia (60 % air saturated water) compared to larvae exposed to hyperoxia (150 % air saturated water), but CT_{max} was not tested at normoxia in this study (Andreassen et al., 2020). The study did however find that a brain-wide depolarization, taking place at slightly higher temperatures than CT_{max}, occurred at lower temperatures in hypoxia and higher temperatures in hyperoxia compared to normoxia indicating that oxygen availability can affect CT_{max} and related functions in zebrafish.

In Experiment 1 CT_{max} seemed to remain unchanged by hyperoxia for fish acclimated to 28 ℃ and 34 ℃, and this could be due to oxygen independence. However, in Experiment 2 150 % oxygen saturation improved thermal tolerance for fish acclimated to 20 ℃. There were also indications of an effect of hyperoxia for fish acclimated to 20 ℃ in Experiment 1. Thus, it seems like oxygen dependence of CT_{max} is not only species specific as stated by (Ern et al., 2016), but also dependent on acclimation temperature. Moreover, this provides a likely explanation of the contrasting findings by Brijs et al. (2015) and Ekström et al. (2016) in European perch, as the more thermally tolerant population also was acclimated to a 5 ℃ higher water temperature.

Another interesting observation from Experiment 2 is that not only did CT_{max} not increase further with increasing oxygen saturation from 150 % to 250 %, as could be explained by oxygen independence of CT_{max} at this oxygen level, but it declined. This reduction in CT_{max} might be due to harmful effects of high levels of oxygen. For example, McArley et al. (2021) propose that more reactive oxygen species (ROS) could be produced by tissues exposed to higher oxygen levels following hyperoxia and potentially result in oxidative stress. Hyperoxia is also often associated with a hypoventilatory response, that typically results in higher levels of arterial P_{CO2} and a lowering of arterial pH (Gilmour, 2001). It is possible that this also can have harmful effects and result in a small reduction in CT_{max}.

The heating rate for the two experiments was slightly higher than what has been recommended for zebrafish. The same procedure, equipment and amount of water was used as in Morgan et al. (2018), but covering the tank with a plastic plate most likely increased the heating rate slightly. In addition, the amount of cold air, oxygen and nitrogen added to the heating tank could also affect heating rate. Heating rate seemed to affect CT_{max} in the second, but not the first experiment. In addition, when data was combined, a model without heating rate was preferred. In the second experiment a higher heating rate seemed to decrease CT_{max} slightly. This is in contrast to what would have been expected, as several previous studies have reported

that a CT_{max} generally increase with faster heating rates (Allen, Chown, Janion-Scheepers, & Clusella-Trullas, 2016; Illing, Downie, Beghin, & Rummer, 2020; Rezende, Castañeda, & Santos, 2014), and also what was observed in Experiment 1 (even though the effect was not significant). As fish exposed to 50 % air saturated water had the lowest heating rate, a possibility is that the observed effect of heating rate was due to a correlation with oxygen treatment. However, including heating rate in the model did not change the results, so it was kept in the model. In addition, heating rate was generally higher for the second experiment compared to the first experiment. This could partly explain the higher CT_{max} in the second experiment.

4.3 Conclusion

In conclusion, I found that ambient oxygen levels can affect CT_{max} in zebrafish. CT_{max} was considerably reduced when fish were exposed to 30 % air saturated water. Hyperoxia however seemed to only affect CT_{max} for cold acclimated fish. As aerobic scope kept increasing with increasing level of ambient oxygen, upper thermal tolerance did not seem to be limited by a constrained oxygen supply for warm acclimated fish exposed to hyperoxia. Consequently, it seems like the effect of oxygen on upper thermal tolerance is dependent on acclimation temperature in this species. Thus, it seems like there are other physiological constraints limiting the upper thermal tolerance of warm acclimated zebrafish when oxygen availability is high.

5 Literature

Allen, J. L., Chown, S. L., Janion-Scheepers, C., & Clusella-Trullas, S. (2016). Interactions between rates of temperature change and acclimation affect latitudinal patterns of warming tolerance. *Conservation Physiology, 4*(1), cow053. doi:10.1093/conphys/cow053

Andreassen, A. H., Hall, P., Khatibzadeh, P., Jutfelt, F., & Kermen, F. (2020). *Neural dysfunction at the upper thermal limit in the zebrafish*. Cold Spring Harbor Laboratory. Retrieved from https://dx.doi.org/10.1101/2020.12.28.424529

Barton, K., & Barton, M. K. (2015). Package 'mumin'. *Version, 1*, 18.

Becker, C. D., & Genoway, R. G. (1979). Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology of Fishes, 4*(3), 245-256. doi:10.1007/bf00005481

Beitinger, T. L., Bennett, W. A., & McCauley, R. W. (2000). Temperature Tolerances of North American Freshwater Fishes Exposed to Dynamic Changes in Temperature. *Environmental Biology of Fishes, 58*(3), 237-275. doi:10.1023/a:1007676325825

Brijs, J., Jutfelt, F., Clark, T. D., Gräns, A., Ekström, A., & Sandblom, E. (2015). Experimental manipulations of tissue oxygen supply do not affect warming tolerance of European perch. *The Journal of Experimental Biology, 218*(15), 2448-2454. doi:10.1242/jeb.121889

Cadiz, L., Bundgaard, A., Malte, H., & Fago, A. (2019). Hypoxia enhances blood O2 affinity and depresses skeletal muscle O2 consumption in zebrafish (Danio rerio). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 234*, 18-25.

Clark, T. D., Sandblom, E., & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *The Journal of Experimental Biology, 216*(15), 2771-2782. doi:10.1242/jeb.084251

Cortemeglia, C., & Beitinger, T. L. (2005). Temperature Tolerances of Wild-Type and Red Transgenic Zebra Danios. *Transactions of the American Fisheries Society, 134*(6), 1431-1437. doi:10.1577/t04-197.1

Cowles, R. B., & Bogert, C. M. (1944). A preliminary study of the thermal requirements of desert reptiles. *Iguana, 83*, 53.

Crockford, T., & Johnston, I. A. (1990). Temperature acclimation and the expression of contractile protein isoforms in the skeletal muscles of the common carp (Cyprinus carpio L.). *Journal of Comparative Physiology B, 160*(1), 23-30.

Devor, D. P., Kuhn, D. E., O'Brien, K. M., & Crockett, E. L. (2016). Hyperoxia does not extend critical thermal maxima (CTmax) in white-or red-blooded Antarctic notothenioid fishes. *Physiological and Biochemical Zoology, 89*(1), 1-9.

Ekström, A., Brijs, J., Clark, T. D., Gräns, A., Jutfelt, F., & Sandblom, E. (2016). Cardiac oxygen limitation during an acute thermal challenge in the European perch: effects of chronic environmental warming and experimental hyperoxia. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 311*(2), R440-R449.

Ern, R., Johansen, J. L., Rummer, J. L., & Esbaugh, A. J. (2017). Effects of hypoxia and ocean acidification on the upper thermal niche boundaries of coral reef fishes. *Biology Letters, 13*(7), 20170135. doi:10.1098/rsbl.2017.0135

Ern, R., Norin, T., Gamperl, A. K., & Esbaugh, A. J. (2016). Oxygen dependence of upper thermal limits in fishes. *The Journal of Experimental Biology, 219*(21), 3376-3383. doi:10.1242/jeb.143495

Fangue, N. A., Todgham, A. E., & Schulte, P. M. (2021). Thermal Biology. In S. Currie & D. H. Evans (Eds.), *The Physiology of Fishes* (pp. 91-103). Boca Raton: CRC Press.

Fry, F. E. J., & Hart, J. S. (1948). THE RELATION OF TEMPERATURE TO OXYGEN CONSUMPTION IN THE GOLDFISH. *The Biological Bulletin, 94*(1), 66-77. doi:10.2307/1538211

Gilmour, K. M. (2001). The CO2/pH ventilatory drive in fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 130*(2), 219-240.

Grans, A., Jutfelt, F., Sandblom, E., Jonsson, E., Wiklander, K., Seth, H., . . . Axelsson, M. (2014). Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO2 in Atlantic halibut. *Journal of Experimental Biology, 217*(5), 711-717. doi:10.1242/jeb.096743

Hazel, J. R. (1995). Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annual review of physiology, 57*(1), 19-42.

Healy, T. M., & Schulte, P. M. (2012). Factors affecting plasticity in whole-organism thermal tolerance in common killifish (Fundulus heteroclitus). *Journal of Comparative Physiology B, 182*(1), 49-62. doi:10.1007/s00360-011-0595-x

ICES. (2021). Oceanographic Calculator. Retrieved from https://ocean.ices.dk/Tools/Calculator.aspx

Illing, B., Downie, A. T., Beghin, M., & Rummer, J. L. (2020). Critical thermal maxima of early life stages of three tropical fishes: Effects of rearing temperature and experimental heating rate. *Journal of Thermal Biology, 90*, 102582. doi:10.1016/j.jtherbio.2020.102582

IPCC. (2007). *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC)*. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Islam, S. M., Zahangir, M. M., Jannat, R., Hasan, M. N., Suchana, S. A., Rohani, M. F., & Shahjahan, M. (2020). Hypoxia reduced upper thermal limits causing cellular and nuclear abnormalities of erythrocytes in Nile tilapia, Oreochromis niloticus. *Journal of Thermal Biology, 90*, 102604. doi:10.1016/j.jtherbio.2020.102604

Jayasundara, N., & Somero, G. N. (2013). Physiological plasticity of cardiorespiratory function in a eurythermal marine teleost, the longjaw mudsucker, Gillichthys mirabilis. *The Journal of Experimental Biology, 216*(11), 2111-2121. doi:10.1242/jeb.083873

Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D. J., . . . Clark, T. D. (2018). Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. *The Journal of Experimental Biology, 221*(1), jeb169615. doi:10.1242/jeb.169615

Klaiman, J. M., Fenna, A. J., Shiels, H. A., Macri, J., & Gillis, T. E. (2011). Cardiac Remodeling in Fish: Strategies to Maintain Heart Function during Temperature Change. *PLoS ONE, 6*(9), e24464. doi:10.1371/journal.pone.0024464

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2015). Package 'lmertest'. *R package version, 2*(0).

Lagerspetz, K. Y. (2006). What is thermal acclimation? *Journal of Thermal Biology, 31*(4), 332-336.

López-Olmeda, J., & Sánchez-Vázquez, F. (2011). Thermal biology of zebrafish (Danio rerio). *Journal of Thermal Biology, 36*(2), 91-104.

Lüdecke, D., Aust, F., Crawley, S., & Ben-Shachar, M. (2020). Package 'ggeffects'. *Create Tidy Data Frames of Marginal Effects for "ggplot" from Model Outputs, 23*.

Lüdecke, D., & Lüdecke, M. D. (2015). Package 'sjPlot'. In.

McArley, T. J., Hickey, A. J., & Herbert, N. A. (2018). Hyperoxia increases maximum oxygen consumption and aerobic scope of intertidal fish facing acutely high temperatures. *Journal of Experimental Biology, 221*(22).

McArley, T. J., Sandblom, E., & Herbert, N. A. (2021). Fish and hyperoxia—From cardiorespiratory and biochemical adjustments to aquaculture and ecophysiology implications. *Fish and Fisheries, 22*(2), 324-355. doi:10.1111/faf.12522

McBryan, T. L., Anttila, K., Healy, T. M., & Schulte, P. M. (2013). Responses to Temperature and Hypoxia as Interacting Stressors in Fish: Implications for Adaptation to Environmental Change. *Integrative and Comparative Biology, 53*(4), 648-659. doi:10.1093/icb/ict066

Metcalfe, N. B., Van Leeuwen, T. E., & Killen, S. S. (2016). Does individual variation in metabolic phenotype predict fish behaviour and performance? *Journal of Fish Biology, 88*(1), 298-321. doi:10.1111/jfb.12699

Morgan, R., Andreassen, A. H., Asheim, E. R., Finnøen, M. H., Dresler, G., Brembu, T., . . . Jutfelt, F. (Unpublished manuscript). Reduced physiological plasticity in a fish adapted to stable conditions.

Morgan, R., Finnøen, M. H., Jensen, H., Pélabon, C., & Jutfelt, F. (2020). Low potential for evolutionary rescue from climate change in a tropical fish. *Proceedings of the National Academy of Sciences, 117*(52), 33365-33372. doi:10.1073/pnas.2011419117

Morgan, R., Finnøen, M. H., & Jutfelt, F. (2018). CT max is repeatable and doesn't reduce growth in zebrafish. *Scientific Reports, 8*(1), 1-8.

Morgan, R., Sundin, J., Finnøen, M. H., Dresler, G., Vendrell, M. M., Dey, A., . . . Jutfelt, F. (2019). Are model organisms representative for climate change research? Testing thermal tolerance in wild and laboratory zebrafish populations. *Conservation Physiology, 7*(1). doi:10.1093/conphys/coz036

Norin, T., & Clark, T. D. (2016). Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology, 88*(1), 122-151. doi:10.1111/jfb.12796

Norin, T., Malte, H., & Clark, T. D. (2014). Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *Journal of Experimental Biology, 217*(2), 244-251. doi:10.1242/jeb.089755

O'Brien, K. M. (2011). Mitochondrial biogenesis in cold-bodied fishes. *Journal of Experimental Biology, 214*(2), 275-285. doi:10.1242/jeb.046854

Portner, H. O., & Farrell, A. P. (2008). ECOLOGY: Physiology and Climate Change. *Science, 322*(5902), 690-692. doi:10.1126/science.1163156

Pörtner, H.-O., Bock, C., & Mark, F. C. (2017). Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. *The Journal of Experimental Biology, 220*(15), 2685-2696. doi:10.1242/jeb.134585

Rezende, E. L., Castañeda, L. E., & Santos, M. (2014). Tolerance landscapes in thermal ecology. *Functional Ecology, 28*(4), 799-809. doi:10.1111/1365-2435.12268

Roesner, A., Hankeln, T., & Burmester, T. (2006). Hypoxia induces a complex response of globin expression in zebrafish(Danio rerio). *Journal of Experimental Biology, 209*(11), 2129-2137. doi:10.1242/jeb.02243

Rutledge, C. J., & Beitinger, T. L. (1989). The effects of dissolved oxygen and aquatic surface respiration on the critical thermal maxima of three intermittent-stream fishes. *Environmental Biology of Fishes, 24*(2), 137-143. doi:10.1007/bf00001283

Sandblom, E., Clark, T. D., Gräns, A., Ekström, A., Brijs, J., Sundström, L. F., . . . Jutfelt, F. (2016). Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nature Communications, 7*(1), 11447. doi:10.1038/ncomms11447

Schaefer, J., & Ryan, A. (2006). Developmental plasticity in the thermal tolerance of zebrafish Danio rerio. *Journal of fish biology, 69*(3), 722-734. doi:10.1111/j.1095-8649.2006.01145.x

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology, 218*(12), 1856-1866. doi:10.1242/jeb.118851

Seebacher, F., Martin, Paul, Guderley, H., Anthony, & Christopher. (2010). Plasticity of Oxidative Metabolism in Variable Climates: Molecular Mechanisms. *Physiological and Biochemical Zoology, 83*(5), 721-732. doi:10.1086/649964

Spence, R., Fatema, M. K., Reichard, M., Huq, K. A., Wahab, M. A., Ahmed, Z. F., & Smith, C. (2006). The distribution and habitat preferences of the zebrafish in Bangladesh. *Journal of Fish Biology, 69*(5), 1435-1448. doi:https://doi.org/10.1111/j.1095-8649.2006.01206.x

Spence, R., Gerlach, G., Lawrence, C., & Smith, C. (2008). The behaviour and ecology of the zebrafish, Danio rerio. *Biological reviews, 83*(1), 13-34.

Sundin, J., Morgan, R., Finnøen, M. H., Dey, A., Sarkar, K., & Jutfelt, F. (2019). On the Observation of Wild Zebrafish (Danio rerio) in India. *Zebrafish, 16*(6), 546-553. doi:10.1089/zeb.2019.1778

Team, R. C. (2013). R: A language and environment for statistical computing.

Verberk, W. C., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L., & Terblanche, J. S. (2016). Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 192*, 64-78.

Vornanen, M., Shiels, H. A., & Farrell, A. P. (2002). Plasticity of excitation–contraction coupling in fish cardiac myocytes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 132*(4), 827-846. doi:10.1016/s1095-6433(02)00051-x

Wang, T., Lefevre, S., van Cong, N., & Bayley, M. (2009). The effects of hypoxia on growth and digestion. In *Fish physiology* (Vol. 27, pp. 361-396): Elsevier.

Wells, R. M. (2009). Blood-gas transport and hemoglobin function: Adaptations for functional and environmental hypoxia. In *Fish physiology* (Vol. 27, pp. 255-299): Elsevier.

Wickham, H. (2011). ggplot2. *Wiley Interdisciplinary Reviews: Computational Statistics, 3*(2), 180-185.

Appendix A: Water temperature during acclimation periods

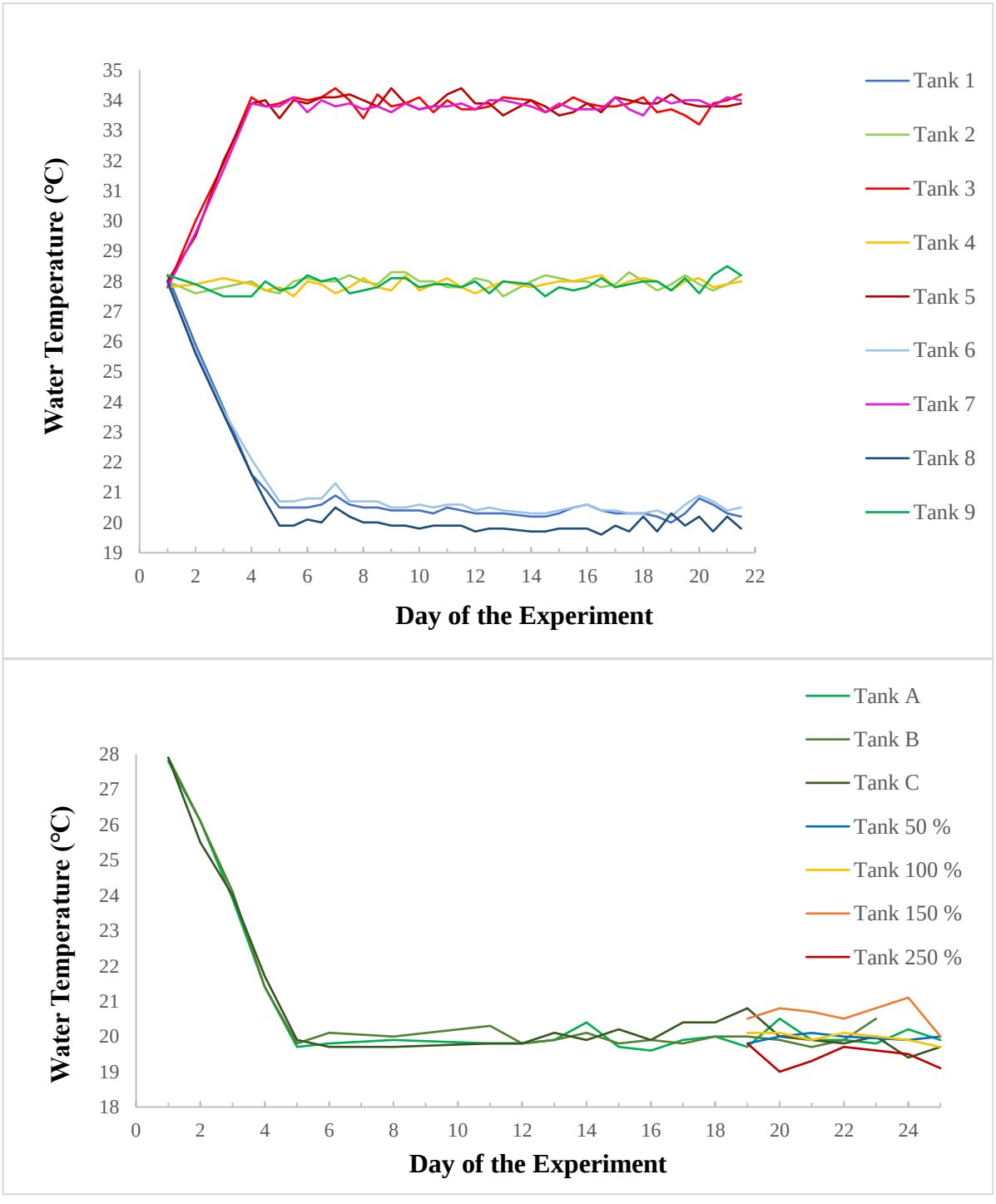


Figure A.1 Water temperature in the aquaria during the acclimation period for Experiment 1 (top panel) and 2 (bottom panel).

Appendix B: Heating rates during CT_{max} measurements

Table B.1 Mean heating rates with standard deviations during CT_{max} measurements for different treatment groups in Experiment 1.

Treatment	Heating rate (°C/min)	SD
Temp. = 20 °C	0.37	0.02
Temp. = 28 °C	0.35	0.02
Temp. = 34 °C	0.33	0.01
O_2 = 30 %	0.35	0.02
O_2 = 100 %	0.33	0.02
O_2 = 200 %	0.36	0.02

Table B.2 Mean heating rates with standard deviations during CT_{max} measurements for different oxygen treatments in Experiment 2.

Treatment	Heating rate (°C/min)	SD
O_2 = 50 %	0.37	0.01
O_2 = 100 %	0.35	0.01
O_2 = 150 %	0.36	0.01
O_2 = 250 %	0.36	0.00

Appendix C: Model selection tables

Table C.1 Model selection table for linear mixed effect models explaining variation in CT_{max} in zebrafish (*Danio rerio*). R^2_m and R^2_c are marginal and conditional R^2s, that is, the amount for variation explained by fixed effects, and both fixed and random effects, respectively. T = acclimation temperature, O = oxygen, BM = body mass and HR = heating rate. The models are ranked by decreasing ΔAIC_c values. df = degrees of freedom. w_i = Akaike weights. Interactions between predictor variables are denoted by (:).

Model	df	ΔAICc	w_i	R^2_m	R^2_c
CT_{max} = T + O + T:O + HR	13	0	0.46	0.90	0.92
CT_{max} = T + O + T:O	12	0.59	0.34	0.89	0.92
CT_{max} = T + O + T:O + BM + HR	14	1.74	0.19	0.90	0.92

Table C.2 Model selection table for linear models explaining variation in $\dot{M}O_{2,routine}$ in zebrafish (*Danio rerio*). R^2 values are adjusted for the number of predictors in the models. T = average temperature, O = oxygen, TBM = total body mass, TA = aquarium, D = day and OR = order (time of day). The models are ranked by decreasing ΔAIC_c values. df = degrees of freedom. w_i = Akaike weights. Interactions between predictor variables are denoted by (:).

Model	df	ΔAICc	w_i	$R^2_{adj.}$
$\dot{M}O_{2,routine}$ = O + T	6	0	0.82	0.41
$\dot{M}O_{2,routine}$ = O	5	4.93	0.07	-0.02
$\dot{M}O_{2,routine}$ = O + T + D	7	5.47	0.05	0.40
$\dot{M}O_{2,routine}$ = O + T + OR	7	6.60	0.03	0.36
$\dot{M}O_{2,routine}$ = O + TBM + T	7	6.63	0.03	0.36
$\dot{M}O_{2,routine}$ = O + T + D + OR	8	13.88	0	0.34
$\dot{M}O_{2,routine}$ = O + TBM + T + D	8	14.04	0	0.34
$\dot{M}O_{2,routine}$ = O + TBM + T + OR	8	15.15	0	0.29
$\dot{M}O_{2,routine}$ = O + TBM + D + OR	8	20.71	0	-0.01
$\dot{M}O_{2,routine}$ = O + TBM + T + D + OR	9	25.29	0	0.26
$\dot{M}O_{2,routine}$ = O + T + TA + D + OR	10	40.65	0	0.19
$\dot{M}O_{2,routine}$ = O + TBM + T + TA + D	10	40.87	0	0.18
$\dot{M}O_{2,routine}$ = O + TBM + T + TA + OR	10	41.63	0	0.14
$\dot{M}O_{2,routine}$ = O + TBM + TA + D + OR	10	46.89	0	-0.20
$\dot{M}O_{2,routine}$ = O + TBM + T + TA + D + OR	11	64.56	0	0.06
$\dot{M}O_{2,routine}$ = O + TBM + T + TA + D + OR + O:TBM	14	389.62	0	0.79
$\dot{M}O_{2,routine}$ = O + TBM + T + TA + D + OR + O:T	14	415.62	0	-0.08

Table C.3 Model selection table for linear models explaining variation in $\dot{M}O_{2,max}$ in zebrafish (*Danio rerio*). T = average temperature, O = oxygen, TBM = total body mass, TA = aquarium, D = day and OR = order (time of day). R^2 values are adjusted for the number of predictors in the models. The models are ranked by decreasing ΔAIC_c values. df = degrees of freedom. w_i = Akaike weights. Interactions between predictor variables are denoted by (:).

Model	**df**	**ΔAICc**	w_i	$R^2_{adj.}$
$\dot{M}O_{2,max}$ = O + TBM	7	0	0.58	0.86
$\dot{M}O_{2,max}$ = O + TBM + T	6	1.41	0.29	0.89
$\dot{M}O_{2,max}$ = O + T	6	3.01	0.13	0.83
$\dot{M}O_{2,max}$ = O + TBM + T + OR	8	9.61	0.01	0.88
$\dot{M}O_{2,max}$ = O + TBM + T + D	8	9.83	0	0.88
$\dot{M}O_{2,max}$ = O + TBM + D + OR	8	12.15	0	0.86
$\dot{M}O_{2,max}$ = O + T + D + OR	8	16.51	0	0.82
$\dot{M}O_{2,max}$ = O + TBM + T + TA + OR	9	20.71	0	0.87
$\dot{M}O_{2,max}$ = O + TBM + T + TA	10	35.64	0	0.86
$\dot{M}O_{2,max}$ = O + TBM + T + TA + D	10	35.65	0	0.86
$\dot{M}O_{2,max}$ = O + TBM + TA + D + OR	10	39.43	0	0.82
$\dot{M}O_{2,max}$ = O + T + TA + D + OR	10	43.14	0	0.78
$\dot{M}O_{2,max}$ = O + TBM + T + TA + D + OR	11	59.63	0	0.84
$\dot{M}O_{2,max}$ = O + TBM + T + TA + D + OR + O:T	14	416.24	0	0.74
$\dot{M}O_{2,max}$ = O + TBM + T + TA + D + OR + O:TBM	14	4.17.62	0	0.71

Table C.4 Model selection table for linear models explaining variation in aerobic scope in zebrafish (*Danio rerio*). R^2 values are adjusted for the number of predictors in the models. AS = aerobic scope, T = average temperature, O = oxygen, TBM = total body mass, TA = aquarium, D = day and OR = order (time of day). The models are ranked by decreasing ΔAIC_c values. df = degrees of freedom. w_i = Akaike weights. Interactions between predictor variables are denoted by (:).

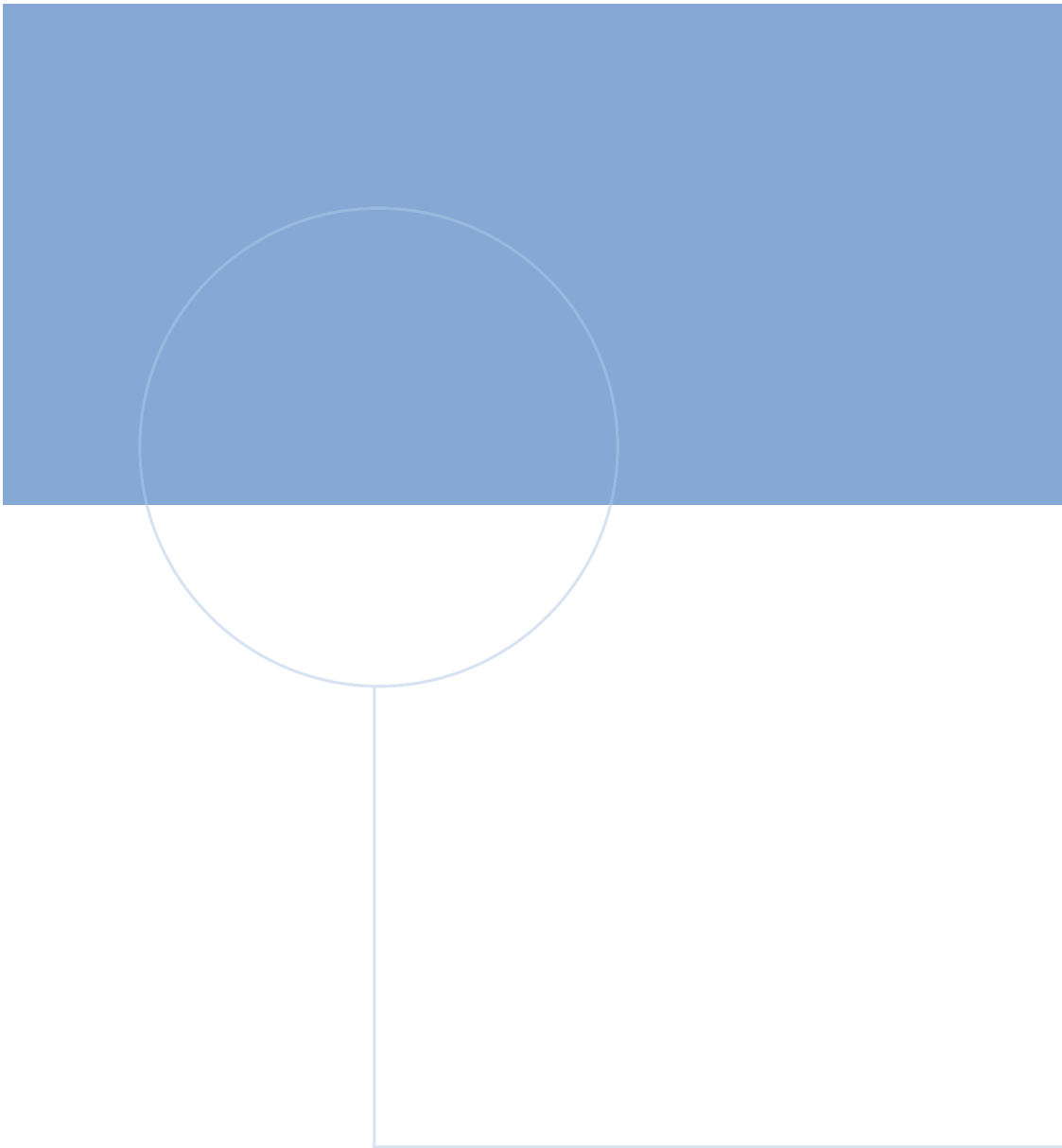
Model	df	ΔAICc	w_i	$R^2_{adj.}$
AS = O + TBM + T	7	0	0.51	0.87
AS = O + T	6	0.23	0.46	0.82
AS = O + TBM + T + D	8	7.04	0.02	0.87
AS = O + TBM + T + OR	8	8.35	0.01	0.86
AS = O + TBM	6	9.17	0	0.68
AS = O + T + D + OR	8	12.29	0	0.82
AS = O + TBM + D + OR	8	17.53	0	0.75
AS = O + TBM + T + D + OR	9	17.76	0	0.86
AS = O + TBM + T + TA + D	10	31.94	0	0.86
AS = O + TBM + T + TA + OR	10	32.76	0	0.85
AS = O + T + TA + D + OR	10	37.93	0	0.79
AS = O + TBM + TA + D + OR	10	44.35	0	0.69
AS = O + TBM + T + TA + D + OR	11	55.83	0	0.83
AS = O + TBM + T + TA + D + OR + O:TBM	14	408.81	0	0.78
AS = O + TBM + T + TA + D + OR + O:T	14	409.00	0	0.78

Table C.5 Model selection table for linear mixed effect models explaining variation in CT_{max} in zebrafish (*Danio rerio*) acclimated to 20 °C. R^2_m and R^2_c are marginal and conditional R^2s, that is, the amount for variation explained by fixed effects, and both fixed and random effects, respectively. O = oxygen, BM = body mass, TE= tested for $\dot{M}O_2$, and HR = heating rate. The models are ranked by decreasing ΔAIC_c values. df = degrees of freedom. w_i = Akaike weights.

Model	df	ΔAICc	w_i	R^2_m	R^2_c
CT_{max} = O + TE + HR	9	0	0.52	0.37	0.47
CT_{max} = O + TE + HR + BM	10	0.94	0.32	0.37	0.47
CT_{max} = O + TE	8	2.72	0.13	0.33	0.47
CT_{max} = O	7	6.65	0.02	0.10	0.47
CT_{max} = O + HR	8	8.88	0.01	0.27	0.48

Table C.6 Model selection table for linear mixed effect models explaining variation in CT_{max} in zebrafish (*Danio rerio*) acclimated to 20 ℃ from Experiment 1 and 2 combined. R^2_m and R^2_c are marginal and conditional R^2s, that is, the amount for variation explained by fixed effects, and both fixed and random effects, respectively. O = oxygen, E = experiment, BM = body mass, TE= tested for $\dot{M}O_2$ and HR = heating rate. The models are ranked by decreasing ΔAIC_c values. df = degrees of freedom. w_i = Akaike weights.

Model	**df**	**ΔAICc**	w_i	R^2_m	R^2_c
$CT_{max} = O + O^2 + E + TE$	8	0	0.58	0.42	0.56
$CT_{max} = O + O^2 + E + HR + TE$	9	2.10	0.20	0.42	0.56
$CT_{max} = O + O^2 + E$	7	3.03	0.13	0.39	0.56
$CT_{max} = O + O^2 + E + HR + TE + BM$	10	4.26	0.07	0.42	0.56
$CT_{max} = O + O^2 + TE$	7	6.47	0.02	0.35	0.55
$CT_{max} = O + E + HR + TE + BM$	9	19.45	0	0.28	0.57



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