Sondre Kristenstuen

The effects of oxygen availability and temperature on appetite, growth and aerobic scope in zebrafish (*Danio rerio*)

Master's thesis in Biology
Supervisor: Fredrik Jutfelt
Co-supervisor: Anna H. Andreassen
May 2022

Master's thesis

NTNU
Norwegian University of Science and Technology
Department of Biology

NTNU
Norwegian University of
Science and Technology

Sondre Kristenstuen

The effects of oxygen availability and temperature on appetite, growth and aerobic scope in zebrafish (*Danio rerio*)

Master's thesis in Biology
Supervisor: Fredrik Jutfelt
Co-supervisor: Anna H. Andreassen
May 2022

Norwegian University of Science and Technology
Department of Biology

NTNU
Norwegian University of
Science and Technology

ABSTRACT

Species diversity and abundance are currently being threatened by extreme heat waves in a world of drastic climate change. Temperature has a dramatic effect on the biology of ectothermic animals, with fishes being directly affected by changes in water temperature. A variety of present hypotheses concerns the ability of fishes to supply tissue with oxygen with increasing temperature. Results from previous studies highlights the mechanistical constraint in capacity to deliver oxygen with increasingly supra-optimal temperatures, leading to reduced growth. In this study, we investigated whether ambient oxygen level (50 %, 100 % and 200 % air saturation) is a limiting factor for appetite, growth and aerobic scope in zebrafish acclimated to 20 °C and 34 °C. We hypothesized that hypoxia would reduce and hyperoxia increase these performances with a greater effect in warm acclimation, as metabolism and O_2-requirement are accelerated with increasing temperature. We found hyperoxia leading to reduced appetite in warm acclimation, and no effect of oxygen level in cold acclimation. We also found that aerobic scope was setting the limits for appetite and growth, and that warm acclimated fish tended to have reduced performance with both hypoxia and hyperoxia. Furthermore, we found growth rate to correlate with aerobic scope in warm acclimation, highlighting the benefit of having a higher oxygen transport capacity at supra-optimal temperatures. In general, the effect of oxygen was dependent on acclimation temperature, and we support the idea of fishes experiencing capacity limitation with increasing temperature, approaching "functional ceilings".

SAMMENDRAG

I en verden preget av ekstreme hetebølger trues artsmangfoldet og biodiversiteten. Temperatur påvirker biologien til ektoterme dyr drastisk, hvorav fisk direkte blir påvirket av vanntemperatur. En rekke hypoteser tar for seg evnen fisk har til å supplere vev med oksygen ved økende temperatur. Resultater fra tidligere studier fremhever kapasitetsbegrensninger ved oksygentransport ved økende temperaturer resulterende i redusert somatisk vekst. I dette studiet har vi undersøkt om oksygennivå (50%, 100% og 200% luftmetning) er en begrensende faktor for appetitt, vekst og aerob kapasitet i sebrafisk akklimert til 20° C og 34° C. Vår hypotese var at hypoksi ville redusere og hyperoksi øke prestasjonen med større effekt i varm akklimasjon siden metabolisme og oksygenopptak øker ved høyere temperatur. Vi fant at hyperoksi ledet til redusert appetitt ved høy temperatur og ingen effekt av oksygennivå i lave temperaturer. I tillegg fant vi at aerob kapasitet begrenset appetitt og vekst og at varmeakklimerte fisk presterte dårligere ved både hypoksi og hyperoksi. Vi fant også at vekstrate korrelerer med aerob kapasitet ved høye temperaturer som understreker fordelen med effektiv oksygentransport ved suboptimale temperaturer. Det viser seg at effekten av oksygennivå er avhengig av temperatur og vi støtter ideen om at økende temperatur setter begrensninger for optimal fysiologisk funksjon.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

OCLTT-hypothesis	Oxygen and capacity limited thermal tolerance
ASP-hypothesis	Aerobic scope protection hypothesis
TPSs	Thermal performance curves
SDA	Specific dynamic action
PRAS	Postprandial aerobic scope
$\mathbf{T_{opt}}$	Optimal temperature
$\mathbf{T_{crit}}$	Critical temperature
ROS	Reactive oxygen species
DO	Dissolved oxygen - % air saturation
$\mathbf{MO_{2,max}}$	Maximum oxygen consumption rate
$\mathbf{MO_{2,min}}$	Minimum oxygen consumption rate
$\mathbf{MO_{2,routine}}$	The lowest oxygen consumption rate of fish during the first 30 minutes after transfer to a respirometer

INTRODUCTION

Ectotherm thermal tolerance

The global environment is currently experiencing unnatural changes, with anthropogenic disturbance leading to extreme conditions. A consequence of climate change is the increased severity of extreme heat waves leading to organisms being exposed to elevated, and often physiologically stressful conditions (Stillman, 2019). Subsequently, heat waves are direct threats to species distribution and abundance, and this recurring pattern is predicted to increase in the future (McBryan, Anttila, Healy, & Schulte, 2012). Ectothermic animals are in particular affected as their body temperature follow the ambient temperature (Schulte, 2015). Ectotherm physiology, and therefor metabolism is directly linked to the surrounding environment, with temperature being the major determinant for crucial processes such as growth, locomotion, and reproduction (Fry, 1967).

Effects of temperature and oxygen

Increasing temperature results in an exponential increase in the rate of chemical and biochemical reactions as well as enzyme activity, leading to higher demand for ATP and thus oxygen requirement. Furthermore, elevated temperature leads to oxygen being progressively transported in the blood to working tissue by increased blood pressure, stroke volume, and for fish – ventilation. Hence, warming requires increased cardiorespiratory work (Morgan, 2021) (Figure 1). Temperature and oxygen availability are hence factors likely to interact, with temperatures above an organism's tolerance typically leading to oxygen deficit. Thermal tolerance is in this regard quantified by minimum and maximum critical temperature (CT_{min} and CT_{max}), of which staying above CT_{max} typically leads to disorganized locomotion (Beitinger et al., 2000). Similarly, exposure to decreased oxygen availability (hypoxia) in for example shallow waters can lead to accelerated physiological stress with warming (Leeuwis et al., 2021).
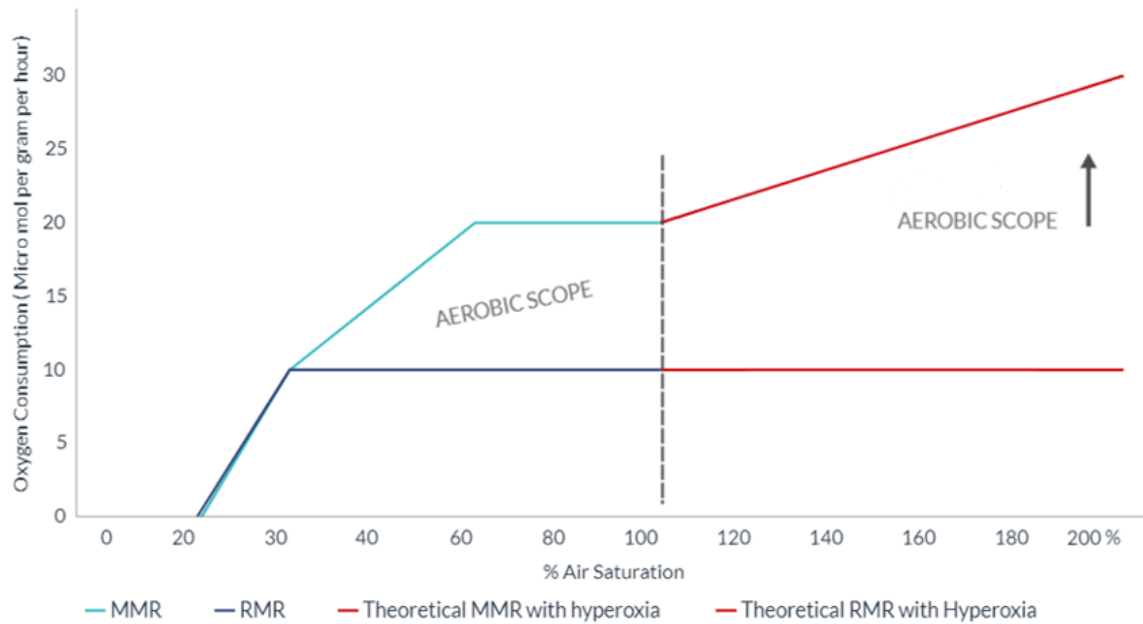
Figure 1: MMR ($MO_{2,max}$), RMR ($MO_{2,routine}$) and aerobic scope with increased air saturation and the effect of hypoxia and hyperoxia on metabolism. The difference between $MO_{2,routine}$ and $MO_{2,max}$ illustrates the available aerobic scope. Predictions from 20 to 100 % air saturation are redrawn from McBryan, 2013. Predictions from 100 to 200 % air saturation are hypothetical and in line with our suggestions. The effect of hyperoxia is suggested to be greater with increasing temperature (Brijs, 2015; Jutfelt, 2021).

Thermal performance, metabolism, and aerobic capacity

One aspect of thermal biology that will be important in predicting how ectotherms will respond to climate change is thermal performance (Schulte et al., 2015). Thermal performance curves (TPCs) can be used to illustrate the performance across a thermal range of traits like metabolism, growth rate, appetite, and maximum swim speed (Figure 2). Typically, TPCs peak at an optimal temperature and decrease above and below this point. Increasing temperature is likely to influence both long-term *thermal performance* and *thermal tolerance,* of which both aspects are important for individuals to persist during heat waves (MacLean et al., 2019).
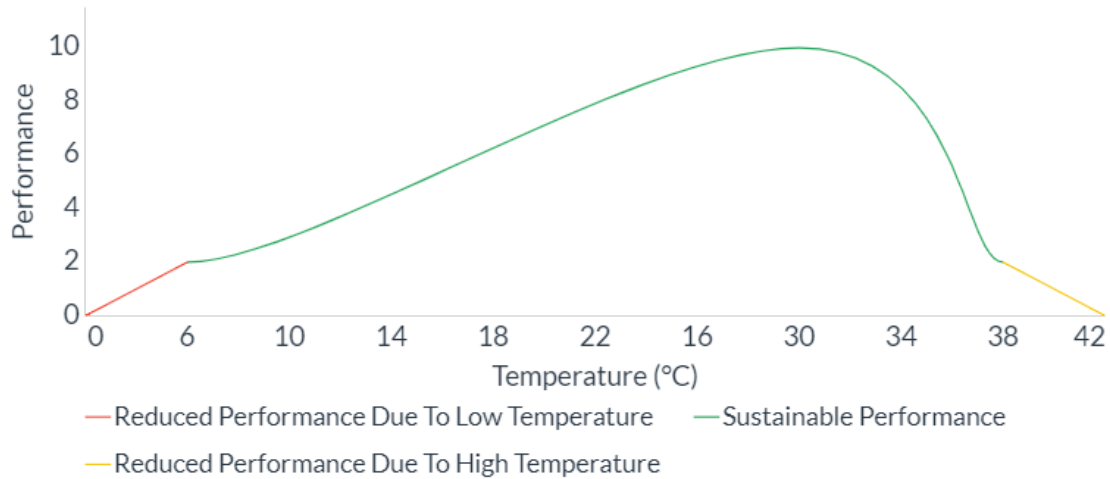
Figure 2: General thermal performance curve. The performance of different bodily functions (metabolism, growth rate, maximum swim speed etc.) in relation to increasing temperature. Performance usually peaks at ~ 28 °C in zebrafish, with sub-optimal temperatures drastically reducing the performance.

Growth and swim speed of laboratory zebrafish (*Danio rerio*) typically peak at about 26-28°C and decrease at both sides of the optimal temperature (T_{opt})(Morgan et al., 2022; Morgan, 2021). A peak in such performance is usually reflected by a maximized metabolism. Further increase of metabolism, especially at temperatures above T_{opt} can potentially cause a mismatch between oxygen supply and demand, resulting in decreased partial pressure of O_2 (pO_2) in the blood, leaving oxygen consuming tissue hypoxic (Frederich and Pörtner, 2000; Pörtner, 2010). Consequently, temperature can have strong effects on aerobic capacity.

Aerobic scope is an animal's capacity to increase its aerobic metabolic rate above maintenance levels, articulated as the difference between maximum metabolic rate ($MO_{2,max}$) and minimum metabolic rate ($MO_{2,min}$) (Seebacher et al., 2010). $MO_{2,max}$ is the maximum rate at which oxygen can be transported from the environment to the tissue mitochondria and $MO_{2,min}$ is the minimum metabolic rate needed to sustain life at a specified temperature. Subsequently, elevated aerobic scope sets the stage for improved work such as food intake and assimilation and thereby increased growth (Schulte, 2015).

Acclimation

With increasing temperatures, organisms can acclimate to compensate for direct thermal effects on physiological functions. The complex mechanisms driving these processes are suggested to include modulation of enzyme activity (Vergauwen, 2010), mitochondrial density (O'Brien, 2011), changes in protein isoforms (Crockford & Johnston, 1990) and membrane lipid composition (Hazel, 1995). This process can be initiated by several other factors than temperature and is commonly called acclimatization in the wild and acclimation in laboratory conditions. The temperature an organism is adapted to affects the scope for its physiological functions, however, higher acclimation temperature can alter the thermal performance breadth and thermal tolerance (Cortemeglia & Beitinger, 2005; Morgan et al., 2019).

Performance parameters: Appetite, growth, and aerobic scope

Due to the asymmetrical relationship between the effect of increasing temperature on $MO_{2,min}$ and $MO_{2,max}$, fishes can potentially approach lethal physiological states, leading to unsustainable cardiorespiratory load and protein denaturation (McBryan, 2013). Appetite, growth, and swim performance sharply declines at temperatures above optimal, proposed to be mediated by oxygen delivery to the body (Pörtner, 2010). The demanding conditions for optimal oxygen uptake and ventilatory function accelerates with increasing temperatures, and with metabolism being temperature-dependent, this oxygen limitation problem is currently known as the "oxygen- and capacity-limited thermal tolerance"-hypothesis (OCLTT-hypothesis; Ege & Krogh, 1914; Pörtner, 2010). In general, the OCLTT-hypothesis implies $MO_{2,min}$ to increase faster than $MO_{2,max}$, leading to reduced aerobic scope. In addition to the OCLTT-hypothesis, the aerobic scope of fishes is suggested to optimize fitness in a specific temperature range, with biochemical and physiological capacities being maximized. As a result of this, aerobic scope is a major determinant for optimal growth rate, locomotion, and reproduction (Clark, Sandblom, & Jutfelt, 2013).

The relationship between aerobic scope, growth and performance in general is not completely understood and different hypotheses have been proposed. While several studies support the OCLTT-hypothesis, pointing to oxygen supply as a limiting factor for heat tolerance (Healy & Schulte, 2012; Islam et al., 2020; Rutledge & Beitinger, 1989; Brijs et al., 2015), other studies contradict the predictions coming out of this hypothesis (Jutfelt et al., 2018; Jutfelt et al., 2021). For example, a study done by Pang in 2010 on catfish *(Silurus meridionalis)* and

goldfish (*Carassius auratus*) suggests voluntarily decline in food intake at high temperatures, despite increased $MO_{2,min}$. Food intake is in general positively correlated with elevated $MO_{2,min}$, however, this process occurring at higher temperatures are rather unexplored (Jutfelt et al, 2021). Moreover, some studies have observed a mismatch between growth rate and aerobic scope, with optimal growth rate at lower temperature than at peak aerobic scope (Brijs et al., 2015; Grans et al., 2014; Schulte, 2015). In addition, a related mismatch has been reported in the relationship between optimal temperature for growth and aerobic scope, with maximum aerobic scope found close to lethal temperatures (Clark et al., 2013; Norin, Malte, & Clark, 2014).

Studies on several fish species have indicated a sharp decline in food intake at temperatures above T_{opt}, most likely explained by deficit in physiological functions. Reduced food intake, combined with elevated $MO_{2,min}$ at high temperatures has for a long time been suggested to result in reduced growth (Jobling, 1997). Food consumption leads to a sharp increase in the metabolism (post-prandial oxygen consumption rate) illustrated as a curve-peak in the relationship between metabolic rate and temperature, and this energy demanding process is called the "specific dynamic action" (SDA) (Figure 3). Increase in post-prandial oxygen consumption rate is temperature sensitive and is considered a product of increased processing and assimilation of nutrients in the gut (Secor, 2009). In addition, the total amount of energy expended to the SDA-response is in general independent of temperature, leading to temporal compression and a greater postprandial peak in metabolic rate. Subsequently, the compressed SDA-response may in extreme conditions leave no postprandial aerobic scope (PRAS), and lead to aerobic deficit (Figure 3).
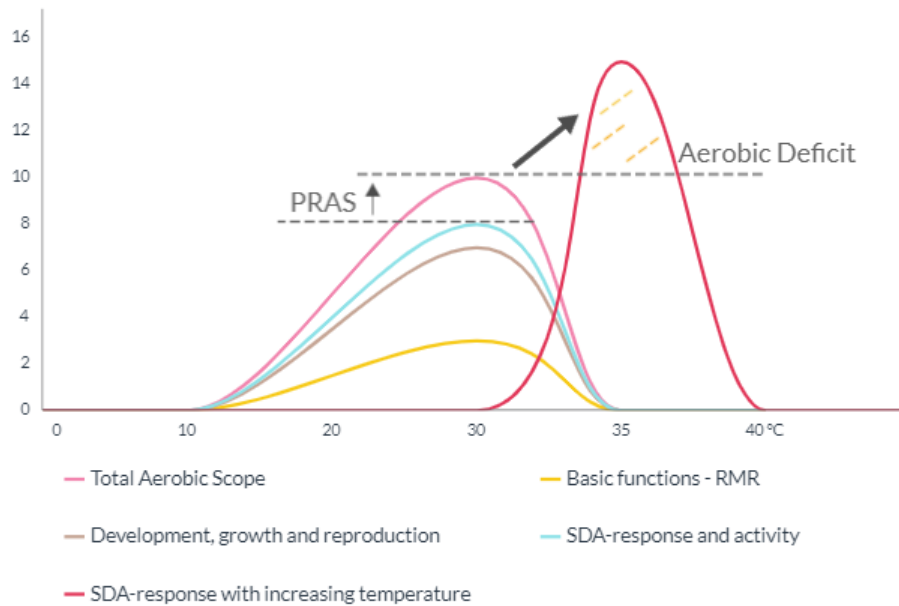
Figure 3: Hypothetical content of the aerobic scope and the effect of the SDA-response with increasing temperature. The total aerobic scope determines the performance of bodily functions as for example growth, reproduction, and the SDA-response. PRAS is illustrated as the available aerobic scope with the SDA-response. With increasing temperature, the SDA-response can get temporally compressed and overcome the total aerobic capacity and lead to aerobic deficit.

The SDA-response is directly linked to meal size, which in theory allows organisms to regulate the magnitude of the metabolic response (Fu et al., 2005; Norin & Clark, 2017; Secor, 2009). In an environment characterized by continually threats, letting the SDA-response reduce the aerobic scope for other oxygen-demanding activities may not be optimal for survival. Besides, studies have shown decreased swim performance during the postprandial period (Alsop & Wood, 1997; Fu et al., 2009; Thorarensen & Farrell, 2006; Zhang et al., 2012), and animals should therefor regulate their food intake based on their need to maintain aerobic scope under challenging conditions (Jutfelt et al., 2021). Due to these observations, Jutfelt and colleagues proposed in 2021 the "aerobic scope protection hypothesis" (ASP-hypothesis), suggesting that water-breathers actively should avoid situations leading to decreased PRAS during warming, leaving sufficient aerobic scope for crucial processes (Jutfelt at al., 2021). The difference between the OCLTT-hypothesis and the ASP-hypothesis lies in its emphasis on the effects of temperature on food intake and highlights different aspects of the underlying mechanisms leading to reduced growth rate of fishes during warming (Jutfelt et al., 2021). The OCLTT-hypothesis has traditionally focused on cardiorespiratory function with limited oxygen availability leading to tissue hypoxia, while

the ASP-hypothesis focuses on the voluntarily act of fish preventing its SDA to occupy the total aerobic scope by reducing appetite with increasing temperature. In essence, both hypotheses concern how oxygen limitation reduces growth, but by different mechanisms.

Study aim

In this study, we compared the effects of high and low ambient oxygen-level (50 and 200% air saturation) among cold and warm acclimated zebrafish (20 °C and 34 °C) on appetite, growth, and aerobic scope. First, we aimed to test the prediction that fish acclimated to 34 °C have higher, and closer to optimal performance in all measurements than fish acclimated to 20 °C in normoxia (Morgan, 2022). This includes higher growth (in terms of both weight and length), higher appetite, and higher aerobic scope. Second, we predict that hypoxia should reduce and hyperoxia increase the performances. Furthermore, we aim to test several of the predictions coming out of the ASP-hypothesis. These predictions are directly retrieved from (Jutfelt, 2021), and reflects the methodology of the experiment.

One prediction is that growth rate of fishes decreases above optimal temperatures due to reduced aerobic scope. As water hyperoxia is shown to increase aerobic scope (Brijs et al., 2015), we predict that the decline is less steep in hyperoxia (Figure 4).
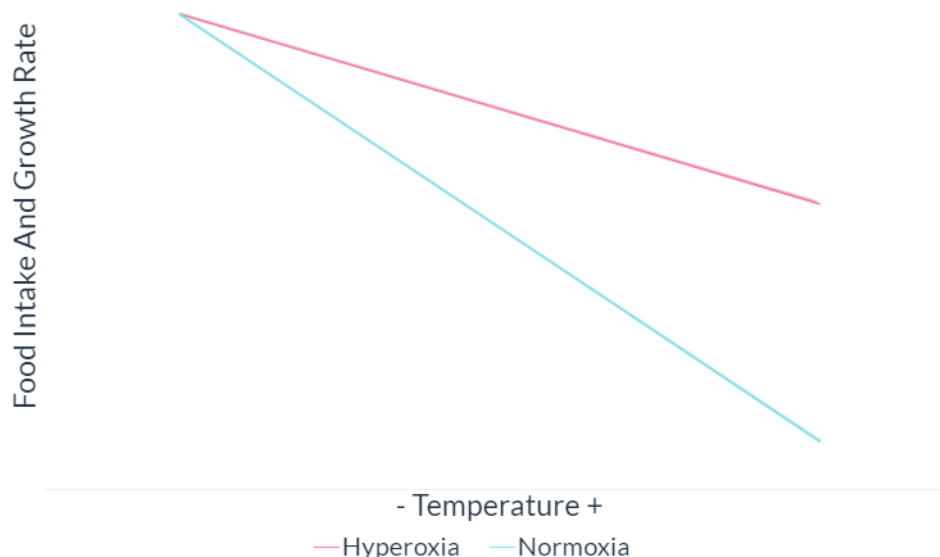
Figure 4: Growth rate as a function of temperature above optimal. Optimal temperature is to the very left, with hyperoxia having no effect. Hyperoxia is suggested to have greater effect with increasing temperature.

We also predict that appetite in general is reduced with increasingly supra-optimal temperature, with hypoxia and hyperoxia having no effect at optimal temperature. However, the effect of air saturation should be greater at elevated temperatures, with hypoxia lowering and hyperoxia increasing the appetite. This prediction uses the peak-centered shape of the temperature-food intake relationship from Figure 5 (Jutfelt et al., 2021). If the reduction in PRAS becomes restrictive to food intake only at higher temperatures, then increased aerobic scope by hyperoxia can be predicted to increase appetite.
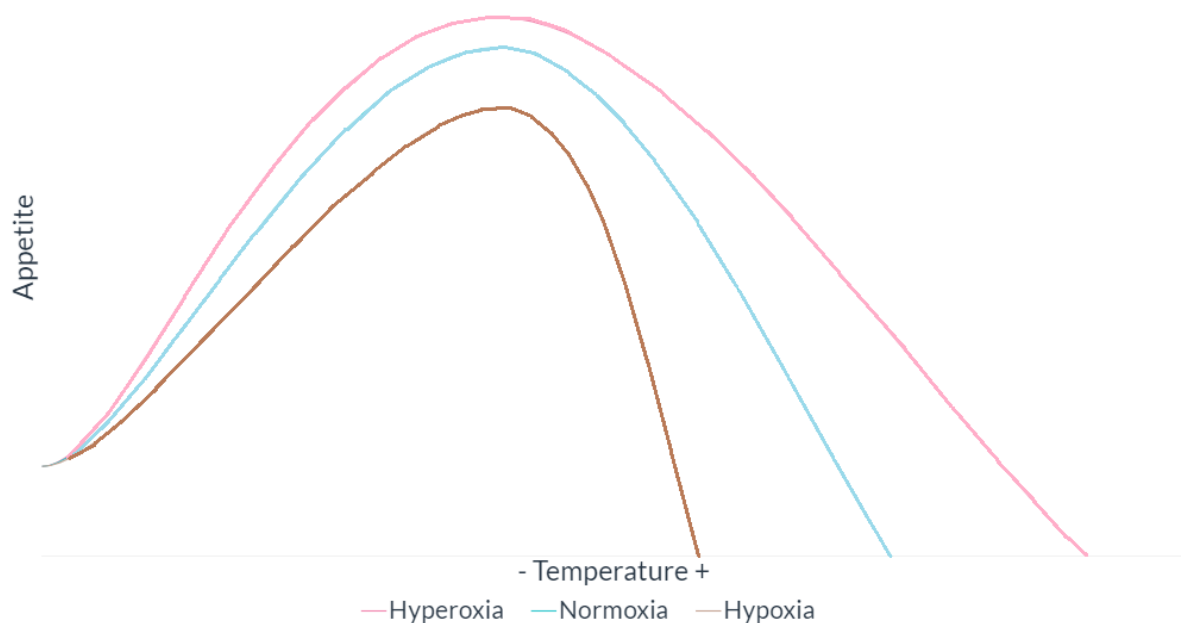
Figure 5: Reduced appetite with increased supra-optimal temperature. Hypoxia and hyperoxia are suggested to having no effect at optimal temperature, and increased effect with elevated temperature.

Lastly, aerobic scope is in general not found to correlate with growth rate at optimal temperature. However, with some support from the field (Auer et al., 2015) the correlation should be temperature-sensitive and positive at supra-optimal temperatures due to its effect on the SDA-response (Figure 3 and 6).
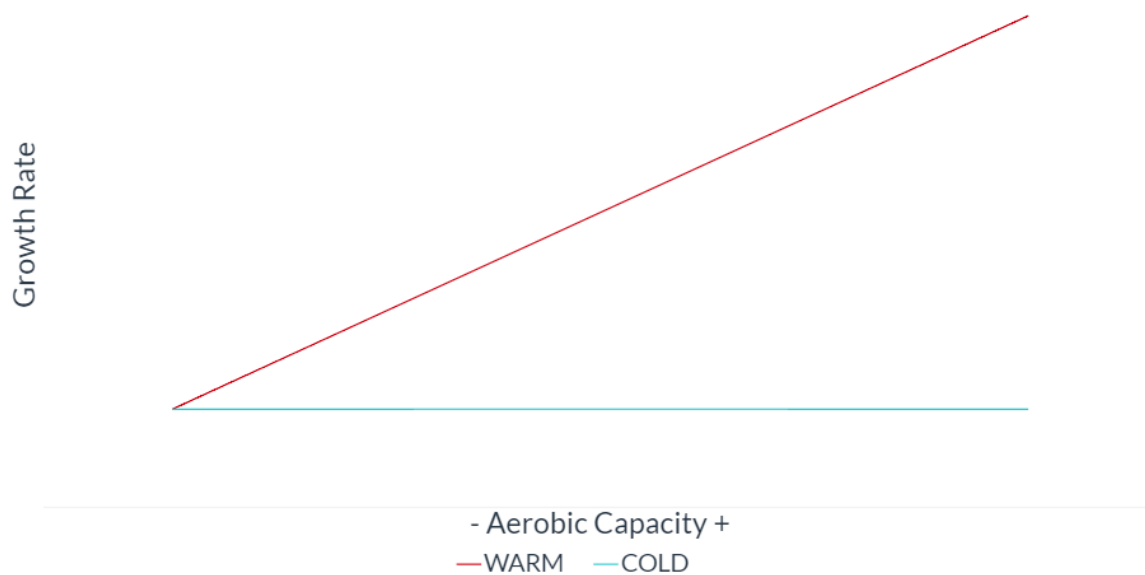
Figure 6: Growth rate in correlation with increasing temperature. No correlation at cold temperatures, but a positive correlation is suggested with increased supra-optimal temperatures.

By testing these predictions, we hope to contribute with crucial insight into the physiology of fishes in a changing world. Mainly, we aim to learn more about the potential effects of long-term acclimation, and how it affects fishes in tolerating non-optimal conditions. By acclimating the fish to both sub- and supra-optimal temperatures and oxygen concentration we allow for testing the potential interacting effects.

METHODOLOGY

The zebrafish (*Danio rerio*) used in this experiment were originating from a wild-caught population in West Bengal, India. The zebrafish is a well-established study species for thermal biology, experiencing huge variations in temperature in the wild. They are found to survive in temperatures ranging from 14 °C to 39 °C, making it a eurythermal species (López-Olmeda and Sánchez-Vázquez, 2011) The fish were kept in glass aquaria (30 x 30 x 50 cm) in a ~16 ° C room at the animal facility at the Norwegian University of Science and Technology (NTNU), Trondheim, Norway. All aquaria were installed with continuous air supply, titanium

heaters (TH-100, Aqua Medic, Germany), thermostats (ITC-306T, Inkbird, China), temperature sensors (type K thermocouple), water pump with a sponge-filter for circulation and filtration (EHEIM universal 300, EHEIM®, Germany) and an ornamental plastic plant. In addition, eight of the aquaria were assigned continuously influx of either N^2 or O_2-gas, to hypoxic (50 % air saturation) or hyperoxic treatments (200 % air saturation) by a ~4 m (4 mm ⌀) plastic tube directly connected to a 20 l N_2 and O_2-gas-tank. The gas-tanks were connected to the aquaria by a flow meter with needle valve (Brooks Instruments, 0.0-0.5 LPM AIR), so that all tanks could be individually adjusted. The hypoxic treatments were maintained at (50 % ± 5 % DO) by a 1:1 ratio of compressed air and N_2-gas. For the hyperoxic treatments a 2:1 ratio of compressed air and O_2-gas were used (200 % ± 10 % DO).

All aquaria were added a robust fiberoptic sensor (Firesting O2, PyroScience, Germany) for continuously monitoring of air saturation (Appendix 1, Figure 11). Temperature sensors were connected to a data logger (Picotech TC-08, Cambridgeshire, UK). The water supplied to the aquariums were retrieved from pre-filled 200 l water barrels containing carbon filtered water and added 0.25 ml/L salt and 0.25 ml/L Aquasafe (Aquasafe, Tetra Sales, Blacksburg, VA, USA). The room was on a 12:12 day:night light regime (Figure 7).

Figure 7: Set up of the experiment. Twelve tanks distributed on the shelf so that neither of the same treatments experienced the same position in the room. Oxygen and nitrogen-tanks to the left supplying hypoxic and hyperoxic aquaria, respectively. All plastic tubes connecting aquaria with gas are joint in a manually adjusted valve-station below the computer. Duplicated treatments resulting in six cold and six warm aquaria combined with three different oxygen treatments.

Experimental description and purpose

The purpose of the experiment was to investigate the effects of hypoxic and hyperoxic acclimation on appetite, growth, and aerobic scope. To investigate whether the effect of oxygen was dependent on acclimation temperature, fish were acclimated to two different temperatures (~ 20 °C and ~ 34 °C). A total of 230 fish (n = ~ 38 per treatment, ~19 per tank), were kept in twelve aquaria. As the experiment commenced each aquarium was assigned one of six acclimation treatments distributed among two replicate tanks per treatment. The aquaria were distributed within the three-shelf unit so that room temperature, light conditions and potential disturbance were approximately the same for every treatment (Figure 4). The aquaria were marked with numbers and color-coded tape according to the assigned temperature treatment.

Experimental procedure – *Prior to acclimation*
Tagging

Over a period of ~ 6 days, 240 individuals were tagged and measured for weight and length
(n = 20 per tank) while anesthetized with 110 mg L-1 buffered tricaine methane sulfonate
(MS-222). The fish were placed in a pre-cut plastic sponge and subcutaneously injected with
two visible implant elastomer tags (Northwest Marine Technologies, Shaw Island, WA, USA)
with a color combination unique to the individual fish within each tank. The tags were located
both sides of the dorsal fin using a 0.5 mm syringe, based on the site shown to be the best
suited for tagging (Hohn and Petrie-Hanson, 2013) (Figure 8).

Weight and length – Measurements and calculations

Individual weight (g) and length (mm) were recorded using a digital weight and length-
caliper. Weight was measured to the nearest 0.01 g and standard length was measured from
the snout to the base of the tail to the nearest 0.01 cm (Figure 8). The second measurement
was performed halfway throughout the experiment over a period of ~ 2 days. The third and
final measurement was performed at the end of the acclimation period after the respirometry
were done. The fish were left to recover in an aerated tank before transferred back to their
previous holding tanks. This way, a continuously weight and length gradient was captured.

Figure 8: Length measuring area and tag-position. Zebrafish (*Danio rerio*).

Experimental procedure – *Acclimation*

Temperature

The acclimation lasted for ~ 8 weeks in total, of which day 43 to 53 were designated respirometry trials. For this experiment, we wanted two temperatures at either side of the optimum temperature for growth and swim performance, where we could expect a close to equal reduction in both measures compared to the optimum. We also wanted the higher temperature close to the highest temperature tolerated by this species (Åsheim et al., 2020; Morgan, 2020). The initial temperature of all aquaria ready for experiment start was ~ 28 °C. The temperature in warm treatment tanks were increased with 3 °C each day for two days until final acclimation temperature of 34 °C. The temperature in cold treatment tanks were decreased by 4 °C each day for two days until final acclimation temperature of 20 °C. Thus, final acclimation temperatures were reached after 2 days.

Oxygen

To test the interacting effects of temperature and air saturation, one O_2-level at each side of the optimum were chosen accordingly to previous studies done in Jutfelt's fish ecophysiology Lab (NTNU, Trondheim) (Reiersen, 2021; Andreassen, 2018) for reasonable comparison. Several studies suggests that 50 % air saturation are a possible breakpoint for oxyconforming in many fish species, especially with increasing temperatures (McBryan, 2013; Burggren 1982; Mandic et al. 2009). Furthermore, 200 % air saturation has previously indicated higher appetite and weight gain in rainbow trout (*Oncorhynchus mykiss*) in comparison with normoxia (Dabrowski et al., 2004). The initial air saturation of all aquaria was ~ 100 % when temperature treatments were reached. At the point of stable temperature, the hypoxia and hyperoxia treatment tanks were manually adjusted to 50 % and 200 % air saturation over the course of ~ 8 hours. Subsequently, the fish were left to acclimate.

Feeding

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) during the acclimation period. Fish were daily controlled for signs of sickness, and dead fish were removed (mortality, n=1).

Appetite and aerobic scope – *Measurements and calculations*

All fish regardless of treatments were starved each third day with a following appetite measurement the next day during the whole acclimation period, resulting in a total of 11 measurements. Subsequently, at the end of the acclimation period the MO_2-tests were conducted over a period of 10 days, starting on day 43 of the experiment (Figure 9). The fish in each aquarium were divided into four groups of five fish. Each group from the same aquarium was assigned the identical treatment as for the acclimation treatment and distributed randomly along the test period. This way, all combinations of temperature and oxygen level were tested (Figure 10). About ~ 6 trails were conducted each day and a total of 48 were performed. Fish were fasted for 2 days prior to the test. This way, fish were in approximately the same state of hunger and the potential effect of lingering SDA-response was minimized. The trials were ordered so that the same tanks were not tested at the same time each day. After metabolic rate measurements the fish were anesthetized with MS-222, measured for weight and length and then dispatched.
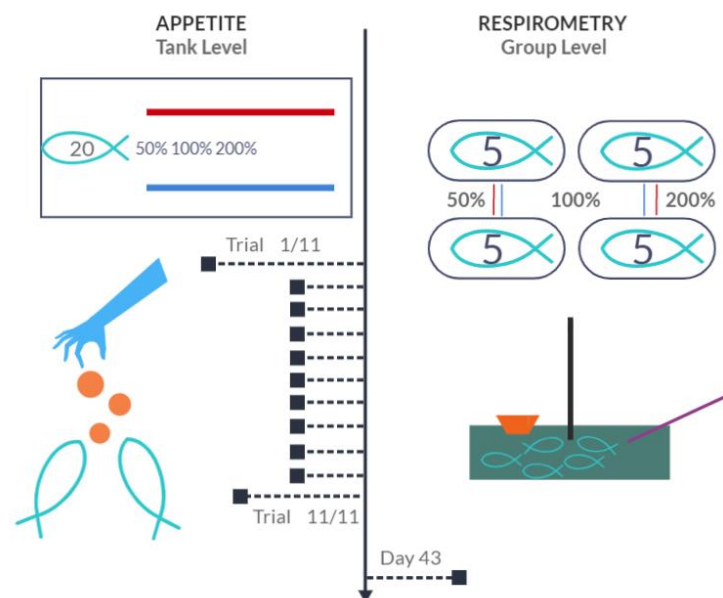
Figure 9: Set-up and timeline of the appetite and respirometry trials. Appetite trials were performed 11 times throughout the acclimation period, on tank level (n=20). Respirometry trials were done on group level (n=5) at the end of the acclimation period.
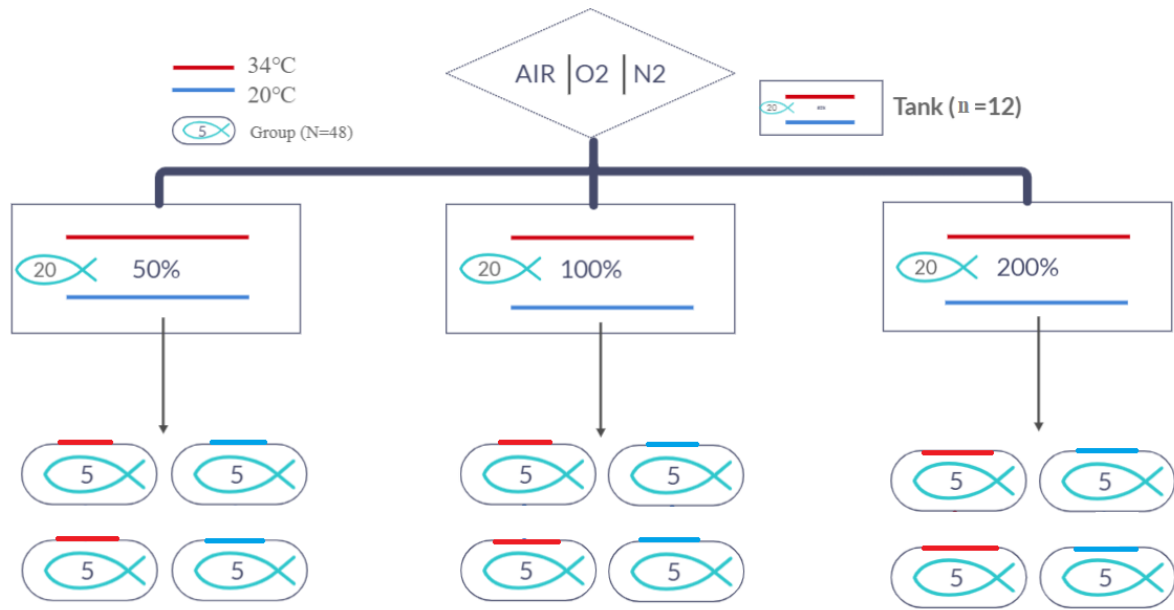
Figure 10: Experimental set up and respirometer group distribution. Air, O_2, and N_2-gas connected to each aquarium (n=12) according to the assigned treatment. Each aquarium was at the end of the acclimation divided into four groups of five fish before performing respirometry. Duplication of set-up resulting in 12 aquaria in total, with half being cold acclimated including 50 %, 100 % and 200 % air saturation. Same applies for warm acclimated fish.

Appetite

Each of the 11 measurements were completed identically and involved feeding of 10 dry flakes (Tetrapro, ~ 0.044 g) through an open lid in the top of the tank, five times (50 flakes given in total), with ~ 10 minutes interval leaving the fish time to consume flakes before next feeding interval. The present measurer left the room at the end of each feeding interval to minimize stress and potential disturbance for the fish. Remaining flakes floating on the water surface or sunk to bottom were visually counted and noted in Table 8. As each count trail led to close encounter between observer and fish in addition to the possibility of visual counting error, the order of tanks observed were changed after each measure. This way, all tanks have a continuously appetite measure throughout the acclimation period. To prevent further consumption of remaining food we used a ~ 1cm ∅ plastic tube to vacuum the bottom of the aquaria following the siphon-principle. Tanks were subsequently re-filled with water to the initial holding level.

We defined appetite as the amount of voluntarily consumed dry flakes out of 50 given, during a feeding period of ~ 60 minutes and was calculated by a linear equation

$$y = aX + b \qquad\qquad \text{(Eq. 1)}$$

where y is the total amount of consumed dry flakes, a is the mean change in dry flakes consumed per day within each treatment over the course of 11 trails, and b is the number of dry flakes consumed at trial 1.

Half-way through the acclimation period we experienced substantially change in water temperature due to power failure in the room. This resulted in a temporary change of appetite among all tanks. We decided to exclude trial 4-8 from our statistical analysis, as illustrated in Table 8.

Difficulties when measuring appetite

Throughout the performance of the appetite-measurements we experienced difficulties in visually counting dry flakes. As dry flakes either maintained on top of the water surface or sunk to the bottom, we found it challenging to keep track of uneaten flakes. In addition, after some time, flakes tended to dissolve into pieces. Lastly, fishes usually ate one flake in one or two "bites", but some individuals however ate $\frac{1}{3}$ or $\frac{1}{2}$ flakes, leading to fragmented pieces randomly distributed at the bottom of the aquarium. This pattern was recurring throughout the whole acclimation period, but we found no differences in eating-patterns among tanks. As a result of this, we predict that the appetite-data and the analyzed statistics appears additionally noisy directly due to sampling error.

Aerobic scope

For the measurements of aerobic scope, we performed intermittent-flow respirometry involving $MO_{2,routine}$ and $MO_{2,max}$. $MO_{2,routine}$ refer to the rate of oxygen consumption when fish is undergoing normal or another predefined activity (Metcalfe, Van Leeuwen, & Killen, 2016) and was defined as the lowest rate of oxygen consumed during 30 minutes in the respirometer at no water current. $MO_{2,max}$ was performed directly after the $MO_{2,routine}$ and was defined as the maximum rate of oxygen consumed at maximum swim speed. Level of air saturation in the respirometer was logged with a sampling frequency of 0.5 Hz for all measurements, and sensors were calibrated to 100 % dissolved oxygen between each round.

An aquarium identical to the acclimation-aquaria (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 fiberoptic

sensor (Firesting O2, PyroScience, Aachen, Germany). Gas concentration was manually adjusted by separate flow meters, temperature was controlled by a titanium heater (TH-100, Aqua Medic, Bissendorf, Germany) and a water pump (Eheim CompactON 300, Deizisau, Germany) ensured a homogenous oxygen concentration (Figure 11).
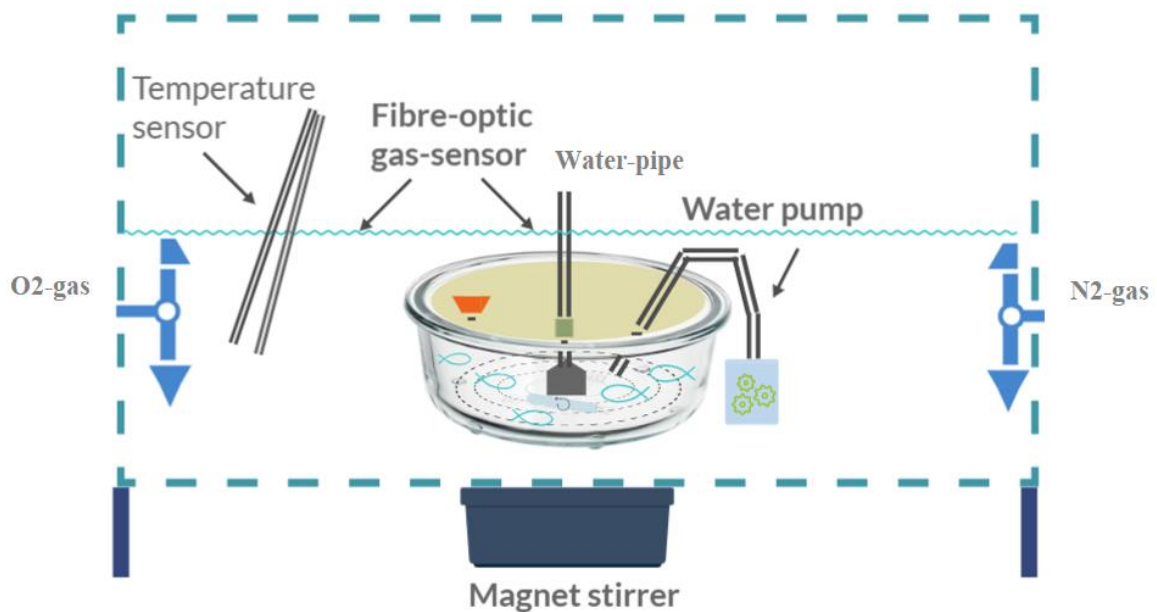
Figure 11: Set-up of the respirometry. The custom-made respirometer was placed in the middle of an aquarium (30 x 30 x 50 cm) on top of a magnet stirrer. The aquarium was filled with water close to the top of the water-pipe. The % air saturation was manually adjusted by valves on O_2 and N_2- holding tanks.

An IKEA circular glass food container (6 x 14 cm, 331.4 mL including equipment) with a custom-made plastic lid was used as respirometer and placed in the large aquarium (Figure 12). A robust fiber-optic sensor (Firesting O2, PyroScience, Aachen, Germany) was placed through the lid into the water outlet to measure oxygen concentration and a flush pump (Eheim CompactON 300, Deizisau, Germany) was attached by a plastic tube through a pre-drilled hole. Fish were transferred into the respirometer by a glass funnel through another pre-drilled hole at the outer edge of the lid.

A magnet drive plate was placed under the aquarium to create an even water current driven by a magnet stir bar at the bottom of the respirometer separated from the fish by an aluminum mesh. Water flow speed was adjusted manually by the magnet stirrer and a 4 cm ϕ plastic cylinder kept the fish swimming in the outer circumference of the respirometer. At the start of each respirometry-trail, the fish group spent 30 minutes in a slow-speed $MO_{2,routine}$-

measurement. Three periods of oxygen consumption were recorded by closing the flush pump for ~ 8 minutes three times with ~ 2 minutes flushing between recordings. As a continuation of the $MO_{2,routine}$-trial the $MO_{2,max}$-measurements were initiated by increasing the water current speed in a stepwise manner. 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 prior to exhaustion. The flush pump restored the oxygen level before the last recording of maximum oxygen consumption, ensuring the fish had the proper oxygen treatment before recording. This method was used based on previous calculations of water speed with the identical equipment (Morgan et al., 2022).
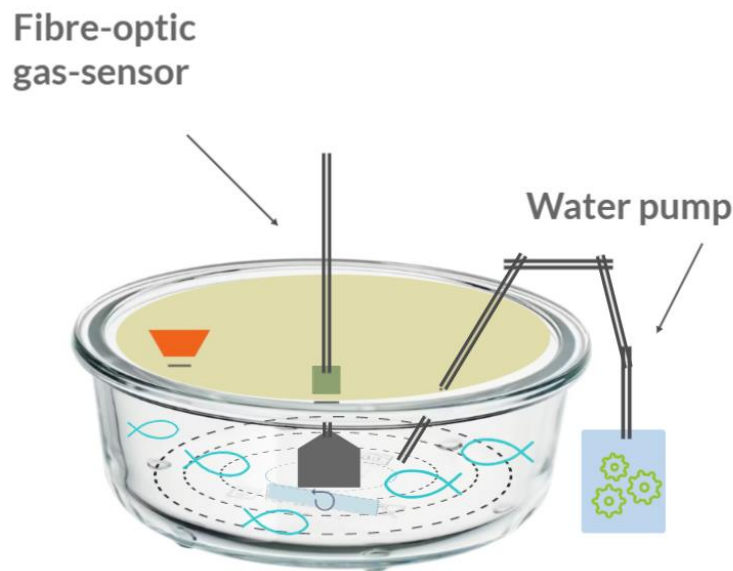
Figure 12*: Respirometer set-up. Five fish per group swimming in opposite direction of the water current. O_2-uptake was monitored by the fiber-optic sensor vertically placed into the water body of the respirometer, sealed from the surrounding aquarium-water. Water-flushing of the respirometer was performed regularly by manually initiating the water-pump.

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

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

(Eq.2).

where V_r and V_f are respirometer and fish volume (1 g fish assumed to be equivalent to 1 ml fish). ΔC_{wO_2} are the change in oxygen concentration in the respirometer water, Δt the change in time over which ΔC_{wO_2} 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 percentage decline in saturation per second was retrieved by the analysis software LabChart Reader (LabChart v.8.1.13, ADInstruments, NSW, Australia) with ~ one-minute intervals to calculate the slopes. $MO_{2,routine}$ was calculated as the mean of the three lowest rate of oxygen consumption slopes and $MO_{2,max}$ was calculated as the highest rate from the last 1-2 minutes at maximum swim speed. The change in oxygen concentration was converted from percent change in level of air saturation per second to mg change per hour (ICES, 2021) in Microsoft Excel.

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), we decided to prioritize models with an interaction term between temperature and oxygen based on our intention to test these interactions. In addition, this made the comparison of effect sizes among models straightforward (Appendix 1). 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 and standard error of the mean (mean ± SE).

Growth

To test the effect of temperature and oxygen availability on growth, all fish were measured in grams(g) and millimeter (mm) and transformed to % per day by the equation

$$\left(\frac{(W_{time}-W_{initial})}{W_{initial}}\times100\right)/(T_{time}-T_{initial})=\% \ day^{-1}$$

(Eq.3).

here W_{time} and $W_{initial}$ is the final weight and initial length, and T_{time} and $T_{initial}$ is the final time (days) and start time, respectively.

For each of the three growth measurements individual tag, growth and length, treatment, measure person, date and order were recorded. To test whether water temperature and environmental hypoxia/hyperoxia treatments could affect growth, that is, if individual weight or length differed between treatments, data were analyzed using linear models. Effects of measure person (student), holding tank and interactions between oxygen and temperature were controlled for by comparing models with different fixed factors and interactions included. Growth rate was defined as the percentage increase in body mass during the experiment (% increase in weight per day), calculated using the initial, half-way, and final weight, thus accounting for initial size differences.

Only weight was further used as proxy in the analysis since weight and length was found to be highly correlated (r = 0.79). In addition, a related zebrafish study found a similar correlation to be (r = 0.76) (Morgan, 2021).

Appetite

For the appetite measurements, treatment, number of flakes, and measurer were recorded for each tank, in addition to feeding interval number and date. To test whether appetite was affected by acclimation temperature and level of oxygen, data from cold acclimated fish was analyzed using a linear model. Level of oxygen saturation and measurement number (6 in total) were set as explanatory variables affecting the amount of feed consumed as response variable. For warm acclimated fish, data were analyzed using a mixed-effect model, as tank

influenced the variation in appetite. An interaction term was included to investigate whether oxygen-level was dependent on measurement number.

Aerobic scope

For the oxygen consumption measurements of $MO_{2,routine}$ and $MO_{2,max}$, oxygen and temperature treatment, individual growth rate, holding tank and respirometry group were recorded. Aerobic scope was calculated as the difference between $MO_{2,routine}$ and $MO_{2,max}$. To test the effect of temperature and oxygen level on aerobic scope, and the potential influence of weight and holding tank, data were analyzed using linear models. Whether there was an interaction between oxygen and temperature was tested by including an interaction term in the model.

Correlations

Correlation between growth rate and aerobic scope were tested using a linear model with % increase in weight per day as response variable and aerobic scope and oxygen level as explanatory variables. No random effect of tanks on growth rate was found, so we chose to exclude mean-centered values and used only raw values in the analysis. The correlation was expressed with a R^2-value between 0 and 1, indicating the variance of the dependent variable that the independent variable explains in percent. We evaluated our sample size in this regard (44 groups and n = 5 in each group) to be sufficient to consider R^2-values above 0.20 to have a "biological significant meaning".

RESULTS

Growth – Weight

Throughout the acclimation period, hypoxia and hyperoxia including the additional effect of temperature on growth were investigated. To test the effect of temperature and oxygen availability on weight, all fish were individually measured before, during, and after the acclimation period.

Initial weight, final weight and % per day increase - Warm acclimated fish (34 °C)

Mean initial weight of all fish were 0.102 ± 0.01 g and there was no statistically significant difference between the treatments. Fish acclimated to 34 °C had a mean increase in weight of 1.96 ± 0.17 % day^{-1} over the eight weeks of acclimation. The hypoxic treatment had a 33.2 % lower growth rate (t = - 2.79, p = <0.05). Hyperoxia also seemed to have a slight reducing effect, although not significant (t = - 1.23, p = 0.22). The final weight of normoxic fish was 0.18 ± 0.01 g. Neither hypoxia (0.17 ± 0.01) nor hyperoxia (0.17 ± 0.01) significantly affected the final weight at 34 °C (t = - 0.98, p = 0.33 and t = - 1.45, p = 0.15, respectively).

Cold acclimated fish (20 °C)

Mean increase in weight for normoxic 20 °C acclimated fish were 0.79 ± 0.16 % day^{-1}. Nor hypoxic or hyperoxic fish had any significant different weight increase, with only slightly reduced growth rate (t = - 0.83, p = 0.41 and t = - 0.61, p = 0.54) respectively.

Mean final weight of normoxic 20 °C acclimated fish were 0.13 ± 0.006 g. Hypoxic fish had no significant different weight than the control group (t = 0.16, p = 0.87). On the other hand, hyperoxic fish had a significant different weight than the control group, with a final weight of 0.15 ± 0.007 g, and thus increased the mean weight by 15.4 % (t = 2.44, p = 0.02) (Table 1, Figure 13).
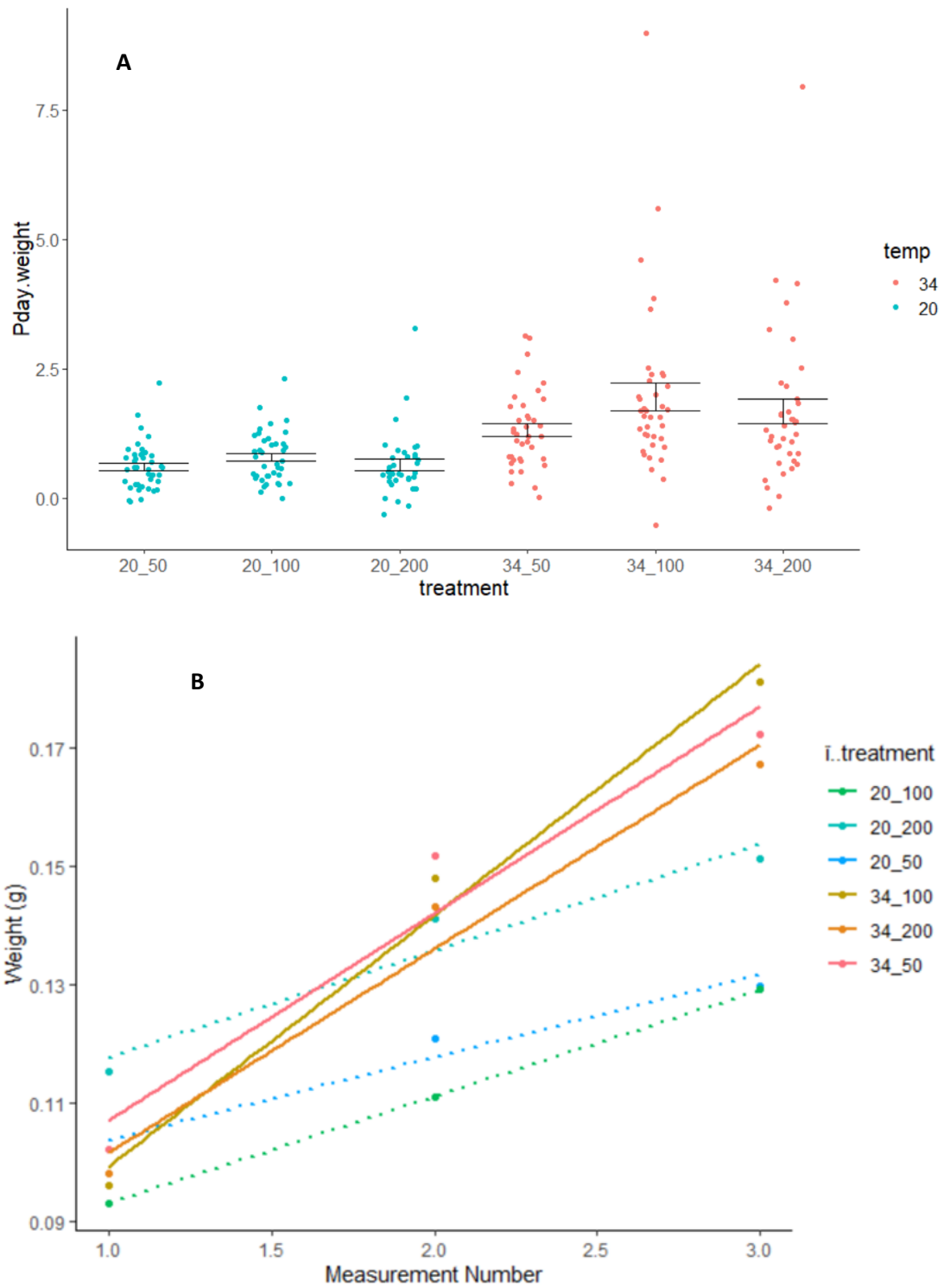
Figure 13*:* A: Growth rate of all treatments illustrated as % increase in weight throughout the acclimation period. Blue color is cold acclimated and red color is warm acclimated fish (including oxygen level). Error bars are mean of each group with standard error. B: Linearity of the three weight measurements performed.

Overall, 34 °C acclimated fish had consistently higher growth rate and final weight than 20 °C acclimated fish with warm acclimated fish experiencing reducing effects of change in oxygen level. The effect of temperature on growth rate were substantially larger in warm acclimated fish than in cold, with cold fish experiencing a ~ 60% lower growth rate (Table 1, Figure 13).

MO2 – $MO_{2,routine}$, $MO_{2,max}$ and Aerobic Scope

At the end of the acclimation period, hypoxia and hyperoxia including the additional effect of temperature on aerobic scope were investigated. To test the effect of temperature and oxygen availability on metabolic rates, all fish groups were exposed to their identical acclimation treatment during the respirometry. $MO_{2,routine}$, $MO_{2,Max}$ and aerobic scope were measured.

Initially there were 48 groups of fish running the respirometry trials. Due to technical errors, we lost data on group 1-4. The following analysis was done on the remaining 44 groups.

Warm acclimated fish (34 °C)

$MO_{2,routine}$ for normoxic warm acclimated fish was 1.31 ± 0.07 mg O_2 $h^{-1}g^{-1}$, and was not significantly different from the hypoxic treatment (t = - 1.59, p = 0.12). Hyperoxic fish had a significantly higher $MO_{2,routine}$, and was increased by 22.9 % (t = 2.88, p = 0.007) (Table 2 and 3, Figure 14).

$MO_{2,max}$ for normoxic fish were 3.00 ± 0.17 O_2 $h^{-1}g^{-1}$ and $MO_{2,max}$ for hypoxic fish was reduced by 25 % from normoxic (t = - 2.94, p = <0.05). Furthermore, $MO_{2,max}$ for hyperoxic fish was not significantly reduced from the normoxic group (t = - 0.47, p = 0.64). Aerobic scope was 1.69 ± 0.16 mg O_2 $h^{-1}g^{-1}$ for normoxic fish, and significantly reduced in hypoxia by 34.3 % (t = - 2.56, p = 0.01). On the other hand, aerobic scope was reduced by 24.9 % in hyperoxia (t = - 1.86, p = 0.07). Although not significant throughout the full gradient, aerobic scope increased with oxygen availability from hypoxia to normoxia and was substantially reduced in hyperoxia (Table 2 and 3, Figure 14).

Cold Acclimated Fish (20 ℃)

$MO_{2,routine}$ for normoxic cold acclimated fish was 0.59 ± 0.07 mg O_2 $h^{-1}g^{-1}$, and was not significantly increased when treated with hyperoxia ($t = 0.25$, $p = 0.80$). Furthermore, hypoxic fish had significantly lower $MO_{2,routine}$, and was reduced by 33.9 % ($t = -2.05$, $p = 0.047$) (Table 5 and 6, Figure 14).

$MO_{2,max}$ for normoxic fish was 1.18 ± 0.17 mg O_2 $h^{-1}g^{-1}$. $MO_{2,max}$ for hypoxic fish was not significantly different from normoxic ($t = -1.04$, $p = 0.30$), on the other hand, $MO_{2,max}$ for hyperoxic fish increased $MO_{2,max}$ with 51 % ($t = 2.25$, $p = 0.03$).

Aerobic scope was 0.59 ± 0.16 mg O_2 $h^{-1}g^{-1}$ for normoxic fish, and only marginally lower in hypoxia ($t = -0.21$, $p = 0.84$). On the other hand, aerobic scope was increased by 95 % in hyperoxia ($t = 2.38$, $p = 0.02$). Although not significant throughout the full gradient, aerobic scope increased with oxygen availability (Table 3 and 4, Figure 14)
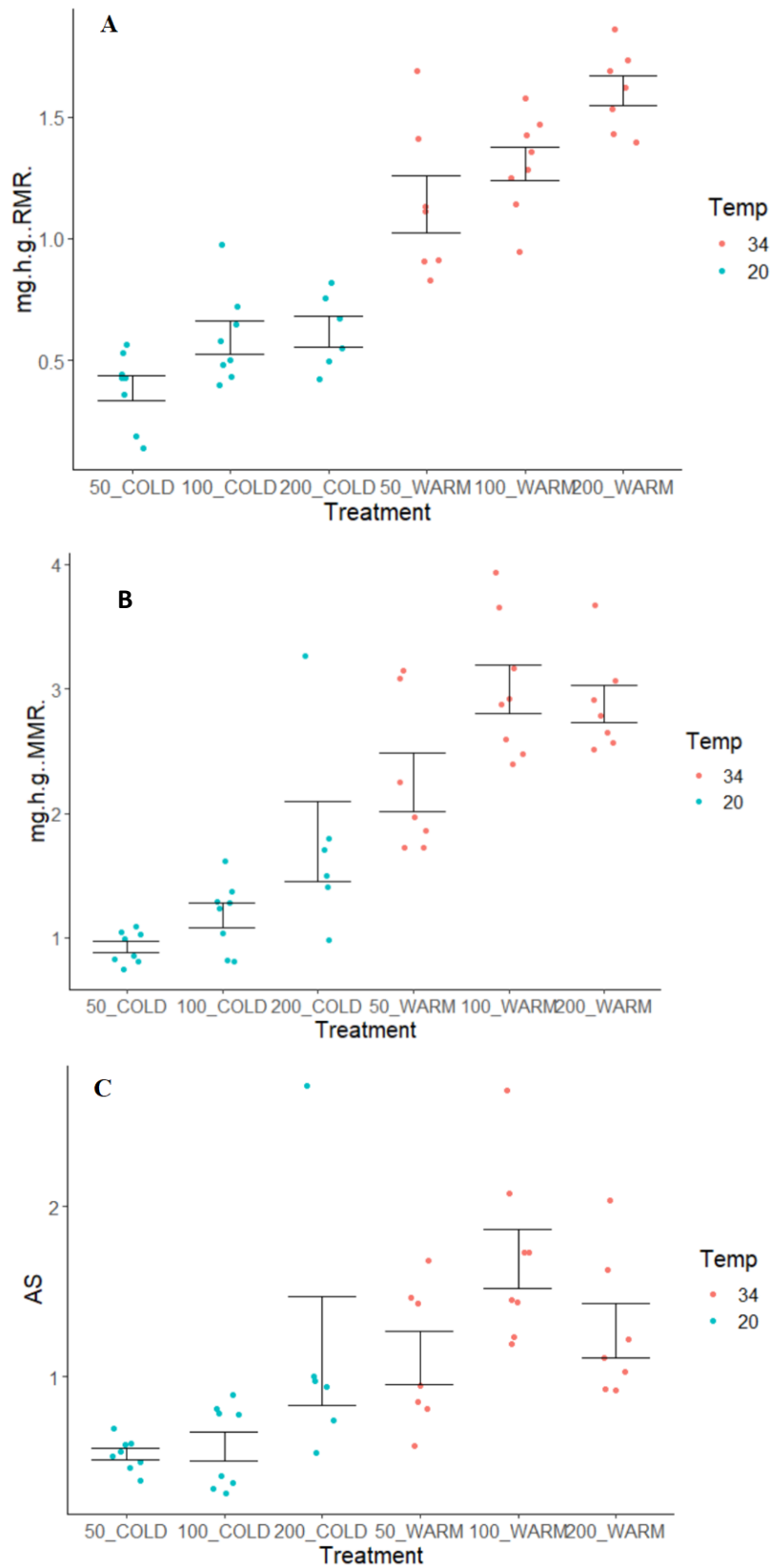
Figure 14: A: Routine metabolic rate ($MO_{2,routine}$), B: Maximum metabolic rate ($MO_{2,max}$) and C: Aerobic scope. Measurements are done on group level (N=48). Blue colour is cold and red color is warm acclimated fish (including oxygen level). Error bars are given as mean and standard error.

Overall, the effect of increased temperature was positive, and nearly all measures of $MO_{2,routine}$ and $MO_{2,max}$ and average aerobic scope were higher in 34 °C than in 20 °C (Table 2,3,4 and 5, Figure 14).

Appetite

Appetite was throughout the acclimation period estimated by 11 trails, resulting in a measure of the development of appetite. Only the three first and the three last trails are included in the analysis.

Warm acclimated fish (34 °C)

Mean appetite of normoxic fish were 0.33 ± 1.02 increase in consumed dry flakes day^{-1} (Table 7, Figure 15). The effect of hypoxia decreased the appetite slightly by $- 1.17 \pm 1.45$ dry flakes day^{-1} although not significantly ($t = - 0.84$, $p = 0.41$). On the other hand, hyperoxia reduced the appetite to $- 2.29 \pm 1.45$ dry flakes day^{-1} ($t = - 1.80$, $p = 0.08$). In addition, holding tank influenced the variation in appetite with 13.7 %.

Cold acclimated fish (20 °C)

Mean appetite of all fish was $- 1.27 \pm 0.55$ decrease in dry flake consumed day^{-1} with no difference between oxygen treatments (Table 6, Figure 15)

In total, cold acclimated fish had lower appetite than warm acclimated fish (Table, 6 and 7, Figure 15), and responded substantially lower to change in oxygen availability. Only normoxic warm acclimated fish developed a positive appetite-trend.
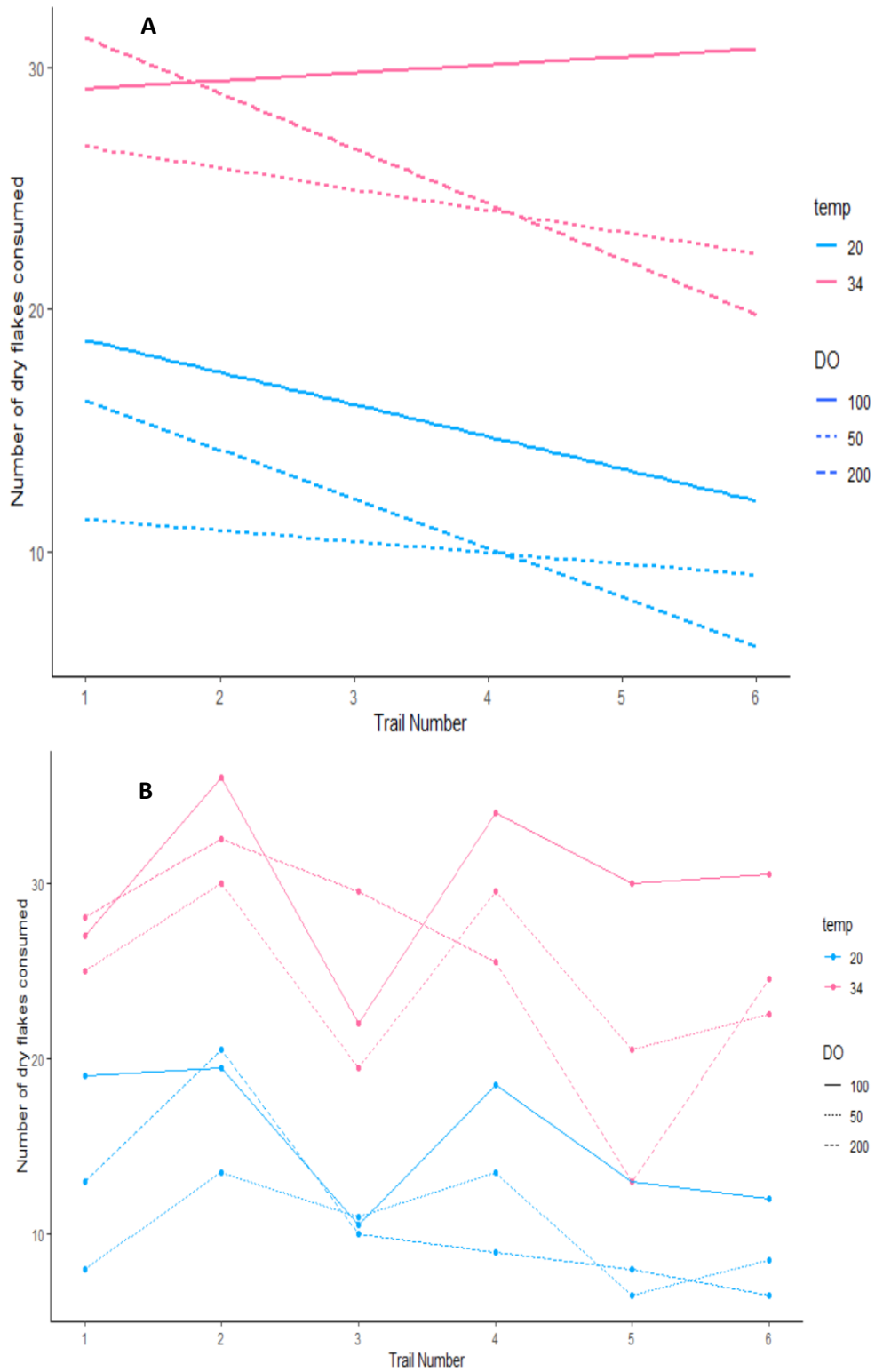
Figure 15*:* A: Linear transformed development of appetite throughout six trails of appetite measurements. B: Raw-data of the appetite measurements. Blue color is cold acclimated fish and pink color is warm acclimated fish (oxygen level are illustrated with linetype).

Correlations – Growth rate and aerobic scope
Weight and length

We found % weight increase day^{-1} and % length increase day^{-1} to correlate significantly by (R^2= 0.79, p= < 0.05) including all treatments.

Growth rate and aerobic scope

For the correlation between growth rate and aerobic scope, average growth rate in weight and average aerobic scope for all fish groups were used.

The overall correlation between aerobic scope and growth rate including both temperatures and oxygen level was positive and statistically significant (r = 0.27, p = < 0.005). In both temperatures separately, this correlation was also positive, and higher correlated in 34 °C than 20 °C (r = 0.22, p = 0.21 and r = 0.15, p = 0.38) respectively. Nor hypoxia or hyperoxia had any statistically significant influence on this correlation in neither 20 °C or 34 °C. However, our data suggests oxygen level to influence the correlation to a greater extent, and more positive in 34 °C (Table 9 and 10, Figure 16).
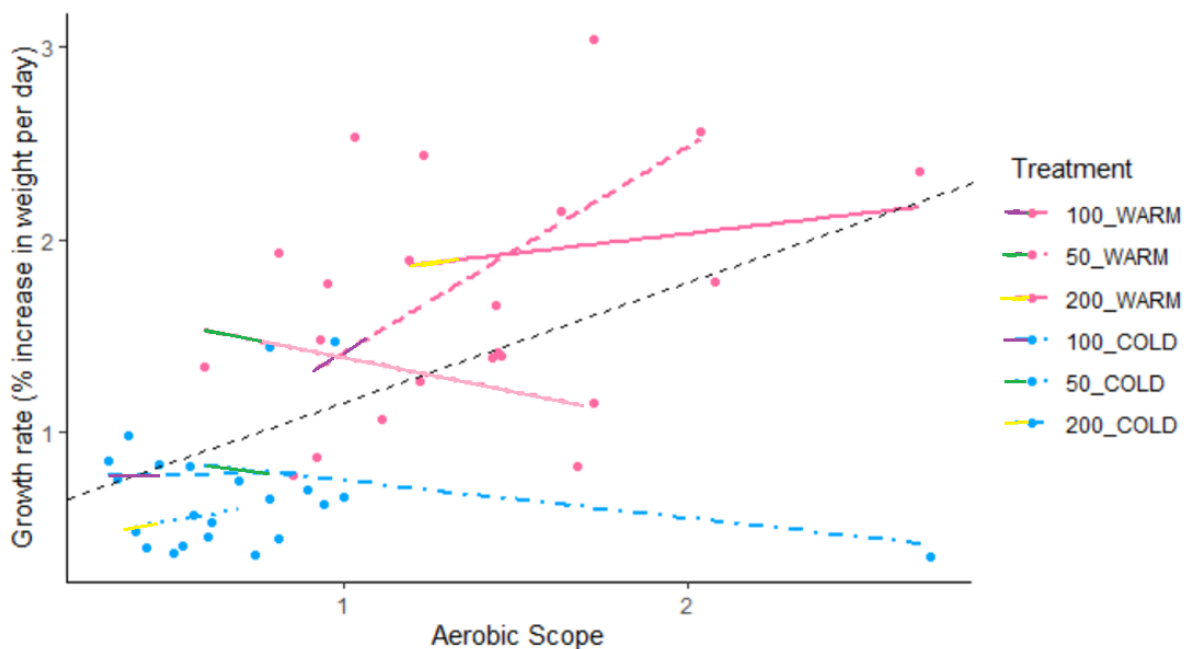
Figure 16*:* Correlation between growth rate (% increase in weight per day) and aerobic scope. The colored lines represent the different treatments with blue being cold acclimated and pink being warm acclimated fish. Black dotted line is the overall correlation trend ($R^2 = 0.27$).

DISCUSSION

In this study, we aimed at identifying and functionally dissecting the mechanisms underlying the processes of reduced growth with increasing temperatures in zebrafish. The thermal and O_2-related parameters measured was then linked together to form a complete picture of these complex processes.

The difference between the oxygen- and capacity-limited thermal tolerance hypothesis (OCLTT-hypothesis) and the aerobic scope protection hypothesis (ASP-hypothesis) as proposed by (Jutfelt et al., 2021), is that cardiorespiratory oxygen limitations only develop at high temperatures if the animal allows its SDA to occupy the majority of its aerobic scope. The potential of temporal compression of the PRAS will be increased with increasing temperature, and the animal should respond by lowering the food intake. Accordingly, temperature should be a major interacting factor in the correlation between growth rate and aerobic scope. If reductions in growth occurs through reduction in food intake, its predicted that the appetite would be more sensitive to water hypoxia in warm than in cold. In addition, fish should experience increased oxygen limitation on appetite with increasing temperature. However, this effect could potentially be reduced with hyperoxia (Jutfelt er al., 2021) (Figure 5).

Fish acclimated to a supra-optimal temperature had higher growth rate than cold acclimated fish, and both hypoxia and hyperoxia negatively affected this growth. However, not significant in all treatments. Thus, we find contrary to our prediction, that growth rate in temperatures above T_{opt} could not be enhanced by hyperoxia. In contrast to our prediction (Figure 4), we found hyperoxia to reduce growth rate as temperature approached T_{crit}. In general, the bulk of existing evidence shows no effect of hyperoxia on growth (McArley, 2021). Contrary, Brijs et al (2015) found aerobic scope to increase with hyperoxia in European perch (*Perca fluviatilis Linneaus*), and further suggested that hyperoxia could allow for higher food intake and thus growth. Our data did not support these suggestions in zebrafish when including supra-optimal temperature as a factor, as the zebrafish tended to have decreased aerobic scope when approaching T_{crit}. In the studied zebrafish, aerobic scope declined at high acclimation temperature contradicting other studies that suggests high aerobic scope and growth at high temperatures (Gräns et al., 2014; Norin et al., 2014; Brijs et al., 2015).

In accordance with Vergauwen (2010) it is suggested that exposure to supra-optimal temperature is particularly stressful for fishes. Our findings could possibly indicate that long term acclimation of 34 °C is stressful and that the effect of stress would overcome the effect of physiological acclimation. Moreover, both hypoxia and hyperoxia are both acting as additional stressful factors. The interaction between high temperature and change in oxygen level is then resulting in more extreme differences in warm acclimated fish. As temperature rise beyond T_{opt}, the accelerated metabolism can overcome the ability for efficient feed conversion and appetite (Imsland and Jonassen, 2001; Volkoff, 2020; Volkoff and Rønnestad, 2020). However, studies on killifish (*Fundulus heteroclitus*) and southern flounder (*Paralichthys lethostigma*) have suggested a shift in T_{opt} toward lower temperature in hypoxia, indicating that the interactions between temperature and hypoxia have characteristics of a threshold interaction and of synergism (Del Toro-Silva et al. 2008; Healy & Schulte, 2012; McBryan et al., 2013).

Within the OCLTT framework, and in the context of chronic exposure, hypoxia would be expected to limit growth as a result of the reduced height of the aerobic scope-temperature relationship (McBryan et al., 2013). In addition, hypoxia is suggested to be poorer tolerated in warm than cold due to the right shift in the Hb-O_2 saturation curve. Our findings support the idea that the available energy for growth is reduced when aerobic scope in warm hypoxic environments is decreased (McBryan et al., 2013). In addition, tissue hypoxia should restrict growth due to increased anaerobic metabolism (Pörtner & Farrell, 2008; Pörtner & Knust, 2007), but only in temperatures close to lethal.

Overall, within warm acclimated fish, both hypoxic and hyperoxic fish had lower aerobic scope than normoxic fish. Our results suggests both hypoxia and hyperoxia to be stressful agents in terms of growth and metabolism, thus acting by different processes; hypoxia leading to relatively low $MO_{2,routine}$, and greatly reduced $MO_{2,max}$, and hyperoxia leading to high $MO_{2,routine}$ and close to no change in $MO_{2,max}$, both resulting in reduced growth and aerobic scope. From Pörtner (2010), the effects of hypoxia on $MO_{2,routine}$, $MO_{2,max}$ and aerobic scope are in stark contrast to the effect of temperature. Hypoxia acts to limit oxygen supply and would be affecting $MO_{2,max}$ over a much wider oxygen-gradient than $MO_{2,routine}$. While $MO_{2,routine}$ is suggested to only be affected under severe hypoxia, the aerobic scope in hypoxia would experience different patterns depending on the extent of the available O_2. According to our findings, with both hypoxia and hyperoxia lowering the aerobic scope in

warm acclimated fish, hyperoxia almost doubled (+ 95 %) aerobic scope in cold acclimation. Despite this extreme increase, one of the fish groups had remarkably high aerobic scope elevating the average aerobic scope substantially. In line with Brijs et al (2015), hyperoxia (200%) has been shown to double the aerobic scope in European perch (*Perca fluviatilis*) at optimal temperature (Bris et al, 2015). Similar to the predictions made by McBryan (2013), $MO_{2,max}$ was reduced in hypoxia, but we also found a slight reduction when treated with hyperoxia in 34 ° C. Tissue exposed to high oxygen levels has been suggested to produce reactive oxygen species (ROS), leading to oxidative stress (McArley et al., 2021). It is therefore plausible that there is a harmful effect of the interaction between high temperature and hyperoxia since we only found decreased effect on all performance parameters. Lastly, our findings support the general effects of temperature on $MO_{2,min}$ and $MO_{2,max}$ proposed in McBryan (2013), where both $MO_{2,routine}$ and $MO_{2,max}$ were expected to increase with higher temperature.

As in growth and metabolism, we find in line with the ASP-hypothesis higher appetite in warm acclimated fish, with oxygen level affecting warm fish to a greater extent than cold fish. These findings partly support the suggestion made by Jutfelt et al (2021), stating a bigger difference in appetite among oxygen treatments in warmer temperature, and no difference in cold. Interestingly, this effect was most pronounced in warm acclimated fish having reduced appetite in hyperoxia. This seemingly contradicts the specific predictions (Figure 4 and 5) with hyperoxia leading to higher appetite and growth rate with warming. Limited aerobic scope apparently leads to reduced appetite in hyperoxia, again highlighting the potential harmful effects when fish are exposed to elevated temperature and oxygen levels.

Compilation of data on fish physiology suggests a sharp decline in appetite at temperatures above optimal, and this decline in appetite most likely relates to a change in appetite-regulating hormones (Rønnestad et al., 2017; Volkoff, 2016). With an increasing $MO_{2,routine}$ at higher temperature, in addition to the interacting effects of hyperoxia and temperature, the limited aerobic scope for growth and food processing are therefor though to be part of the explanation. On the other hand, cold acclimated fish developed a negative appetite-trend during the whole acclimation-period, resulting in substantially lower appetite than in warm acclimation. Oxygen level nor increased or decreased the appetite of cold fish, in contrary to the warm. Previously, a few studies on rainbow trout tested these effects, although with ambiguous results.

In one of the studies hyperoxia showed to increase appetite in temperatures way above optimal and no effect at normal temperatures. However, the other study did not find the same benefit of hyperoxia (Dabrowski et al., 2004; Caldwell & Hinshaw, 1994). Furthermore, including temperature as a systematic factor when aiming to repeat these findings, we failed to find any positive effect of hyperoxia. In relation to this, fishes tend to hypoventilate when exposed to hyperoxia. Hypoventilation will in turn lead to retention of CO_2 and respiratory acidosis, in addition to oxidative stress and concomitant changes to antioxidant defense systems (McArley, 2021). On the other hand, some evidence exists on increased aerobic capacity, improved cardiac performance and mitigation of anaerobic stress with hyperoxia during acute warming (Clark et al., 2008). As revealed by McArley (2021), an exponential decay in total ventilation with increasing O_2 levels have been found across species when exposed to hyperoxia (Mark et al., 2002; McArley et al., 2018). With reduced ventilation of about 50 % at 200 % air saturation, retention of bicarbonate may be highly impaired leading to acid-base disturbances. Despite hypoventilation, PaO_2 (partial pressure in arterioles) has been found to increase in hyperoxia. Counterintuitively, this effect was not found to enhance O_2-uptake, exemplified by that increased PaO_2 came along with decreased blood pH in a study in common carp (Takeda, 1990). Together, this resulted in a 6 % decrease in the Hb-O_2 saturation. In general, the majority of studies demonstrate no effect of hyperoxia on blood oxygen-carrying capacity as reflected by Hb and hematocrit, but direct testing of long-term exposure of hyperoxia and supra-optimal temperature still lacks (Jutfelt et al., 2021).

Furthermore, and with mixed support in the field (Jutfelt et al., 2021; Blier et al., 1997; Auer et al., 2015) we find aerobic scope to correlate with growth rate in supra-optimal temperatures, as predicted in figure 6. Aerobic scope is in general not considered to correlate with growth rate (Blier et al., 1997), although this should be temperature-dependent (Jutfelt et al., 2021). The effects of oxygen level on this correlation seemed to be of little importance in both warm and cold, although slight differences among oxygen level in warm acclimation, with hypoxia reducing and hyperoxia increasing the correlation. Contrary to the study of the correlation between aerobic scope and growth rate in cod (Blier, 1997), there is evident that supra-optimal temperatures led to a higher correlation in our study. In optimal temperature, the cod showed no correlation between either gill surface area, muscle aerobic capacity or conversion efficiency in the digestive tract with growth rate. On the other hand, Auer (2015) found aerobic scope to correlate with maximum food consumption ($R^2 = 0.35$) in juvenile brown trout (*Salmo trutta*). The author suggested that given the cost of SDA increases with

meal size, aerobic scope constrains the SDA-response and thus food consumption rates. With the correlation of ($R^2 = 0.22$), we find aerobic scope and growth rate to correlate in supra-optimal temperatures. These findings could potentially indicate to a «aerobic scope protection»-like process with increased aerobic scope allowing a substantially PRAS to consume food in warm temperatures.

Overall, the general interacting effects of temperature with hypoxia and hyperoxia above T_{opt} on all the performance-parameters measured in this study may suggest more mechanical-physiological explanations. As both the OCLTT and ASP-hypothesis predicts, O_2 may be limiting when approaching unsustainable temperatures. The question is by which processes the accumulating O_2-debt is avoided and repaid. In general, O_2 transfer to the tissue is a process of complex physiology, including factors as hemoglobin-affinity, cardiac output, ventilation, and hormone regulation (Perry and Gilmour, 2002). As our findings indicate that the potential positive effects of hyperoxia disappears in supra-optimal temperatures, this may suggest the integrative physiology behind O_2- and thermal regulation reaches functional ceilings, indicating capacity limitation (Gollock et al., 2006; Clark et al., 2008; Mendonça and Gamperl, 2010).

Several studies suggests that the additional cardiorespiratory load associated with exposure to higher temperature is partially offset in hyperoxia by reduced circulatory and ventilatory costs (Mark et al., 2002; Berschick et al., 1987; McArley, 2018). Although there in theory may be more accessible O_2 in hyperoxia, the metabolism of warm fish already reached its physiological peak, and hence the effect of increased available O_2 disappears (Gollock et al., 2006; Clark et al., 2008; Mendonça and Gamperl, 2010). If so, these processes would affect the simple idea originating from the ASP-hypothesis, that higher aerobic scope would allow for a higher postprandial peak metabolic rate during the SDA-response without markedly restricting postprandial residual aerobic scope or tissue oxygenation. The temperature-sensitive effect of hyperoxia would in turn not increase appetite at higher temperatures. Consequently, the general assumptions coming from the ASP-hypothesis with hyperoxia having increased effect at increasing supra-optimal temperatures and thus leading to increased appetite may need closer attention.

Overall, we find all the performance parameters to change in correlation with aerobic scope. In addition, it seems that appetite and growth are strongly correlated, with aerobic scope setting the limits for change in those. Aerobic scope declines at high acclimation temperatures

(Morgan, 2020; Gräns et al., 2014; Norin et al., 2014) thus supporting two of the predictions of the OCLTT-hypothesis, that when aerobic scope peak and declines, other performances follow (Lefevre, 2016). With aerobic scope in warm temperatures reacting to changing oxygen levels, we find that other performances follow this oxygen availability related aerobic scope-change in the same directions. Same applies to cold, where aerobic scope in general remains unchanged with changing oxygen level, and other performances remain unchanged.

CONCLUSION

In conclusion, our study suggests better growth, appetite and aerobic scope in warm acclimated fish compared to cold acclimated fish, with warm fish to a certain degree responding to changing oxygen levels. We find aerobic scope to set limits for appetite and growth, with hypoxia in general lowering the performance. We predicted that hyperoxia could lead to elevated aerobic scope and better appetite, however, our results indicates that warm acclimated fish experiences no or negative effect of hyperoxia. Consequently, it seems like the effect of oxygen on appetite, growth and aerobic scope is dependent on acclimation temperature in this species. Accordingly, we found growth rate to correlate with aerobic scope to a greater extent in warm acclimation, with close to no correlation in cold acclimation. Overall, our findings confirm that increased temperature close to lethal are in particular stressful for fishes, with changing oxygen levels having additional negative effects of important fitness factors. Increasingly supra-optimal temperatures in interaction with hypoxic environments are especially demanding and should receive considerable attention in the future. We do not support the prediction of hyperoxia leading to neither higher appetite nor growth in warm acclimated fish, which in turn remains the underlying processes of an aerobic scope protection-like behavior unknown.

With our method we were not able to directly test appetite in relation to SDA, and the potential voluntarily avoidance of large meals with increasing temperature. Overall, we found cold acclimated fish to represent a slower life pace than warm acclimated fish, with little response to change in oxygen level. Ideally, a temperature gradient starting at optimal temperature to ~ 35 ° C would be interesting to test. Lastly, with our findings of poor effect of hyperoxia in all performance parameters, future focus on hypoxia would probably bring more realistic knowledge to the field.

Remarks and future directions

First of all, we recommend future researchers to include optimal temperature ($\sim 28\,°C$) in their study. Initially, we aimed to test the standard metabolic rate more explicitly with increasing temperature. The original idea was to run 24-hour standard metabolic rate trials with an automatic water-flushing concept. Unfortunately, we found this method way to complex with the available equipment. Therefore, we must note that our 30-minute $MO_{2,routine}$ −trails could potentially be influenced by general handling stress. In addition, we fasted the fish for the same amount of time independent of acclimation temperature. According to Jutfelt et al (2021), the SDA-response are suggested to last for a longer period in cold than in warm. This could potentially result in lingering SDA-response in cold acclimated fish, affecting all metabolic rate measurements. Therefore, there are several limitations to our study regarding the effect of temporally compressed SDA-response.

LITTERATURE

Alsop, D. H., & Wood, C. M. (1997). *The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (Oncorhynchus mykiss).* Journal of Experimental Biology, 200(17), 2337–2346. https://doi.org/10.1242/ jeb.200.17.2337

Andreassen, H. A. (2018). *Development of an automated annular arena and thermal preference of zebrafish selected for thermal tolerance*. (Master thesis). NTNU

Auer, S. K., Salin, K., Anderson, G. J., & Metcalfe, N. B. (2015). *Aerobic scope explains individual variation in feeding capacity.* Biology Letters, 11(11), 20150793–20150803. https://doi.org/10.1098/ rsbl.2015.0793

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

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

Berschick, P., Bridges, C., & Grieshaber, M. (1987). *The influence of hyperoxia, hypoxia and temperature on the respiratory physiology of the intertidal rockpool fish Gobius cobitis Pallas.* Journal of Experimental Biology, 130(1), 368–387.

Blier, P. U., Pelletier, D., & Dutil, J.-D. (1997). *Does aerobic capacity set a limit on fish growth rate?* Reviews in Fisheries Science, 5(4), 323–340. https://doi.org/10.1080/10641269709388604

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

Burggren W. 1982. *''Air gulping'' improves blood oxygen transport during aquatic hypoxia in the goldfish Carassius auratus.* Physiol Zool 55:327–34.

Caldwell, C. A., & Hinshaw, J. (1994). *Physiological and haematological responses in rainbow trout subjected to supplemental dissolved oxygen in fish culture.* Aquaculture, 126(1–2), 183–193. https://doi. org/10.1016/0044-8486(94)90259-3

Clark TD, Sandblom E, Cox GK, Hinch SG, Farrell AP (2008). *Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (Oncorhynchus tshawytscha).* Am J Physiol Regul Integr Comp Physiol 295: R1631-1639

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

R.J. Collier, L.H. Baumgard, R.B. Zimbelman, Y. Xiao. *Heat stress: physiology of acclimation and adaptation.* Anim. Front., 9 (2019), pp. 12-19, 10.1093/af/vfy031

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

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.

Dabrowski, K., Lee, K.-J., Guz, L., Verlhac, V., & Gabaudan, J. (2004). *Effects of dietary ascorbic acid on oxygen stress (hypoxia or hyperoxia), growth and tissue vitamin concentrations in juvenile rainbow trout (Oncorhynchus mykiss).* Aquaculture, 233(1–4), 383–392. https://doi.org/10.1016/j.aquaculture.2003.09.047

Del Toro-Silva FM, Miller JM, Taylor JC, Ellis TA. 2008. *Influence of oxygen and temperature on growth and metabolic performance of Paralichthys lethostigma (Pleuronectiformes: Paralichthyidae).* J Exp Mar Biol Ecol 358:113–23.

Ege, R., & Krogh, A. (1914*). On the relation between the temperature and the respiratory exchange in fishes*. Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie, 7(1), 48–55. https://doi. org/10.1002/iroh.19140070105

Frederich M, Pörtner HO (2000). *Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, Maja squinado.* Am J PhysiolRegul Integr Comp Physiol 279: R1531–R1538

Fu, S. J., Xie, X. J., & Cao, Z. D. (2005*). Effect of meal size on postprandial metabolic response in southern catfish (Silurus meridionalis).* Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 140(4), 445–451. https://doi.org/10.1016/j. cbpb.2005.02.008

Gollock MJ, Currie S, Petersen LH, Gamperl AK. (2006).*Cardiovascular and haematological responses of Atlantic cod (Gadus morhua) to acute temperature increase*. J Exp Biol 209: 2961–2970

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

Hohn, C. and Petrie-Hanson, L. (2013). *Evaluation of visible implant elastomer tags in zebrafish (Danio rerio).* Biol. Open 2, 1397-1401. doi:10.1242/bio. 20136460

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

Imsland, A.K. and T.M. Jonassen, (2001). *Regulation of growth in turbot (Scophthalmus maximus Rafinesque) and atlantic halibut (Hippoglossus hippoglossus L.):* aspects of environment genotype interactions. Rev. Fish Biol. Fish. 11:71-90.

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

Jobling, M. (1997). *Temperature and growth: Modulation of growth rate via temperature change.* In C. M. Wood, & D. G. McDonald (Eds.), Global warming implications for freshwater and marine fish (pp. 1–32). Cambridge University Press.

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

Jutfelt, F., Norin, T., Åsheim, E.R., Rowsey, L.E., Andreassen, A.H., Morgan, R. et al. (2021) *'Aerobic scope protection' reduces ectotherm growth under warming*. Functional Ecology, 35(7), 1-11.

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).

Lefevre S. (2016). *Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO_2 and their interaction*. Conserv Physiol 4. doi:10.1093/conphys/cow009

Leeuwis Robine H. J., Zanuzzo Fábio S., Peroni Ellen F. C. and Gamperl A. Kurt (2021). *Research on sablefish (Anoplopoma fimbria) suggests that limited capacity to increase heart function leaves hypoxic fish susceptible to heat waves*. Proc. R. Soc. B.2882020234020202340. http://doi.org/10.1098/rspb.2020.2340

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.

MacLean HJ, Sørensen JG, Kristensen TN, Loeschcke V, Beedholm K, Kellermann V, Overgaard J. (2019). *Evolution and plasticity of thermal performance: an analysis of variation in thermal tolerance and fitness in 22 Drosophila species*. Philos Trans R Soc B Biol Sci 374: 2018054

Mandic M, Todgham AE, Richards JG. 2009. *Mechanisms and evolution of hypoxia tolerance in fish.* Proc R Soc Lond B Biol Sci 276:735–44.

Mark FC, Bock C, Pörtner HO. (2002). *Oxygen-limited thermal tolerance in Antarctic fish investigated by MRI and 31P-MRS*. Am J Physiol-Regul Integr Comp Physiol 283: R1254–R1262.

McArley, T. J., Hickey, A. J., & Herbert, N. A. (2018). *Hyperoxia increases maximum oxygen consumption and aerobic scope of intertidal fish facing acutely high temperature*s. 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

Mendonça PC, Gamperl AK (2010). *The effects of acute changes in temperature and oxygen availability on cardiac performance in winter flounder (Pseudopleuronectes americanus).* Comp Biochem Physiol A Mol Integr Physiol 155: 245–252

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. (2020). *Physiological plasticity and evolution of thermal performance in zebrafish* [PHD]. NTNU, Norway.

Morgan, R., Andreassen, A. H., Asheim, E. R., Finnøen, M. H., Dresler, G., Brembu, T., Adrian Loh., Joanna J. Miest., Fredrik Jutfelt. (2022). *Reduced physiological plasticity in a fish adapted to stable conditions*. (Unpublished manuscript).

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., 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., 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

Perry, S.F. and Gilmour, K.M. (2002). *Sensing and transfer of respiratory gases at the fish gill.* J. Exp. Zool., 293: 249-263. https://doi.org/10.1002/jez.10129

Pörtner. H. O. (2010*). Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems.* J Exp Biol 213: 881–893

Pörtner, H. O., & Farrell, A. P. (2008). ECOLOGY: *Physiology and Climate Change.*

Science, 322(5902), 690-692. doi:10.1126/science.1163156

Pörtner, H. O., & Knust, R. (2007). *Climate change affects marine fishes through the oxygen limitation of thermal tolerance*. Science, 315(5808), 95–97. https://doi.org/10.1126/science.1135471

Reiersen, M. (2021). *The effects of oxygen availability and acclimation temperature on upper thermal tolerance in zebrafish (Danio rerio.* (Master thesis). NTNU

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

Rønnestad, I., Gomes, A. S., Murashita, K., Angotzi, R., Jönsson, E., & Volkoff, H. (2017). *Appetite-controlling endocrine systems in teleosts*. Frontiers in Endocrinology, 8(3), 661–724. https://doi. org/10.3389/fendo.2017.00073

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

Secor, S. M. (2009). Specific dynamic action: *A review of the postprandial metabolic response*. Journal of Comparative Physiology B, 179(1), 1– 56. https://doi.org/10.1007/s00360-008-0283-

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

Somero, G. N. (2004). Adaptation of enzymes to temperature: *searching for basic ''strategies''*. Comparative Biochemistry and Physiology, Part B 139 (2004) 321 – 333. 10.1016/j.cbpc.2004.05.003

J.H. Stillman. (2019). Heat waves, the new normal: *Summertime temperature extremes will impact animals, ecosystems, and human communities*. Physiology, 34, pp. 86-100, 10.1152/physiol.00040.2018

Takeda, T. (1990). *Ventilation, cardiac output and blood respiratory parameters in the carp, Cyprinus carpio, during hyperoxia*. Respiration Physiology, 81(2), 227–239. https://doi.org/10.1016/0300- 9629(90)90243-L

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

Thorarensen, H., & Farrell, A. P. (2006). *Postprandial intestinal blood flow, metabolic rates, and exercise in Chinook Salmon (Oncorhynchus tshawytscha)*. Physiological and Biochemical Zoology, 79(4), 688–694. https://doi.org/10.1086/505512

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.

Volkoff, H. (2016). *The neuroendocrine regulation of food intake in fish: A review of current knowledge*. Frontiers in Neuroscience, 10, 236– 331. https://doi.org/10.3389/fnins.2016.00540

Volkoff, H., & Rønnestad, I. (2020). *Effects of temperature on feeding and digestive processes in □sh. Temperature*, 7(4), 307–320. https://doi.org/10.1080/ 23328940.2020.1765950

Vergauwen, L., Benoot, D., Blust, R & Knappen, D. (2010). *Long-term warm or cold acclimation elicits a specific transcriptional response and affects energy metabolism in zebrafish.* Comparative biochemistry and physiology, Part A. 157. 149-157. 10.1016/j.cbpa.2010.06.160

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

Zhang, W., Cao, Z.-D., & Fu, S.-J. (2012). *The effects of dissolved oxygen levels on the metabolic interaction between digestion and locomotion in Cyprinid fishes with different locomotive and digestive performances*. Journal of Comparative Physiology B, 182(5), 641–650. https://doi.org/10.1007/s00360-012-0644-0

Åsheim ER, Andreassen AH, Morgan R, Jutfelt F (2020). *Rapid-warming tolerance correlates with tolerance to slow warming but not growth at non-optimal temperatures in zebrafish.* J Exp Biol 223: 1–7.

APPENDIX

(1) Oxygen values

50% air saturation

Figure 1: Overview of dissolved oxygen level (50%) in hypoxic tanks. The graph shows slight variations throughout the acclimation period. Different colors represent different aquaria.

200 % air saturation

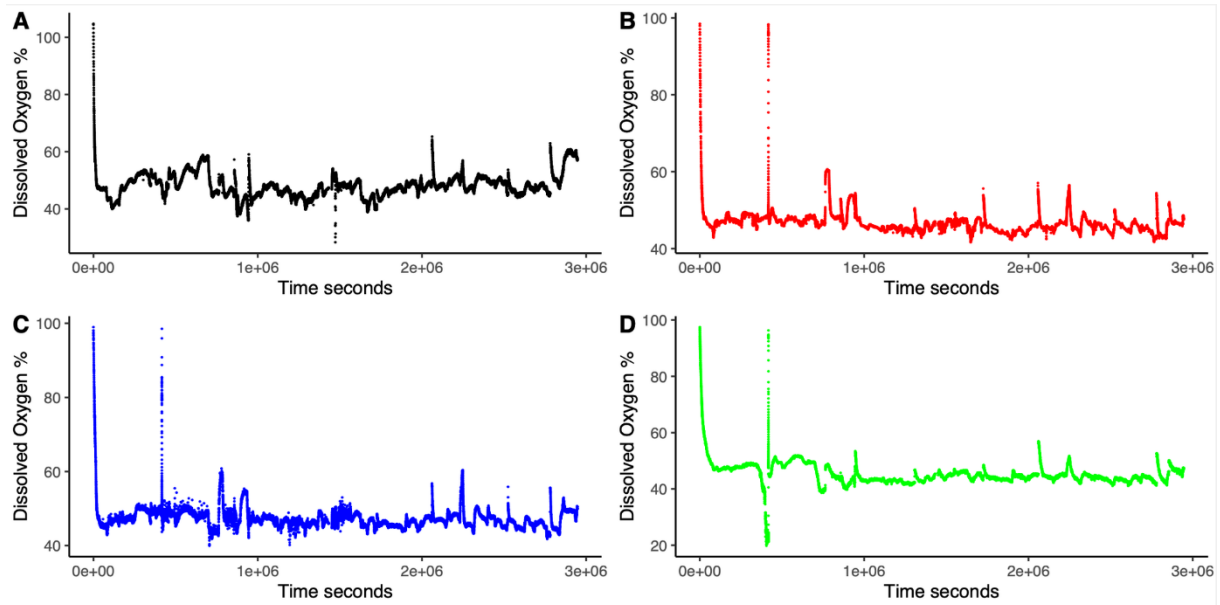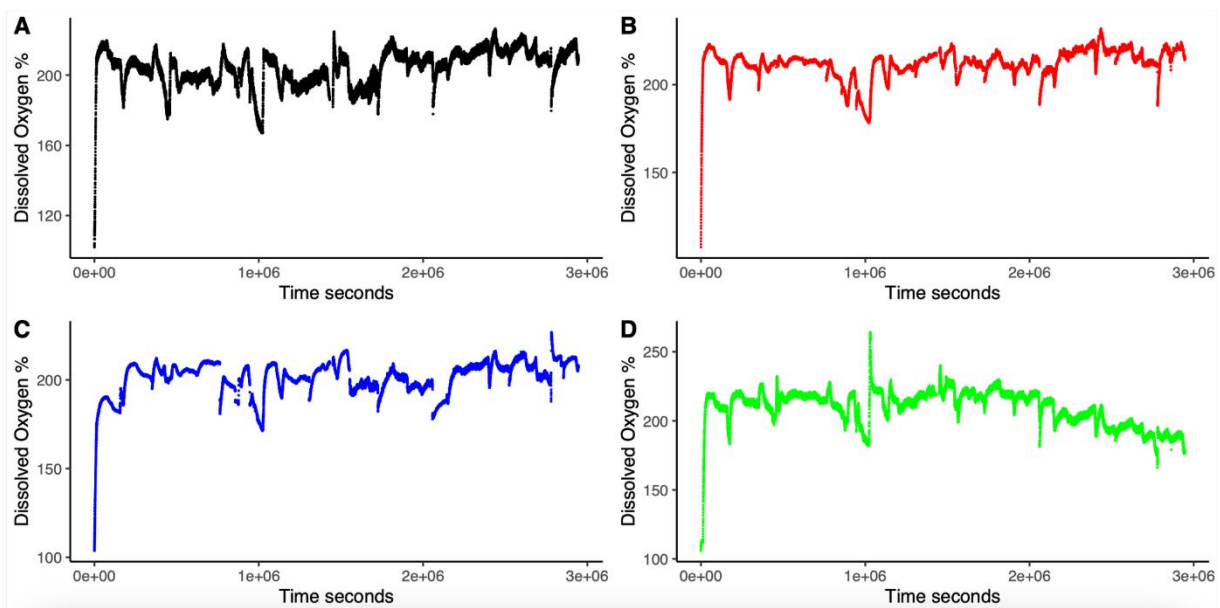
Figure 2: Overview of dissolved oxygen level (200%) in hyperoxic tanks. The graph shows slight variations throughout the acclimation period. Different colors represent different aquaria.

(2) Model selection tables

For simplicity, only the most biological meaningful models are included in the tables.

Aerobic Scope

Table 1: Model selection table for linear models explaining variation in aerobic scope in zebrafish (*Danio rerio*). AS_INT is model fitted with an interaction term and AS_ADD is model fitted with an additive term. Delta_AICc is the difference in AICc-units (adjusted for sample size) between models. Both models included oxygen level and temperature as explanatory variables and aerobic scope as response variable.

	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
AS_INT	7	63.05	0.00	0.88	0.88	-22.97
AS_ADD	5	67.01	3.96	0.12	1.00	-27.72

$MO_{2,max}$

Table 2: Model selection table for linear models explaining variation in $MO_{2,max}$ in zebrafish (*Danio rerio*). MMR_INT is model fitted with an interaction term and MMR_ADD is model fitted with an additive term. Delta_AICc is the difference in AICc-units (adjusted for sample size) between models. Both models included oxygen level and temperature as explanatory variables and $MO_{2,max}$ as response variable.

	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
MMR_ADD	5	71.72	0.00	0.63	0.63	-30.07
MMR_INT	7	72.75	1.03	0.37	1.00	-27.82

$MO_{2,routine}$

Table 3: Model selection table for linear models explaining variation in $MO_{2,routine}$ in zebrafish (*Danio rerio*). RMR_INT is model fitted with an interaction term and RMR_ADD is model fitted with an additive term. Delta_AICc is the difference in AICc-units (adjusted for sample size) between models. Both models included oxygen level and temperature as explanatory variables and $MO_{2,routine}$ as response variable.

	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
RMR_ADD	5	-6.55	0.00	0.68	0.68	9.07
RMR_INT	7	-5.07	1.49	0.32	1.00	11.09

Growth – Weight % per day

Table 4: Model selection table for linear models explaining variation in growth rate in zebrafish (*Danio rerio*). pday_weightINT is model fitted with an interaction term and pday_weightADD is model fitted with an additive term. Delta_AICc is the difference in AICc-units (adjusted for sample size) between models. Both models included oxygen level and temperature as explanatory variables and growth rate (weight) as response variable. The other models tested (irrespective of low AIC-value) was evaluated to not have any biological meaning.

	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
pdayw3	6	665.33	0.00	0.45	0.45	-326.48
pday_weightINT	5	665.96	0.63	0.33	0.77	-327.85
pday_weightADD	7	668.10	2.77	0.11	0.88	-326.80
pdayw6	8	668.23	2.90	0.10	0.99	-325.79
pdayw1	14	674.92	9.58	0.00	0.99	-322.48
pdayw4	14	674.92	9.58	0.00	1.00	-322.48
pdayw2	13	675.82	10.49	0.00	1.00	-324.06
pdayw5	13	675.82	10.49	0.00	1.00	-324.06

Appetite – 20 ℃

Table 5: Model selection table for linear models explaining variation in appetite for 20 °C acclimated zebrafish (*Danio rerio*). APP_INT is model fitted with an interaction term and APP_ADD is model fitted with an additive term. Delta_AICc is the difference in AICc-units (adjusted for sample size) between models. Both models included oxygen level and temperature as explanatory variables and appetite (consumed dry flakes) as response variable.

	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
APP_ADD	9	240.45	0.00	1	1	-107.77
APP_INT	19	293.37	52.92	0	1	-103.94

Appetite – 34 ℃

Table 6: Model selection table for mixed effect linear models explaining variation in appetite for 34 °C acclimated zebrafish (*Danio rerio*). lmer_app2_INT is model fitted with an interaction term and lmer_app2_ADD is model fitted with an additive term. AIC is the difference in AIC-units between models. Both models included oxygen level and temperature as explanatory variables and appetite (consumed dry flakes) as response variable. Holding tank was set as random factor in both models.

	df	AIC
lmer_app2_INT	20	161.0807
lmer_app2_ADD	10	209.5544

Correlations – Growth rate and aerobic scope

Table 7*: Model selection table for linear models explaining the correlation between growth rate and aerobic scope in zebrafish (*Danio rerio*). GrowthRate_AS_INT is model fitted with an interaction term and GrowthRate_AS_ADD is model fitted with an additive term. Delta_AICc is the difference in AICc-units (adjusted for sample size) between models. Both models included aerobic scope as explanatory variable and growth rate (weight) as response variable.*

```
                   K  AICc Delta_AICc AICcWt Cum.Wt      LL
x2_int             9 71.35       0.00   0.62   0.62 -24.03
GrowthRate_AS_ADD  8 72.36       1.00   0.38   1.00 -26.12
GrowthRate_AS_INT 13 82.03      10.68   0.00   1.00 -21.95
```

TABLES

Growth – Weight

Tabell 1: Overview of average initial, final and % increase per day weight. Estimates are given as mean with standard error. * indicate statistically significant differences from normoxia.

Treatment	Avg Initial weight (g)	Avg End weight	Avg % per day
20 ℃ - 50%	0.102±0.01	0.13±0.006	0.60±0.16
20 ℃ - 100%	0.093±0.01	0.13±0.006	0.79±0.16
20 ℃ - 200%	0.115±0.01	0.15±0.007 *	0.65±0.17
34 ℃ - 50%	0.102±0.01	0.17±0.006	1.31±0.16 *
34 ℃ - 100%	0.096±0.01	0.18±0.006	1.96±0.17
34 ℃ - 200%	0.098±0.01	0.17±0.006	1.67±0.17

Metabolism – Warm acclimated fish

Tabell 2: Overview of $MO_{2,routine}$, $MO_{2,max}$ and aerobic scope of warm acclimated fish. Estimates are mean of respirometer-group, given with standard error. * indicate statistically significant difference from normoxia.

Oxygen treatment	$MO_{2,routine}$	$MO_{2,max}$	Aerobic Scope
50%	1.14 ± 0.08	2.25 ± 0.19 *	1.11 ± 17 *
100%	1.31 ± 0.07	3.0 ± 0.17	1.69 ± 0.16
200%	1.61 ± 0.08 *	2.88 ± 0.19	1.27 ±0.17 *

Tabell 3: Overview of $MO_{2,routine}$, $MO_{2,max}$ and aerobic scope of warm acclimated fish. Estimates are given as effect size, in difference from 100% air saturation with standard error. * indicate statistically difference from normoxia.

Oxygen treatment	$MO_{2,routine}$	$MO_{2,max}$	Aerobic Scope
50%	-0.17 ± 0.10	-0.75 ± 0.25 *	-0.58 ± 0.23*
100%	1.31 ± 0.07	3.0 ± 0.17	1.69 ± 0.16
200%	+0.30 ± 0.10 *	-0.12± 0.25	-0.42 ± 0.23*

Cold acclimated fish

Tabell 4: Overview of $MO_{2,routine}$, $MO_{2,max}$ and aerobic scope of cold acclimated fish. Estimates are mean of respirometer-group, given with standard error. * indicate statistically difference from normoxia.

Oxygen treatment	$MO_{2,routine}$	$MO_{2,max}$	Aerobic Scope
50%	0.39 ± 0.10 *	0.93 ± 0.25	0.54 ± 0.22
100%	0.59 ± 0.07	1.18 ± 0.17	0.59 ± 0.16
200%	0.62 ± 0.11	1.78 ± 0.26 *	1.15 ± 0.24 *

Tabell 5: Overview of $MO_{2,routine}$, $MO_{2,max}$ and aerobic scope of cold acclimated fish. Estimates are given as effect size, in difference from 100% air saturation with standard error. * indicate statistically difference from normoxia.

Oxygen treatment	$MO_{2,routine}$	$MO_{2,max}$	Aerobic Scope
50%	-0.21 ± 0.10 *	-0.26 ± 0.25	-0.05 ± 0.22
100%	0.59 ± 0.07	1.18 ± 0.17	0.59 ± 0.16
200%	+0.03 ± 0.11	+0.60 ± 0.26 *	+0.56 ± 0.24 *

Appetite – Cold acclimated fish

Tabell 6: Overview of appetite measurements in cold acclimated fish. Estimates are given as number of consumed dry flakes with standard error. Intercept is the mean consumed dry flakes at trial one. The average slope is the mean decrease in dry flakes consumed per day. * indicate statistically significant difference from normoxia.

Intercept 100% DO	19.87±2.52
50% DO	14.62±2.30 *
200% DO	15.62±2.30 *
Avg Slope for all treatments	-1.27±0.55
	CI= -2.39 / -0.15

Warm acclimated fish

Tabell 7: Overview of appetite measurements in warm acclimated fish. Estimates are given as number of consumed dry flakes with standard error. Intercept is the mean consumed dry flakes at trial one. The different slopes are the mean decrease in dry flakes consumed per day per oxygen-treatment with normoxia as reference. * indicate statistically significant difference from normoxia.

Intercept 100% DO	28.77±4.34	
50% DO	27.60	-1.17±6.13
200% DO	33.50	+4.73±6.13
Slope 100% DO	0.33	0.33±1.02
50%DO	-0.89	-1.17±1.44
200% DO	-2.29 *	-2.61±1.44 *

Tabell 8 Overview of appetite measurements. Green color indicates data used in analysis. Each measurement represents counts of dry flakes per tank per trial.

Tank	Temp	% Air Saturation	m1	m2	m3	m4	m5	m6	m7	m8	m9	m10	m11
1	34	50	23	28	20	16	17	23	9	17	24	19	22
2	20	100	15	19	5	17	5	13	18	10	21	15	11
3	34	200	32	32	33	22	19	25	19	20	31	12	25
4	20	50	12	10	7	13	9	14	11	9	13	6	7
5	20	200	19	34	9	13	15	12	12	12	6	6	5
6	34	50	27	32	19	17	18	21	22	27	35	22	23
7	20	200	7	7	11	13	5	10	11	13	12	10	8
8	34	100	31	35	19	28	20	15	11	9	28	24	23
9	34	100	23	37	25	34	30	34	30	18	40	36	38
10	20	50	4	17	15	7	8	13	8	7	14	7	10
11	34	200	24	33	26	28	20	13	20	11	20	14	24
12	20	100	23	20	16	11	17	10	9	9	16	11	13

Correlations - Growth rate and aerobic scope

Tabell 9: Linear model of growth rate (weight) as a function of aerobic scope in 20 °C acclimated fish. Estimates are given ass effect sizes different from normoxia, with standard error. Correlation is given with R^2-value.

Intercept	0.88 ± 0.15
Aerobic scope	-0.16 ± 0.16, p= 0.35
50% - 20°C	-0.23 ± 0.15, p=0.15
200% - 20°C	0.03 ± 0.19, p=0.88
R^2	0.15, p= 0.38

Tabell 10: Linear model of growth rate (weight) as a function of aerobic scope in 34 °C acclimated fish. Estimates are given ass effect sizes different from normoxia, with standard error. Correlation is given with R^2-value.

Intercept	1.47 ± 0.56
Aerobic scope	0.29 ± 0.31, p= 0.35
50% - 34°C	-0.45 ± 0.36, p=0.22
200% - 34°C	-0.14 ± 0.33, p=0.68
R^2	0.22, p= 0.21