UNIVERSITY OF GOTHENBURG

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

HEART DEFORMITIES, IMPAIRED PHYSIOLOGICAL PERFORMANCES AND REDUCED STRESS TOLERANCE IN ADULT RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FARMED UNDER INTENSE CONDITIONS

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Abstract

In salmonid aquaculture, an increasingly important food production sector, intense and warm rearing conditions boosting growth rate and reducing production time may cause irreparable consequences. In juvenile rainbow trout (*Oncorhynchus mykiss*), warm rearing inducing rapid growth has resulted in abnormal and deviating heart morphologies compared to juvenile trout reared at cooler temperatures. This was associated with reduced cardiac function, metabolic performance and an impaired warming tolerance. However, it is unknown how the negative consequences of warm rearing develop over time and if they persist in adult fish. In this project, I studied differences between warm reared adult and juvenile (assessed in a previous experiment) rainbow trout in terms of their physiological performances and stress tolerance. Fish were instrumented with electrodes to determine heart rate and cardiac activity, and fish metabolic rate was assessed using respirometry. The fish were subjected to a chase protocol where the maximum cardiac and metabolic capacities were evaluated, to determine their scope to elevate heart rate and metabolic rate above resting levels when needed during stress. Fish were then subjected to an acute warming protocol to determine the warming tolerance of the fish. The heart of each fish was then dissected for morphometrical analyses. My results showed that cardiac deviations persisted in adult rainbow trout. Adult fish exhibited lower maximum heart rate and metabolic rate following physical stress, reducing their heart rate and metabolic scopes. Adult fish also presented a lower warming tolerance than juvenile fish. This was associated with an inability to maintain a high heart rate and metabolism at critically high temperatures in the adult rainbow trout. The impaired physiological capacities and reduced stress resilience caused by warm rearing observed here constitute an animal welfare concern and could explain mortalities and production losses during stressful events of salmonid fish, including rainbow trout, in aquaculture.

Sammanfattning

Inom laxodling, en alltmer viktig livsmedelssektor, kan intensiva och varma uppfödningsförhållanden som ökar tillväxten och minskar produktionstiden orsaka negativa konsekvenser. I juvenil regnbåge (*Oncorhynchus mykiss*), så har varma uppfödningstemperaturer som inducerar snabb tillväxt visat sig resultera i onormala och avvikande hjärtmorfologier jämfört med regnbåge som fötts upp i kallare temperaturer. Detta var associerat med reducerad hjärtfunktion, metabolisk prestation och en nedsatt värmetolerans. Dock är det okänt hur dessa negativa konsekvenser av varm uppfödning utvecklas över tid och om de kvarstår i vuxen fisk. I detta projekt studerade jag skillnaderna mellan varm uppfödd vuxen och juvenil (utvärderat i ett tidigare experiment) regnbåge i form av fysiologiska prestationer samt stresstolerans. Fiskarna var instrumenterade med elektroder för att utvärdera hjärtfrekvens och hjärtats arbetskapacitet, och fiskarnas ämnesomsättning (metabolism) uppmättes genom respirometri. Fiskarna utsattes för ett fysiskt stressprotokoll där maximal hjärt-, och metabola kapacitet uppmättes, för att avgöra fiskens kapacitet att höja hjärtats arbetskapacitet och ämnesomsättningen vid behov under fysisk stress. Fiskarna utsattes sedan för ett akut värmeprotokoll för att avgöra deras värmetolerans. Fiskens hjärtan dissekerades sedan ut för morfometriska analyser. Mina resultat visade att hjärtavvikelserna bestod i vuxen regnbågslax. De vuxna fiskarna uppvisade även en lägre maximal hjärtfrekvens och ämnesomsättning i förhållande till fysisk stress, som reducerade deras hjärtfrekvens och metabola kapacitet. Vuxen fisk uppvisade också en lägre värmetolerans än juvenil fisk. Detta sammanföll med en oförmåga att bibehålla hög hjärtfrekvens och ämnesomsättning vid kritiskt höga temperaturer i vuxen regnbåge. De försämrade fysiologiska kapaciteterna och minskad stresstålighet orsakat av varm uppfödning som setts här utgör ett djurvälfärdproblem, och kan kanske bidra till dödlighet och produktionsbortfall under stressfulla förhållanden hos laxfisk, inkluderat regnbåge, inom fiskodlingsindustrin.

1. Introduction

The aquaculture industry is important since it provides humans with large amounts of food. Farming of salmonids (*Salmonidae spp*.), including rainbow trout (*Oncorhynchus mykiss*) account for a large portion of the fish farming productions globally (Pandey et al., 2023). Rainbow trout is one of the most common species farmed within the Swedish aquaculture industry (Statistiska centralbyrån [SCB], 2018). Salmonids are commonly subjected to high rearing temperatures with the intent of boosting the growth rate, particularly during the juvenile life stages (Brijs et al., 2020; Frisk et al., 2020). Rapid growth causes the salmonid heart to undergo morphological plasticity (Frisk et al., 2020; Engdal et al., 2024), and it has been shown that farmed Atlantic salmon (*Salmo salar*) and rainbow trout have developed problems relating to abnormal heart morphology (Frisk et al., 2020; Vindas et al., 2024; Brijs et al., 2020; Engdal et al., 2024; Hvas et al., 2025; Poppe et al., 2003).

1.1. The importance of normal cardiac morphology and physiological functions in fish

Regarding the standard cardiac morphology in salmonids, including a pyramid-shaped ventricle along with an aligned outflow tract known as the *bulbus arteriosus* are correlated with normally operating cardiac functions (Frisk et al., 2020; Poppe et al., 2003). Grave environments, however, can contribute to multiple cardiac deformities including rounded ventricles (lower ventricular height:width, H:W, ratio) rather than the normal pyramid-shape of the hearts of wild salmon and rainbow trout. Salmonids also develop a deviated, skewed cardiac outflow tract, the *bulbus arteriosus*, relative to the ventricle (a higher ventricle:*bulbus arteriosus* angle). These abnormal cardiac morphologies presumably impair cardiac health and may contribute to increased mortality (Brocklebank & Raverty, 2002; Rodger & Mitchell, 2011). How these cardiac deformities develop over time in rainbow trout is currently unknown.

Fishes, such as rainbow trout, rely heavily on proper heart function to provide blood supply to body tissues, to sustain physiological, metabolic as well as whole animal performances. The heart is the most vital organ in the body, driving circulatory blood flow which in turn controls the oxygen availability and nutrients throughout all tissues. Maintenance of the heart activity is then important in terms of maintaining metabolism, i.e. the conversion of energy needed to fuel all processes in the cells and organs in the body (Farrell & Smith, 2017; Gamperl et al., 2017). The ability to increase heart activity and metabolism during highly demanding activities determines how well the fish can endure physical stress. This is determined by heart rate and metabolic (aerobic) scope, that is, the capacity to increase heart rate and metabolic rate above resting levels when fish are exposed to energetically demanding situations (Clark et al., 2013).

Resting and maximum metabolism, as well as aerobic scope, increases with body size, and this has been shown in several salmonid species, including rainbow trout (Norin & Malte, 2011; Kuchenmüller et al. 2024, Roze et al. 2013). However, it was recently shown that the ability to increase and maintain high heart rate scope and metabolic (aerobic) scope, that is the capacity to increase these performances above baseline (resting) levels, was lower in warm reared juvenile rainbow trout (the same juvenile group of trout used in this study, more details below), compared to cold reared juvenile trout (reared at 4°C lower temperature) (Ekström et al., unpublished observations).

1.2. Effects of environmental factors on morphology and physiology in rainbow trout

In salmonids exhibiting abnormal heart shapes, impairment of heart function and metabolic performance would likely have detrimental consequences for whole animal performances. For example, a study showed that rainbow trout with rounder ventricles were less tolerant to confinement stress (Brijs et al. 2020). Another study had revealed that rainbow trout with rounder ventricles was worse at swimming, which was in turn associated with a lower cardiac output than good swimmers (Claireaux et al., 2005).

Warm rearing may also be detrimental for animal tolerance to various stressors in their environment. For example, in warm water temperatures, the heart is subjected to a higher workload due to increased metabolism (Eliason & Anttila, 2017; Ekström et al., 2017; Morgenroth et al., 2021). As the cardiac working capacity, which is a factor that can dictate the upper thermal tolerance limit in fish (Ekström et al., 2017; Morgenroth et al., 2021), an abnormal heart may not have the capacity to endure the rough conditions during acute warming exposures, thus limiting the upper thermal tolerance. Indeed, both cardiac and metabolic performance and scope were shown to decrease during acute warming in warm reared juvenile rainbow trout, which resulted in a lower thermal tolerance compared to cold reared juvenile trout (Ekström et al., unpublished observations). It is unknown, however, how the resilience and tolerance to environmental stress develops over time in trout reared at warm regimes.

1.3. Aims and hypotheses

The overarching aim of this study was to determine the effects that warm rearing temperatures and accelerated growth rates have on physiological performance, cardiac morphology and temperature tolerance in rainbow trout and how these traits develop over time, from juvenile to adult fish. To address this aim, I performed assessments of cardiac morphology, metabolic and heart performance, and determined temperature tolerance limits of a group of warm reared adult rainbow trout. I then compared my results with previously recorded data in juvenile trout that was collected using identical experimental protocols through November-December 2024.

My hypotheses for this study are; 1) Warm reared adult rainbow trout will have more severe cardiac deformities compared to the warm reared juvenile rainbow trout, e.g., more abnormally shaped heart chambers as well as abnormally skewed heart chambers and how the chambers align with each other, 2) Warm reared adult rainbow trout will exhibit higher heart rates and lower metabolic rates than the warm reared juvenile rainbow trout, and 3) Warm reared adult rainbow trout will have a lower warming tolerance than the warm reared juvenile trout.

2. Materials and methods

2.1. Ethical permits

All experiments were carried out under ethical permit #5.8.18-14181-2025, issued by the regional animal ethics committee in Gothenburg.

2.2. Experimental animals, rearing and holding conditions

The fish used in the experiment with a total of 31 individuals were adult rainbow trout (*Oncorhynchus mykiss*) of mixed sexes obtained from a Swedish fish farm (Vänneåns Fiskodling

AB) in which they had been reared in warm water conditions since May 1st, 2024. The fish were transported to the animal facility in Natrium on September 5th, 2025. Prior to the start of the experiment, the fish were held in separate freshwater tanks (1000 L; 112 x 112 x 80) supplied with aerated 10-12°C freshwater and were acclimated to the laboratory conditions for 4 weeks at under a 12:12 h dark:light cycle. The fish were fed with pellets (Skretting) daily until the start of the experiments, at which the fish fasted for 2 days prior to experiments.

To address the hypotheses, the data from the adult group was compared with a juvenile group of fish with a total of 32 individuals. These fish were part of the same cohort of fish as the adult fish, and were brought to the animal facility on October 30th, 2024, were housed and acclimated in an identical way as the adult rainbow trout. The experiment on juvenile fish was conducted during November-December 2024, following the protocols described below.

2.3. Experimental and data setups

There were two experimental setups with water reservoirs containing custom-made cylindrical, transparent, respirometers (Length: 60 cm; Diameter: 15 cm; 10,2 L) in which the fish was held during the experiment. Setup 1 had 2 basins (342 L; Length: 99 cm; Width: 78 cm; Height: 21 cm) housing 3 respirometers in total, while setup 2 (227 L; Length: 139 cm; Width: 78 cm; Height: 21 cm) contained two respirometers. In between the two respirometer setups, there was a heating water tank (159 L; Length: 103 cm; Width: 103 cm; Height: 15 cm) used for the thermal ramping protocol (described below), was connected to the experimental setups and contained two 3 kW titanium water heaters controlled by a custom-made EW 7221 thermostat unit. When used, water was pumped out from the heat tank into the experimental setups, which then drained back into the heat tank, thus comprising a re-circulating system.

2.4. Data acquisition

Prior to placing the fish into the respirometers, a slope was recorded in the empty respirometers to acquire the background, or bacterial respiration. In the respirometers, the fish recovered from surgery overnight while MO_2 (oxygen consumption rate) data was collected continuously. Freshwater was constantly circulating the respirometers, and a O_2 optode (Pyroscience, Aachen, Germany) in the circulation loop enabled continuous recordings of percentage of the oxygen saturation inside the respirometer. The respirometers were also connected to a flush pump which perfused the respirometer with aerated water from the experimental tank. When turned off, either manually or via a timer, recordings of the rate of the decline in air saturation of the water in the respirometer (generating a downward slope in air saturation per unit of time) provided a measure of oxygen consumption rate of the fish (in mg O_2 kg^{-1} h^{-1}). A Firesting O_2 system (PyroScience GmbH, Aachen, Germany) that connected the respirometer oxygen sensors to the data collection system allowed for recordings of the air saturation percentage of the water in the respirometers via the Pyro oxygen logger software. Each oxygen sensor was two-point calibrated at 0% and 100% prior to the experiment.

The electrodes were connected to a Bio Amplifier FE231 (ADInstruments, Australia) which allowed for the recording of the electrical biopotentials arising from the electrical activity of the heart when generating the ECG. Heart rate (HR) was then measured through the counting of the R waves of the ECG (ventricular depolarization initiating heart contractions) per minute. The best

ECG signal qualities were achieved by adjusting through the frequency filters in order to remove unwanted disturbances.

To record the water temperature in the experimental setups, a PT100 temperature sensor was used. Each experimental setup had their own computer setup that communicated the signals from the data collection hardware through a Powerlab system (AdInstruments, Australia). The data was analyzed using the software Labchart (v. 7.3.8, AdInstruments, Australia).

2.5. Experimental protocols

2.5.1. Day 1: Surgical protocol

Fish were anesthetized in a 10-12°C freshwater containing MS222 (tricaine mesylate) at a concentration of 150 mg L^{-1}, buffered with 300 mg L^{-1} $NaHCO_3$. Once the opercular, i.e., gill ventilation movements ceased, the fish was considered anaesthetized and was placed on a measuring board on top of a scale. The weight and standard length were documented for each fish. Then, fish were placed belly up on wet foam on a surgery table on which they were continuously perfused with aerated freshwater containing 75 mg L^{-1} MS222 and 150 mg L^{-1} $NaHCO_3$. Electrodes were placed into the muscle of the abdominal wall ventral to the position of the heart in a triangular formation (positive, negative and ground leads). The positive lead I placed at the base of the right pectoral fin while lead the negative lead was placed at the base of the left pectoral fin. The ground lead was placed ~1 cm in a posterior direction, along the ventral midline. The cords of the flow probe and electrode were then fixed to the fish with several sutures on the ventral side, and on the dorsal side anterior to the dorsal fin. After the surgery, the fish was placed in a respirometer and were left there overnight for recovery and resting, during which electrocardiogram (ECG) and, through 5:10 min closed:open flush pump cycles, heart rate and metabolic rate (see details below) were recorded.

2.5.2. Day 2: Chase protocol

Before commencing the chase protocol, recovery status was checked so the fish were not already stressed when being chased. If the fish were entangled in cords, they were detangled and given time (typically 30-60 minutes) to calm down and reach low steady-state values of heart and metabolic rate. Then, one by one fish was taken out of its respirometer in a plastic bag and put into the chase tank. The big, round tank designated for the chase protocol was of 138 liters (Diameter: 105 cm, Height: 16 cm). The fish was then encouraged to swim for 3 min until exhaustion, according to a standardized chase protocol (Clark et al. 2013), by tapping on the walls of the basin as well as touching it and pinching the caudal peduncle. The fish was then placed back in the respirometer and the air saturation in the respirometer was allowed to decline to ~80% at which the flush pump was turned back on. After 1-5 manual slope of the decline in air saturation in water per unit of time had been generated, the timed cycles (5:5 min closed:open) were initiated. Then, the fish were monitored for at least 5 h, after the flush pump cycles were set to 5:10 min closed:open cycles prior to the overnight recovery.

2.5.3. Day 3: Warming protocol

The next day, a thermal ramping protocol was executed. After checking on and disentangling the fish if needed, the fish were allowed to recover to obtain stable resting values. When steady state resting values had been acquired, the flush pumps were set to be constantly running and flushing the respirometers. The temperature was then increased to 15 °C for over one hour. The fish were closely monitored using a camera mounted above the experimental setup. When the water temperature had reached 15 °C, the flush pumps were manually turned off in order to obtain slopes depicting increased oxygen consumption rates, during the last 10 minutes of the 1 hour period. The heart rate was recorded during this period. Then, the water temperature increased to 20°C for 1 h, during which two slopes was recorded as described above. The temperature of the water was then increased by 2°C h^{-1} , during which two slopes are generated during the last 10 min of the 30 min temperature increments, during which the heart rate was recorded. The temperature was increased until the point at which the fish lost its ability to maintain an upright body posture (loss of equilibrium, LOE) which was determined as the critical thermal maximum. The exact temperature at which this occurred was noted using a highly accurate handheld thermometer (Testo 735, Testo Ltd, United Kingdom).

Afterwards, the fish was anesthetized with MS222 while in the respirometer and then euthanized through a sharp blow to the head with a fishing bat once taken out of the respirometer.

anesthetized, removed and euthanized. The fish was also dissected, and the heart was taken out. The heart was then flushed with phosphate buffered saline solution (PBS) (pH = 7.4) followed by 20 mM potassium chloride solution. It was later fixated in a 10% phosphate buffered formalin solution for 24-48 h after which the tissue was placed in 70% ethanol.

2.6. Imaging of the hearts

Each heart was placed in a Petri dish containing water and was fixed in different positions by inserting a fixation needle glued to the bottom of the dish. Images of ventrodorsal side, and the left and right lateral sides of the heart were taken using a Canon HX540 HS camera. These were then transferred to a computer, and the metamorphic analysis was done by using ImageJ (Rasband, W., USA), an image-processing program. The values for ventricle height, ventricle width, bulbus width, ventricular bulbus alignment, ventricular symmetry and bulbus alignment were collected for every single heart, according to Engdal and colleagues (2024). The remaining data categories were calculated; height:width as ventricle height divided by ventricle width and ventricle:bulbus width ratio as bulbus width divided by ventricle width. All these data values were then pasted into an excel file for further data analysis.

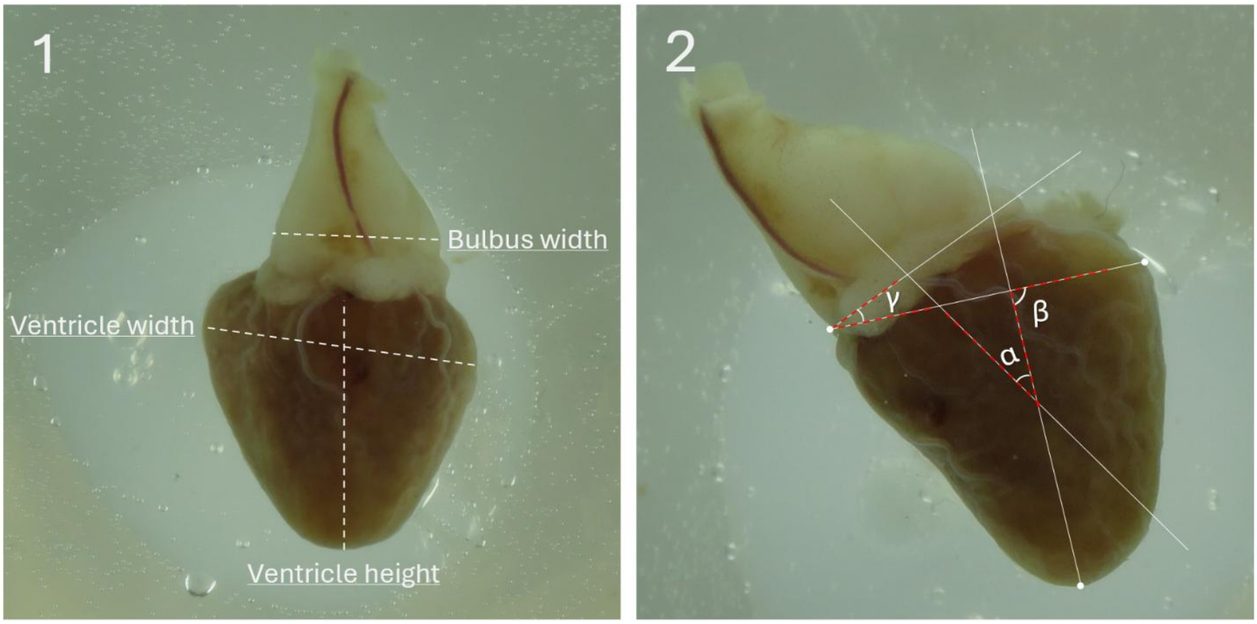

Figure 1. Example of morphometrics through ImageJ for one individual fish. Image 1 shows the ventrodorsal side of the heart, along with lines for ventricle height, ventricle width and bulbus width. Image 2 depicts the right lateral side of the heart including three angles (α-γ) (α: the ventricular bulbus alignment; β: ventricular symmetry; γ: bulbus alignment).

2.7. Data analysis and statistics

The programs used for data analysis were Microsoft Excel and IBM Statistics SPSS (v.30). The first values recorded and collected were all slopes for resting heart rate and metabolic rate (5 min closed: 10 min flushing cycles) overnight between 9pm and 8 am, being that the lowest values across the night were counted as resting states. During each slope with a decline in % air saturation, the more angled the slope was, the more oxygen the fish consumed. The resting metabolic rate was determined as the 20th percentile of all slopes recorded overnight. Resting heart rate was determined as the average of the lowest 5-10 heart rate values acquired overnight. Maximum metabolic rate and heart was determined within 10 min after the chase, when the fish was put back in its respirometer. The steepest slope, i.e. the highest MO_2 value, was used as the maximum metabolic rate, which was in all cases the first slope after the chase and the highest heart rate was attained within 10 min after the chase. The MO_2 values were calculated through a specific formula:

$$MO_2 = [(V_r - V_f) \times (\Delta\%Sat/t) \times \alpha]/(M_b)$$

where V_r is the volume of the respirometer, V_f is the volume of the fish assuming that 1 g of fish equals 1 ml of water, Δ%Sat/t is the change in % O_2 saturation per unit time, α is the solubility coefficient of O_2 in freshwater and adjusted for the different experimental temperatures, and M_b is the body mass of the fish (Clark et al., 2013).

All these values were then collected and put into an excel file alongside the data from the previous experiment performed on the warm reared juvenile rainbow trout. This enabled further statistical data analyses conducted through IBM SPSS (v.30). To assess whether there were any differences between juvenile and adult fish in critical thermal maximum, cardiac- and body morphometry, metabolic- and heart rate variables, an independent t-test was performed. A Shapiro-Wilk test was used to test the assumption normality, in which the significance value (p) indicated whether there was or was not a normal distribution across the data values. If the significance values are $p<0.05$, the data was further explored to check for abnormalities, outliers, unreliable datapoints etc. Then, a Levene's test was performed to test if the variances of the two groups were homogenous or not. If any of the assumptions were violated, a non-parametric Mann-Whitney U test was done instead. This test gives the value of asymptotic significance, and if $p<0.001$, there is significant difference between the two groups and vice versa. Differences in heart rate and metabolic rate responses during the temperature protocol were analysed using a linear mixed model with temperature as a repeated measure, within subjects' factor, treatment and the interaction between the within-subject factor and treatment as fixed factors, and fish ID as subject variable. A first-order autoregressive covariance structure was used (as indicated by the lowest Akaike's information criterion, AIC). Significant main effects or interactions were explored with pair-wise comparisons, where confidence intervals were adjusted for multiple testing.

3. Results

3.1. Body morphometrics

There were significant differences regarding all morphometrical variables between the warm reared juvenile and warm reared adult group, as shown in table 1 below. The adult group was larger in weight and length in comparison to the juvenile group ($p<0.05$). The condition factor (ratio between weight and length) was also shown to be higher in the adult relative to juvenile group of fish, and the adult fish exhibited a more than two-fold higher absolute growth rate ($p<0.05$).

Table 1. Body morphometrics of warm reared juvenile (n=32) and adult group (n=31) of rainbow trout (Oncorhynchus mykiss) used in this study. Asterisk signifies statistically significant (p<0.05) differences between the two groups. The values are stated as mean±S.E.M.

	Juveniles	Adults
Body weight (g)	188.5±9.3	967±26.7*
Standard length (mm)	233.6±3.8	389.7±3.8*
Condition factor	1.4±0.02	1.6±0.03*
Absolute growth rate (g day^{-1})	0.8±0.04	1.8±0.05*

3.2. Cardiac morphometrics

There were no significant differences between the two groups regarding the height:width ratio ($p>0.05$), which was 1.01 ± 0.02 cm for the juvenile group and 1 ± 0.02 cm for the adult group ($T_{61}=1.3$; Fig. 2A). Concerning the ventricular symmetry, the average for the juvenile group was 101.2 ± 1.2° and 88.7 ± 1.2° for the adult group ($T_{61}=7.5$; $p<0.05$; Fig. 2B). There were significant differences in the ventricular bulbus alignment between the two groups, in which the juvenile group average was 42.3 ± 1.1° and 33.9 ± 0.9° ($T_{61}=5.7$; $p<0.05$; Fig. 2C) in the adult group. There was also no significant difference between the groups regarding bulbus:ventricle width ratio ($p>0.05$), in which it was 0.6 ± 0.02 cm for the juvenile group and 0.6 ± 0.01 cm ($T_{60}= 1.38$; Fig. 2D) for the adult group. The groups also differed significantly in the bulbus alignment, being higher in juvenile compared to adult trout (21.03 ± 0.8° vs. 26.9 ± 0.9°, respectively; $T_{61}=-4.7$; $p<0.001$; Fig. 2E).

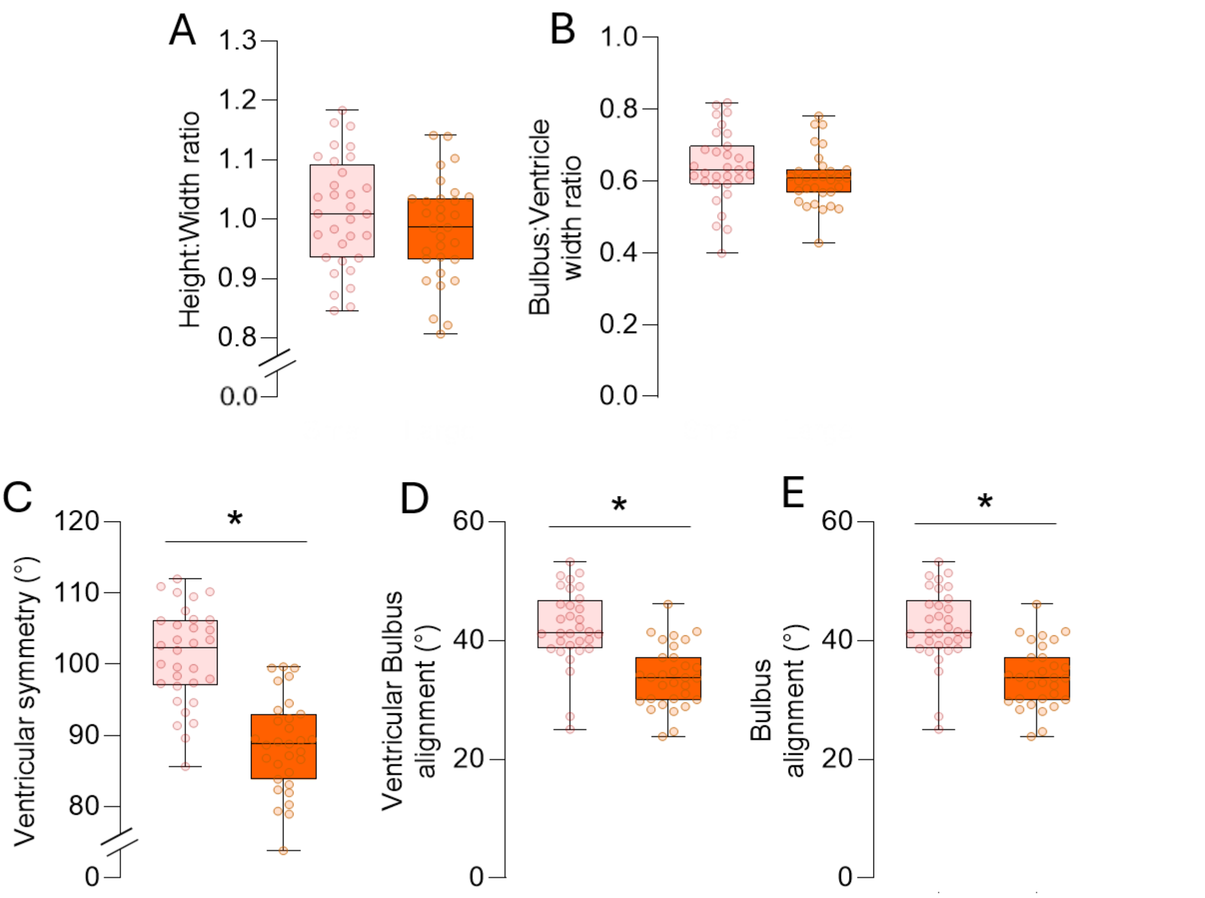

Figure 2. Cardiac morphometry in warm reared juvenile (n=32) and adult rainbow trout (n=31). The figure shows A) height:width ratio, B) bulbus ventricle width ratio, C) ventricular symmetry, D) ventricular bulbus alignment, E) bulbus alignment in warm reared juvenile (pink) and adult (orange). Asterisk signifies statistically significant (p<0.05) differences between the two groups.

3.3. Metabolic responses in juvenile and adult trout

There were significant differences in overall metabolic activity between the two groups as seen in Fig. 3. The resting metabolic rate for the juvenile group was 87 ± 4.4 mg O_2 kg^{-1} h^{-1}, while the adult group had an average of 54.3 ± 3.2 mg O_2 kg^{-1} h^{-1} (T_{54}=5.9; $p<0.05$). The maximum metabolic rate was also significantly different between the two groups. The adult group presented a lower average by 290.2 ± 9.4 mg O_2 kg^{-1} h^{-1} (T_{58}=9.9; $p<0.05$) and it being 433 ± 11 mg O_2 kg^{-1} h^{-1} for the juvenile group. The juvenile group and adult group also significantly differed in terms of aerobic scope, which was also lower in the adult group. For the juvenile group, the aerobic scope was 341 ± 9 mg O_2 kg^{-1} h^{-1} while it was 237 ± 9 mg O_2 kg^{-1} h^{-1} (T_{53}= 8.08; $p<0.05$) for the adult group.

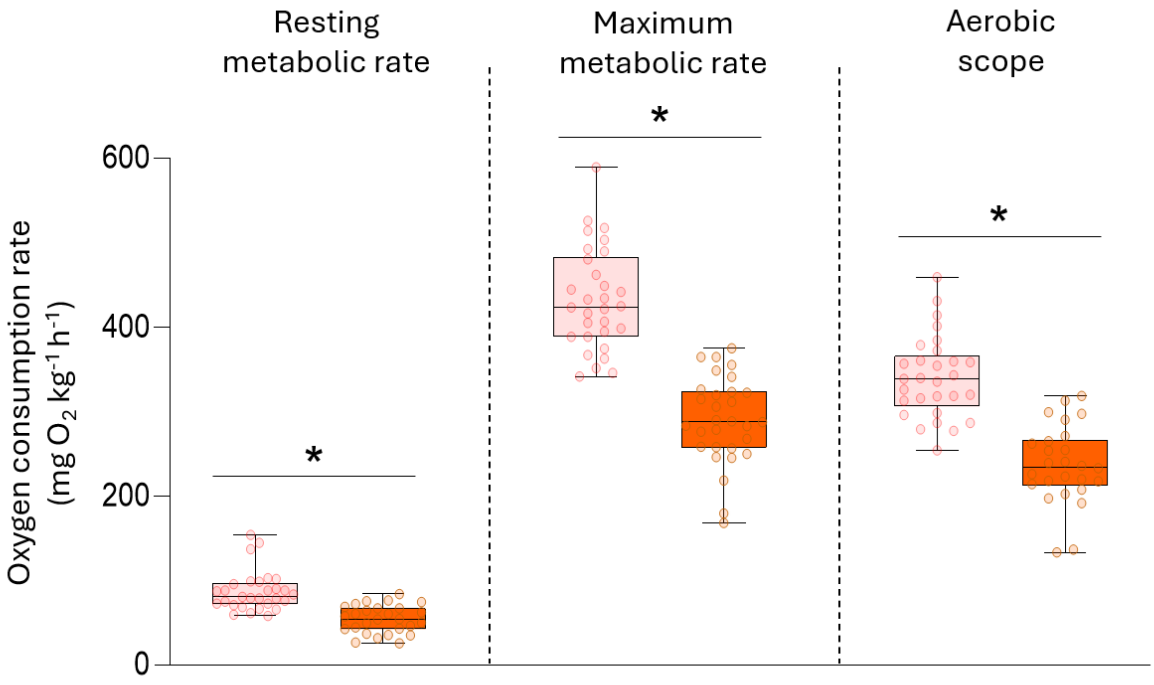

Figure 3. Metabolic performances in warm reared juvenile (n=32) and adult rainbow trout (n=31). Resting and maximum metabolic rate as well as aerobic scope in warm reared juveniles (in pink) and warm reared adults (in orange). Asterisk signifies statistically significant (p<0.05) differences between the two groups.

3.4 Differences in heart rate performance between juvenile and adult trout

As shown in Fig. 4, there were significant differences in heart rate performances between the two groups. Regarding the resting heart rate, it different vastly between the juvenile and the adult group, with it being 50.1 ± 1.5 beats min^{-1} for the juvenile group and 60.5 ± 1.0 beats min^{-1} (T_{54}=-5.5; $p<0.05$) for the adult group. The adult group did present a lower maximum heart rate, being 71.1± 0.9 beats min^{-1} (T_{51}=6.02; $p<0.05$), while the maximum heart rate for the juvenile group was 78 ± 0.8 beats min^{-1}. The heart rate scopes also revealed significant differences between the two groups where the adult group also had a lower value, with 10.3 ± 1.3 beats min^{-1} (T_{50}=9.6 ; $p<0.05$) and 28.2 ± 1.3 beats min^{-1} for the juvenile group.

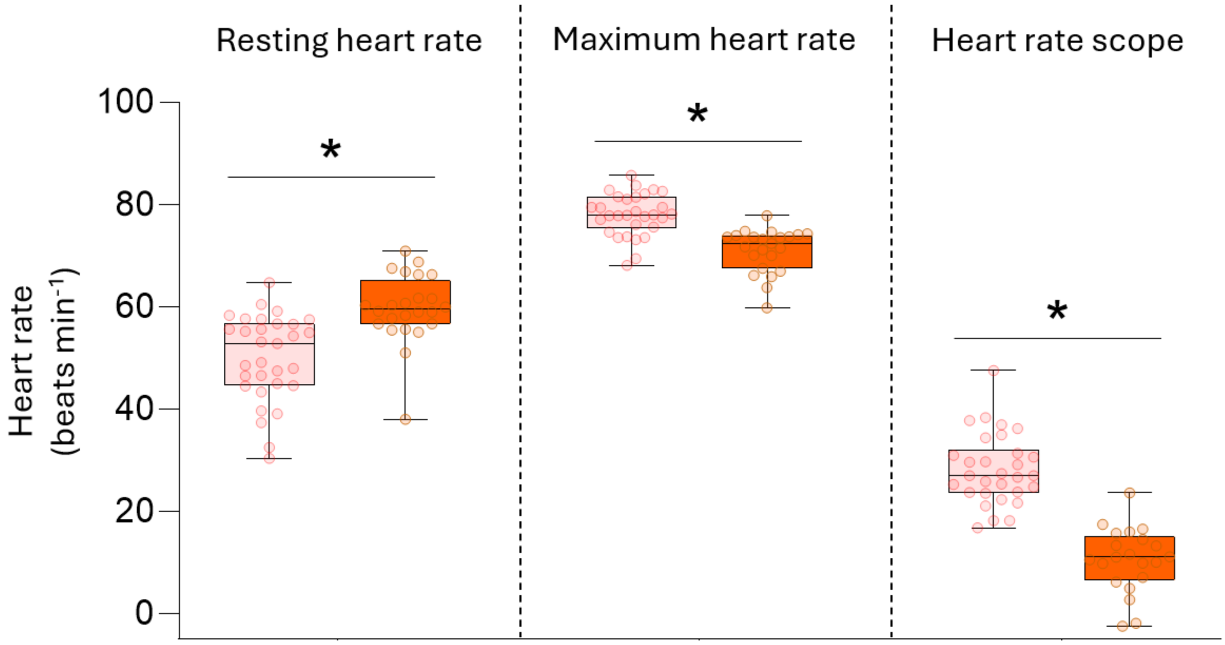

Figure 4. Heart rate responses during rest and following an exhaustive stress protocol in warm reared juvenile (n=32) and adult trout (n=31). Resting and maximum heart rate as well as heart rate scope in warm reared juveniles (in pink) and warm reared adults (in orange). Asterisk signifies statistically significant differences (p<0.05) between the two groups.

3.5. Critical thermal maximum, heart rate and metabolic rate during acute warming

The adult group had a significantly lower critical thermal maximum, with an average of 24.8 ± 0.3℃ in adult trout (T_{44}=5.09; $p<0.05$; Fig. 5) and 26.4 ± 0.2℃ for the juvenile group. Both heart rate (Fig. 6A) and metabolic rate (Fig. 6B) increased significantly with warming in both juvenile and adult trout. Adult fish exhibited lower heart rates at higher temperatures prior to reaching critical thermal maximum compared to juvenile fish (Fig. 6A). The MO_2 average was also significantly lower in the adult group at higher temperatures before reaching critical thermal maximum (Fig. 6B).

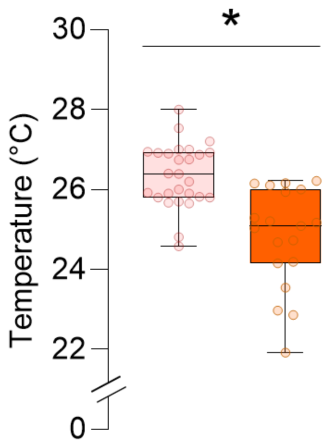

Figure 5. Acute warming tolerance in warm reared juvenile (n=32) and juvenile rainbow trout (n=31). The critical thermal maximum during an acute warming protocol in warm reared juveniles (pink) and warm reared adults (orange). Asterisk signifies statistically significant differences (p<0.05) between the two groups.

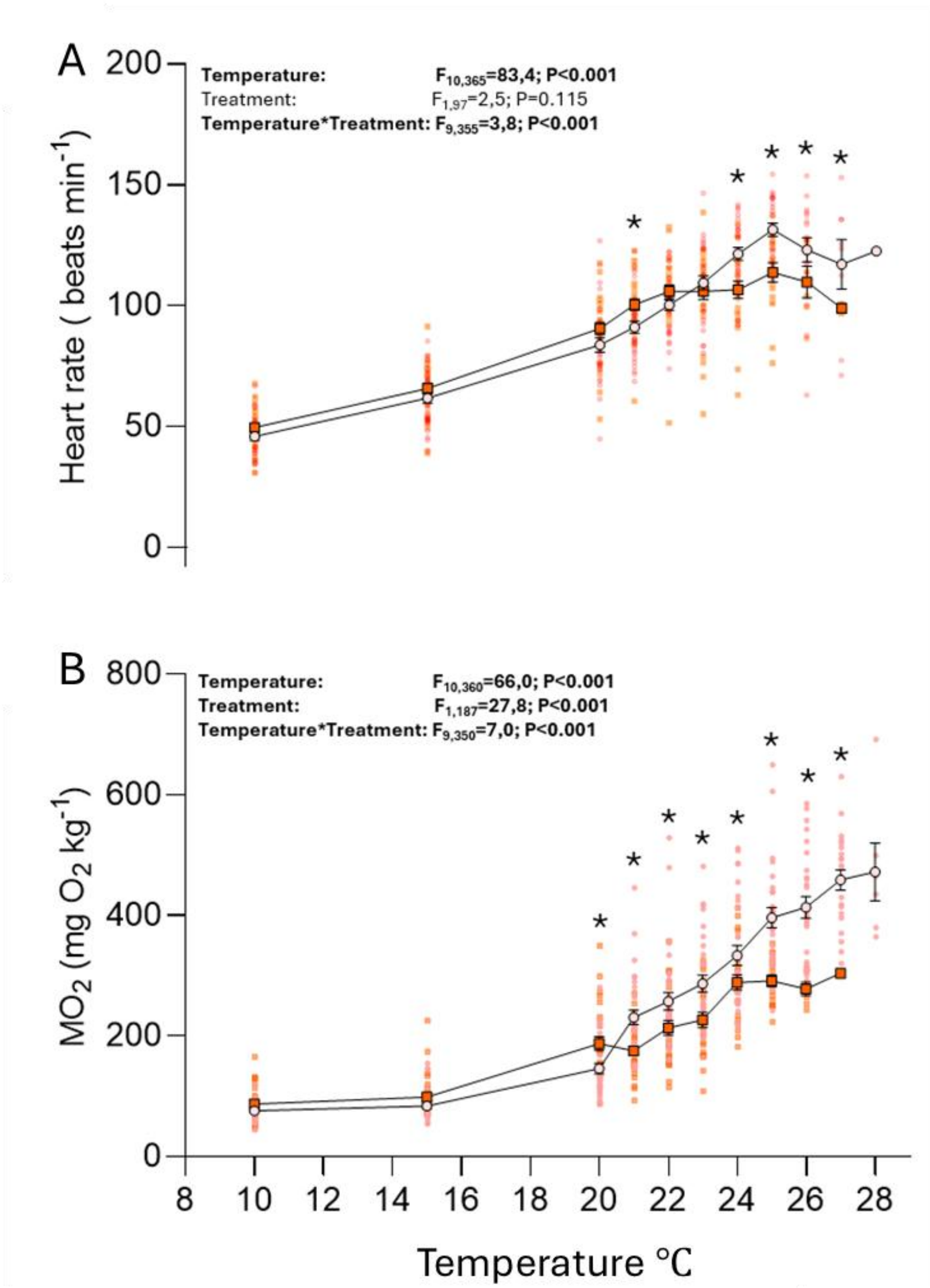

Figure 6. Cardiac and metabolic performance during acute warming in warm reared juvenile (n=32) and adult rainbow trout (n=31). A) Heart rate and B) maximum metabolic rate during an acute warming protocol. Warm reared juveniles in pink circles and warm reared adults in orange squares. Asterisks show significant differences (p<0.05)between the two groups during each temperature.

4. Discussion

As the results indicate, the adult and juvenile groups differed vastly regarding the multiple variable categories that were mentioned previously as the aimed assessments for this study. The results indicated that the adult trout had a higher absolute growth rate relative to the juvenile trout. The cardiac morphometrical ratios were, however, not significantly different when comparing the adult trout and the juvenile trout, however, the angles including the ventricular symmetry, ventricular bulbus alignment and bulbus alignment differed between the two groups. The adult trout had a vastly lower maximum metabolic rate after the exhaustive stress protocol, thus resulting in the lower aerobic scope in comparison to the juvenile trout. Cardiac capacity did also decline over development time in warm rearing temperatures, in that adult fish had a lower maximum heart rate, resulting in lower heart rate scope. The adult trout had a lower warming tolerance than the juvenile trout, and cardiac and metabolic performance was impaired in the adult trout while undergoing warming.

4.1. Heart morphologies are more deviating in adult rainbow trout

My results for cardiac morphometry enable me to support proof for my first hypothesis, that the adult trout exhibited more deviating heart morphology compared to the juvenile trout. In fact, the results show that the alignment of heart chambers (ventricular symmetry, bulbus:ventricle alignment and bulbus alignment) differed between juvenile and adult fish, but the ratio between heart chambers (H:W ratio and bulbus:ventricle width ratio) did not. Since there were no significant differences in H:W ratio between adult trout and juvenile trout in this study, this suggests ventricles are rounded already at the juvenile stage and that this trait does not worsen (i.e. lower H:W ratio) over time. My results did however not present a higher ventricle bulbus alignment, but a lower alignment, meaning that the adult trout did in fact have a less skewed bulbus relative to the ventricle. Nevertheless, Engdal et al. (2024) showed that heart deviations worsen during the development when comparing wild and farmed Atlantic salmon at different life stages (fry to adults). The heart deviations in farmed salmon included rounded ventricles (i.e. low H:W ratio), a skewed bulbus (high ventricular bulbus alignment) and the ventricular (symmetry) angle was lower in farmed salmon as compared to wild salmon. Here, the ventricular symmetry was lower in adult trout, which corresponds to what the Engdal et al. (2024) also showed in farmed salmon compared to wild salmon. Note, however, that this study on warm reared rainbow trout did not involve comparisons with wild rainbow trout with a “normal” heart morphology, which could possibly explain why my results differ from what has been observed in farmed and wild Atlantic salmon. Also, cardiac morphologies can vary between species which may explain the discrepancies.

4.2. Adult trout exhibits metabolic depression relative to juvenile trout

My findings did present that adult trout retained lower metabolic scope than juvenile trout, which support my second hypothesis. The adult trout showed a lower aerobic scope due to a lower maximum metabolic rate. However, a possible confounding factor regarding the comparison between juvenile and adult fish is the known effects of body size on metabolism in fish. As previously mentioned, the resting and maximum metabolic values including aerobic scopes should increase with the body size of the fish in rainbow trout. My results showed, however, that the metabolic values were lower in the adult group which correlate with my predictions in the second hypothesis. This could possibly signify that the metabolic activity was counteracted, despite subjection to high metabolically demanding activities This would potentially be a result of the warm rearing conditions, during the development from the juvenile to the adult life stage in rainbow trout itself.

Although, it is noteworthy that these results could have been influenced by differences in the degree of instrumentation of the fish. The adult fish were also instrumented with a blood flow probe around the ventral aorta (to record cardiac output as part of another study), which required more invasive surgery, as compared to juvenile trout that were only instrumented ECG electrodes which are less invasive. This could have impacted the recovery from the surgical intervention and physiological variables, by lowering the performance somewhat. However, I believe that the negative effects seen in terms of the metabolism of adult trout observed here mainly reflect the impacts of warm rearing over time.

4.3. Warm rearing leads to declined cardiac activity in adult rainbow trout

The data from heart rate performances supports my hypothesis that the adult trout would present higher heart rates than juvenile trout. A lower heart rate scope, as seen in the adult trout, was a result of a lower maximum heart rate. Adult trout did present a higher resting heart rate. These results could also imply that the cardiac contractility and stroke volume (volume pumped per heartbeat) was impaired, and that the heart had to beat faster to achieve the same amount of cardiac output, that is, the amount of blood pumped by the heart per minute (Farrell and Smith, 2017; Morgenroth 2021, Ekström et al, 2023). It could also signify that the adult trout were worse at rehabilitating themselves in terms of surgical recovery due to inferior hearts. However, it is noteworthy that the adult trout could have possibly recovered slower following a more invasive surgical intervention, and the instrumentation with ventral aortic blood flow probes. The cardiac pumping ability increases as a result of higher oxygen demand to tissues during high load activities. If the heart is unable to perfuse oxygen due to declined function, the tissues will not meet their oxygen demands. Warm rearing likely gives fish the inability to maintain sufficient blood supply to the tissues which are likely to explain the impaired metabolism, lower maximum metabolic rate and aerobic scope in adult trout.

4.4. Adult rainbow trout presented reduced warming tolerance

My third hypothesis is strongly supported by my results, which were that the adult trout would have a lower warming tolerance than the juvenile trout. During warming of the water, the critical thermal maximum for the adult trout was lower than in the juvenile trout. The findings of a previous study showed that rapidly growing rainbow trout exhibited a lower warming tolerance when compared to slow growing trout, this correlates with my results being that the adult trout, who had a higher absolute growth rate, presented a lower thermal tolerance (Roze et al., 2013). Kuchenmüller et al. (2024) specified that the larger a fish is, the worse it is in coping with high temperatures in difference to smaller individuals of the same species. With higher water temperatures that induce stress responses and in turn increased metabolic activity, if the body is unable to supply oxygen to all tissues with such deformed hearts (as seen in the adult trout) and it won’t be long until the body cannot endure anymore. The adult trout did not perform as well during the warming protocol and presented a lower-than-normal heart rate, and heart rate did not increase as much as juvenile trout at the higher temperatures close to critical thermal maximum, as presented in my data. In relation to the increase of water temperature, adult trout were not as efficient as the juvenile trout in maintaining high heart and metabolic rate, and both heart rate and metabolic rate declined at higher temperatures in the adult trout, which likely caused the lower warming tolerance. This has been observed before in other contexts, where the hearts oxygen supply and function were deliberately negatively manipulated, which resulted in a deteriorated cardiac function and critical thermal maximum (Morgenroth et al., 2021; Ekström et al., 2017).

4.5. Conclusions and future perspectives

My results have shown that warm rearing of rainbow over development time cause progressively deformed heart morphologies, lowered cardiac and metabolic capacities as well as reduced warming tolerances. I conclude that warm farming conditions do more harm than good and it is detrimental to fish welfare over time. I believe that these physiological factors play an important role in how fish survive in challenging environments. Rainbow trout are essentially not fit to handle grave conditions such as physical and warming stress, and I believe that these conditions could lead to elevated mortality in aquaculture of rainbow trout

I suggest that further research on this specific topic is done, for better understanding of how these certain variables affect the living conditions of rainbow trout, both in their natural habitats but also in their farming conditions. The continuous heating of the earths' atmosphere causing warmer water climates can lead to negative consequences for farming of rainbow trout and salmonids in aquaculture system that rely on flow-through natural water temperatures. Furthermore, implementation of improvements in animal husbandry within the fish farming industry as well as changing of conditions that the fish are brought up in should be set in motion. A decrease in intensive farming conditions could lead to impaired stress resilience and reduced mortality rates which would at least stagnate the production within the aquaculture industry.

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