Journal of Thermal Biology 132 (2025) 104231

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Acute stress-induced mortality in big Atlantic salmon at high temperatures is associated with insufficient oxygen uptake capacity

Malthe Hvas [a,*], André Morin [b], Ida B. Johansen [c], Tone Vågseth [a]

[a] Animal Welfare Research Group, Institute of Marine Research, Matre, Norway
[b] Sustainable Aquaculture Laboratory – Temperate and Tropical (SALTT), Deakin University, Australia
[c] Department of Preclinical Sciences and Pathology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway

ARTICLE INFO

Keywords:
Aerobic scope
Cardiac morphology
Delayed mortality
Hypoxia tolerance
Lamellar density
Respirometry

ABSTRACT

Thermal studies on fish can help us to understand their robustness to warming climates. Most experiments are performed on smaller individuals and may not represent larger life-stages owing to physiological scaling effects, particularly with regards to thermal tolerance and respiratory capacities. In this study, respirometry experiments were performed on big Atlantic salmon (*Salmo salar*) (≈4 kg) following 3-weeks acclimation to seawater of 9 °C or 19 °C. Additionally, gill and heart morphology traits were assessed. At 9 °C metabolic rates resembled earlier work on smaller fish. However, at 19 °C following stress exposure, 81 % died unexpectedly within ≈6 h while surviving fish struggled to recover a baseline metabolic rate. Most noteworthy was that maximum metabolic rates remained similar across temperature whereas smaller Atlantic salmon previously were found to increase their maximum metabolic rates until near-lethal temperatures. As standard metabolic rates also inevitably increases with temperature, aerobic scopes become reduced at 19 °C. Meanwhile lamellar density was unaffected, indicating similar gill surface areas. However, acclimation to 19 °C reduced ventricle roundness and symmetry, while bulbus width to ventricle width ratios increased. These changes presumably reflect adaptive responses to more metabolically demanding environments. Yet the fish appeared unable to supply sufficient oxygen at 19 °C during stress, which we attribute to physiological scaling constraints. Big Atlantic salmon were therefore more susceptible to stress-induced mortality at elevated temperatures, indicating reduced thermal tolerance relative to smaller individuals. This highlights the need to include larger fish in experiments as the underlying basis for thermal tolerance changes across large differences in body size.

1. Introduction

Climate change may favor smaller fish sizes owing to faster developmental rates and earlier reproduction ages, together with more limited food resources to support increased energetic demands in warmer environments (Daufresne et al., 2009; Gardner et al., 2011; Sheridan and Bickford, 2011).

A mechanistic framework to explain shrinking body sizes in a warmer future is the gill-oxygen limitation theory which proposes that geometrical scaling constraints of the two-dimensional surface of gill lamellae cannot keep up with the increasing oxygen demand of growing three-dimensional bodies (Pauly and Cheung, 2018; Pauly, 2021). However, this idea has been debated (Lefevre et al., 2017). The main opposing argument is that since gill lamellae are folded surfaces, they are well able to increase appropriately with body size, and moreover, physiological data shows that the respiratory surface areas in fish reflects species-specific adaptations in metabolic requirements and not the other way around (Lefevre et al., 2017; Scheuffele et al., 2021; Skeeles and Clark, 2024).

Another widespread framework to assess thermal tolerance and effects of climate change in fish is aerobic scope models (Pörtner and Farrell, 2008; Clark et al., 2013; Lefevre, 2016). The aerobic scope is the capacity to increase metabolic rates above resting baseline levels, either reported as the absolute or factorial differences between maximum and standard metabolic rates (MMR and SMR, respectively) (Fry and Hart, 1948; Brett, 1971; Claireaux and Lefrancois, 2007; Clark et al., 2013). All physiological functions have an energetic cost, and the aerobic scope then represents the capacity to engage in activities such as foraging, growth, predator avoidance, and reproduction. Environmental conditions that reduce aerobic scopes are therefore less optimal as activities

* Corresponding author.
E-mail address: malthe.hvas@hi.no (M. Hvas).

https://doi.org/10.1016/j.jtherbio.2025.104231
Received 26 February 2025; Received in revised form 2 August 2025; Accepted 8 August 2025
Available online 13 August 2025

become energetically more restricted. How the aerobic scope is affected by temperature in species of fish has received much scrutiny (Lefevre, 2016). Generally, SMR increases steadily with temperature whereas MMR also tends to increase throughout the thermal niche and sometimes plateaus or even declines at extreme temperatures (Fry and Hart, 1948; Brett, 1964; Claireaux et al., 2006; Norin et al., 2014; Hvas et al., 2017; Leonard and Skov, 2022). The resultant aerobic scope therefore tends to increase with temperature when expressed in absolute units but may decrease when expressed as a factor, leading to some confusion in its interpretation (Halsey et al., 2018). Furthermore, whether ecologically relevant thermal limits of fish can be explained by oxygen supply limitations and thus declining aerobic scopes has been debated (Pörtner et al., 2017; Jutfelt et al., 2018).

Meanwhile, an often overlooked key feature of aerobic scope models is that they change with ontogeny and body size, where the earliest life-stages and the final sexual mature life-stages are expected to have lower aerobic scopes (Killen et al., 2007; Pörtner and Farrell, 2008). Those critical life-stages should therefore be more sensitive to climate change effects. Despite of this, experimental work on thermal fish physiology has mainly been performed on juvenile intermediate life-stages of fish, as it can be technically difficult to experiment on small fish larvae and even more so on larger individuals weighing several kilograms.

A relevant species to consider in terms of temperature effects across body size is the Atlantic salmon (*Salmo salar*) owing to its cultural importance in recreational fishing, its economic importance in aquaculture, and conservation concerns of wild populations (Krkošek et al., 2007; Dadswell et al., 2021; Vollset et al., 2021). The thermal niche and limits along with numerous miscellaneous biological temperature effects have been widely studied in this species. Briefly summarized, Atlantic salmon are eurythermal anadromous fish that naturally may encounter temperatures from 0 to 3 °C in their northern distribution (Lacroix, 2013; Reddin, 1985), and occasionally to above 20 °C in summer (Valiente et al., 2011). While they may spend most of their life in nature below 10 °C, the growth optimum under cultured conditions is 10.5 °C–14 °C, depending on size (Handeland et al., 2003, 2008; Fraser et al., 2025). Above 18 °C growth declines owing to lower appetite and feed conversion efficiency (Hevrøy et al., 2015; Kullgren et al., 2013; Wade et al., 2019). Behaviorally Atlantic salmon appear to avoid environments above 16 °C if possible (Johansson et al., 2009; Lacroix, 2013). Long-term survival is generally impossible at chronic temperatures above 22 °C (Hvas et al., 2017; Gamperl et al., 2020), although some genetic variation in upper thermal limits have been documented (Ignatz et al., 2023).

Most experimental data on fish thermal physiology has been obtained on smaller sized individuals and may not represent larger individuals owing to size-scaling constraints in oxygen supply. The purpose of this study was therefore to measure metabolic rate traits and aerobic scopes in larger sized Atlantic salmon (≈4 kg) acclimated to midrange (9 °C) and suboptimal (19 °C) seawater temperatures. Morphological metrics of gills and hearts were also assessed to infer potential links between form and function in respiratory capabilities as both organs are known to display substantial phenotypic plasticity in response to the environment (Crispo and Chapman, 2010; Nilsson et al., 2012; Vindas et al., 2024). Earlier work on smaller sized Atlantic salmon found preserved aerobic scopes at high suboptimal seawater temperatures (Hvas et al., 2017; Hvas, 2022). However, if size-scaling constraints in fact becomes a limiting factor for gill oxygen uptake (e.g., Pauly, 2021), we hypothesized that big Atlantic salmon would display an impaired aerobic scope at the higher acclimation temperature.

2. Materials and methods

2.1. Fish husbandry

Big Atlantic salmon post-smolts of a cultured genotype (Aquagen) hatched and reared on-site at the Matre Research Station, Institute of Marine Research, Norway was used in this study. Two months prior to the experimental trials, approximately 120 fish were transferred and distributed into four indoor circular holding tanks (3 m in diameter, 5.3 m^3 in volume). Aerated, filtered, and UV-C treated seawater of 34 ppt was supplied into the holding tanks at a continuous flow-through of 120 l min^{-1} to provide normoxia and remove waste products. A 12h light/dark photoperiod between 08.00 and 20.00 was used and the fish were fed size appropriate commercial feed pellets (Skretting, Norway) in excess daily via automatic feeding devices. The water temperature was initially maintained at 9 °C in all tanks and was also the test temperature used for the first treatment group. To acclimate fish to the second test temperature of 19 °C in two of the holding tanks, the temperature was first increased from 9 °C to 13 °C, then two days later increased further to 16 °C, and after another two days finally increased to 19 °C. The fish were then maintained at 19 °C for a minimum of three weeks before experimental trials at this temperature. To provide adequate oxygen conditions (>85 %PO_2) at 19 °C, oxygen diffusers were added in the holding tanks. The desired water temperatures were maintained precisely via custom made computer software (SDMatre, Normatic AS, Nordfjordeid, Norway) that controlled the automatic mixing of ambient and heated water reservoirs in header tanks above the holding tanks.

This experiment was conducted between February and April 2023 and ethical approval for the use of animals in scientific research was obtained from The Norwegian Food Safety Authorities under permit number 29883.

2.2. Respirometry setup

To measure MO_2 in big Atlantic salmon, an automatic static intermittent-flow respirometry system was used (Loligo Systems, Viborg, Denmark). The system consisted of four cylindrical shaped acrylic chambers submerged in their own water tanks so that four individual fish could be tested simultaneously and independently. The respirometry chambers were 125 cm long with a 30 cm internal diameter and were connected to an internal loop with gas-tight PVC tubes that ran through a circulation pump and a flow-through oxygen sensor cell (measuring at 1 Hz). The total closed volume including tubes was 88.88 l. Each chamber was connected to an open loop with a flush pump (Eheim, 20 l min^{-1}) to facilitate intermittent flush periods. Flush pumps, a temperature probe, and oxygen fiber cables were connected to a computer running the AutoResp software (Loligo Systems), and each oxygen sensor had been carefully calibrated according to the manufacturer's instructions before experimental trials started and was checked again prior to the second treatment group. Each water tank containing a respirometer was 150 x 150 cm square shaped and 60 cm high, with an adjusted water height of 45 cm (volume of 1013 l). Furthermore, each tank had its own flow-through water supply of ≈40 l min^{-1}, using the same water supply from the header tank that supplied the holding tanks, ensuring stable temperatures as well as a continuous exchange with clean water. Each respirometry setup was covered with black plastic sheets once a trial with a fish had started. To further mitigate potential disturbances to the fish, the room lights were turned off and other activities in the laboratory hall were not allowed while trials were running.

2.3. Experimental protocols

Before each trial feed was withheld from a holding tank for one day to mitigate confounding metabolic effects associated with feeding and digestion for estimating the SMR (Chabot et al., 2016). Then, at the start of each trial a fish was netted from a holding tank and transferred into a respirometer chamber. Since a major study aim was to capture the initial peak stress response from being handled, air-exposed, and subsequently introduced to a novel confined space as a proxy of the MMR, the fish were not anaesthetized before movement. Owing to the fish weighing ≈3–5 kg with fork lengths of ≈65–72 cm, they were quite powerful and safe transfer into the respirometers therefore required careful

coordination of two people. The respirometry setup was located adjacent to the holding tanks and the duration of air-exposure and handling of the fish was therefore brief and consistent between replicates (<1 min).

Regarding estimations of the MMR, a common method is to perform a chase protocol before introducing the fish to the respirometer to capture a peak MO_2 immediately following exhaustion (Norin and Clark, 2016; Little et al., 2020). In the case of Atlantic salmon, a higher MO_2 can be achieved by omitting the chase protocol and instead relying on the acute stress imposed from being handled and confined (Hvas and Oppedal, 2019a). We therefore decided not to use a chase protocol before introducing fish to the respirometers in the present study. Alternatively, the MMR can also be measured in swim tunnels while the fish is swimming at its highest aerobic capacity (Norin and Clark, 2016). However, this method may not be feasible for testing big athletic fish owing to the required laminar flow speeds while also providing ample space for burst swimming. For instance, in a previous study in our laboratory, we were unable to consistently exhaust Atlantic salmon of 3 kg when using a large swim tunnel system with flow capacities of up to 140 cm s^{-1} (Oldham et al., 2019). When planning the present study, we therefore concluded that the approach chosen would be the best available method to infer max oxygen uptake capacity in big Atlantic salmon.

Once a fish was inside the respirometer, the chamber was sealed off as quickly as possible while also ensuring that any air bubbles were removed so that logging of MO_2 could commence. An automated intermittent-closed measurement cycle was then repeated over the next 24 h. In the 9 °C treatment group this cycle was 12 min long and consisted of a 6-min closed measurement period followed by a 5.5-min flush period to reestablish oxygen levels and a 0.5-min closed wait period to stabilize flow conditions before repeating the cycle. At 19 °C a higher MO_2 was anticipated meaning that a shorter measurement period still should allow for robust measurements while a longer flushing period likely would be needed to adequately reestablish oxygen levels between cycles. In the first 8 fish tested at 19 °C the measurement period was therefore reduced to 4 min and the flush period increased to 7.5 min allowing for maintaining a 12-min cycle. On the latter 8 fish tested at 19 °C the flush period was further increased to 10.5 min, changing the cycle to 15 min. This was done as a precaution owing to initial unexpected mortalities in this treatment group, although it only marginally increased ambient oxygen levels at the end of flush periods, so it presumably did not make much of a difference, further evidenced from the occurrence of additional mortalities (see results). Generally, oxygen levels remained within normoxic ranges, dropping to ≈80 % at the end of closed periods during periods of peak MO_2.

After 24 h in the respirometers, the fish were subjected to progressive hypoxia by omitting the flush period from the measurement cycle. When MO_2 estimates started to decrease approximately linearly with decreasing oxygen levels, and prior to loss of equilibrium and cessation of active gill ventilation, the fish were removed from the setup and euthanized in an overdose of anesthetics (Tricaine mesylate, Finquel vet.). After fish removal, the empty chambers were resealed to account for background respiration rates where a minimum of three cycles were completed in normoxia.

Meanwhile the weight and fork length of the fish were measured. Then the heart was dissected and momentarily submerged in a saline solution (137 mM NaCl, 2.7 mM KCl, 10 mM Phosphate buffer) to help empty the heart of blood and was then carefully cleaned with paper towels before being preserved in a 70 % ethanol solution in preparation for morphological analyses. Additionally, the second left gill arch was cut out and first stored in a 5 % buffered formalin solution and later preserved in a 70 % ethanol solution at 4 °C in preparation for histological analyses. Then, gonads were dissected out and weighed allowing for sex determination and the calculation of the gonadosomatic index (GSI) in females. All males tested were deemed immature. Lastly, in preparation for the next trials, the water tanks were emptied, and the respirometers were cleaned. Sixteen big Atlantic salmon were tested at both acclimation temperatures.

2.4. Lamellar density

Tissue was prepared with a benchtop histoprocessor (Leica TP 1020) according to the following protocol: 2 × 70 %, 80 %, 2 × 96 %, and 2 × 100 % ethanol, 2 × xylene, 2 × paraffin. Paraffin-embedded tissue blocks were cut with a rotary microtome (Thermo Fisher Microm HM 355s) into 3 μm cross-sections which were mounted on glass slides for haematoxylin-eosin-safranin staining and subsequent scanning (Hamamatsu Photonics NanoZoomer® S60). Morphometric analysis of lamellae was performed using NDP.view2 version 2.9.29 (Hamamatsu Photonics, 2022). Five gill filaments were randomly selected and 10 consecutive lamellae from each filament were chosen using stratified random sampling (2 proximal, 1 middle, and 2 distal regions). The lamellar density could then be calculated by measuring the distance spanned by the 10 consecutive lamellae in each of the 5 regions, averaging these measurements, and extrapolating the density per mm.

2.5. Heart morphology

Fixed hearts were placed in water, attached to a pushpin at the bottom of a transparent plastic box on a light plate (Slimlite LED,32 × 22.8 cm, Kaiser, Buchen, Germany). Photographs of the ventrodorsal and left lateral views of the heart were captured from above utilizing a Canon Powershot SX540 HS (Canon Inc., Tokyo, Japan) camera for morphometric examination. Morphological cardiac measurements were conducted following the procedures outlined by Engdal et al. (2024).

In summary, in the ventrodorsal projection the ventricular height to width ratio was quantified by dividing ventricle length by ventricle width, and relative bulbus size was expressed as bulbus width divided by ventricle width. In the same projection, the apex angle was measured by first dividing the ventricular height into four equal segments, starting from the apex, and extending to the bulbus, followed by drawing a horizontal line at the boundary between the first and second segments, starting at the right and ending at the left longitudinal ventricular ridges. The apex angle was then found from the three points where the horizontal line intersected the right longitudinal ventricular ridge, the apex, and the point where the horizontal line intersected the left longitudinal ventricular ridge.

Thereafter, in the left lateral projection, the ventricular bulbus angle was defined as the angle between the bulbular horizontal axis and the ventricular vertical axis. The ventricular symmetry was quantified by measuring the angle between the ventricular vertical axis and the axis running from the ventriculobulbar groove to the left dorsal ventricular apex. Finally, the bulbus alignment was quantified as the angle between the lines running from the ventriculobulbar groove to the left dorsal ventricular apex and from the ventriculobulbar groove to the atriobulbar incision.

2.6. Calculations and statistics

The MO_2 (mg O_2 kg^{-1} h^{-1}) was calculated in each closed measurement period from the decreasing oxygen level over time:

$$MO_2 = \frac{\frac{\Delta O_2}{\Delta t}\left(V_{res} - V_f\right)}{W_f}$$

Where $\Delta O_2/\Delta t$ is the change in mg O_2 per hour, V_{res} is the volume of the respirometer, V_f is the volume of the fish, assuming a density of 1 kg l^{-1}, and W_f is the weight of the fish.

The SMR was calculated as the mean of the 10 % lowest MO_2 data point from the initial 24-h measurement period prior to the hypoxia test, after having removed potential outliers defined as exceeding ±2 standard deviations of the mean (Clark et al., 2013). The MMR was defined as the highest MO_2 measured, which coincided with the beginning of the

trial when the fish were maximally stressed (Hvas and Oppedal, 2019a). The absolute aerobic scope was expressed as MMR minus SMR, and the factorial aerobic scope was expressed as MMR divided by SMR. The critical oxygen tension (P_{crit}) was defined as the ambient oxygen level before MO_2 decreased below the estimated SMR in normoxia (Ern et al., 2016; Reemeyer and Rees, 2019).

As a standardized morphometric parameter, the condition factor of all fish was calculated as 100 × (weight (g)/length(cm)^3). The relative ventricular mass (RVM) of fixated hearts and the GSI were both expressed as a percentage of total body weight.

To express the factorial change in metabolic rate traits with a 10 °C change in temperature, the temperature quotient, Q_{10}, was calculated as:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

Where R_1 and R_2 are the rates at the first temperature, T_1 and the second temperature, T_2, respectively.

Statistical differences between the two treatment groups in measured parameters were assessed with a *t*-test after having checked for equal variance and normal distribution of the data with Levene's mean test and Shapiro-Wilks tests, respectively. In the case of weight and P_{crit} data, a log transformation was required to adhere to test assumptions, and if this did not satisfy test assumptions the Mann-Whitney Rank Sum Test was used instead. A P-value below 0.05 was considered significant and data reported in the text are mean ± s.e.m. unless stated otherwise.

3. Results

The weight, fork length, condition factor, RVM, and GSI of big Atlantic salmon tested with respirometry were all statisticaly similar between treatment groups (T-test, DF = 30, P > 0.05 for weight, condition factor, RVM and Mann-Whitney Rank Sum Test, P > 0.05 for fork length and GSI) (Table 1).

No fish died while being maintained in their holding tanks, including the acclimation period at 19 °C. However, during the respirometry trials at 19 °C, 13 out of the 16 fish tested died unexpectedly in the respirometers within 5.5 ± 0.6 h. This corresponded to an accumulated mortality of 81.25 %. In comparison all fish tested at 9 °C survived the entire trial (Fig. 1).

At 9 °C the fish showed a consistent pattern in MO_2 over time following introduction into the respirometers where MO_2 peaked initially and then gradually decreased towards a baseline level that stabilized after 12–15 h (Fig. 2A). Of the three fish that survived at 19 °C only one fish showed a similar response pattern as the fish at 9 °C where MO_2 decreased substantially after the initial peak, reaching a baseline after approximately 12 h (Fig. 2B). The two other surviving fish at 19 °C maintained a high MO_2 over the entire 24-h trial period suggesting they were struggling to recover from the acute stress experienced at the start of the trial (Fig. 2B). For dying fish at 19 °C the initial peak MO_2 was similar to the survivors whereafter it decreased steadily over the following hours and then suddenly jumped to zero, coinciding with death (Fig. 2C). On a couple of occasions dying fish were removed shortly after MO_2 became zero, and immediate dissections revealed that the hearts were still beating, showing that mortality was not caused by a sudden cardiac arrest.

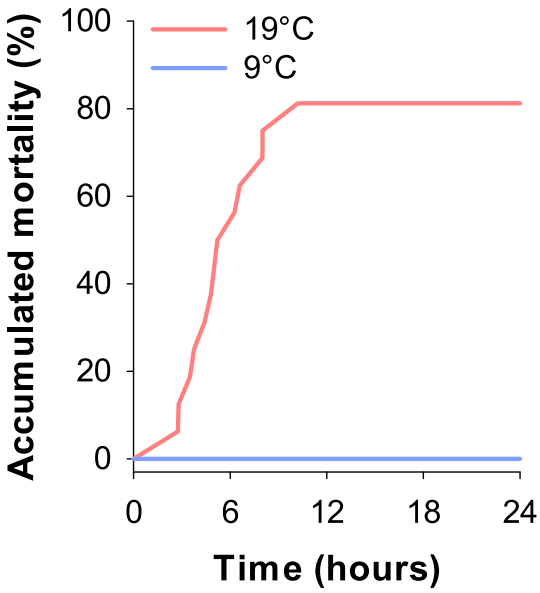

Fig. 1. Accumulated mortalities following transfer into respirometry chambers. 13 out of 16 fish died at 19 °C while all 16 fish survived 24 h at 9 °C. No mortalities occurred when acclimating in holding tanks at either temperature.

The MMR was unaffected by acclimation temperature being 404 ± 11 and 397 ± 14 mg O_2 kg^{-1} h^{-1} at 9 °C and 19 °C, respectively (T-test, DF = 30, P = 0.685) (Fig. 3A). Owing to the high mortality rate at 19 °C, an SMR could only be calculated in the three surviving fish. Since the group estimate then becomes less robust owing to low replication and owing to uncertainty regarding whether measurements represented true resting states, we therefore cautiously refer to a routine metabolic rate (RMR) rather than a SMR (Fig. 3B). Furthermore, the P_{crit} as well as the absolute and factorial aerobic scopes could only be derived from surviving fish. Those estimates therefore also become less robust at 19 °C. The RMR at 9 °C was 94.6 ± 3.4 mg O_2 kg^{-1} h^{-1} while it was 244 ± 45 mg O_2 kg^{-1} h^{-1} at 19 °C which was significantly higher (Mann-Whitney Rank Sum Test, P = 0.009) (Fig. 3B). Similarly, the P_{crit} was significantly higher at 19 °C being 53.9 ± 7.9 %PO_2 compared to 22.9 ± 1.2 %PO_2 at 9 °C (T-test, DF = 17, P < 0.0001) (Fig. 3C).

The surviving fish at 19 °C had significantly lower aerobic scopes (Fig. 4). Specifically, the absolute aerobic scope was 309 ± 12.0 and 195 ± 33 mg O_2 kg^{-1} h^{-1} at 9 °C and 19 °C, respectively (T-test, DF = 17, P = 0.002) (Fig. 4A), and the factorial aerobic scope was 4.36 ± 0.20 at 9 °C and 1.92 ± 0.32 at 19 °C (T-test, DF = 17, P = 0.0001) (Fig. 4B).

The lamellar density did not differ between groups being 18.8 ± 0.3 and 18.4 ± 0.3 lamellae mm^{-1} at 9 °C and 19 °C, respectively (T-test, DF = 30, P = 0.317) (Fig. 5).

For heart morphology metrics, significant differences were found in three of the six parameters assessed (Fig. 6). Specifically, the ventricle height to width ratio and the bulbus width to ventricle width ratio were both significantly higher in the 19 °C group (T-test, DF = 30, P = 0.0214 and P = 0.0207, respectively) (Fig. 6A and B). Meanwhile, the ventricular symmetry was lower in the 19 °C group (T-test, DF = 30, P = 0.00159) (Fig. 6E). The apex angle, the ventricular bulbus angle, and the bulbus alignment did not differ between the two acclimation

Table 1
Morphometric parameters. RVM = Relative ventricular mass and GSI = Gonadosomatic index in females. All size parameters were statistically similar (T-test, P > 0.05). N = 16 for both groups and data are mean ± s.e.m. The RVM were measured on hearts fixated and dehydrated in ethanol, yielding lower values.

Temperature	Weight (g)	Length (cm)	Condition factor	RVM (%)	Female/Male	Female GSI (%)
9 °C	3722 ± 115	68.7 ± 0.41	1.14 ± 0.02	0.052 ± 0.004	9/7	0.19 ± 0.01
19 °C	3766 ± 159	68.4 ± 0.77	1.17 ± 0.03	0.054 ± 0.005	13/3	0.51 ± 0.15

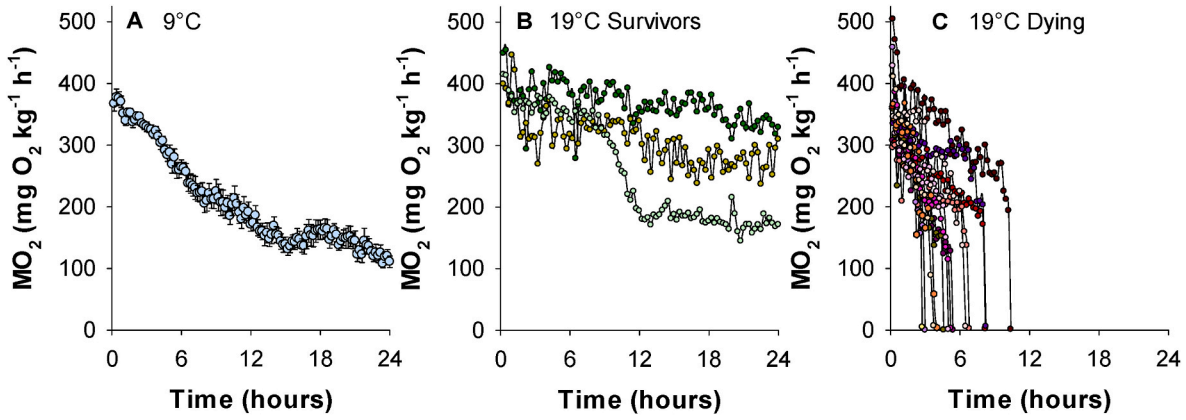

Fig. 2. Oxygen uptake rates (MO_2) over a 24-h period following movement into respirometers. A: The mean ± s.e.m. values of all fish at 9 °C (N = 16). **B**: Individual data series from each fish that survived the trials at 19 °C. **C**: Individual data series for each fish that died during the trials at 19 °C.

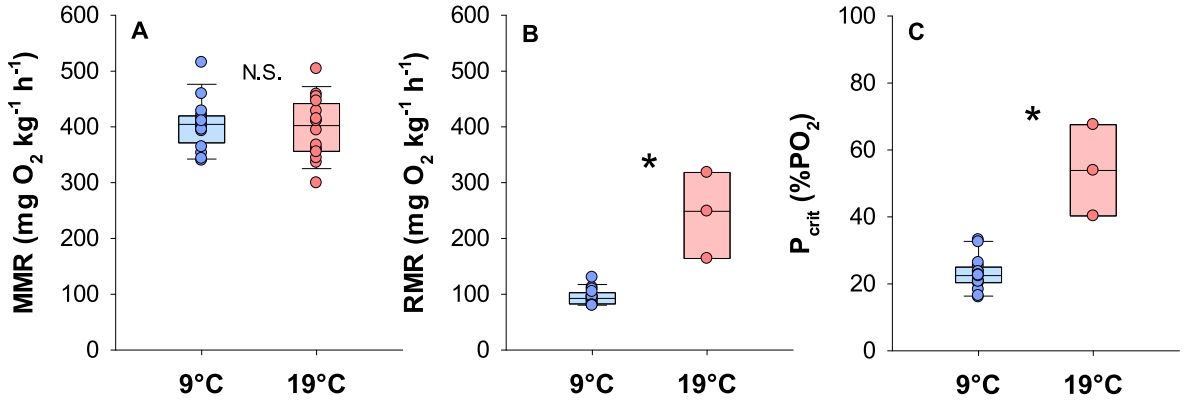

Fig. 3. Metabolic rate parameters. Maximum metabolic rate (MMR) (**A**), Routine metabolic rate (RMR) (**B**), and Critical oxygen tension (P_{crit}) (**C**). Data are shown as boxplots together with individual data points. N.S. indicates a non-significant difference between groups and asterisks a significant difference (T-test, $P < 0.05$). Only three estimates of SMR and P_{crit} were obtained at 19 °C owing to high mortality rates.

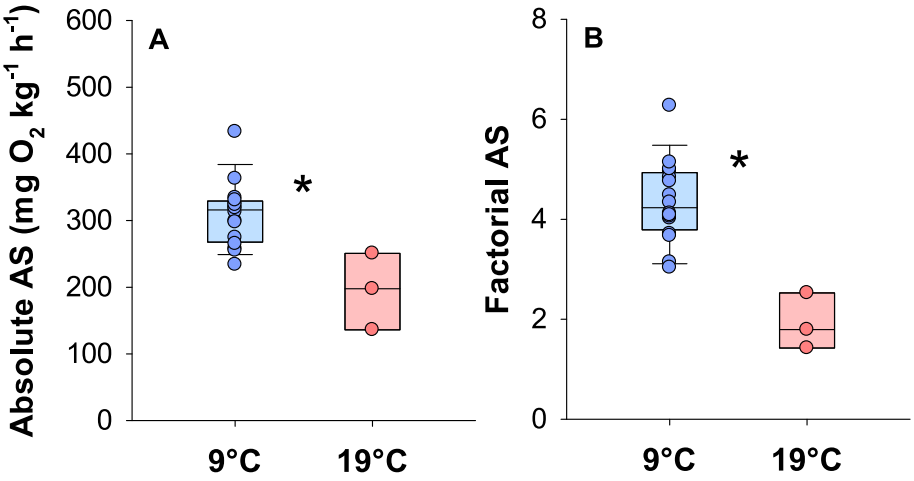

Fig. 4. The absolute aerobic scope (AS) (A) and the factorial AS (B). Data are shown as boxplots together with individual data points and asterisks indicate a significant difference (T-test, $P < 0.05$). Owing to the high mortality rate at 19 °C only three aerobic scope estimates were obtained.

temperatures (T-test, DF = 30, $P > 0.05$) (Fig. 6C,D,F).

To illustrate size dependent effects across acclimation temperature on MMR, data from two previous studies using smaller sized seawater adapted Atlantic salmon (≈0.5 kg) were compared with the present study (Fig. 7) (Hvas et al., 2017; Hvas, 2022). These studies utilized the same genetic strain of Atlantic salmon, the same research facility, and a thermal acclimation period of three weeks minimum. Notably, smaller Atlantic salmon increased their MMR substantially with a Q_{10} of 1.57 and 1.54 within a similar thermal interval as in the present study. Meanwhile, the Q_{10} of the MMR in big Atlantic salmon was 0.98, signifying no change across temperature (Fig. 7).

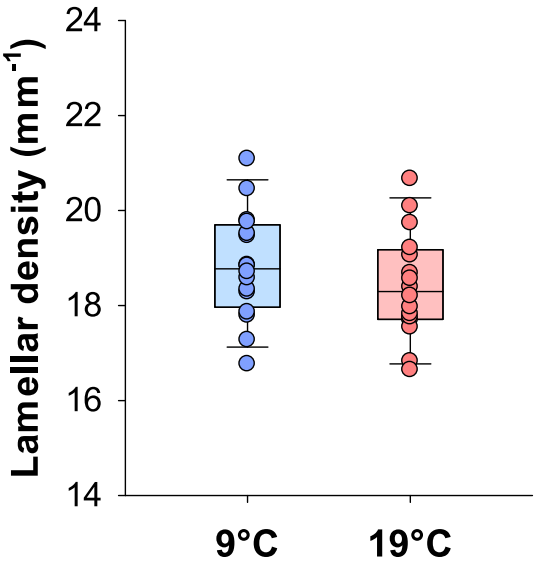

Fig. 5. Lamellar density of Atlantic salmon acclimated to 9 °C and 19 °C. Data are shown as boxplots together with individual datapoints (N = 16). The groups were statistically similar (*t*-test, P > 0.05).

4. Discussion

4.1. Oxygen supply limitation in big Atlantic salmon at high temperatures

Based on the idea that fish may become restricted in oxygen supply capacity as they grow larger (Pauly, 2021), it was hypothesized that larger sized Atlantic salmon (≈4 kg) would struggle to maintain adequate aerobic scopes at higher acclimation temperatures. This hypothesis was supported by the results as the MMR remained unaffected between 9 °C and 19 °C while the RMR inevitably increased, leading to lower aerobic scopes at 19 °C. Furthermore, in smaller Atlantic salmon the MMR increased substantially with temperature, allowing for a preserved aerobic scope (Hvas et al., 2017; Hvas, 2022, Fig. 7).

The P_{crit} in big Atlantic salmon at 9 °C was comparable to previous reports on Atlantic salmon of 150–300 g at similar temperatures (Remen et al., 2016; Hvas and Oppedal, 2019b). The tolerance to hypoxia worsens at higher temperatures, and in smaller Atlantic salmon regression analyses estimated a P_{crit} of 40 % PO_2 at 19 °C for fish weighing 300 g (Remen et al., 2016). Meanwhile, the P_{crit} of big Atlantic salmon at 19 °C in the present study was notably higher at 53 % PO_2. This may be ascribed to less robust SMR estimates that are better described as RMR as it is uncertain whether the fish had recovered to a true resting state at 19 °C. Although, if larger sized Atlantic salmon become more restricted in gill oxygen uptake at higher temperatures, they would be expected to

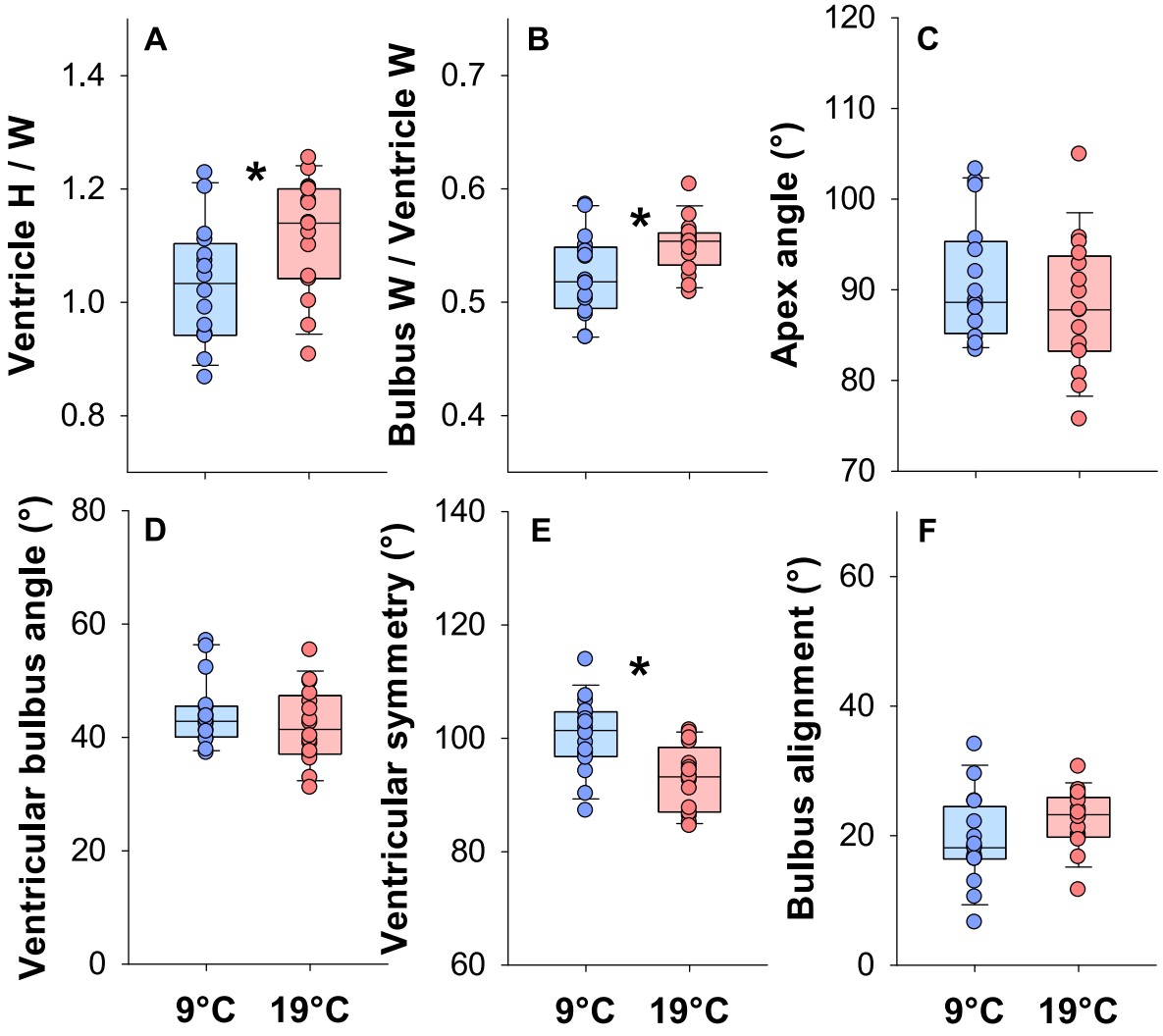

Fig. 6. Heart morphology parameters of Atlantic salmon acclimated to 9 °C and 19 °C. Panels show boxplots together with individual datapoints (N = 16). H = height and W = width. Statistical differences between groups are indicated with an asterisk (T-test, P < 0.05).

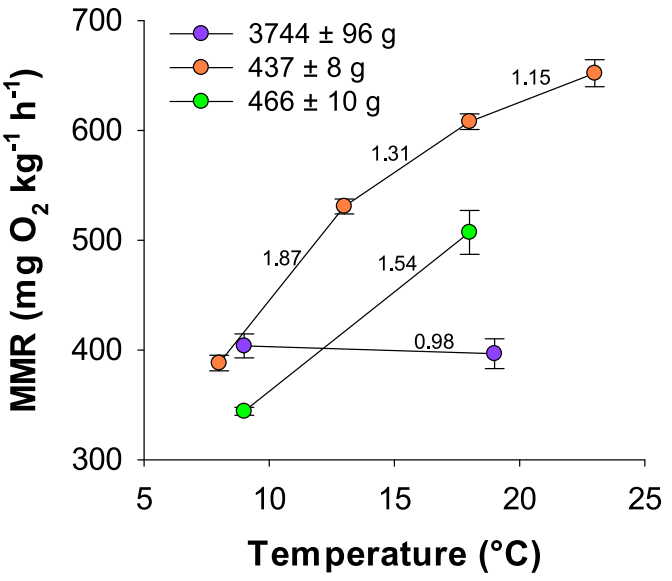

Fig. 7. Maximum metabolic rate (MMR) of Atlantic salmon across acclimation temperature and size class. Purple: Present study, Orange: modified from Hvas et al. (2017), and Green: modified from Hvas (2022). The numbers between data points within studies are Q_{10} values. The studies compared on the figure were performed on the same genetic strain of Atlantic salmon maintained in similar husbandry condition at the Matre Research Station, Norway. All fish had been acclimated for 3 weeks minimum at their respective test temperature. The data shown are mean ± s.e.m.

become less tolerant to hypoxia relative to smaller sized individuals.

In other species of fish assessed with respirometry across different acclimation temperatures, the pattern has often been similar to those of smaller sized Atlantic salmon where MMR was well able to increase with temperature. Notable examples of this pattern include European sea bass (*Dicentrarchus labrax*) (Claireaux et al., 2006), barramundi (*Lates calcarifer*) (Norin et al., 2014), Atlantic halibut (*Hippoglossus hippoglossus*) (Gräns et al., 2014), lumpfish (*Cyclopterus lumpus*) (Hvas et al., 2018), and ballan wrasse (*Labrus bergylta*) (Yuen et al., 2019). Common for these experiments are that they used small juvenile fish weighing <0.5 kg. Furthermore, in a comprehensive meta-analysis on metabolic rate effects across temperature, it was found that in most fish species investigated the MMR increased with temperature, showing no or sometimes a minor decline at extreme temperatures (Lefevre, 2016). However, it should be emphasized that in the present study, 19 °C was not supposed to represent an extreme temperature, but rather a suboptimal yet ecologically relevant acclimation temperature well below known lethal limits in Atlantic salmon (Hevrøy et al., 2015; Gamperl et al., 2020).

When considering size as a factor for MMR across different acclimation temperatures, a study on Atlantic cod (*Gadus morhua*) found patterns similar to the present study (Tirsgaard et al., 2015). These authors used five acclimation temperatures and body sizes spanning 30 g–460 g and showed that in smaller Atlantic cod MMR increased with temperature, but in larger individuals MMR plateaued (Tirsgaard et al., 2015). Moreover, in a study on Nile tilapia (*Oreochromis niloticus*) ranging in size from 21 g to 313 g the tolerance to acute warming while exercising declined with mass and was associated with lower oxygen uptake prior to fatigue in the larger individuals (Blasco et al., 2022). Interestingly, these restrictions in MMR at increasing temperatures in Atlantic cod and Nile tilapia occurred at body sizes approximately one order of magnitude smaller than in Atlantic salmon, suggesting that size thresholds for temperature-dependent limitations in oxygen uptake capacity can be highly species-specific. Meanwhile, a study on mature coho salmon (*Oncorhynchus kisutch*) weighing ≈2 kg reported statistically similar MMRs between 9 °C, 14 °C, and 18 °C (Kraskura et al., 2021). A pattern similar to large Atlantic salmon in the present study. As such, the inability to preserve aerobic scopes at higher temperatures by further increasing oxygen uptake capacities in larger individuals has also been observed in other fish species.

4.2. Stress-induced mortality in Atlantic salmon at high temperatures

The high mortality rates following acute stress exposure at 19 °C were unexpected. Meanwhile, no fish died at 9 °C and no fish died during the acclimation period in the holding tanks at 19 °C spanning several weeks. The mortalities at 19 °C must therefore have been caused by the combination of acute stress and high temperature. The experimental protocol involved a combination of stressors, starting with the fish being netted from its holding tank, followed by air exposure while being transferred in the net, and then being placed inside the respirometer, an unfamiliar and highly confined environment. At both test temperatures, the initial behavioural response inside the respirometer was a short period of frantic movements, as an attempt to escape, followed by visible exhaustion where the fish would lie still and hyperventilate.

It is well-known that fish, particularly salmonids, can die from exhaustive stress, where mortality is associated with failure of physiological recovery as inferred from high lactate levels and a high intracellular acidosis in moribund individuals (Black, 1958; Wood et al., 1983; Holder et al., 2022). Stress-induced mortality is also termed delayed or post-exercise mortality and may occur within a few hours or up to several days after having encountered a stressful event (Wood et al., 1983; Clark et al., 2017). For instance, in another study on Atlantic salmon delayed mortality occurred 4–7 days after exhaustive exercise stress and was associated with the inability to re-attain normal heart rates while surviving fish had fully recovered resting heart rates within 24 h (Hvas et al., 2021).

Fish mortalities after stressful events have several important implications. In commercial fisheries undesired fish are typically discarded and owing to exhaustive capture stress, they may later die (Turunen et al., 1994; Davis, 2002; Cook et al., 2019). Similarly, catch-and-release angling can also cause delayed mortality where guidelines are established to help facilitate post-release survival (Cooke and Suski, 2005; Bartholomew and Bohnsack, 2005; Pollock and Pine, 2007; Smukall et al., 2019). In Atlantic salmon aquaculture delayed mortality is associated with transportation, crowding, and parasite treatments where efforts are made to improve handling strategies by reducing stress levels during farm operations (Iversen et al., 2005; Overton et al., 2019). Furthermore, near-harvest sized Atlantic salmon can be at higher risk of stress-induced mortality which has been linked with heart disease and deviating heart morphology (Garseth et al., 2018; Frisk et al., 2020). In light of the present study, it is therefore interesting that a body mass >3 kg increases mortality risk in salmon aquaculture.

A recent review concluded that an all-encompassing mechanistic explanation for post-exercise mortality in fish has not been found, although several ideas have been proposed involving oxygen delivery, ion regulation, hormone signaling, and cardiac function (Holder et al., 2022). While hearts were observed to still be beating upon dissection of recently dead fish in the present study, irregular heart rates were previously associated with delayed mortality in Atlantic salmon (Hvas et al., 2021). Meanwhile, in moribund rainbow trout, strong heartbeats and normal aortic blood pressure were measured following the cessation of active gill ventilation (Wood et al., 1983). In the present study, the cause of mortality was presumably related to the inability to take up sufficient oxygen at 19 °C. If oxygen requirements cannot be met in highly stressed Atlantic salmon for several hours, a cascade of detrimental consequences will follow as tissues become more hypoxic and acidotic, eventually resulting in death.

Two key risk factors for stress-induced mortality seem to be body size and elevated temperatures. It has also been shown in coho salmon that larger sizes make physiological recovery more difficult when subjected

to a simulated capture event where it was found that larger fish accumulated a higher oxygen debt and were slower at reattaining osmoregulatory homeostasis (Clark et al., 2012). Increased risks of delayed mortality at higher temperatures have also been found in leopard coral grouper (*Plectropomus leopardus*) where exhaustive exercise stress caused mortality in some individuals after 3–13 days at 30 °C, while at 33 °C mortality rates increased, and they occurred much sooner after 2–15 h (Clark et al., 2017). Furthermore, in a study on triploid brook trout (*Salvelinus fontinalis*) subjected to exhaustive exercise at 19 °C, 90 % of the fish died within 4 h of recovery while all the diploid counterparts survived (Hyndman et al., 2003). Triploids have larger cells with lower surface to volume ratios and thus may be more diffusion limited. Similar to larger sized fish, triploids can therefore also become restricted in gill-oxygen uptake at high temperatures (Hansen et al., 2015), which could explain the delayed mortalities reported by Hyndman et al. (2003).

4.3. Thermal plasticity in gill and heart morphology - links between form and function

The gill and the heart of fish are well-known for their morphological plasticity in response to environmental factors and may provide an anatomical link to explain respiratory capabilities. Here, the lamellar density provides an indicator of gill surface area (Laporte et al., 2016; Skeeles and Clark, 2024). A similar lamellar density across acclimation temperature in the present study may therefore aid in explaining why MMR was unable to increase further at 19 °C, assuming that the gill surface area is closely correlated with the oxygen uptake capacity. Perhaps a longer acclimation period than 3 weeks to high temperatures would have allowed for more adequate gill remodeling to better adapt to more metabolically demanding environments in big Atlantic salmon.

Contrary to the gills, the heart morphology did change in response to a 3-week acclimation period at 19 °C. Notably, ventricle height to width ratio increased which signify less rounded ventricles. Less rounded and more pyramid shaped ventricles is a characteristic of active fish that rely on rapid and efficient pumping of blood (Agnisola and Tota, 1994; Sanchez-Quintana et al., 1995). Furthermore, in rainbow trout (*Oncorhynchus mykiss*), fish with triangular ventricles were better swimmers, had higher maximum oxygen uptake capacities, and a larger cardiac output than individuals with more rounded ventricles (Claireaux et al., 2005). In the present study, fish at 19 °C also had a lower ventricular symmetry. Lower ventricular symmetry is a trait associated with wild salmon (Poppe et al., 2003; Engdal et al., 2024; Vindas et al., 2024), while higher ventricular symmetry is typical for intensive culture conditions and has been associated with morbidity in larger-sized farmed Atlantic salmon (Frisk et al., 2020). Less rounded ventricles and lower symmetry in the 19 °C acclimated fish could therefore be an adaptive plastic response to improve cardiac function in warmer and more challenging environments. Warm acclimated fish also had a higher bulbus width to ventricle width ratio, which signifies a relatively larger bulbus. This trait is associated with intense growth conditions in aquaculture, contrasting wild salmon that have a relatively smaller bubus (Vindas et al., 2024). Whether a phenotypic change of this trait is functional advantageous or maladaptive is therefore less clear but could reflect remodeling to adjust compliance in response to higher temperatures (Keen et al., 2021).

The reported RVM in the present study was similar between the two acclimation temperatures, but the values of ≈0.5 % were substantially smaller than typically reported for Atlantic salmon of various sizes where RVM generally ranges from 0.8 % to 1.2 % of the total body weight (Hvas and Oppedal, 2017; Frisk et al., 2020; Vindas et al., 2024). However, those measurements were taken as wet weights in recently dissected fish while RVM in the present study were measured after the hearts had been fixated in ethanol for little over a year. Curiously, owing to the dehydrating properties of ethanol, prolonged fixation can reduce muscle tissue weights by ~ 60 % (Leonard et al., 2021). The initial wet weights of the ventricles from the present study would therefore presumably have been similar to earlier reports.

4.4. Ethical and method considerations when experimenting on larger-sized fish

In the present study, high mortality rates unfortunately occurred following acute stress in the larger-sized Atlantic salmon acclimated to 19 °C. Based on previous studies with smaller Atlantic salmon (e.g., Hansen et al., 2015; Hvas et al., 2017), mortalities were not expected at 19 °C. When unforeseen mortalities occur in laboratory experiments, thorough ethical and methodological considerations are required to evaluate potential causes and whether to continue, modify, or prematurely terminate the experiment. After the first mortalities occurred, we attempted to find errors in the setup and modified the test protocol for subsequent trials. We eventually ended up testing the planned number of animals but were unable to prevent additional mortalities in the latter trials.

Meanwhile, Norway is the world's largest producer of farmed salmon. In 2023 the mortality rates in Norwegian salmon aquaculture were the highest ever recorded where 63 million (16.7 %) Atlantic salmon died during the seawater growth phase (Sommerset et al., 2024). A worrisome trend is that mortality of larger sized fish appears to increase. This is associated with stressful handling operations at farms, particularly following lice-treatments and crowding events (Overton et al., 2019; Sommerset et al., 2024). Furthermore, summer heatwaves are becoming a greater concern in Norway, and persistent summer temperatures approaching and exceeding 19 °C are not unusual. These higher temperatures also increase proliferation of sea lice (Hamre et al., 2019), and thus the need to perform additional stressful de-lousing operations. The severity level imposed in our study therefore resemble conditions fish may experience in commercial scale aquaculture in a warmer climate. We therefore believe that the mortalities imposed in the present study have some justification from a utilitarian ethics point of view. That is, a low number of fish suffered so that a high number of fish in the future may not have to.

There are generally little empirical data on environmental limits in larger sized fish weighing several kilograms. Our present study suggests that larger sized Atlantic salmon have very different stress tolerances at elevated temperatures compared to previous studies on smaller fish. This highlights the timely relevance of incorporating larger fish sizes in experiments. Although, it is more challenging to maintain and study bigger fish in the laboratory.

Provided that larger sized fish generally are less robust in coping with various experimental procedures, and more so at elevated temperatures, additional precautions when designing protocols are warranted. Based on what we have learned in the present study, this may involve refinements in how the fish are handled and the choice of test conditions used. As such, 19 °C was evidently too high a temperature for a respirometry trial that sought to measure SMR in 4 kg Atlantic salmon. Even the surviving fish appeared to struggle with recovering from the initial stress. A longer test period than the conventional 24 h would presumably then be needed to more robustly infer true resting states in these bigger fish. Previously established techniques may also not be logistically feasible on larger fish. A relevant example are swim tunnel respirometers to measure MMR in fish exercising at the aerobic limit. Presently available tunnel designs do not provide ample space and adequate laminar flow properties at high velocities to reliably exhaust Atlantic salmon of 3 kg and above (Oldham et al., 2019). Alternative and more pragmatic approaches are therefore needed to infer physiological capacities and environmental limits in bigger fish. For instance, the monitoring of MO_2 while gradually increasing temperatures could be a useful approach to assess interactions between body size, thermal tolerance, and aerobic capacity while also avoiding stress-related mortalities (Paschke et al., 2018).

5. Conclusion

Whether the underlying cause for an insufficient MMR as well as stress-induced mortality in big Atlantic salmon at a high acclimation temperature was driven by oxygen supply limitation was not directly discerned in the present study. Although, the most proximal explanation would seem to involve limitations in physiological scaling properties of the cardio-respiratory system. In a recent study on rainbow trout exposed to hyperoxia, it was concluded that the MMR was gill diffusion limited after having assessed several factors of the oxygen transport system (McArley et al., 2022). Moreover, these authors proposed that gill-diffusion limitation could constrain MO_2 in various stressful situations such as during high-intensity swimming and exposure to high temperatures (McArley et al., 2022). One could here speculate that the consequence of gill diffusion limitation would become more pronounced in larger sized individuals under stressful conditions. Additionally, the limitation in oxygen uptake and transport capacity in salmonids has also been argued to originate from insufficient cardiac function where a reduced heart rate scope can lead to cardiac collapse and mortality during strenuous activities at high temperatures (Eliason et al., 2013).

Regardless of the underlying cause, big Atlantic salmon were unable to increase their MMR between 9 °C and 19 °C, contrasting the results from smaller sized Atlantic salmon. Furthermore, this inability of larger sized fish to increase their MMR at higher temperatures seem to also be the case in other species (Tirsgaard et al., 2015; Kraskura et al., 2021; Blasco et al., 2022). The consequences of being restricted in oxygen supply during acute stress at elevated temperatures are clearly dire as evidenced by the high occurrence of delayed mortality observed in the present study.

In conclusion, larger sized seawater adapted Atlantic salmon appear less tolerant to high temperatures and hypoxia. This study also highlights that when elucidating ecologically relevant thermal limitations of fish species, it is important to consider natural size ranges despite the added logistical challenge imposed by experimenting on larger size classes.

CRediT authorship contribution statement

Malthe Hvas: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **André Morin:** Writing – review & editing, Visualization, Software, Methodology, Investigation. **Ida B. Johansen:** Writing – review & editing, Validation, Methodology, Funding acquisition, Formal analysis, Data curation. **Tone Vågseth:** Writing – review & editing, Validation, Methodology, Investigation, Data curation, Conceptualization.

Funding

This wok was funded by the Research Council of Norway (project numbers 295200 and 334819).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the staff at the Matre research station for excellent animal husbandry and for helpful technical assistance with an oversized and inconvenient experimental setup.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2025.104231.

Data availability

The data has been shared as supplementary material.

References

Agnisola, C., Tota, B., 1994. Structure and function of the fish cardiac ventricle: flexibility and limitations. Cardioscience 5, 145–153.
Bartholomew, A., Bohnsack, J.A., 2005. A review of catch-and-release angling mortality with implications for no-take reserves. Rev. Fish Biol. Fish. 15, 129–154.
Black, E.C., 1958. Hyperactivity as a lethal factor in fish. J. Fish. Res. Board Can. 15, 245–251.
Blasco, F.R., Taylor, E.W., Leite, C.A.C., Monteiro, D.A., Rantin, F.T., McKenzie, D.J., 2022. Tolerance of an acute warming challenge declines with body mass in Nile tilapia: evidence of a link to capacity for oxygen uptake. J. Exp. Biol. 225 (16), jeb244287.
Brett, J.R., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21, 1183–1226.
Brett, J.R., 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am. Zool. 11, 99–113.
Chabot, D., Steffensen, J.F., Farrell, A.P., 2016. The determination of standard metabolic rate in fishes. J. Fish. Biol. 88, 81–121.
Claireaux, G., Lefrancois, C., 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. Phil. Trans. Biol. Sci. 362, 2031–2041.
Claireaux, G., McKenzie, D.J., Genge, A.G., Chatelier, A., Aubin, J., Farrell, A.P., 2005. Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. J. Exp. Biol. 208, 1775–1784.
Claireaux, G., Couturier, C., Groison, A.-L., 2006. Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). J. Exp. Biol. 209, 3420–3428.
Clark, T.D., Donaldson, M.R., Pieperhoff, S., Drenner, S.M., Lotto, A., Cooke, S.J., Hinch, S.G., Patterson, D.A., Farrell, A.P., 2012. Physiological benefits of being small in a changing world: responses of coho salmon (*Oncorhynchus kisutch*) to an acute thermal challenge and a simulated capture event. PLoS One 7, e39079.
Clark, T.D., Sandblom, E., Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J. Exp. Biol. 216, 2771–2782.
Clark, T.D., Messmer, V., Tobin, A.J., Hoey, A.S., Pratchett, M.S., 2017. Rising temperatures may drive fishing-induced selection of low-performance phenotypes. Sci. Rep. 7 (1), 40571.
Cook, K.V., Reid, A.J., Patterson, D.A., Robinson, K.A., Chapman, J.M., Hinch, S.G., Cooke, S.J., 2019. A synthesis to understand responses to capture stressors among fish discarded from commercial fisheries and options for mitigating their severity. Fish Fish. 20, 25–43.
Cooke, S.J., Suski, C.D., 2005. Do we need species-specific guidelines for catch-and-release recreational angling to effectively conserve diverse fishery resources? Biodivers. Conserv. 14, 1195–1209.
Crispo, E., Chapman, L.J., 2010. Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. J. Evol. Biol. 10, 2091–2103.
Dadswell, M., Spares, A., Reader, J., McLean, M., McDermott, T., Samways, K., Lilly, J., 2021. The decline and impending collapse of the Atlantic salmon (*Salmo salar*) population in the North Atlantic Ocean: a review of possible causes. Rev. Fish. Sci. Aquac. 30, 215–258.
Daufresne, M., Lengfellner, K., Sommer, U., 2009. Global warming benefits the small in aquatic ecosystems. Proc. Natl. Acad. Sci. U. S. A 106 (31), 12788–12793.
Davis, M.W., 2002. Key principles for understanding fish bycatch discard mortality. Can. J. Fish. Aquat. Sci. 59, 1834–1843.
Eliason, E.J., Clark, T.D., Hinch, S.G., Farrell, A.P., 2013. Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. Conserv. Physiol 1, cot008.
Engdal, V.A., Dalum, A.S., Kryvi, H., Frisk, M., Torsvik, H., Hodne, K., Romstad, H., Johansen, I.B., 2024. State of the heart: anatomical annotation and assessment of morphological cardiac variation in Atlantic salmon (*Salmo salar* L.). Aquaculture 578, 740046.
Ern, R., Norin, T., Gamperl, A.K., Esbaugh, A.J., 2016. Oxygen dependence of upper thermal limits in fishes. J. Exp. Biol. 219, 3376–3383.
Fraser, T.W.K., Sambraus, F., Remø, S.C., Stien, L.H., Hansen, T.J., Fjelldal, P.G., 2025. Growth, sexual maturation, cataracts, and blood biochemistry in diploid and triploid Atlantic salmon post-smolts reared at one of eight constant temperatures from 3.0 to 20.5 °C for 140 days. Aquaculture, 742323.
Frisk, M., Høyland, M., Zhang, L., Vindas, M.A., Øverli, Ø., Johansen, I.B., 2020. Intensive smolt production is associated with deviating cardiac morphology in Atlantic salmon (*Salmo salar* L.). Aquaculture 529, 735615.
Fry, F., Hart, J., 1948. The relation of temperature to oxygen consumption in the goldfish. Biol. Bull. 94, 66–77.
Gamperl, A.K., Ajiboye, O.O., Zanuzzo, F.S., Sandrelli, R.M., Peroni, E.F.C., Beemelmanns, A., 2020. The impacts of increasing temperature and moderate

hypoxia on the production characteristics, cardiac morphology and haematology of Atlantic salmon (*Salmo salar*). Aquaculture 519, 734874.
Gardner, J.L., Peters, A., Kearney, M.R., Joseph, L., Heinsohn, R., 2011. Declining body size: a third universal response to warming? Trends Ecol. Evol. 26 (6), 285–291.
Garseth, Å.H., Fritsvold, C., Svendsen, J.C., Bang Jensen, B., Mikalsen, A.B., 2018. Cardiomyopathy syndrome in Atlantic salmon *Salmo salar* L.: a review of the current state of knowledge. J. Fish. Dis. 41, 11–26.
Gräns, A., Jutfelt, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Einarsdottir, I., 2014. Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO_2 in Atlantic halibut. J. Exp. Biol. 217, 711–717.
Halsey, L.G., Killen, S.S., Clark, T.D., Norin, T., 2018. Exploring key issues of aerobic scope interpretation in ectotherms: absolute versus factorial. Rev. Fish Biol. Fish. 28, 405–415.
Hamre, L., Bui, S., Oppedal, F., Skern-Mauritzen, R., Dalvin, S., 2019. Development of the salmon louse *Lepeophtheirus salmonis* parasitic stages in temperatures ranging from 3 to 24°C. Aquaculture Environment Interactions 11, 429–443.
Handeland, S.O., Björnsson, B.T., Arnesen, A.M., Stefansson, S.O., 2003. Seawater adaptation and growth of post-smolt Atlantic salmon (*Salmo salar*) of wild and farmed strains. Aquaculture 220, 367–384.
Handeland, S.O., Imsland, A.K., Stefansson, S.O., 2008. The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. Aquaculture 283, 36–42.
Hansen, T.J., Olsen, R.E., Stien, L., Oppedal, F., Torgersen, T., Breck, O., Remen, M., Vågseth, T., Fjelldal, P.G., 2015. Effect of water oxygen level on performance of diploid and triploid Atlantic salmon post-smolts reared at high temperature. Aquaculture 435, 354–360.
Hevrøy, E.M., Tipsmark, C.K., Remø, S.C., Hansen, T., Fukuda, M., Torgersen, T., Vikeså, V., Olsvik, P.A., Waagbø, R., Shimizu, M., 2015. Role of the GHIGF-1 system in Atlantic salmon and rainbow trout postsmolts at elevated water temperature. Comp. Biochem. Physiol. Mol. Integr. Physiol. 188, 127–138.
Holder, P.E., Wood, C.M., Lawrence, M.J., Clark, T.D., Suski, C.D., Weber, J.-M., Danylchuk, A.J., Cooke, S.J., 2022. Are we any closer to understanding why fish can die after severe exercise? Fish Fish. 6, 1400–1417.
Hvas, M., 2022. Swimming energetics of Atlantic salmon in relation to extended fasting at different temperatures. Conservation Physiology 10 (1), coac037.
Hvas, M., Oppedal, F., 2017. Sustained swimming capacity of Atlantic salmon. Aquaculture Environment Interactions 9, 361–369.
Hvas, M., Oppedal, F., 2019a. Influence of experimental set-up and methodology for measurements of metabolic rates and critical swimming speed in Atlantic salmon *Salmo salar*. J. Fish. Biol. 95, 893–902.
Hvas, M., Oppedal, F., 2019b. Physiological responses of farmed Atlantic salmon and two cohabitant species of cleaner fish to progressive hypoxia. Aquaculture 512, 734353.
Hvas, M., Folkedal, O., Imsland, A., Oppedal, F., 2017. The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon Salmo salar. J. Exp. Biol. 220, 2757–2764.
Hvas, M., Folkedal, O., Imsland, A., Oppedal, F., 2018. Metabolic rates, swimming capabilities, thermal niche and stress response of the lumpfish, *Cyclopterus lumpus*. Biology Open 7 (9), bio036079.
Hvas, M., Folkedal, O., Oppedal, F., 2021. Heart rates of Atlantic salmon *Salmo salar* during a critical swim speed test and subsequent recovery. J. Fish. Biol. 98 (1), 102–111.
Hyndman, C.A., Kieffer, J.D., Benfey, T.J., 2003. Physiology and survival of triploid brook trout following exhaustive exercise in warm water. Aquaculture 221 (1), 629–643.
Ignatz, E.H., Sandrelli, R.M., Vadboncoeur, É., Zanuzzo, F.S., Perry, G.M.K., Rise, M.L., Gamperl, A.K., 2023. The Atlantic salmon's (*Salmo salar*) incremental thermal maximum is a more relevant and sensitive indicator of family-based differences in upper temperature tolerance than its critical thermal maximum. Aquaculture, 739628.
Iversen, M., Finstad, B., McKinley, R.S., Eliassen, R., Carlsen, K.T., Evjen, T., 2005. Stress responses in Atlantic salmon (*Salmo salar* L.) smolts during commercial well boat transports, and effects on survival after transfer to sea. Aquaculture 243, 373–382.
Johansson, D., Ruohonen, K., Juell, J.-E., Oppedal, F., 2009. Swimming depth and thermal history of individual Atlantic salmon (*Salmo salar* L.) in production cages under different ambient temperature conditions. Aquaculture 290, 296–303.
Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D.J., Lefevre, S., Nilsson, G.E., Metcalfe, N.B., Hickey, A.J.R., Brijs, J., Speers-Roesch, B., Roche, D.G., Gamperl, A.K., Raby, G.D., Morgan, R., Esbaugh, A.J., Gräns, A., Axelsson, M., Ekström, A., Sandblom, E., Binning, S.A., Hicks, J.W., Seebacher, F., Jørgensen, C., Killen, S.S., Schulte, P.M., Clark, T.D., 2018. Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. J. Exp. Biol. 221, jeb169615.
Keen, A.N., Mackrill, J.J., Gardner, P., Shiels, H.A., 2021. Compliance of the fish outflow tract is altered by thermal acclimation through connective tissue remodeling. J. R. Soc. Interface 184, 10492.
Killen, S.S., Costa, I., Brown, J.A., Gamperl, A.K., 2007. Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. Proc. R. Soc. A B. 274, 431–438.
Kraskura, K., Hardison, E.A., Little, A.G., Dressler, T., Prystay, T.S., Hendriks, B., Farrell, A.P., Cooke, S.J., Patterson, D.A., Hinch, S.G., Eliason, E.J., 2021. Sex-specific differences in swimming, aerobic metabolism and recovery from exercise in adult coho salmon (*Oncorhynchus kisutch*) across ecologically relevant temperatures. Conservation Physiology 9, coab016.
Krkošek, M., Ford, J.S., Morton, A., Lele, S., Myers, R.A., Lewis, M.A., 2007. Declining wild salmon populations in relation to parasites from farm salmon. Science 318, 1772–1775.
Kullgren, A., Jutfelt, F., Fontanillas, R., Sundell, K., Samuelsson, L., Wiklander, K., Kling, P., Koppe, W., Larson, D.G.J., Björnsson, B.T., et al., 2013. The impact of temperature on the metabolome and endocrine metabolic signals in Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. A 164, 44–53.
Lacroix, G.L., 2013. Population-specific ranges of oceanic migration for adult Atlantic salmon (*Salmo salar*) documented using pop-up satellite archival tags. Can. J. Fish. Aquat. Sci. 70, 1011–1030.
Laporte, M., Dalziel, A.C., Martin, N., Bernatchez, L., 2016. Adaptation and acclimation of traits associated with swimming capacity in Lake Whitefish (*Coregonus clupeaformis*) ecotypes. BMC Evol. Biol. 16 (1), 160.
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, cow009.
Lefevre, S., McKenzie, D.J., Nilsson, G.E., 2017. Models projecting the fate of fish populations under climate change need to be based on valid physiological mechanisms. Glob. Change Biol. 23, 3449–3459.
Leonard, J.N., Skov, P.V., 2022. Capacity for thermal adaptation in Nile tilapia (*Oreochromis niloticus*): effects on oxygen uptake and ventilation. J. Therm. Biol. 105, 103206.
Leonard, K.C., Worden, N., Boettcher, M.L., Dickinson, E., Hartstone-Rose, A., 2021. Effects of long-term ethanol storage on muscle architecture. Anat. Rec. 305, 184–198.
McArley, T.J., Morgenroth, D., Zena, L.A., Ekström, A.T., Sandblom, E., 2022. Experimental hyperoxia (O_2 supersaturation) reveals a gill diffusion limitation of maximum aerobic performance in fish. Biol. Lett. 18, 20220401.
Nilsson, G.E., Dymowska, A., Stecyk, J.A.W., 2012. New insights into the plasticity of gill structure. Respir. Physiol. Neurobiol. 184, 214–222.
Norin, T., Clark, T.D., 2016. Measurement and relevance of maximum metabolic rate in fishes. J. Fish. Biol. 88, 122–151.
Norin, T., Malte, H., Clark, T.D., 2014. Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. J. Exp. Biol. 217, 244–251.
Oldham, T., Nowak, B., Hvas, M., Oppedal, F., 2019. Metabolic and functional impacts of hypoxia vary with size in Atlantic salmon. Comp. Biochem. Physiol. A 231, 30–38.
Overton, K., Dempster, T., Oppedal, F., Kristiansen, T., Gismervik, K., Stien, L., 2019. Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review. Rev. Aquacult. 11, 1398–1417.
Paschke, K., Aguero, J., Gebauer, P., Diaz, F., Mascaro, M., Lopez-Ripoll, E., Re, D., Caamal-Monsreal, C., Tremblay, N., Pörtner, H.-O., Rosas, C., 2018. Comparison of aerobic scope for metabolic activity in aquatic ectotherms with temperature related metabolic stimulation: a novel approach for aerobic power budget. Front. Physiol. 9, 1438.
Pauly, D., 2021. The gill-oxygen limitation theory (GOLT) and its critics. Sci. Adv. 7, eabc6050.
Pauly, D., Cheung, W.W.L., 2018. Sound physiological knowledge and principles in modeling shrinking of fishes under climate change. Glob. Change Biol. 24, e15–e26.
Pollock, K.H., Pine, W.E., 2007. The design and analysis of field studies to estimate catch-and-release mortality. Fish. Manag. Ecol. 14, 1–8.
Poppe, T.T., Johansen, R., Gunnes, G., Torud, B., 2003. Heart morphology in wild and farmed Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss*. Dis. Aquat. Org. 57, 103–108.
Pörtner, H.O., Farrell, A.P., 2008. Physiology and climate change. Science 322, 690–692.
Pörtner, H.-O., Bock, C., Mark, F.C., 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. J. Exp. Biol. 220, 2685–2696.
Reddin, D.G., 1985. Atlantic salmon (*Salmo salar*) on and east of the grand bank. J. Northwest Atl. Fish. Sci. 6, 157–164.
Reemeyer, J.E., Rees, B.B., 2019. Standardizing the determination and interpretation of P_{crit} in fishes. J. Exp. Biol. 222, jeb210633.
Remen, M., Sievers, M., Torgersen, T., Oppedal, F., 2016. The oxygen threshold for maximal feed intake of Atlantic salmon post-smolts is highly temperature-dependent. Aquaculture 464, 582–592.
Sanchez-Quintana, D., Garcia-Martinez, V., Climent, V., Hurle, J.M., 1995. Morphological analysis of the fish heart ventricle: myocardial and connective tissue architecture in teleost species. Ann. Anat. 177, 267–274.
Scheuffele, H., Jutfelt, F., Clark, T.D., 2021. Investigating the gill-oxygen limitation hypothesis in fishes: intraspecific scaling relationships of metabolic rate and gill surface area. Conservation Physiology 9, coab040.
Sheridan, J.A., Bickford, D., 2011. Shrinking body size as an ecological response to climate change. Nat. Clim. Change 1, 401–406.
Skeeles, M.R., Clark, T.D., 2024. Fish gill surface area can keep pace with metabolic oxygen requirements across body mass and temperature. Funct. Ecol. 38 (4), 755–764.
Smukall, M.J., Shaw, A., Behringer, D.C., 2019. Effect of simulated catch-and-release angling on postrelease mortality and egg viability in sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 76 (12), 2390–2395.
Sommerset, et al., 2024. Fiskehelserapporten 2023. Veterinærinstituttets rapportserie nr 8a (In Norwegian) 2024.
Tirsgaard, B., Behrens, J.W., Steffensen, J.F., 2015. The effect of temperature and body size on metabolic scope of activity in juvenile Atlantic cod Gadus morhua. Comp. Biochem. Physiol. A 179, 89–94. https://pubmed.ncbi.nlm.nih.gov/25281351/.
Turunen, T., Käkelä, A., Hyvärinen, H., 1994. Trawling stress and mortality in undersized (< 40 cm) brown trout (*Salmo trutta* L). Fish. Res. 19, 51–64.
Valiente, A.G., Juanes, F., Garcia-Vazquez, E.G., 2011. Increasing regional temperatures associated with delays in Atlantic salmon sea-run timing at the southern edge of the European distribution. Trans. Am. Fish. Soc. 140, 367–373.
Vindas, M.A., Engdal, V.A., Kavaliauskiene, S., Folkedal, O., Höglund, E., Moyano, M., Øverli, Ø., Frisk, M., Johansen, I.B., 2024. Importance of environmental signals for

proper cardiac morphological development in Atlantic salmon. J. Exp. Biol. 227 (20), jeb247557.
Vollset, K.W., Lennox, R.J., Davidsen, J.G., Eldøy, S.H., Isaksen, T.E., Madhun, A., Karlsson, S., Miller, K.M., Pernet, F., 2021. Wild salmonids are running the gauntlet of pathogens and climate as fish farms expand northwards. ICES J. Mar. Sci. 78, 388–401.
Wade, N.M., Clark, T.D., Maynard, B.T., Atherton, S., Wilkinson, R.J., Smullen, R.P., Taylor, R.S., 2019. Effects of an unprecedented summer heatwave on the growth performance, flesh colour and plasma biochemistry of marine cage-farmed Atlantic salmon (*Salmo salar*). J. Therm. Biol. 80, 64–74.
Wood, C.M., Turner, J.D., Graham, M.S., 1983. Why do fish die after severe exercise? J. Fish. Biol. 22, 189–201.
Yuen, J.W., Dempster, T., Oppedal, F., Hvas, M., 2019. Physiological performance of ballan wrasse (*Labrus bergylta*) at different temperatures and its implication for cleaner fish usage in salmon aquaculture. Biol. Control 135, 117–123.