Aerobic scope is sustained through a heatwave in juvenile Atlantic salmon

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Lay Summary

In juvenile Atlantic salmon, standard metabolic rate increases but there is no difference in maximum metabolic rate and aerobic scope with increasing temperatures. Considering the increased metabolic costs of activities at higher temperatures, our results show that juvenile salmon are vulnerable to deteriorating performance at temperatures commonly experienced in present-day heatwaves.

Abstract

Aquatic ectotherms are vulnerable to heatwave-induced physiological stress, which arises from increased energy demands and reduced dissolved oxygen content in warmer waters. Understanding thermal physiology is critical for predicting how commercially and ecologically important populations could be affected by the increasing risk of rising temperatures. Heatwave risk assessments often examine extremities of time scales: immediate impacts or long-term consequences. However, little is known about how consistently increasing mid-term thermal stress shapes aerobic performance in commercially important species such as Atlantic salmon (*Salmo salar*), which may face heat stress in rivers, especially at the juvenile life stage. By measuring how salmon juveniles manage their aerobic capacity at 16, 19 and 22°C using intermittent respirometry, we test if their thermal performance curve shows a decline at temperatures commonly occurring during heatwaves. Whole-animal metabolism was measured from control individuals kept at 16 °C before and after the heatwave, and after 4-5 days exposure at 19 and 22°C during the heatwave. We show that standard metabolic rate increases with temperature, but maximum metabolic rate and aerobic scope do not change between these temperatures. These findings suggest that juvenile Atlantic salmon may have limited capacity to increase aerobic performance during moderate heatwaves, leaving them vulnerable to cumulative effects of oxygen limitation to vital functions such as growth and stress responses. As climate change intensifies, incorporating thermal performance curves into conservation strategies can be utilized for predicting population resilience and informing effective management.

Keywords

Respirometry, thermal performance, ecophysiology, energetics

Introduction

With climate change increasing the severity and frequency of heatwaves (IPCC, 2023), organisms may experience physiological thermal stress and even mortality due to extreme temperature fluctuations. Understanding organisms' thermal physiology is therefore critically important for conservation (Clark et al., 2013; Deutsch et al., 2008). Heatwaves frequently surpass the temperature range that species have been adapted to, often cumulatively and repeatedly, imposing selection on thermal performance. This is especially true for ectotherms, whose internal temperature are regulated by the surrounding environment. Some species may demonstrate adaptive or resilient responses to heatwaves including behavioural adjustments, physiological acclimation, or shifts in geographical ranges (Breedveld et al., 2023; Burraco et al., 2020; Jørgensen et al., 2022). However, these responses may not always be sufficient to counteract the negative consequences of extreme weather fluctuation (Perry et al., 2005; Pörtner & Farrell, 2008). Most physiological processes in ectotherms, including development, reproduction, and metabolism, are temperature-dependent (Angilletta et al., 2010; Hochachka & Somero, 1973; Schulte, 2015; Seebacher, 2009). These processes can be described using thermal performance curves (TPCs), which illustrate how a biological rate, such as oxygen consumption or activity per unit time, changes with temperature (Schulte et al., 2011).

TPCs can be estimated using thermal windows, critical temperatures, and preferred temperature ranges (Jørgensen et al., 2021; Ørsted et al., 2022; Peralta-Maraver & Rezende, 2021). TPCs can be used to compare inter- and intraspecies responses to anticipated temperatures under a future climate and how this might vary across life stages (Lefevre et al., 2021; Seebacher & Little, 2021). In water-breathing aquatic ectotherms, such as fishes, TPCs linked to demand, uptake and delivery of oxygen

are particularly important because of the decreasing solubility but increasing oxygen consumption in warmer water (Little et al., 2020; Little & Seebacher, 2021; Seebacher & Little, 2021; Verberk et al., 2022). Consequently, TPCs related to metabolic rates and aerobic performance are pivotal to understanding the effects of heatwaves on fishes.

Metabolic rate is a fundamental aspect of fish physiology, reflecting the energy expenditure associated with basic life processes such as respiration, digestion, and movement (Brett, 1964; Fry et al., 1947). Standard metabolic rate (SMR) of an animal represents the baseline rate of aerobic metabolism required to sustain life in a post-absortive, resting state, while the maximum capacity for aerobic performance is set by the maximum metabolic rate (MMR) (Sandblom et al., 2016). The difference between SMR and MMR represents the aerobic scope (AS) of an animal and theoretically determines the capacity of aerobic metabolism to support key life-history attributes such as activity, growth and reproduction, each of which has a specific oxygen cost (Fry, 1971; Fry et al., 1947). Therefore, reductions in aerobic scope can impair physical performance (Johansen & Jones, 2011; Pörtner & Farrell, 2008; Priede, 1985). Given that the SMR of ectotherms rises with temperature and often no such increase is detected in MMR (Fry et al., 1947; Norin et al., 2014; Sandblom et al., 2016), increases in temperature beyond optimum can result in a decline in AS, as depicted by the TPC (Farrell, 2016; Fry, 1971; Lefevre et al., 2021). Increased temperature, and decreasing AS, potentially causes declining growth, health, or survival of individuals (Alfonso et al., 2021; Jutfelt et al., 2021; Navarro et al., 2019; Sadoul & Vijayan, 2016). Specifically in salmonids, which include economically important, cold-water adapted species, reduction of AS has already been linked to reduced growth, survival, or collapse of aerobic capability during heatwaves (Eliason, Wilson, et al., 2013; Pörtner & Farrell, 2008; Wade et al., 2019).

The Atlantic salmon (*Salmo salar* L.) is faced with supra-optimal temperatures especially at riverine life stages, including juveniles (parr) and adults during their spawning migration, with reports of present-day river temperatures up to 28 °C (O'Sullivan et al., 2023; Strøm et al., 2019). Juvenile Atlantic salmon rear for one to five years in freshwater before migrating to the sea (Aas et al., 2010), with potential to encounter multiple heatwaves. These can have direct effects on survival, but also cascading effects on population dynamics through reduced growth rates. Faster freshwater growth in salmon is linked to higher survival and faster maturation at sea (Hutchings & Jones, 1998; Simpson, 1992; Thorpe, 2007). In some populations, a negative association between freshwater growth and river temperature has been detected (Alioravainen et al., 2023). For the majority of their life cycle at sea, Atlantic salmon will experience temperatures below 8°C (Jensen et al., 2014; Lacroix, 2013; Reddin, 1985). Atlantic salmon tend to avoid temperatures above 15°C (Fisher & Elson, 1950; Johansson et al., 2009; Lacroix, 2013), and their optimal temperature range for feeding and normal behaviour in the wild has been suggested to be 6–20°C, with peak growth rates occurring at 16–17°C in aquaculture conditions (Dwyer & Piper, 1987; Elliott, 1982). Previous work assessing thermal performance in Atlantic salmon has often focussed on upper limits during acute exposure (Desforges et al., 2023 and papers within), or long-term stable temperature increases (Anttila et al., 2014; Del Rio et al., 2021; Hvas et al., 2017). When aerobic scope has been measured in a heatwave-context in a related species, (Casselman et al., 2012) found AS peaked at 17 °C, and decreased until testing finished at 21 °C in juvenile coho salmon *(Oncorhynchus kisutch*). In post-smolt Atlantic salmon, AS tended to increase (a non-significant effect) as temperature increased from 13 °C to 23 °C, but swimming ability and feeding rate were greatly decreased and mortality increased, when metabolic rates of salmon were tested in groups without a cumulative exposure (Hvas et al., 2017). Further,

thermal variability in acclimation regime had no effect on salmon AS compared to a stable acclimation when metabolic rates were tested acutely at the same temperature (Morissette et al., 2021). Still, information on the cumulative effects of thermal fluctuations over several days or weeks, which better reflect natural heatwave patterns, are limited (Morash et al., 2021; Nuic et al., 2024). This information is pivotal for the conservation of Atlantic salmon. The diminishing of salmon populations has already reduced opportunities for commercial and recreational fishery, cultural traditions, and increased the risk of local extinctions (ICES, 2024, Dadswell et al., 2022)

Here, we test how mid-term increasing temperatures that are prevalent in rivers during heatwaves impact metabolic rates and AS in hatchery-reared juvenile Atlantic salmon. We hypothesise that SMR will increase as temperature rises. We expect MMR to show little variation between temperatures, and therefore a decrease in AS as temperature increases. These findings will provide insight into the physiological constraints juvenile salmon may face during heatwaves, using an ecologically relevant temperature regime.

Methods

Fish Husbandry

The experiment was performed with a permit granted by the Finnish Project Authorisation Board (no. ESAVI/16748/2023). Families of Atlantic salmon were bred using a hatchery broodstock originating from River Iijoki, maintained by Natural Resources Institute of Finland (Luke), in Taivalkoski, Finland. The broodstock was established in 2012 from parents that were originally stocked into the river as juveniles/smolts, and therefore had completed half of their life cycle in the wild. Individuals from the

124 broodstock were crossed to make full-sib families of offspring in October 2022. The embryos were
125 incubated in Taivalkoski hatchery in family-specific flow-through trays at natural temperatures of
126 incoming water from River Ohtaoja, and dead embryos were removed regularly.

127 Embryos were disinfected by iodine bath (Buffodine) on 9th March 2023, and transported to a LUKE
128 hatchery in Laukaa, Finland, where experiments were conducted. Survival between transportation
129 and start of the experiment was 22%. Excess mortality may have been caused by the closeness to the
130 embryo's hatching date and transporting to the fish farm (no disease was observed in the offspring).
131 Embryos from three unrelated families were incubated and reared in separate circular tanks
132 (diameter 80 cm) and supplied with a continuous flow of filtered water from a mix of local lake
133 Peurunkajärvi and river Peurunkajoki. Feeding of alevins was started with powdered feed (BioMar
134 Group) when most of the egg yolk had been consumed at approximately 25 May, 2023. Tanks were
135 cleaned by scrubbing surfaces and siphoning excess food twice a week until 10 June, 2023, and then
136 daily until the end of the experiment. Feed rations were calculated from growth predictions assuming
137 feed conversion efficiency of 0.8 (Elliott & Hurley, 1997), and adjusted such that a small amount of
138 feed was left uneaten. Fish were fed using 12h belt feeders with the daily ration, except for one day
139 preceding metabolic measurements, when fish were fasted. During the summer and the experiment,
140 fish were reared under constant light. Rearing temperature raised gradually with natural water
141 temperatures over approximately three months until the experiment was started; temperature varied
142 from 9.2 to 15.7 °C between first feeding and experimental period.

143 At the start of the experiment 27 July, 2023, families were divided into two groups, control and
144 heatwave, with approximately same number of individuals in each group within a family (N = 24 to 62

145 within family /tank). Fish were kept in three tanks each for control and heatwave groups. Densities
146 within families were kept similar between control and heatwave tanks throughout the experiment by
147 simultaneously removing fish from control tanks to match densities in of heatwave tanks. Control
148 tanks were kept at close to 16 °C (mean = 16.1, SD = 0.5) throughout the experimental period by
149 regulating the incoming water flows from the river and the lake with a natural temperature
150 difference. Temperatures in one control tank (the same water source used in all three tanks) and the
151 three heatwave tanks were measured every 30 min with Hobo 64K Pendant temperature loggers
152 (Hobo, Bourne, MA, USA).

153 Temperatures of the heatwave tanks were regulated via a heating tank to offset the colder
154 temperatures of the room and incoming water using a temperature controller (TS1000, H-Tronic
155 GmbH, Germany) with a temperature probe PT-1000 located in each tank, and an Eheim 600 or 300
156 pump (Eheim, Deizisau, Germany) depending on the distance from the heating tank. The pumps
157 circulated water through stainless steel coils in the heating tank, in which temperature fluctuated
158 between 30-35 °C. Heatwave tank water was aerated constantly to prevent declines in dissolved O_2
159 related to increasing temperatures, as the flow-through rate of fresh water to the tanks was reduced
160 (approx. 1 L min^{-1}) during the heatwave to maintain temperatures.

161

162 *Experimental Heatwave*

163 Fish in the heatwave treatment were exposed to three test temperatures: 16 °C, 19 °C, and 22 °C
164 (Figure 1). First, 16 °C was maintained as a control temperature for eight days, after which 3-8
165 individuals from each family were randomly picked for measurements of metabolic rate (similar

sample size across temperatures within a family), and moved into a fasting tank, where they were unfed until the measurements started the following day. Temperature of the fasting tank was the same as the heatwave tanks. After the measurement of MMR and SMR at 16 °C, temperature was increased by one degree per day for three days, and maintained for 4-5 days, after which metabolic rate measurements were taken from a new set of fish at 19 °C. This was repeated and metabolic rate measurements were taken at 22°C. Due to a malfunctioning temperature controller at 19 °C (after moving fish to acclimation for B3 and B4), there was an increase in temperature in one of the heatwave tanks to 21.5 °C (Figure 1). Metabolic rate experiments were conducted once per individual, and fish were euthanised after the SMR measurement.

To measure MMR, fish were transferred from the fasting tank into 50 L buckets filled with 5 cm of water at the testing temperature using plastic cups of water to reduce air exposure. Fish were encouraged to swim by hand around the circular container (Prokkola et al., 2022; Raby et al., 2020). Chasing lasted 2 min, and all fish were fatigued by the end. The fish were then rapidly transferred to respirometry chambers using plastic cups with water. Two people chased one fish each at once, and each batch of max. 16 individuals was processed within 2 h. Fish were left in respirometry chambers overnight in order to capture the MMR and SMR for each fish, as per (Chabot, McKenzie, et al., 2016). Chambers were flushed with fresh water from the tank for 5 min every 15 min, allowing fully aerated water to enter the chamber. The lowest recorded oxygen level in any chamber was 6.7 mg L^{-1} at 22 °C. After SMR measurements, fish were euthanised using an overdose of MS-222, measured, and weighed.

186 Immediately before and after metabolic measurements, background respiration was measured in
187 empty chambers for three measurement cycles (5 min flush, 15 min measurement) and an average of
188 these slopes was used to adjust the data for MMR and SMR. For those trials which took place
189 immediately after the previous, the post-trial background data of the previous trial was used as the
190 pre-trial background values. To prevent bacterial build-up, the system was bleached between
191 temperature changes, and all chamber parts scrubbed to limit bacterial growth and rinsed thoroughly.
192 Additionally, the water in the respirometry tank was continuously circulated through a UV filter.

193 A total of 120 fish were subjected to intermittent flow respirometry after a 24h fasting period to
194 provide estimates of metabolic rate (SMR, MMR, AS see table S1) (Killen et al., 2021; Svendsen et al.,
195 2016). Due to death in respirometer chambers, exclusion due to outliers, incomplete data recording,
196 and due to computer failure, 72 fish were included in the analysis (Table S2).

197

198 *Respirometer design*

199 For a summary of the respirometry design and measurement protocol, see Supplementary Table 1,
200 after (Killen et al., 2021). A 16-chamber intermittent flow respirometer, each chamber containing an
201 individual fish, was submerged in a temperature-regulated water bath (16, 19 & 22 ± 0.1 °C; 200 L) that
202 was saturated with air. The oxygen content of the water in the chambers was measured every two
203 seconds using a 4-channel fibre optic oxygen meter with associated oxygen sensors and software
204 (FireStingO2; PyroScience GmbH, Aachen, Germany). All 16 O_2 sensors were calibrated for 0% oxygen
205 saturation simultaneously with O_2-free water, made using sodium sulphate, and simultaneous for 100%
206 oxygen saturation with air-saturated water using an air stone before the first measurements at each

207 temperature. Temperature-compensation for O_2 saturation was based on PT100 temperature sensors,
208 which were placed in the middle of the respirometer tank and connected to each of the Firesting
209 meters. The chambers were shielded from disturbance and light using an opaque plastic cover during
210 the measurement of SMR. Flush and recirculation pumps were controlled using PumpResp controllers
211 (4-channel model, FishResp, Finland, https://github.com/embedded-sergey/PumpResp, (2024)). The
212 temperature of the respirometer tank was maintained using a temperature controller (as in the
213 heatwave tanks) and a reservoir connected to a TECO 2000 Chiller/heater. The reservoir received
214 constant inflow of water (approx. 1 L min^{-1}) from the same source as the rearing tanks to maintain good
215 water quality. Chambers for the respirometer were made using glass tubing (120 mm length, inner
216 diameter 38 mm, wall thickness 3.2 mm) and plastic caps that were 3D-printed on PA2200 polyamide.
217 The 'HeiBer'-caps were designed by Heidrikur Bergsson, University of Copenhagen
218 (https://zenodo.org/record/4062429#.YMSW7h1RVTY). Volume of chambers and tubing was 131.86 ±
219 1 mL (the exact volumes per chamber are reported in an online data repository, see Data Availability).
220 Water inside the chambers was mixed by a plastic disk attached to each cap to distribute the flow. The
221 disks were 3D printed using the same method as the respirometry caps and attached with stainless
222 steel screws. Caps were sealed using rubber O-rings and connected to the flush and recirculation pumps
223 and valves with gas-impermeable Tygon tubing (Tygon S3 E-3603, Satint-Gobain, Paris. France). The
224 recirculation system was confirmed to be waterproof by filling with water and plugging the flush pump
225 and probe connections. Water was recirculated using one submersible pump (12V DC, 6W 2 L min^{-1}
226 pump, Qingdao Xinhui Hardware Machinery Co., Ltd., Qingdao) per channel within the recirculation
227 loop. Chambers were flushed using a second pump (same as previous), using an inflow of water from
228 the tank into the chambers, and excess water was flushed through the chamber to a flush pipe which

229 was placed with the end above the water surface. This allowed fully aerated water to enter the
230 chamber. Flow rate through both the flush line and recirculation loop could be controlled by the valves
231 attached within the flow. Flow rate of the respirometers was approximately 0.1 (± 0.008) L min^{-1}
232 without fish. Information about time and pump phase (either flush or measurement) was recorded by
233 the PumpResp controllers and stored on a computer. Flow had no apparent effect on movement of fish
234 within the chambers in SMR or MMR measurements.

235 *Data Analysis*

236 All data and statistical analyses were done in R environment (R Core Team ,2022). The FishResp
237 package (Morozov et al., 2019) was used to filter and calculate metabolic rate estimates. Slopes of
238 oxygen consumption were adjusted for bacterial oxygen consumption using pre-and post-background
239 measurements, assuming a linear change. For SMR, oxygen consumption (mg O_2 h^{-1}) for each
240 measurement phase was derived from the slope of linear regression of dissolved oxygen
241 concentration over time. The SMR slopes were quality filtered and smoothed before analysis to
242 exclude non-linear declines of oxygen: first, slopes were filtered based on $R^2 > 0.95$, then slopes that
243 did not meet the R^2 criteria were smoothed using a running mean of 29 seconds (Chabot et al., 2021),
244 then R^2 filter was re-applied, and finally, linearity was checked visually from all slopes that met the
245 criteria of $R^2 > 0.95$. The first and last 60 seconds of measurement periods were excluded to account
246 for mixing of water and changes in flow due to flushing. The mean of the lowest normal distribution
247 (MLND) was used to estimate SMR for each individual from the extracted slopes (Chabot, Steffensen,
248 et al., 2016). Slopes for MMR were extracted using a derivative of a polynomial curve fitted on each
249 measurement (function *smooth.spline,* d.f. = 10) as in the *spline-MMR* method (Prokkola et al., 2022),
250 i.e. from the beginning of the MMR measurement, when respiration was the highest. The slopes were

251 then used to calculate MMR (mg O_2 h^{-1}) using *FishResp*. AS was calculated as the difference between
252 the absolute MMR and SMR.

253 A linear mixed effect model was fitted (estimated using restricted maximum likelihood and *nloptwrap*
254 optimizer) to predict SMR, MMR and a non-linear mixed effect model was fitted to predict AS, all of
255 which were log transformed. Temperature and log-transformed mass as fixed effects (formula:
256 Metabolic Measurement ~ Temperature + Mass). The model included batch as a random effect
257 (formula:~1|Batch) (Zuur et al., 2009). Homoscedasticity and normality of residuals were assessed by
258 visual inspection of residual plots. 95% Confidence Intervals (CIs) and p-values were computed using a
259 Wald t-distribution approximation. Post-hoc comparisons of temperatures were performed using
260 Tukey method of comparing estimates, and P value < 0.05 was considered significant. Statistical
261 models were fitted using *nlme*, *lme4* and *lmerTest* packages in R, and post-hoc analysis was
262 performed in *emmeans* package (Bates et al., 2015; Kuznetsova et al., 2017; Lenth, 2017; Pinheiro et
263 al., 1999). For visual representation, SMR, MMR, and AS data were mass-adjusted to account for the
264 hypo-allometric scaling of metabolic rates by linear regression of log_{10}-transformed metabolic rates
265 against log_{10}-transformed body mass (Figure 2). Finally, Pearson's correlations were calculated among
266 the either absolute or mass-adjusted metabolic variables.

267

268 **Results**

269 In total, the data of 72 fish was used at three temperatures across four consecutive weeks: 16°C,
270 19°C, and 22°C and 16 °C again (Table 1, Table S3). The metabolic rates and AS scaled positively with
271 body mass, with scaling exponents from 0.86 to 1.1 (Figure 2, Table 2, Table S4).

272 We found temperature to be a significant predictor of SMR (16–19 °C: $t(66) = 4.80$, $P < .001$; 16–22 °C:
273 $t(66) = 7.43$, $P < .001$, Table 3). There were significant differences in SMR between all temperature
274 groups, except between 19 °C and 22 °C (Figure 3, Table 4). There was a 57% increase in predicted
275 SMR for 6 °C increase in temperature (Table 4). However, there was no significant change in MMR or
276 AS between temperatures (Figure 3, Table 2).

277 *Correlations among metabolic variables*

278 At all temperatures, MMR and AS were highly positively correlated ($r > 0.9$) in both raw and mass
279 adjusted data. Absolute SMR and MMR were positively correlated at 16 and 22 °C. Absolute SMR and
280 AS were positively correlated at 16 °C. However, mass adjusted SMR and AS were negatively
281 correlated at 19 and 22 °C (Table S5, Figure S1).

282 **Discussion**

283 This study demonstrates that rising temperatures lead to an increase in standard metabolic rate
284 (SMR) in juvenile Atlantic salmon. However, there was no change in maximum metabolic rate (MMR)
285 or, contrary to our hypothesis, in aerobic scope (AS) across the tested temperatures. While an
286 increase in SMR with temperature is well established in ectotherms due to the effect of temperature
287 on biochemical rates (Clark et al., 2013; McKenzie et al., 2021; Raby et al., 2016), there are more
288 mixed results on the relationship of MMR, AS and increasing temperatures in fishes.

289 The lack of differences between the MMR within temperatures in this study was not surprising, given
290 that the individuals were similar in age, with identical handling and rearing environment (Farrell et al.,
291 2009a; Killen et al., 2016). MMR represents the maximum rate at which aerobic physiological

292 processes can occur, and at 16 °C the salmon may have already reached their maximum capacity for
293 oxygen uptake and delivery (Clark et al., 2011; McKenzie & Claireaux, 2010; Norin & Clark, 2016;
294 Sandblom et al., 2016). However, there may be among-individual variation in thermal reaction norms
295 of SMR and MMR that was not possible to detect with our experimental design, which could explain
296 why AS was not decreasing even though on average MMR was stable while SMR was increasing (Norin
297 et al., 2014; Norin & Metcalfe, 2019; Réveillon et al., 2019). In gobies (*Neogobius melanostomus)*,
298 MMR increased at the very highest temperatures tested; Christensen et al. (2020) speculate that
299 increases in MMR could be due to the onset of physiologically demanding processes for coping with
300 high temperatures such as heat shock protein and enzyme production (Christensen et al., 2020;
301 Loughland & Seebacher, 2020). Other studies have shown that MMR can increase with increasing
302 temperatures (*Lates clacarifer*: Norin et al 2014; *Dicentrarchus labrax* Claireux et al. 2006)(Claireaux
303 et al., 2006; Norin et al., 2014), however these studies are based on acute temperature increases. In
304 *O. nerka* and *O. kisutch*, MMR continuously increased in acclimation until 22-25 °C, where it plateaued
305 (Eliason et al., 2011). This plateau may reflect the species' maximum aerobic capacity, similar to what
306 may be occurring in the present study.

307

308 The TPC of AS in fish has been suggested to be a bell shaped curve (Pörtner & Farrell, 2008). For
309 example, in pink salmon (*O. gorbuscha*), AS peaked at 21 °C and declined with further warming,
310 although this temperature increase occurred over a maximum of 2 days (Clark et al., 2011). Other
311 studies note a decrease in AS, which could elude to the post peak decrease of the AS curve, as these
312 are tested at higher temperatures or after longer acclimations, for example, in a recent study on
313 salmon parr, AS decreased with temperature from 15 to 21°C after a three-week acclimation (Nuic et

al., 2024). This could suggest long term exposure to a heatwave leading to aerobic collapse. In contrast in murray cod (*Maccullochella peelii peelii*) and temperatures that increased from 14 – 29 °C, where fish were acclimated for at least 18 h to each increase, resulted in a continuous increase in aerobic scope (Clark et al., 2005).

Other studies report a plateau of AS at high temperatures. For example, there were no differences in AS between acclimation temperatures in minnows (*Phoxinus phoxinus*) after eight months (Závorka et al., 2020). Morissette et al. (2021) found that below 23 °C, there was no difference between any metabolic rates after different temperature acclimations, however in their study all metabolic rates were measured at the same temperature, which complicates the interpretation of the results. In gobies acclimated for three weeks, AS was seen to plateau between 15 and 28 °C after initially increasing (Christensen et al., 2021). Further, in Atlantic salmon post-smolts, aerobic scope was increased with 4-week acclimations at temperatures from 3–13 °C, and plateaued from 13 °C up to a temperature of 23 °C. Interestingly, fish condition and swim speed dropped and mortality increased (Hvas et al., 2017). Upper thermal limit of salmon has been widely reported at 23 °C (Elliott & Elliott, 2010), and notable, often cited work (Jensen et al., 1989) found optimal salmon temperature for growth to be 16 °C. Our results agree with Hvas et al. (2017) as we found that AS plateaued at 16–22°C when measured at acclimation temperature. However, even though the expectation of reduced AS with increasing temperature was not met in this study, it is likely that oxygen supply can limit performance of salmon as temperature increases. For example, maintaining growth and other aerobic functions with increasing temperature would require an increasing AS, because of the higher costs of activities such as digestion and assimilation and swimming (Jahn & Seebacher, 2022; Jutfelt et al.,

336 2021). Also the stress-induced peak oxygen consumption increases exponentially with temperature
337 (Madaro et al., 2018). An absence of increase suggests physiological systems supporting oxygen
338 uptake such as cardiac function, gill function and haemoglobin oxygen affinity are no longer able to
339 scale with increasing thermal pressure (Anttila et al., 2014; Schulte & Healy, 2022). As AS plateaus but
340 SMR and the costs of aerobic activities continue to increase, fish might become more constrained in
341 their physiological state and experience cumulative thermal stress, leading to mortality.

342

343 AS is proposed to be of ecological significance because it defines the upper limit for oxygen allocation
344 by a fish to sustain aerobic activities such as foraging, digestion, tissue deposition, migration,
345 reproduction, and so forth (Claireaux & Lefrançois, 2007; Farrell et al., 2009b; Fry, 1971; H.-O.
346 Pörtner, 2010; Schulte, 2015). For example, reduced appetite could be behaviourally driven to avoid
347 anaerobic metabolism, as suggested by the reduction of feed intake during low AS (review by (Jutfelt,
348 2020). In support of this, reduced growth and lower condition factor at higher temperatures has
349 previously been documented in Atlantic salmon, when comparing increases to temperatures up to 19
350 °C (Hevrøy et al., 2015; Kullgren et al., 2013). By measuring AS as an indicator of thermal
351 performance, we can predict that at high temperatures, Atlantic salmon in the wild would either have
352 to seek thermal refuge, or compromise aspects of their performance in order to cope with the high
353 temperatures, which could result in poor growth or health (Koskela et al., 1997).

354

355 It is important to note that both TPCs and upper thermal limits are dependent on acclimation
356 duration and rate of warming (Currie et al., 2014; Lefevre et al., 2021; McKenzie et al., 2021). Most

research in fishes has been conducted with stable thermal profiles, whereby each individual experiences only one acclimation temperature (Hvas et al., 2017). In contrast, our study accounts for a cumulative effect of exposure to higher temperatures, using a gradual heating regime. While some acclimation is expected in the MMR over short-term temperature increases ((Norin & Clark, 2016); and papers within), each temperature exposure in this study occurred over 10 days, which may not be enough time to see notable plasticity in salmon's metabolic ceiling. Sandblom et al. (2017) found no difference in MMR or AS between acute (24 h) or chronic warming (years) regime in perch (*Perca fluviatilis*), but that all warm-acclimated fish (5 days acclimation, 23 °C) have a higher MMR than fish acclimated at 18 °C, their reference temperature. However, an ecologically relevant scenario for salmon in their environments is a heatwave lasting from a few days to few weeks, thus these time frames are the most relevant to study from a conservation perspective. By testing the cumulative effect of a heatwave mirroring the temperatures seen in the wild, we can better predict how salmon could cope in a realistic exposure to sub-lethal temperatures (Morash et al., 2018).

The general expectation is that longer acclimation with a slower increase of temperature should be beneficial to survival, giving the fish time to make compensatory physiological modifications such as displaying plasticity in SMR or MMR, enabling AS to be maintained over a broad range of temperatures. However, in barramundi (*L. calcarifer*) MMR and AS increased when exposed to acute 10 °C warming, due to a higher increase in MMR than SMR, but after 5 weeks of acclimation, AS was similar, mainly due to a reduction in MMR (Norin et al., 2014). A very similar pattern was observed in black sea bass (*Centropristis striata)* and common triplefin (*Forsterygion lapillum*), where short term

378 exposure caused a raise in AS, before it decreased after some weeks of exposure (Khan et al., 2014;
379 McArley et al., 2017; Slesinger et al., 2019). Additionally, in salmon post smolts, post-stress peak
380 cortisol levels significantly increased with higher temperatures (Madaro et al., 2018). This suggests
381 that despite a stable aerobic capacity to cope with temperature increases, costly physiological
382 mechanisms may be occurring, and therefore a plateau in AS does not necessarily suggest a broad
383 optimum temperature range.

384

385 More accurate predictions of species response to heatwaves under climate change can be achieved
386 using bioenergetics modelling rather than assuming physiological performance inferred by historical
387 distribution data (Baltar et al., 2019; Lefevre et al., 2021). For example, in sockeye salmon, the
388 decrease of AS at high temperatures was associated with a collapse in cardiac performance, and
389 applying these data to seasonal river temperatures can be used to predict population collapse
390 (Eliason, Clark, et al., 2013; Eliason et al., 2011; MacNutt et al., 2006). With detailed TPCs, thermal
391 safety margins can be defined in terms of functional success—such as swimming, feeding, and
392 reproducing—rather than mortality alone (Pinsky et al., 2019). Interestingly, (Morgan et al., 2022)
393 discuss plasticity of laboratory fish to temperatures, and show that wild fish have a different TPC to
394 multigenerational laboratory fish population. Building on this, we propose that our results could be
395 used to design more detailed studies on individual-level reaction norms and performance metrics at
396 the temperature range used in this study, which could ultimately inform both management and
397 conservation strategies.

398

399 Recent advances in conservation physiology demonstrate how individual-level physiological metrics
400 can be used to guide policy and management strategies, as shown in studies on sockeye salmon in
401 Canada (Patterson et al., 2016). Integrating TPCs and stock specific information, such as life history
402 genotypes and developmental stages, can inform stakeholders of specific vulnerabilities in
403 populations and how they vary in location and season (Ward et al., 2017). Temperature-dependent
404 fisheries management strategies have already been successfully implemented, for example, in
405 Canadian rivers, and could be adapted globally to account for population-specific TPCs (Van Leeuwen
406 et al., 2023). Management strategies, such as adjusting harvest timing and identifying thermal refuges
407 for protection and restoration efforts, could help mitigate the impact of rising temperatures on
408 vulnerable salmon populations.

409

410 In recent years, extreme heat events have had catastrophic impacts on migration success and survival
411 across species of salmonids, indicating that a better understanding of their thermal performance is
412 critical for conservation (Baisez et al., 2011; Hinch et al., 2012; Martins et al., 2011, 2012; Muñoz et
413 al., 2021). Our study showed that aerobic scope was not increased between 16 and 22°C in juvenile
414 Atlantic salmon despite a significant increase in SMR. This, combined with the increasing metabolic
415 costs of activities, such as swimming and digestion, indicate that the stable aerobic scope may be
416 leaving juvenile salmon vulnerable to deteriorating performance at temperatures commonly
417 experienced in present-day heatwaves.

418

419 **Ethical Declarations**

The experiment was performed with permit granted by the Finnish Project Authorisation Board no. ESAVI/16748/2023.

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Author Contributions

LC and JP: experimental design, conducting experiment, data analysis and writing the manuscript. JP: supervision, management, and funding acquisition. SM: equipment and experimental design. MR and ES: fish care, equipment maintenance and assisting with data collection.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability

Data is hosted on Zenodo data repository (https://doi.org/10.5281/zenodo.15308028) and code for analysis is hosted on Github (https://github.com/jprokkola/Salmon-aerobic-scope-2023-public.git).

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Figures and Tables

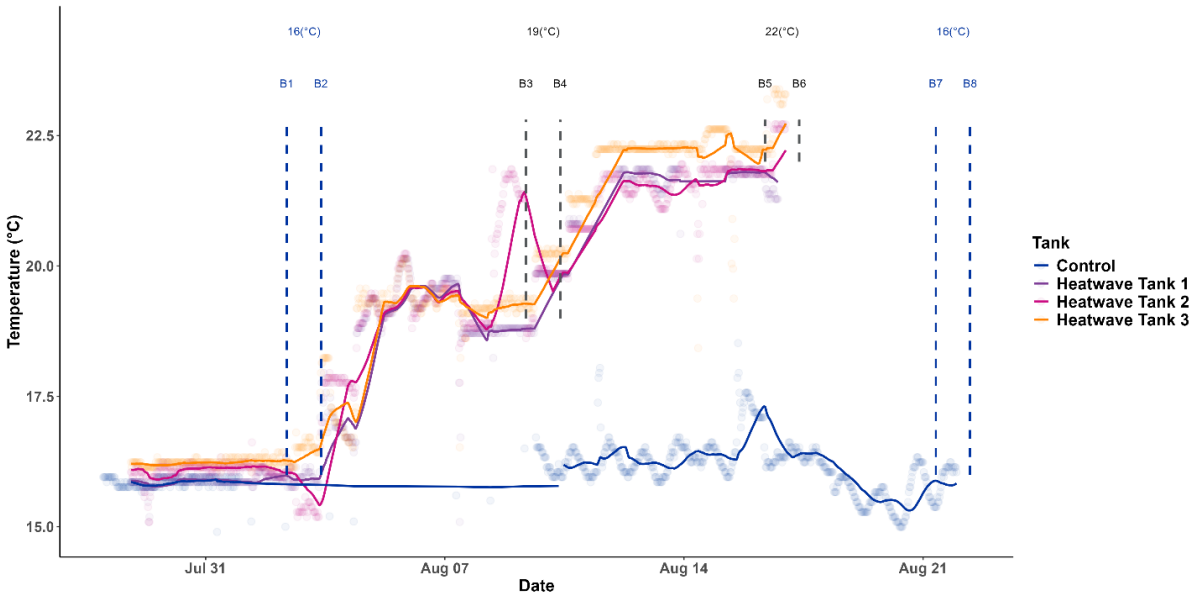

Figure 1: Outline of the experiment temperature regime. Solid lines and points represent temperatures (mean of 16, 19 and 22 °C) of the heatwave tanks (three tanks with independent temperature regulation) and control tanks (three tanks with an identical temperature, 16 °C) during the experiment. Lines fitted using a 40-point rolling average, where each point is one hour. Dashed vertical lines (B1 – B8) indicate timing of metabolic rate measurements: two batches measured each week. Fish were removed from heatwave tanks into H-Tronic controlled acclimation tank 24 hours before testing. Variation in temperature profiles at each step is related to natural mixing in the incoming lake water.

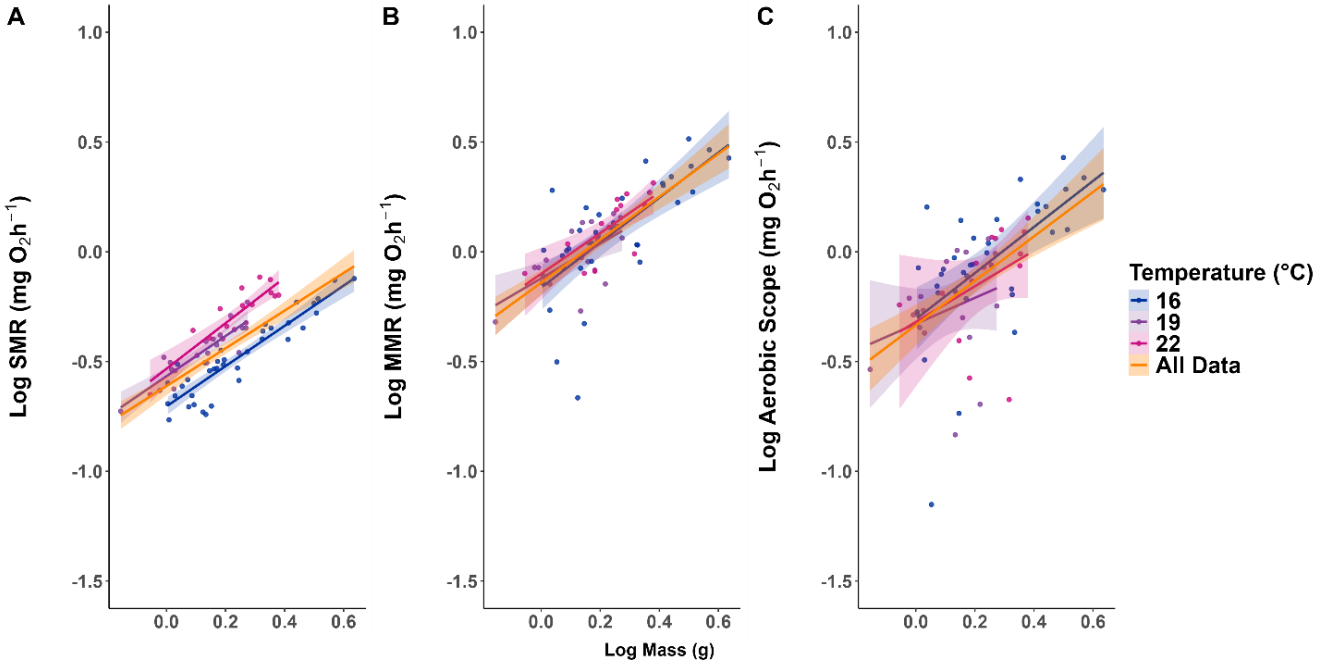

Figure 2: Scatter plots to show the relationship of log transformed metabolic rates with log transformed mass (A: Standard metabolic rate (SMR), B: Maximum Metabolic rate (MMR), C: Aerobic Scope (AS)). Each point represents individual fish, with different colours representing different tested temperatures (16 °C: Blue, 19 °C: Purple, 22 °C: Pink). Goodness of fit lines indicate linear regression and shading shows 95% confidence intervals for each temperature, with orange colour representing fit of all data.

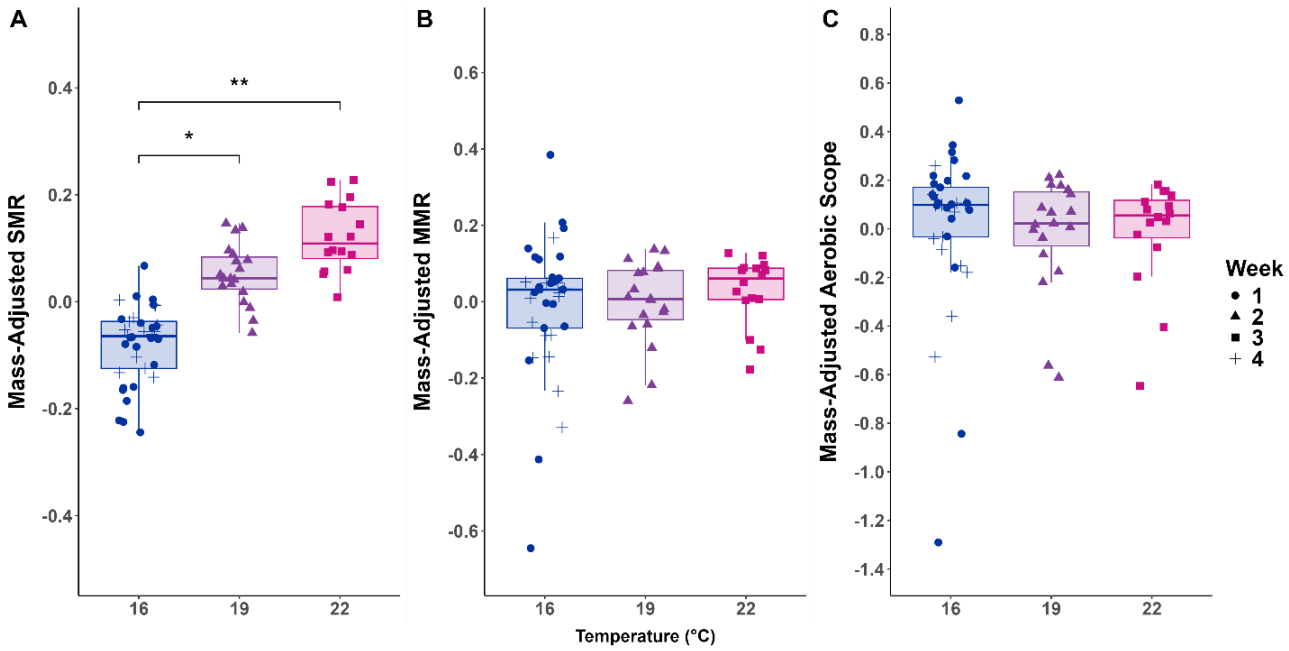

Figure 3: Boxplots to show the relationship between temperature and mass-adjusted metabolic rate measurements: SMR (a), MMR (b) and AS (c). Different point shapes indicate different weeks of measurement and different colours indicate different temperatures. Asterix and brackets show significant differences (* $p < 0.05$ and ** $p < 0.01$).

Table 1: Descriptive statistics for Mass (g) and Length (mm) for juvenile salmon tested at different temperatures. n = number of individuals, $\bar{x}$ = mean metabolic rate, σ = standard deviation, Min = minimum value, Max = maximum value.

		Mass (g)				*Length (mm)*			
Temperature (°C)	***n***	$\bar{x}$	***σ***	***Min***	***Max***	$\bar{x}$	***σ***	***Min***	***Max***
16 (week 1)	*22*	*1.42*	*0.41*	*1.01*	*2.59*	*57.64*	*5.38*	*50*	*69*
19	*19*	*1.35*	*0.35*	*0.70*	*1.88*	*56.84*	*4.71*	*47*	*64*
22	*16*	*1.79*	*0.42*	*0.88*	*2.40*	*61.00*	*4.35*	*54*	*68*
16 (week 4)	*15*	*2.56*	*0.88*	*1.40*	*4.32*	*68.53*	*8.22*	*56*	*82*
Total	*72*	*1.72*	*0.70*	*0.70*	*4.32*	*60.44*	*7.16*	*47*	*82*

Table 2: Model summaries for metabolic rate estimates, including fish body mass and temperature (16, 19 and 22 °C) as independent variables. Batch is included as random effect. Brackets indicate standard error, *asterisks* indicates significance values with $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$).

	log10(SMR)	***log10(MMR)***	***log10(AS)***
log10(mass)	0.922^{***}	0.978^{***}	1.044^{***}
	(0.057)	(0.120)	(0.229)
19 °C	0.131^{***}	0.011	-0.038
	(0.027)	(0.046)	(0.086)
22 °C	0.198^{***}	0.040	-0.033
	(0.027)	(0.046)	(0.086)
Intercept	-0.704^{***}	-0.152^{***}	-0.342^{***}
	(0.021)	(0.038)	(0.072)
Random Effects			
Intercept SD (Batch)	0.0215	0.0116	0
Residual SD	0.0638	0.1492	0.2878
Marginal R^2 / Conditional R^2	0.849 / 0.864	0.521 / 0.524	0.261 / NA

Table 3: Post-hoc ANOVA contrasts from the results of the SMR model described in Table 2, comparing the temperature treatments 16 °C, 19 °C, and 22 °C. SE shows standard error, df degrees of freedom and the associated *t* ratio and *p* (significance) with $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

Comparison	*Estimate*	*SE*	*df*	*t*	*p*
16 °C - 19 °C	-0.13	0.03	4.66	-4.71	0.014*
16 °C - 22 °C	-0.20	0.03	4.73	-7.42	0.002***
19 °C - 22 °C	-0.07	0.03	4.94	-2.13	0.178

Table 4: Table showing predicted SMR (mg O_2 h^{-1}) and 95% confidence intervals (CI) for a mean mass fish (1.72g) at different temperatures.

Temperature (°C)	*Predicted SMR (for a 1.72g fish)*	*Lower CI*	*Upper CI*
16	0.33	0.24	0.44
19	0.44	0.32	0.58
22	0.52	0.39	0.70