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 (Farrell, 2016; Fry, 1971; Lefevre et al., 2021). However, maintaining growth and other aerobic functions with increasing temperature requires an increasing AS, because of the higher costs of activities such as digestion and assimilation and swimming (Jahn & Seebacher, 2022; Jutfelt et al., 2021). When AS declines at increased temperatures, this may cause declining growth, health, or survival of individuals (Alfonso et al., 2021; Jutfelt et al., 2021; Navarro et al., 2019; Sadoul & Vijayan, 2016). Alternatively, AS can plateau at higher temperatures suggesting that oxygen supply can limit performance as temperature increases (hvas et al 2017, Závorka et al., 2020, Christensen et al 2012). As AS plateaus but SMR and the costs of aerobic activities continue to increase, fish might become more constrained in their physiological state and experience cumulative thermal stress, leading to mortality. Both declines and plateaus of AS pose significant risks for salmonids, which include economically important, cold-water adapted species. In these fish, limitations of AS has already been linked to reduced growth, survival, or collapse of aerobic capability during heatwaves (Hvas 2017 (Eliason, Wilson, et al., 2013; Pörtner & Farrell, 2008; Wade et al., 2019).

88 The Atlantic salmon (*Salmo salar* L.) is faced with supra-optimal temperatures especially at riverine life stages,
89 including juveniles (parr) and adults during their spawning migration, with reports of present-day river
90 temperatures up to 28 °C (O'Sullivan et al., 2023; Strøm et al., 2019). Juvenile Atlantic salmon rear for one to
91 five years in freshwater before migrating to the sea (Aas et al., 2010), with potential to encounter multiple
92 heatwaves. These can have direct effects on survival, but also cascading effects on population dynamics
93 through reduced growth rates. Faster freshwater growth in salmon is linked to higher survival and faster
94 maturation at sea (Hutchings & Jones, 1998; Simpson, 1992; Thorpe, 2007). In some populations, a negative
95 association between freshwater growth and river temperature has been detected (Alioravainen et al., 2023).
96 For the majority of their life cycle at sea, Atlantic salmon will experience temperatures below 8°C (Jensen et
97 al., 2014; Lacroix, 2013; Reddin, 1985). Atlantic salmon tend to avoid temperatures above 15°C (Fisher &
98 Elson, 1950; Johansson et al., 2009; Lacroix, 2013), and their optimal temperature range for feeding and
99 normal behaviour in the wild has been suggested to be 6–20°C, with peak growth rates occurring at 16–17°C in
100 aquaculture conditions (Dwyer & Piper, 1987; Elliott, 1982). Previous work assessing thermal performance in
101 Atlantic salmon has often focussed on upper limits during acute exposure of CTmax experiments under less
102 than 24 hr(Desforges et al., 2023 and papers within), or long-term stable temperature increases over several
103 months (Anttila et al., 2014; Del Rio et al., 2021; Hvas et al., 2017). When aerobic scope has been measured in
104 a heatwave-context in a related species, (Casselman et al., 2012) found AS peaked at 17 °C, and decreased
105 until testing finished at 21 °C in juvenile Coho salmon *(Oncorhynchus kisutch*). In post-smolt Atlantic salmon,
106 AS tended to increase (a non-significant effect) as temperature increased from 13 °C to 23 °C, but swimming
107 ability and feeding rate were greatly decreased and mortality increased, when metabolic rates of salmon were
108 tested in groups without a cumulative exposure (Hvas et al., 2017). Further, thermal variability in acclimation
109 regime had no effect on salmon AS compared to a stable acclimation when metabolic rates were tested
110 acutely at the same temperature (Morissette et al., 2021). Still, information on the cumulative effects of
111 thermal fluctuations over several days or weeks, which better reflect natural heatwave patterns, are limited
112 (Morash et al., 2021; Nuic et al., 2024). This information is pivotal for the conservation of Atlantic salmon. The

113 diminishing of salmon populations has already reduced opportunities for commercial and recreational fishery,
114 cultural traditions, and increased the risk of local extinctions (ICES, 2024, Dadswell et al., 2022)

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

120

121 **Methods**

122 *Fish Husbandry*

123 The experiment was performed with a permit granted by the Finnish Project Authorisation Board (no.
124 ESAVI/16748/2023). Families of Atlantic salmon were bred using a hatchery broodstock originating from
125 River Iijoki, maintained by Natural Resources Institute of Finland (Luke), in Taivalkoski, Finland. The broodstock
126 was established in 2012 from parents that were originally stocked into the river as juveniles/smolts, and
127 therefore had completed half of their life cycle in the wild. Individuals from the broodstock were crossed to
128 make full-sib families of offspring in October 2022. The embryos were incubated in Taivalkoski hatchery in
129 family-specific flow-through trays at natural temperatures of incoming water from River Ohtaoja, and dead
130 embryos were removed regularly.

131 Embryos were disinfected by iodine bath (Buffodine) on 9th March 2023, and transported to a LUKE hatchery
132 in Laukaa, Finland, where experiments were conducted. Survival between transportation and start of the
133 experiment was 22%. Excess mortality may have been caused by the closeness to the embryo's hatching date
134 and transporting to the fish farm (no disease was observed in the offspring). Embryos from three unrelated
135 families were incubated and reared in separate circular tanks (diameter 80 cm) and supplied with a continuous

136 flow of filtered water from a mix of local lake Peurunkajärvi and river Peurunkajoki. Feeding of alevins was
137 started with powdered feed (BioMar Group) when most of the egg yolk had been consumed at approximately
138 25 May, 2023. Tanks were cleaned by scrubbing surfaces and siphoning excess food twice a week until 10
139 June, 2023, and then daily until the end of the experiment. Feed rations were calculated from growth
140 predictions assuming feed conversion efficiency of 0.8 (Elliott & Hurley, 1997), and adjusted such that a small
141 amount of feed was left uneaten. Fish were fed using 12h belt feeders with the daily ration, except for one day
142 preceding metabolic measurements, when fish were fasted. During the summer and the experiment, fish were
143 reared under constant light. Rearing temperature raised gradually with natural water temperatures over
144 approximately three months until the experiment was started; temperature varied from 9.2 to 15.7 °C
145 between first feeding and experimental period.

146 At the start of the experiment 27 July, 2023, families were divided into two groups, control and heatwave,
147 with approximately same number of individuals in each group within a family (N = 24 to 62 within family
148 /tank). Fish were kept in three tanks each for control and heatwave groups. Densities within families were
149 kept similar between control and heatwave tanks throughout the experiment by simultaneously removing fish
150 from control tanks to match densities in of heatwave tanks. Control tanks were kept at close to 16 °C (mean =
151 16.1, SD = 0.5) throughout the experimental period by regulating the incoming water flows from the river and
152 the lake with a natural temperature difference. Temperatures in one control tank (the same water source
153 used in all three tanks) and the three heatwave tanks were measured every 30 min with Hobo 64K Pendant
154 temperature loggers (Hobo, Bourne, MA, USA).

155 Temperatures of the heatwave tanks were regulated via a heating tank to offset the colder temperatures of
156 the room and incoming water using a temperature controller (TS1000, H-Tronic GmbH, Germany) with a
157 temperature probe PT-1000 located in each tank, and an Eheim 600 or 300 pump (Eheim, Deizisau, Germany)
158 depending on the distance from the heating tank. The pumps circulated water through stainless steel coils in
159 the heating tank, in which temperature fluctuated between 30-35 °C. Heatwave tank water was aerated

160 constantly to prevent declines in dissolved O_2 related to increasing temperatures, as the flow-through rate of
161 fresh water to the tanks was reduced (approx. 1 L min^{-1}) during the heatwave to maintain temperatures.

162

163 *Experimental Heatwave*

164 Fish in the heatwave treatment were exposed to three test temperatures: 16 °C, 19 °C, and 22 °C (Figure 1).
165 First, 16 °C was maintained as a control temperature for eight days, after which 3-8 individuals from each
166 family were randomly picked for measurements of metabolic rate (similar sample size across temperatures
167 within a family), and moved into a fasting tank, where they were unfed until the measurements started the
168 following day. Temperature of the fasting tank was the same as the heatwave tanks. After the measurement
169 of MMR and SMR at 16 °C, temperature was increased by one degree per day for three days, and maintained
170 for 4-5 days, after which metabolic rate measurements were taken from a new set of fish at 19 °C. This was
171 repeated and metabolic rate measurements were taken at 22°C. Due to a malfunctioning temperature
172 controller at 19 °C (after moving fish to acclimation for B3 and B4), there was an increase in temperature in
173 one of the heatwave tanks to 21.5 °C (Figure 1). Metabolic rate experiments were conducted once per
174 individual, and fish were euthanised after the SMR measurement.

175 To measure MMR, fish were transferred from the fasting tank into 50 L buckets filled with 5 cm of water at the
176 testing temperature using plastic cups of water to reduce air exposure. Fish were encouraged to swim by hand
177 around the circular container (Prokkola et al., 2022; Raby et al., 2020). Chasing lasted 2 min, and all fish were
178 unresponsive to the touch of caudal fins, thus determined fatigued. The fish were then rapidly transferred to
179 respirometry chambers using plastic cups with water. Two people chased one fish each at once, and each
180 batch of max. 16 individuals was processed within 2 h. Fish were left in respirometry chambers overnight in
181 order to capture the MMR and SMR for each fish, as per (Chabot, McKenzie, et al., 2016). Chambers were
182 flushed with fresh water from the tank for 5 min every 15 min, allowing fully aerated water to enter the

183 chamber. The lowest recorded oxygen level in any chamber was 6.7 mg L^{-1} at 22 °C. After SMR
184 measurements, fish were euthanised using an overdose of MS-222, measured, and weighed.

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

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

196

197 *Respirometer design*

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

on PT100 temperature sensors, which were placed in the middle of the respirometer tank and connected to each of the Firesting meters. The chambers were shielded from disturbance and light using an opaque plastic cover during the measurement of SMR. Flush and recirculation pumps were controlled using PumpResp controllers (4-channel model, FishResp, Finland, https://github.com/embedded-sergey/PumpResp, (2024)). The temperature of the respirometer tank was maintained using a temperature controller (as in the heatwave tanks) and a reservoir connected to a TECO 2000 Chiller/heater. The reservoir received constant inflow of water (approx. 1 L min^{-1}) from the same source as the rearing tanks to maintain good water quality. Chambers for the respirometer were made using glass tubing (120 mm length, inner diameter 38 mm, wall thickness 3.2 mm) and plastic caps that were 3D-printed on PA2200 polyamide. The 'HeiBer'-caps were designed by Heidrikur Bergsson, University of Copenhagen (https://zenodo.org/record/4062429#.YMSW7h1RVTY). Volume of chambers and tubing was 131.86 ± 1 mL (the exact volumes per chamber are reported in an online data repository, see Data Availability). Water inside the chambers was mixed by a plastic disk attached to each cap to distribute the flow. The disks were 3D printed using the same method as the respirometry caps and attached with stainless steel screws. Caps were sealed using rubber O-rings and connected to the flush and recirculation pumps and valves with gas-impermeable Tygon tubing (Tygon S3 E-3603, Satint-Gobain, Paris. France). The recirculation system was confirmed to be waterproof by filling with water and plugging the flush pump and probe connections. Water was recirculated using one submersible pump (12V DC, 6W 2 L min^{-1} pump, Qingdao Xinhui Hardware Machinery Co., Ltd., Qingdao) per channel within the recirculation loop. Chambers were flushed using a second pump (same as previous), using an inflow of water from the tank into the chambers, and excess water was flushed through the chamber to a flush pipe which was placed with the end above the water surface. This allowed fully aerated water to enter the chamber. Flow rate through both the flush line and recirculation loop could be controlled by the valves attached within the flow. Flow rate of the respirometers was approximately 0.1 (± 0.008) L min^{-1} without fish. Information about time and pump phase (either flush or measurement) was recorded by the PumpResp controllers and stored on a computer. Flow had no apparent effect on movement of fish within the chambers in SMR or MMR measurements.

231 *Data Analysis*

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

248 A linear mixed effect model was fitted (estimated using restricted maximum likelihood and *nloptwrap*
249 optimizer) to predict SMR, MMR and a non-linear mixed effect model was fitted to predict AS, all of which
250 were log transformed. Temperature and log-transformed mass as fixed effects (formula: Metabolic
251 Measurement ~ Temperature + Mass). The model included batch as a random effect (formula:~1|Batch) (Zuur
252 et al., 2009). Homoscedasticity and normality of residuals were assessed by visual inspection of residual plots.
253 95% Confidence Intervals (CIs) and p-values were computed using a Wald t-distribution approximation. Post-
254 hoc comparisons of temperatures were performed using Tukey method of comparing estimates, and P value <

255 0.05 was considered significant. Statistical models were fitted using *nlme*, *lme4* and *lmerTest* packages in R,
256 and post-hoc analysis was performed in *emmeans* package (Bates et al., 2015; Kuznetsova et al., 2017; Lenth,
257 2017; Pinheiro et al., 1999). For visual representation, SMR, MMR, and AS data were mass-adjusted to account
258 for the hypo-allometric scaling of metabolic rates by linear regression of $\log_{10}$-transformed metabolic rates
259 against $\log_{10}$-transformed body mass (Figure 2). Finally, Pearson's correlations were calculated among the
260 either absolute or mass-adjusted metabolic variables.

261

262 **Results**

263 In total, the data of 72 fish was used at three temperatures across four consecutive weeks: 16°C, 19°C, and
264 22°C and 16 °C again (Table 1, Table S3). The metabolic rates and AS scaled positively with body mass, with
265 scaling exponents from 0.86 to 1.1 (Figure 2, Table 2, Table S4).

266 We found temperature to be a significant predictor of SMR (16–19 °C: $t(66) = 4.80$, $P < .001$; 16–22 °C: $t(66) =$
267 7.43, $P < .001$, Table 3). There were significant differences in SMR between all temperature groups, except
268 between 19 °C and 22 °C (Figure 3, Table 4). There was a 57% increase in predicted SMR for 6 °C increase in
269 temperature (Table 4). However, there was no significant change in MMR or AS between temperatures (Figure
270 3, Table 2).

271 *Correlations among metabolic variables*

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

276 **Discussion**

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

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

301 at 23 °C (Elliott & Elliott, 2010), and notable, often cited work (Jensen et al., 1989) found optimal salmon
302 temperature for growth to be 16 °C. Our results agree with Hvas et al. (2017) as we found that AS plateaued at
303 16–22°C when measured at acclimation temperature, and it is likely that oxygen supply can limit performance
304 of salmon as temperature increases. An absence of increase suggests physiological systems supporting oxygen
305 uptake such as cardiac function, gill function and haemoglobin oxygen affinity are no longer able to scale with
306 increasing thermal pressure (Anttila et al., 2014; Schulte & Healy, 2022). As AS plateaus but SMR and the costs
307 of aerobic activities continue to increase, fish might become more constrained in their physiological state and
308 experience cumulative thermal stress, leading to mortality.

309

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

320 It is important to note that both TPCs and upper thermal limits are dependent on acclimation duration and
321 rate of warming (Currie et al., 2014; Lefevre et al., 2021; McKenzie et al., 2021). Most research in fishes has
322 been conducted with stable thermal profiles, whereby each individual experiences only one acclimation
323 temperature (Hvas et al., 2017). In contrast, our study accounts for a cumulative effect of exposure to higher

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

334 The general expectation is that longer acclimation with a slower increase of temperature should be beneficial
335 to survival, giving the fish time to make compensatory physiological modifications such as displaying plasticity
336 in SMR or MMR, enabling AS to be maintained over a broad range of temperatures. However, in barramundi
337 MMR and AS increased when exposed to acute 10 °C warming, due to a higher increase in MMR than SMR, but
338 after 5 weeks of acclimation, AS was similar, mainly due to a reduction in MMR (Norin et al., 2014). A very
339 similar pattern was observed in black sea bass (*Centropristis striata)* and common triplefin (*Forsterygion*
340 *lapillum*), where short term exposure caused a raise in AS, before it decreased after some weeks of exposure
341 (Khan et al., 2014; McArley et al., 2017; Slesinger et al., 2019). Additionally, in salmon post smolts, post-stress
342 peak cortisol levels significantly increased with higher temperatures (Madaro et al., 2018). This suggests that
343 despite a stable aerobic capacity to cope with temperature increases, costly physiological mechanisms may be
344 occurring, and therefore a plateau in AS does not necessarily suggest a broad optimum temperature range.

345

346 MMR did not differ across temperatures in this study, which may be due to the fish being of similar age and
347 reared under uniform hatchery conditions (Farrell et al., 2009a; Killen et al., 2016). MMR reflects the

maximum rate at which aerobic physiological processes can occur, and at 16 °C the salmon may already have been operating near their upper capacity for oxygen uptake and delivery (Clark et al., 2011; McKenzie & Claireaux, 2010; Norin & Clark, 2016; Sandblom et al., 2016). Although no treatment-level differences in MMR were detected, individual variation in thermal reaction norms of SMR and MMR may still exist but could not be resolved with our experimental design. This limitation may help explain why AS did not decline despite SMR increasing while MMR remained stable (Norin et al., 2014; Norin & Metcalfe, 2019; Réveillon et al., 2019). Sample size was reduced from 128 to 72 fish due to lost data, largely caused by malfunction of the water circulation and temperature control system. This uneven sample distribution across temperature treatments may have limited our ability to detect patterns, particularly at 19 and 22 °C. In addition, one extreme outlier was removed from SMR, MMR, and AS analyses based on visual inspection of histograms, and while this was a single fish, its exclusion should be acknowledged. Mortality also occurred in the respirometers (one, three, and two fish at 16, 19, and 22 °C, respectively), and while this represented <10% of the initial sample size for each group, removing these data introduces some potential for survival bias. Importantly, our experiment tested only a single hatchery stock and one juvenile age class; responses could differ in other populations or life stages, especially given that juvenile parr are often more sensitive than eggs or spawners. Together, these limitations highlight the need for caution in extrapolating our findings and suggest that broader testing across stocks, life stages, and larger sample sizes will be important for future studies.

Recent advances in conservation physiology demonstrate how individual-level physiological metrics can be used to guide policy and management strategies, as shown in studies on Sockeye salmon in Canada (Patterson et al., 2016). Integrating TPCs and stock specific information, such as life history genotypes and developmental stages, can inform stakeholders of specific vulnerabilities in populations and how they vary in location and season (Ward et al., 2017). With detailed TPCs, thermal safety margins can be defined in terms of functional success—such as swimming, feeding, and reproducing—rather than mortality alone (Pinsky et al., 2019). Temperature-dependent fisheries management strategies have already been successfully implemented, for

example, in Canadian rivers, and could be adapted globally to account for population-specific TPCs (Van Leeuwen et al., 2023). Management strategies, such as adjusting harvest timing and identifying thermal refuges for protection and restoration efforts, could help mitigate the impact of rising temperatures on vulnerable salmon populations.

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

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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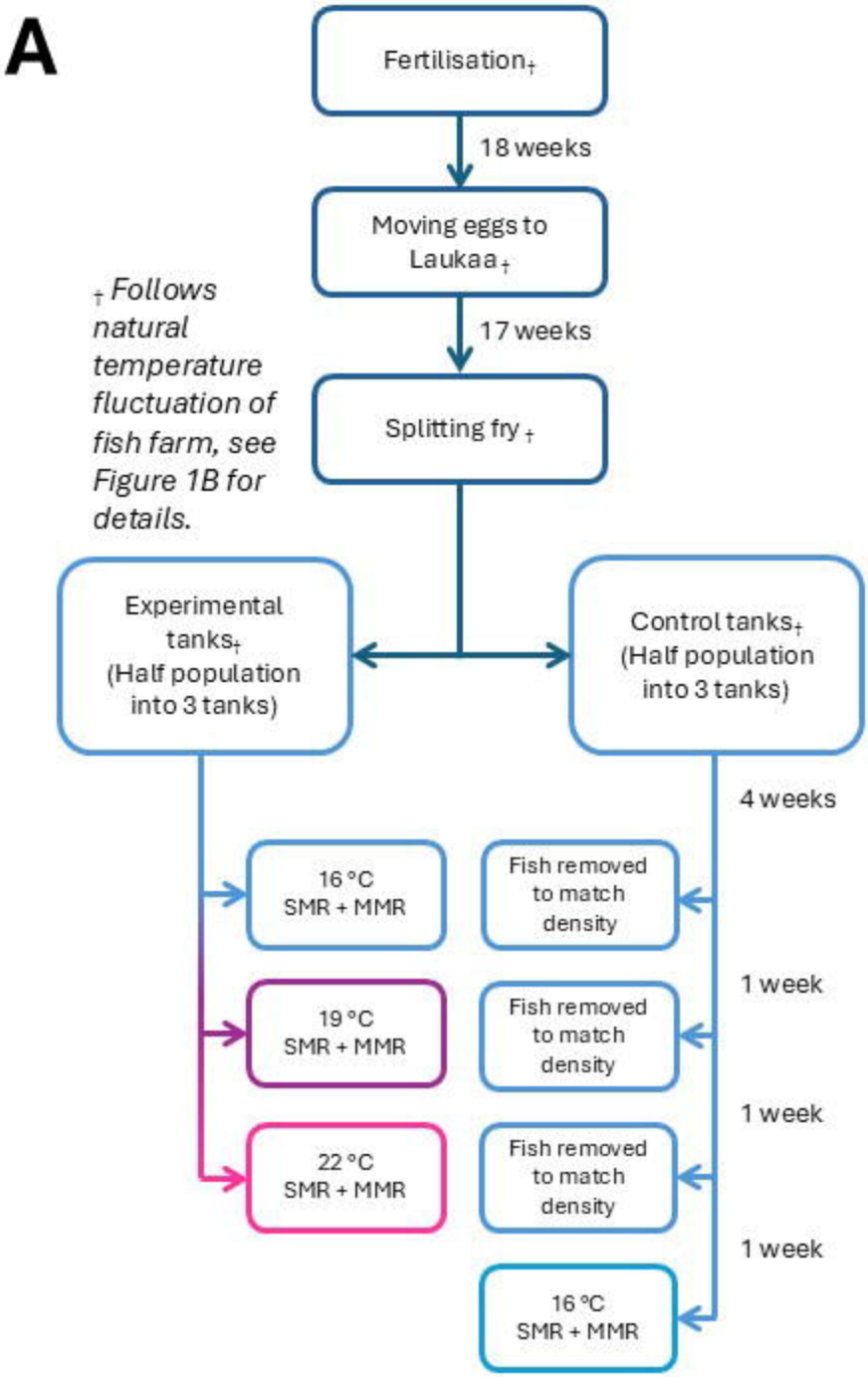
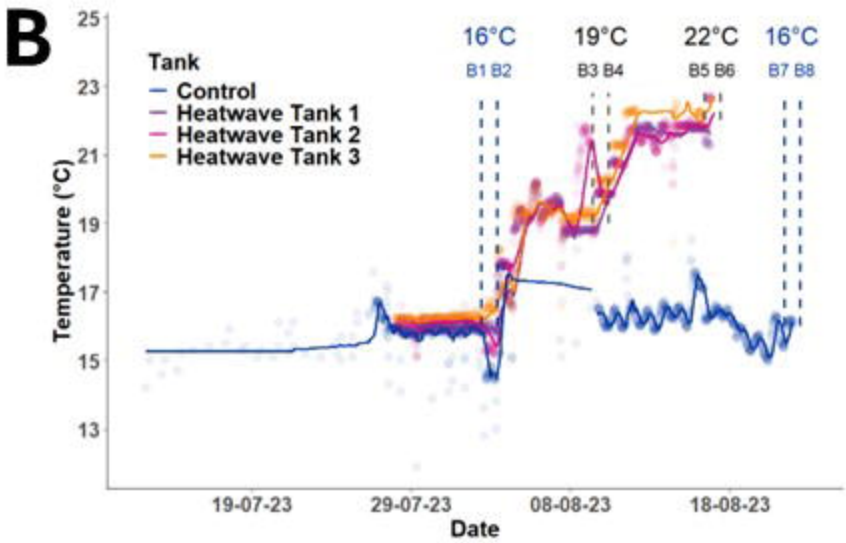
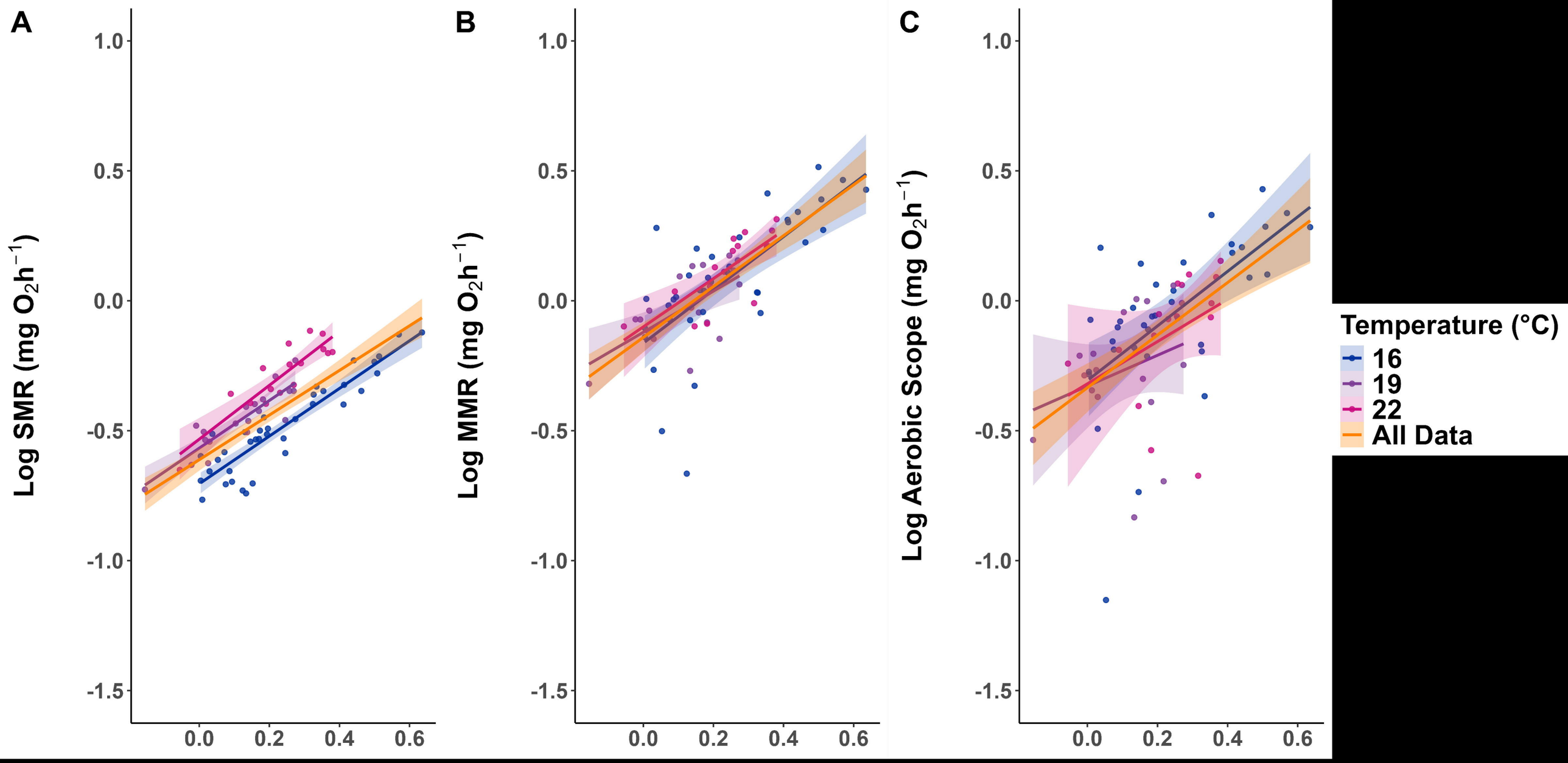

A
Fertilisation†
18 weeks
Moving eggs to Laukaa†
17 weeks
Splitting fry†
† Follows natural temperature fluctuation of fish farm, see Figure 1B for details.
Experimental tanks† (Half population into 3 tanks)
Control tanks† (Half population into 3 tanks)
4 weeks
16 °C SMR + MMR
Fish removed to match density
1 week
19 °C SMR + MMR
Fish removed to match density
1 week
22 °C SMR + MMR
Fish removed to match density
1 week
16 °C SMR + MMR


B
Tank
Control
Heatwave Tank 1
Heatwave Tank 2
Heatwave Tank 3
16°C
B1 B2
19°C
B3 B4
22°C
B5 B6
16°C
B7 B8
Temperature (°C)
25
23
21
19
17
15
13
19-07-23
29-07-23
08-08-23
18-08-23
Date


A
B
C
Log SMR (mg O2h−1)
Log MMR (mg O2h−1)
Log Aerobic Scope (mg O2h−1)
1.0
0.5
0.0
-0.5
-1.0
-1.5
0.0
0.2
0.4
0.6
Temperature (°C)
16
19
22
All Data

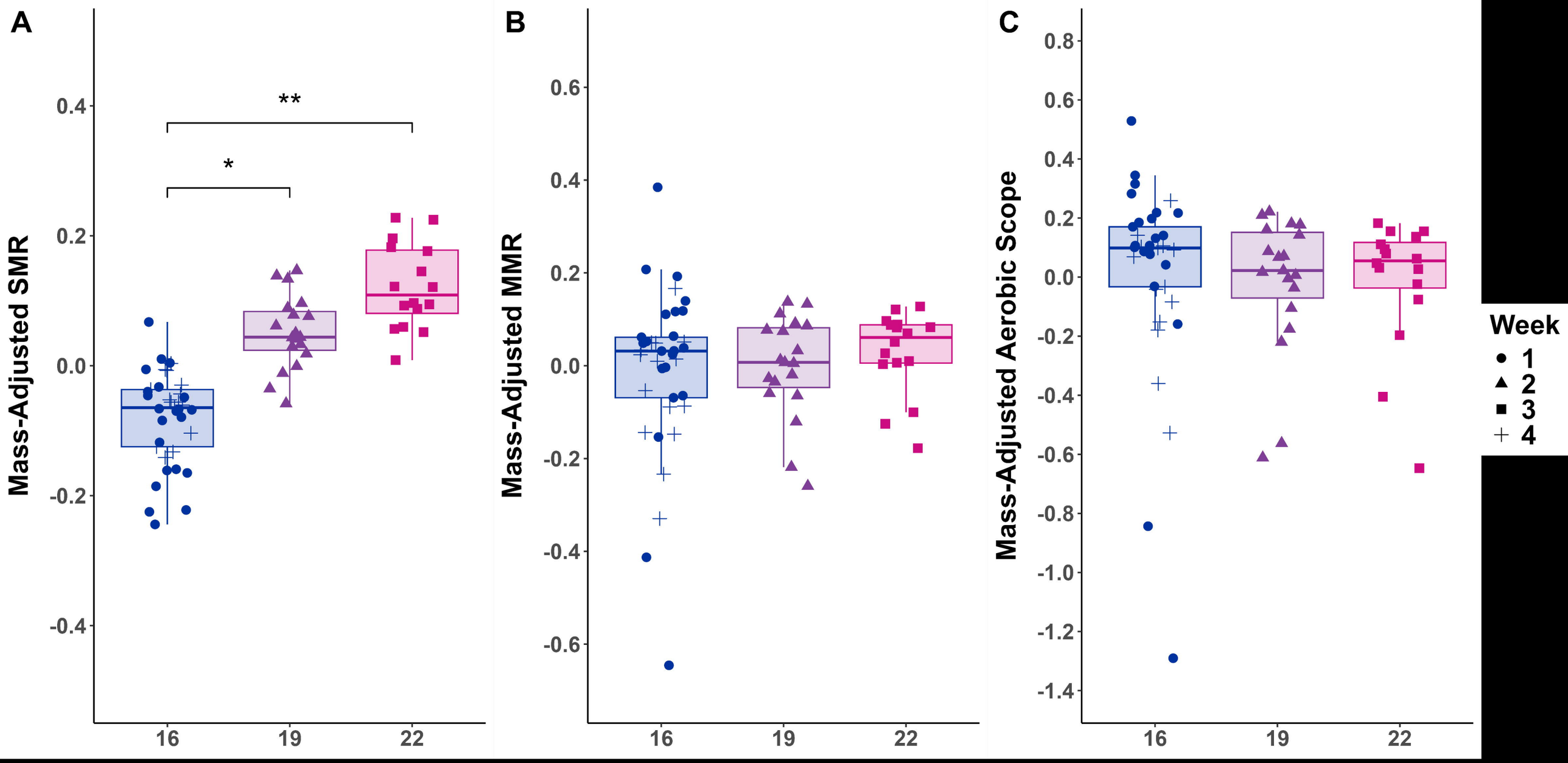

A
B
C
Mass-Adjusted SMR
Mass-Adjusted MMR
Mass-Adjusted Aerobic Scope
**
*
0.4
0.2
0.0
-0.2
-0.4
0.6
-0.6
0.8
-0.8
-1.0
-1.2
-1.4
16
19
22
Week
1
2
3
4

Table 1: Descriptive statistics for Mass (g) and Length (mm) for juvenile salmon tested at different temperatures. n = number of individuals, 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)*		
	Est.	***CI***	***p***	***Est.***	***CI***	***p***	***Est.***	***CI***	***p***
log10(mass)	**0.922**	**0.057**	**<0.001**	**0.978**	**0.120**	**<0.001**	**1.044**	**0.229**	**<0.001**
19 °C	**0.131**	**0.027**	**0.004**	0.011	0.046	0.826	-0.038	0.086	0.674
22 °C	**0.198**	**0.027**	**<0.001**	0.040	0.046	0.424	-0.033	0.086	0.717
Intercept	**-0.704**	**0.021**	**<0.001**	**-0.152**	**0.038**	**0.004**	**-0.342**	**0.072**	**<0.001**
Random Effects									
Intercept SD (Batch)		0.0215			0.0116			0.0116	
Residual SD		0.0638			0.1492			0.1492	
Marginal R^2		0.849			0.521			0.521	
Conditional R^2		0.864			0.524			0.524	

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