

## RESEARCH ARTICLE

# The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar*

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## ABSTRACT

The Atlantic salmon is extensively studied owing to conservation concerns and its economic importance in aquaculture. However, a thorough report of their aerobic capacity throughout their entire thermal niche has not been described. In this study, Atlantic salmon (~450 g) were acclimated for 4 weeks at 3, 8, 13, 18 or 23°C, and then tested in a large Brett-type swimming respirometer in groups of 10 per trial. Both standard metabolic rate and active metabolic rate continued to increase with temperature, which resulted in an aerobic scope that also increased with temperature, but was statistically similar between 13, 18 and 23°C. The critical swimming speed peaked at 18°C (93.1±1.2 cm s<sup>-1</sup>), and decreased significantly at the extreme temperatures to 74.8±0.5 and 84.8±1.6 cm s<sup>-1</sup> at 3 and 23°C, respectively. At 23°C, the accumulated mortality reached 20% over 4 weeks, while no fish died during acclimation at colder temperatures. Furthermore, fish at 23°C had poor appetite and lower condition factor despite still having a high aerobic scope, suggesting that oxygen uptake was not the limiting factor in the upper thermal niche boundary. In conclusion, Atlantic salmon were able to maintain a high aerobic capacity and good swimming capabilities throughout the entire thermal interval tested, thus demonstrating a high level of flexibility in respiratory capacity towards different temperature exposures.

**KEY WORDS:**  $U_{crit}$ , Respirometry, Scope for activity, Temperature

## INTRODUCTION

Nearly all species of fish are poikilothermic animals and therefore must cope with environmentally induced fluctuations in temperature of various magnitudes (Nelson, 2016). Because temperature affects the rate of physiological processes, fish have a preferred thermal window in which they can function optimally, which is highly dependent on species-specific adaptations (Scholander et al., 1953; Fry, 1958; Beamish, 1981). Species from environments in which temperature is nearly constant all year, such as in arctic regions and tropical coral reefs, may only tolerate a very narrow range of temperatures (Somero and DeVries, 1967; Nilsson et al., 2009), whereas fish from more variable environments have broader and more flexible thermal windows (Fry and Hart, 1948; Claireaux et al., 2006; Norin et al., 2014). Flexibility in thermal tolerance is partly due to the capacity for acclimation, which typically occurs within days or weeks (Hazel and Prosser, 1974). Among other

factors, acclimation involves a range of biochemical changes in enzyme functioning, alterations in the composition of membrane lipids and adjustments of haematological parameters (Roots, 1968; Houston and DeWilde, 1968, 1969; Somero and Hochachka, 1971).

Atlantic salmon (*Salmo salar* Linnaeus 1758) naturally reside in the Atlantic Ocean and adjacent river systems from Svalbard at 78°N to Northern Spain at 42–43°N (Jensen et al., 2014; Horreo et al., 2011). Within this geographical area, Atlantic salmon encounter seawater temperatures down to 0–3°C in the far north (Reddin, 1985; Lacroix, 2013) and above 20°C during river migration in the south (Valiente et al., 2004). Local populations have distinct genetic identities, which may aid in their adaptation to the wide range of habitats in which they are found (Bourret et al., 2013; Jensen et al., 2014). However, genotypic differences appear negligible when evaluating cardiac function at different temperatures, where Atlantic salmon show a remarkable ability to improve cardiac performance through thermal acclimation regardless of genetic origin (Anttila et al., 2014). This suggests that an Atlantic salmon primarily relies on phenotypic plasticity when it encounters different thermal environments throughout its natural distribution.


A widely used concept for the assessment of whole-animal performance is the scope for activity, coined by Fry (1947) as the aerobic scope (AS), which is the difference between the standard metabolic rate (SMR) and the active metabolic rate (AMR). SMR represents the minimum resting metabolic state, while AMR is the maximum rate of aerobic metabolism. The AS thereby provides a quantification of oxygen available for fitness related traits such as growth, foraging, locomotion and fecundity (Priede, 1985; Claireaux and Lefrançois, 2007).

Metabolism is greatly influenced by temperature, where SMR increases inexorably from acceleration of all biochemical processes, whilst AMR tends to either reach a plateau or decrease at higher temperatures dictated by the maximal capacity of the cardio-respiratory system. Consequently, the scope for activity exhibits a thermal optimum (Fry and Hart, 1948; Brett, 1971; Eliason and Farrell, 2016). The temperature at which AS is maximized is associated with optimum growth (Brett, 1971; Claireaux et al., 2000; Mallekh and Lagardère, 2002; Khan et al., 2014) and the highest critical swimming speed ( $U_{crit}$ ) in incremental swim speed protocols, which also coincides with maximum cardiac function (Brett, 1965; Farrell, 2002). Furthermore, fish populations are expected to occupy habitats where AS is high (Pörtner and Peck, 2010; Asbury and Angilletta, 2010).

AS may thereby provide an indication of how well a species of fish is coping in a given environment. However, some studies have found discrepancies in this assumption where growth optima did not correlate with the highest AS (Gräns et al., 2014), and where AS continued to increase up to near-lethal temperatures, while the fish behaviourally preferred colder waters (Norin et al., 2014). Furthermore, a recent review found that fish species do not always have a clear thermal optimum in AS (Lefevre, 2016).

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**List of symbols and abbreviations**

AMR	active metabolic rate
AS	aerobic scope
COT	cost of transport
FAS	factorial aerobic scope
$\dot{M}_{O_2}$	oxygen consumption rate
$Q_{10}$	temperature coefficient
SMR	standard metabolic rate
$U_{crit}$	critical swimming speed

Atlantic salmon are an extensively studied species because of their economic importance in aquaculture and for conservation concerns from declining natural populations (e.g. Horreo et al., 2011; Bourret et al., 2013). To complete their anadromous life cycle, Atlantic salmon undoubtedly need to function at a wide range of temperatures, and appear to possess an impressive phenotypic plasticity in response to thermal acclimation (Anttila et al., 2014). However, a thorough description of the aerobic capacity and swimming performance throughout their thermal niche is lacking.

The aim of the present study was therefore to quantify the scope for activity by measuring AMR and SMR, while also obtaining  $U_{crit}$  and monitoring swimming behaviour, at five different acclimation temperatures from 3 to 23°C. This was achieved by performing swim tunnel respirometry, where oxygen uptake provides an indirect measure of aerobic metabolism. It was hypothesized that Atlantic salmon would be able to maintain a high scope for activity and therefore also a high  $U_{crit}$  in a wide thermal interval, wherein the optimum temperature for these parameters should correspond with the optimum growth of post-smolts at 13°C (Handeland et al., 2003, 2008).

**MATERIALS AND METHODS****Animals and environmental monitoring**

Atlantic salmon post-smolts (Aquagen, Norway) were held in the Tank Environmental Laboratory at the Institute of Marine Research, Matre, Norway, in indoor large circular tanks (3 m diameter, 5.3 m<sup>3</sup>) with a water flow of 120 litres min<sup>-1</sup> in full-strength seawater (34‰) under a natural photoperiod. The fish were fed standard commercial feed (Nutra, 3 mm pellet size, Skretting, Norway) in excess through automatic feeding devices from 10:00 to 14:00 h each day. The temperature in the holding tanks was continuously monitored and kept within ±0.1°C by custom software at the research facility. Dissolved oxygen was also monitored, and was not allowed to fall below 85% saturation. To achieve this at the highest acclimation temperatures during daytime, it was necessary to add an inlet of hyperoxic water. All experiments were performed in accordance with the Norwegian laws and regulations for procedures and experiments on live animals under permit number 9776.

**Swim tunnel setup**

A thorough description of the large Brett-type swim tunnel respirometer used for the experiments is provided in Remen et al. (2016). The main specifications of the swim respirometer are briefly summarized as follows. The swim section of the tunnel had an internal diameter of 36 cm and was 248 cm long, which provided a volume of 252 litres. The volume of the entire swim tunnel system was 1905 litres. Water currents were generated by a motor-driven propeller (Flygt 4630, 11° propeller blade, Xylem Water Solutions

Norge AS, Nesttun, Norway), and the propeller speed (rpm) was controlled with a frequency converter (ITT Monitoring and Control, Norway). To determine the relationship between propeller speed and the water current velocity, an acoustic Doppler velocimeter (Vectrino Lab Velocimeter, Nortek AS, Rud, Norway) was mounted at the rear of the swim section. Water was added into the tunnel opposite of the swim section through an adjustable inlet that drew from the same reservoir used for the holding tanks, where temperature was tightly regulated. The maximum flow capacity into the tunnel was ~280 litres min<sup>-1</sup>, which was more than enough for rapid flushing of the setup, to restore oxygen levels and to maintain constant temperatures during swim trials. A camera was placed at the rear of the swim section to monitor the fish without disturbing them. An oxygen sensor was deployed adjacent to the camera (RINKO ARO-FT, JFE Advanced Co., Japan), which was connected to a computer where measurements were logged every 2 s (MiniSoft SD200W, SAIV A/S Environmental Sensors & Systems, Norway). Fatigued fish were easily removed from the tunnel at the rear where the top opening could be partially removed.

The main advantage of using a relatively large swim tunnel setup is that underestimations of  $U_{crit}$  are avoided, since fish are freely able to change between steady and burst and glide swimming gaits (e.g. Tudorache et al., 2007; Deslauriers and Kieffer, 2011; Remen et al., 2016). However, to obtain reliable oxygen consumption rate ( $\dot{M}_{O_2}$ ) measurements within a reasonable time in this setup, groups of fish had to be tested simultaneously to reduce the ratio between biomass and volume, which means that measurements represent a group average. Because the mass-specific  $\dot{M}_{O_2}$  decreases with size, it is imperative to use fish within the same size class.

The fish used in the experiments had a cross-sectional area of ~30 cm<sup>2</sup>, meaning that more than three fish would have to overlap to exceed 10% of the cross-sectional area of the swim tunnel, which was 1017 cm<sup>2</sup>. Generally, the fish were evenly spread out in the swim section during swim trials and would not overlap for long periods. Exceptions were at the lowest speed initially, and infrequently when individuals fatigued simultaneously at the highest speeds. Therefore, the effect of solid blocking was not corrected for here (Bell and Terhune, 1970; Plaut, 2001). Because groups of fish were tested simultaneously in this setup, it is likely that individuals experienced either slightly higher or lower current velocities depending on their relative position in the swim tunnel, owing to hydrodynamic interaction with other fish and the tunnel itself. However, the set current speed should still be representative for the actual current speeds experienced by the fish and therefore sufficient for the purpose of this study.

**Experimental protocols**

Acclimation procedures and swim tunnel respirometry were conducted between June and September 2016. Prior to swim trials, the fish had been acclimated for a minimum of 4 weeks to 3, 8, 13, 18 or 23°C. When targeting 3 and 23°C, the fish had previously been exposed to 8 and 18°C, respectively, for 4 weeks to better accommodate the transition to these more extreme temperatures. Groups of 10 fish were used in each swim trial, and six replicate trials were conducted for each of the five temperature groups.

In the afternoon, random fish were gently netted and transferred to the swim tunnel. Because the swim tunnel was situated in the same room as the holding tanks, movement only took a few seconds, which presumably minimised handling stress. The fish were left to acclimate in the tunnel overnight at 15 cm s<sup>-1</sup>

(~0.45 body lengths  $s^{-1}$ ) with moderate flushing to maintain temperature and oxygen levels.

Swim trials commenced the following day. Current velocity was increased incrementally by 15  $cm\ s^{-1}$  every 30 min until all fish reached fatigue. Fatigue was defined as when fish no longer were able to remove themselves from the rear grid, even with human tactile stimulation. The time was noted, and then fatigued fish were quickly removed from the tunnel and euthanized with a blow to the head. Fork length ( $L_f$ ) and mass were measured for every individual after removal from the tunnel.

To measure oxygen uptake, the swim tunnel system was kept closed for the first 20 min of each increment interval and subsequently flushed for the remaining 10 min, to re-establish oxygen levels for the following increment speed. Oxygen was not allowed to fall below 85% saturation during closed periods. To accomplish this at 23°C during high current velocities, the closed period was occasionally reduced to 15 min. Furthermore, because 10 fish were tested simultaneously, the period of oxygen measurement was sometimes shortened when the first fish started to fatigue and thus had to be removed from the tunnel. Because this only occurred at the highest velocities, it was still possible to obtain a good trace of oxygen consumption in these reduced measurement intervals. If six or more fish remained at the onset of the next increment speed, oxygen measurements were continued. With fewer fish it became increasingly more difficult to obtain a proper trace within reasonable time because of the large volume of the experimental setup relative to fish biomass. However, the fish generally reached fatigue within one increment interval, so final  $\dot{M}_{O_2}$  measurements represented the majority of the fish, while the best swimmers in each trial were already near fatigue when reliable oxygen measurements were no longer possible. No noteworthy background oxygen consumption from bacterial respiration was detected after swim protocols.

Tail-beat frequency was measured in three randomly selected fish at each current velocity by counting 100 tail beats with a stopwatch, and changes from steady to burst and glide swimming as the current speed increased were recorded.

### Calculations

For each closed period in the swim trials, a linear regression was fitted to the decrease in oxygen concentration as a function of time. The slope was then used to calculate the mass-specific  $\dot{M}_{O_2}$  of the fish at the given swimming speed:

$$\dot{M}_{O_2} = \frac{\Delta O_2}{\Delta t} \frac{(V_{sys} - V_b)}{M_b}, \quad (1)$$

where  $\Delta O_2/\Delta t$  is the change in oxygen over time,  $V_{sys}$  is the volume of the system, and  $V_b$  and  $M_b$  are the volume and mass of the fish, respectively, where a density of 1  $kg\ l^{-1}$  is assumed. SMR was calculated by exponentially fitting  $\dot{M}_{O_2}$  as a function of swimming speed and extrapolating back to zero swimming speed (Brett, 1964; Beamish, 1978). Theoretically, oxygen consumption should increase exponentially with swimming speed (Beamish, 1978). However, because the anaerobic component becomes more substantial at high current velocities when the fish approaches its maximum oxygen uptake, the curve tended to reach a plateau in the present study. The final one or two measurements were therefore omitted from the SMR calculation because they skewed the curve and caused a substantial reduction to the  $R^2$  of the fit. A similar plateauing in  $\dot{M}_{O_2}$  prior to  $U_{crit}$  has also been seen in other salmonids

with high anaerobic capacities (Lee et al., 2003). AMR was defined as the highest  $\dot{M}_{O_2}$  measured which coincided with the current velocity where the majority of the fish reached fatigue. Because  $\dot{M}_{O_2}$  was not measured in the few fish that required one more velocity increment to fatigue than the majority, AMR may have been slightly underestimated for the best swimmers. However, a plateau in  $\dot{M}_{O_2}$  was observed between the highest speeds where some fish had been removed in between, indicating that the reported AMR is representative for all the fish.

$U_{crit}$  was calculated according to Brett (1964):

$$U_{crit} = U_f + \frac{t_f U_i}{t_i}, \quad (2)$$

where  $U_f$  is the last completed current velocity,  $t_f$  is the time spent at the current velocity where fatigue was reached,  $t_i$  is the time spent at each velocity and  $U_i$  is the magnitude of the velocity increment.

To assess swimming efficiency, the gross and net cost of transport (COT) was calculated for each swimming speed as the energy required to travel 1 km.  $\dot{M}_{O_2}$  was converted to joules using an oxycaloric coefficient of 14.2  $J\ mg^{-1}\ O_2$  (Videler, 1993; Ohlberger et al., 2006), and then divided by the swimming speed. Only swimming intensities that were considered primarily aerobically fuelled were included in these calculations. The minimum COT was estimated as the minimum of a parabola fitted to the gross COT as a function of swimming speed for each acclimation temperature.

### Statistical analyses

Differences in  $\dot{M}_{O_2}$  between groups were tested with a one-way ANOVA (Sigmaplot 12.3, Systat Software), while a nested two-way ANOVA (Statistica 13.0, Quest Software) was used when measurements for individual fish were available ( $U_{crit}$  and size parameters). A Tukey's *post hoc* test was used to determine which groups differed, while the Shapiro–Wilk test and Levene's mean test were used to confirm normality and equal variance in the data, respectively. *P*-values below 0.05 were considered significant. All data are presented as means±s.e.m.

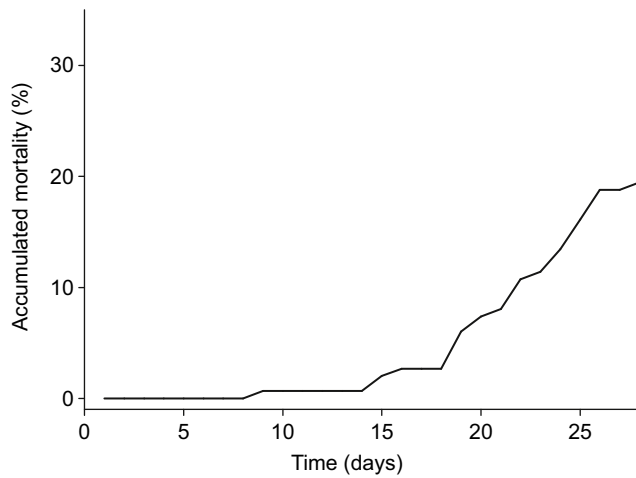
### RESULTS

The fish used in the swim trials were within a similar size range between acclimation groups (Table 1). However, the fish at 3 and 8°C were significantly heavier compared with 13 and 18°C because these trials were carried out 1 month later. Even though trials at 23°C were conducted between the trials at 3 and 8°C, these fish had a significantly lower mass, but similar fork length which resulted in a poor condition factor for this group (Table 1). During the acclimation at 23°C, the fish fed normally in the first week, but in the latter period appetite declined. This coincided with increased mortality where 20% had perished after four weeks of acclimation

**Table 1. Mass, fork length and condition factor for Atlantic salmon used in the swim trials at different acclimation temperatures (N=60)**

Temperature (°C)	Mass (g)	Fork length (cm)	Condition factor
3	479±18 <sup>a</sup>	35.8±0.5 <sup>a,b</sup>	1.02±0.01 <sup>a</sup>
8	491±19 <sup>a</sup>	36.9±0.5 <sup>a</sup>	0.95±0.01 <sup>b</sup>
13	437±16 <sup>a,b</sup>	35.1±0.4 <sup>b,c</sup>	0.98±0.01 <sup>a,b</sup>
18	408±16 <sup>b</sup>	34.0±0.4 <sup>c</sup>	1.01±0.01 <sup>a</sup>
23	413±13 <sup>b</sup>	36.0±0.4 <sup>a,b</sup>	0.87±0.01 <sup>c</sup>

Statistical differences between groups are indicated by superscript letters (Tukey *post hoc* test,  $P<0.05$ ). Data are means±s.e.m.



**Fig. 1. Accumulated mortality of Atlantic salmon in the holding tanks during acclimation to 23°C.**

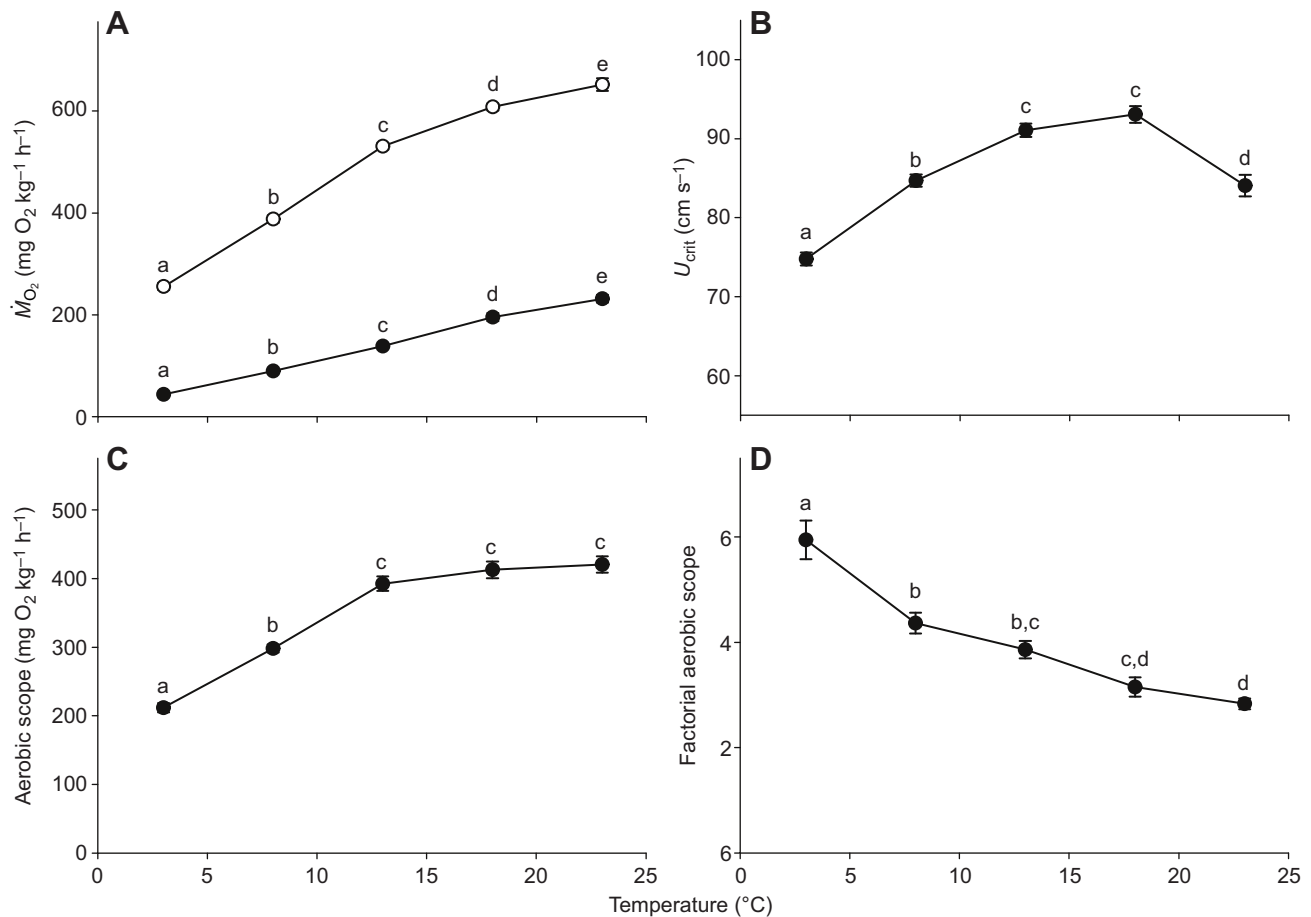
(Fig. 1). At the other temperatures, no fish died except for a small number initially due to side wounds obtained during the transportation from the rearing facilities to the acclimation tanks several weeks before acclimation protocols were initiated.

### Oxygen consumption and aerobic scope

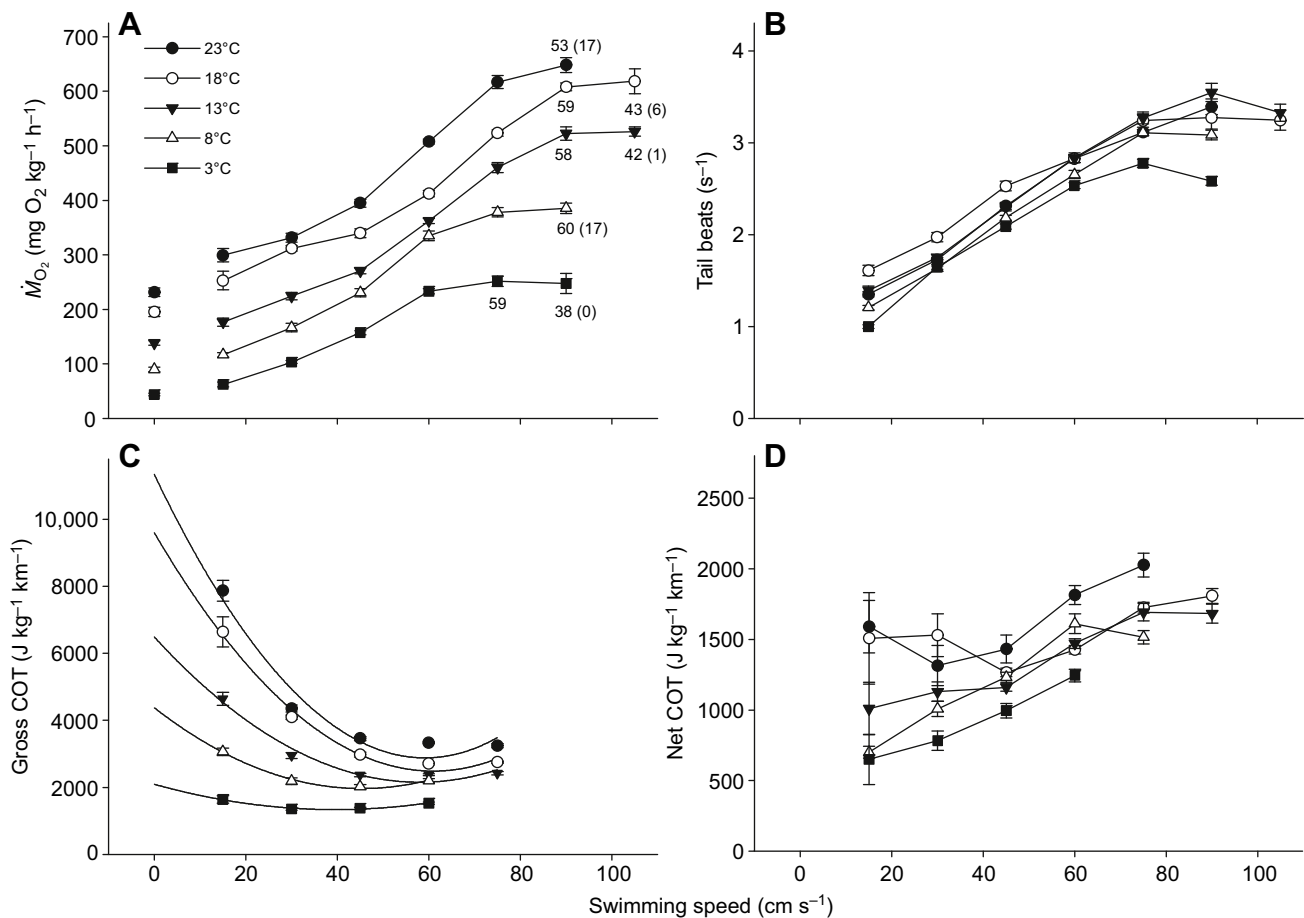
SMR increased steadily with temperature from  $44 \pm 5 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  at 3°C to  $231 \pm 5 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  at 23°C, corresponding to a  $Q_{10}$  of 2.2. The magnitude of the  $Q_{10}$  effect was greatest at the lower temperatures, e.g. 3.1 from 3 to 13°C compared with 1.6 from 13 to 23°C. SMR at all five temperatures were significantly different from each other (Fig. 2A). AMR continued to increase continually to 23°C, with the magnitude of change being greatest from 3 to 13°C (Fig. 2A).

The highest AS (AMR–SMR) was measured at 23°C, where it was  $421 \pm 12$  compared with only  $212 \pm 7 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  at 3°C. However, AS was not significantly different between 13, 18 and 23°C (Fig. 2C). The factorial AS (AMR/SMR) decreased throughout the entire temperature interval from  $5.74 \pm 0.33$  at 3°C down to  $2.95 \pm 0.12$  at 23°C, where a significant difference was found from 3 to 8°C and at every two temperature increments thereafter (Fig. 2C).

An overview of the oxygen uptake rate as a function of swimming speed at different temperatures, including total remaining fish at the highest velocities, is shown in Fig. 3A. Each temperature has a distinct curve owing to differences in SMR and AMR. Oxygen consumption increased with swimming speed until a certain temperature-dependent point where the curve flattens out. When this occurs, continued swimming is not sustainable for long owing to an increasingly greater reliance on anaerobic metabolism to meet



**Fig. 2. Metabolic rate, swimming speed and aerobic scope of Atlantic salmon at different acclimation temperatures.** (A) Standard metabolic rate (SMR) (filled circles) and active metabolic rate (AMR) (open circles), (B) critical swimming speed ( $U_{crit}$ ), (C) aerobic scope (AMR–SMR) and (D) factorial aerobic scope (AMR/SMR).  $N=6$  for A, C and D;  $N=60$  for B. Different letters indicates significant differences between the temperature groups (Tukey *post hoc* test,  $P < 0.05$ ). Data are means  $\pm$  s.e.m.



**Fig. 3. Swimming performance of Atlantic salmon as a function of swimming speed at different acclimation temperatures.** (A) Oxygen consumption rate ( $\dot{M}_{O_2}$ ), (B) tail-beat frequency, (C) gross cost of transport (COT) and (D) net COT.  $N=6$  for A, C and D;  $N=18$  for B. Numbers in A are total remaining fish in the beginning of the corresponding swim speed, while the number in brackets is total fish remaining at the subsequent velocity increment where oxygen uptake was not measured. The isolated points in A represent the calculated SMR from back extrapolation to zero swim speed. Data are means  $\pm$  s.e.m.

energy requirements. Furthermore, the plateauing in  $\dot{M}_{O_2}$  was reached when nearly all the fish still remained in the setup, suggesting that the derived AMR is a representative average for the entire group.

### Swimming performance

$U_{crit}$  increased significantly from  $74.8 \pm 0.6$  cm s<sup>-1</sup> at 3°C to statistically similar peak values of  $91.3 \pm 0.4$  and  $93.1 \pm 1.2$  cm s<sup>-1</sup> at 13 and 18°C, respectively. At 23°C,  $U_{crit}$  had decreased significantly to a similar value as found at 8°C ( $84.9 \pm 1.6$  and  $84.7 \pm 0.4$  cm s<sup>-1</sup>, respectively) (Fig. 2B).  $U_{crit}$  expressed as body lengths s<sup>-1</sup> showed a similar pattern (data not shown).

The tail-beat frequency appeared to be independent of temperature at low and moderate swimming speeds (Fig. 3B). However, at the colder temperatures, the highest attainable tail-beat frequency was impaired, which was most apparent at 3°C, with a peak of  $2.78 \pm 0.04$  tail beats s<sup>-1</sup> when swimming at 75 cm s<sup>-1</sup>. In comparison, at 18°C, the fish reached a tail-beat frequency of  $3.24 \pm 0.07$  tail beats s<sup>-1</sup> when swimming at the same speed. Generally, burst and glide swimming was first observed sporadically at 60 cm s<sup>-1</sup>, and became gradually more pronounced and frequent at higher speeds. Gait transition took place earlier at 3°C ( $45$  cm s<sup>-1</sup>), which in conjunction with the curves for tail-beat frequency and  $\dot{M}_{O_2}$  from this group (Fig. 3B and A, respectively) indicates that they became aerobically challenged at lower speeds compared with the other groups, and hence the lower  $U_{crit}$  (Fig. 2B). Gross COT had a

distinct curve for each acclimation temperature, where the calculated minimum COT was 40, 45, 58, 61 and 59 cm s<sup>-1</sup> at 3, 8, 13, 18 and 23°C, respectively (Fig. 3C). At intermediate and high swimming speeds, net COT was similar between the 8, 13 and 18°C groups, while the 23°C group had a higher net COT from 45 to 75 cm s<sup>-1</sup>, and the 3°C group had a lower net COT from 30 to 60 cm s<sup>-1</sup> compared with the other groups (Fig. 3D).

### DISCUSSION

#### High aerobic capacity throughout the entire thermal niche

Although the pattern of oxygen consumption in Atlantic salmon changed dramatically with temperature, the resulting scope for activity generally remained fairly conserved with statistical similarities from 13 to 23°C, and a reduction by 32% at 8°C compared with the peak at 23°C (Fig. 2C). Similarly, the swimming performance expressed as  $U_{crit}$  also remained high throughout the exposed temperatures.

A drastic impairment in swimming capability and AMR was only measured at 3°C, where an earlier gait transition to burst and glide swimming suggested that the salmon became anaerobic at lower swimming speeds. In support of this, colder temperatures reduce the maximum velocity of shortening of red muscle fibres and their power production in fish, and as a consequence, white muscles are recruited earlier (Rome, 1990; Rome et al., 1992).

Cold acclimation also increases red muscle mass in temperate fish, which improves stride length, and thereby reduces the required tail-beat frequency compared with warm-acclimated fish swimming at similar speeds (Sisson and Sidell, 1987; Sidell and Moerland, 1989; Taylor et al., 1996). It is possible that relatively higher tail-beat amplitudes compensated for the observed lower tail-beat frequencies at 3°C in Atlantic salmon. However, amplitude was not measured in this study. The observed reduction in maximum tail-beat frequency at 3°C could therefore indicate that cold acclimation also increases red muscle mass in Atlantic salmon. Thus, while the maximum swimming capacity is reduced, the energetics of slow or moderate swimming is improved, as illustrated by a reduced net COT compared with the other acclimation groups. Furthermore, the lowered SMR improves the cumulative COT, which may be another important advantage in colder waters (Hoar and Randall, 1978).

Reverse seasonal acclimation has been found to reduce  $U_{crit}$  in brown trout (*Salmo trutta*) (Day and Butler, 2005). This indicates that  $U_{crit}$  may have been underestimated at 3 and 8°C in Atlantic salmon because this experiment was performed during the summer months in a simulated natural photoperiod. The seasonal variation in swimming capacity for Atlantic salmon could therefore be even smaller than suggested by the present study.

Regardless, the aerobic capacity appears to remain adequate in the entire thermal niche, illustrating a high flexibility to changing environmental conditions for this eurythermal species.

### Physiological limitations at high temperatures

When Atlantic salmon were kept at 23°C, their appetite gradually became more reduced, which resulted in a poor condition factor for fish tested in the swim trials. More importantly, a cumulative mortality of 20% in the holding tanks occurred, primarily over the last 2 weeks of the 4-week acclimation period. If they were struggling with meeting their oxygen requirements at 23°C, an overt increase in gill ventilation or even the onset of ram ventilation should be apparent, as seen in triploid Atlantic salmon during moderate hypoxia at 19°C (Hansen et al., 2015). However, this was not observed here, and oxygen saturation in the holding tanks was always kept above 85%. Furthermore, the high AS found in the swim tunnel trials strongly suggests that their routine oxygen requirements were satisfied.

A possible explanation for the high mortality rate could instead be hyperactivity initiated as a form of escape behaviour after prolonged exposure in a suboptimal thermal environment. Delayed death from strenuous activities is a well-documented phenomenon in fish that is associated with high lactate levels and a high intracellular acidosis (Black, 1958; Wood et al., 1983). Furthermore, cortisol was likely elevated during these stressful conditions, and lactate recovery is slower in the presence of cortisol (Pagnotta et al., 1994).

Reduced growth and lower condition factor at high temperatures has previously been documented in Atlantic salmon (Kullgren et al., 2013; Hevrøy et al., 2015). In particular, decreased food intake and lowered feed conversion efficiency in fish acclimated to 18°C compared with 12°C was found to be associated with elevated plasma levels of growth hormone and leptin, while several metabolites in energy metabolism were reduced at 18°C (Kullgren et al., 2013). These authors therefore concluded that impaired growth and appetite at higher temperatures was caused by the anorexigenic function of leptin in conjunction with changes in growth hormone endocrinology (Kullgren et al., 2013).

Higher temperatures increases metabolic rates and the overall maintenance costs of preserving homeostasis, and growth is

impaired if these are not compensated for by an increased feed intake, as seen in rainbow trout (Morgan et al., 2001). If the available AS is reduced at higher temperatures, the relative energetic cost of feeding and growing may be too high. Reduced appetite could therefore also be behaviourally driven to avoid anaerobic metabolism, as suggested by the reduced feed intake in Atlantic cod (*Gadus morhua*) under conditions with low AS (Claireaux et al., 2000). However, in the present study, the AS was not reduced at 23°C in Atlantic salmon; on the contrary, it was higher here than at any other temperature investigated, although statistically similar to values at both 13 and 18°C. The inevitable increase in SMR was accompanied by a similar increase in AMR, which demonstrates that Atlantic salmon are well equipped to meet their oxygen requirements well above temperatures at which growth and appetite decline. This suggests that Atlantic salmon have evolved to cope with extremely high temperatures relative to their preferred thermal niche for periods of time (days), which should be adequate to spatially deal with the problem by searching for lower temperatures or withstanding the duration of exposure, e.g. during river migrations in the summer.

$U_{crit}$  in Atlantic salmon was lower at 23°C compared with 13 and 18°C, despite similar AS. Assuming an increased tendency for hyperactivity as previously discussed, fish at 23°C were perhaps in a state of metabolic recovery during the swim trials, which may have reduced their swimming efficiency and peak performance, considering that AMR was not compromised. This was further supported by a higher net COT, indicating that swimming at 23°C was relatively more costly. Similarly, in warm-acclimated rainbow trout, the repeated swimming performance was found to be reduced because exercise recovery was slower compared with colder acclimated fish (Jain and Farrell, 2003). Therefore, this may partially explain a lower  $U_{crit}$  at 23°C. However, the poor condition factor and apparent lack of appetite during the acclimation period are also likely to have impaired the swimming performance.

The physiological barrier at high temperatures in fish has widely been ascribed to limitations in sufficient oxygen uptake and supply to the tissues (Brett, 1971; Farrell, 2002; Pörtner and Knust, 2007). In Pacific salmonids, AS and cardiac performance decline when the fish approach their upper incipient lethal temperature, which is linked to an insufficient myocardial oxygen supply as returning venous blood to the heart becomes more depleted during exercise (Farrell, 2002, 2007). In warm-acclimated Atlantic salmon, the upper incipient lethal temperature was found to be 27.8°C (Elliott, 1991), which corresponds with cardiac collapse at 27.5°C (Anttila et al., 2014). In theory, AS should therefore also collapse at ~27°C, yet was still preserved at 23°C in the present study.

In barramundi (*Lates calcarifer*), AS was found to increase up to near lethal temperatures (Norin et al., 2014), and likewise in seasonal-acclimated European sea bass (*Dicentrarchus labrax*) where AS continued to increase from 7°C to 30°C (Claireaux et al., 2006), and approached its acute critical temperature at 35°C (Wang et al., 2014). These patterns in thermal performance also describe thermally acclimated Atlantic salmon.

Thus, in ecologically relevant settings, oxygen limitation does not seem to be the proximal factor in defining the upper thermal niche boundary in this species. To thrive and remain competitive in environments with chronically elevated temperatures, other factors concerning hormone balance, stress, behaviour and nerve functioning to ensure proper growth, appetite and fecundity therefore appear to be more important in Atlantic salmon and other species of fish that are able to maintain high AS.

### Thermal optima in Atlantic salmon

Growth in Atlantic salmon post-smolts is maximized at 13°C (Handeland et al., 2003, 2008), and  $U_{crit}$  as a measure of swimming capabilities peaks at 18°C while the AS still is conserved at 23°C (present study). For the majority of their life cycle post smoltification, Atlantic salmon will experience temperatures below 8°C (Reddin, 1985; Lacroix, 2013; Jensen et al., 2014), and may even behaviourally avoid temperatures above 15°C (Johansson et al., 2009; Lacroix, 2013). Therefore, Atlantic salmon fit the idea of multiple optimum temperatures for different physiological processes and life history traits, where it is acknowledged that any factor, in no hierarchical order, may be limiting in either acute or chronic thermal challenges (Clark et al., 2013). Similar multiple optimum temperatures for different traits such as AS, growth and gonadosomatic index have also been documented in other species such as pink salmon (*Oncorhynchus gorbuscha*) (Clark et al., 2011), killifish (*Fundulus heteroclitus*) (Healy and Schulte, 2012) and Atlantic halibut (*Hippoglossus hippoglossus*) (Gräns et al., 2014).

The scope for activity can be expressed either as an absolute value (AMR – SMR) or a factor (AMR/SMR). Absolute AS is arguably more ecologically relevant because it provides a specific value for the aerobic capacity, and various aerobic tasks will have a specific oxygen cost, while it also makes for a better comparison between species and different life stages that may be very different in their energetics despite having similar factorial scopes (Clark et al., 2013; Eliason and Farrell, 2016). The absolute AS has therefore been the focus of this study.

In Atlantic salmon, the factorial AS has an opposing thermal curve (Fig. 2D), as it decreases with temperature, because the increase in SMR makes up a greater fraction of the AMR at higher temperatures. It will therefore lead to different conclusions regarding optimum temperatures. The factorial AS provides additional insights into the relative energy allocations and requirements of a species. A high factorial AS indicates low maintenance costs as seen in Atlantic salmon during colder temperatures, which could be an important advantage in energy savings when food is scarce, during migration or for gonad development. In contrast, the metabolic costs associated with feeding and digestion can be two to three times higher than the SMR, thus a low factorial AS may explain why growth and appetite are reduced in suboptimal environments (Farrell, 2016). For Atlantic salmon, a factorial AS of 2.95 at 23°C may therefore have been too low to support proper digestion and growth while also accommodating other activities. The absolute and the factorial AS evidently provide different important insights, and although a recent review on this topic argued that the absolute AS may be more informative, ideally both should be reported (Clark et al., 2013).

The hypothesis that AS and  $U_{crit}$  should be maximized at the optimum growth rate (13°C) for Atlantic salmon post-smolts was not confirmed. However, at 13°C, ~90% of the maximum AS was still preserved, while  $U_{crit}$  was not significantly reduced. But given the fact that Atlantic salmon at sea predominantly occupy colder waters where AS, swimming capabilities and growth are not maximized, such experimentally obtained values may have limited ecological relevance. Ultimately, marine habitats may simply be selected based on food availability. Furthermore, if prey items in the wild are not overly abundant in conjunction with the migratory nature of Atlantic salmon, improved energy efficiency at colder temperatures to reduce maintenance metabolism and improve COT could be more important during the majority of the species' life cycle, while the improved aerobic capacity and swimming performance at warmer temperatures is crucial during summer river migration.

### Competing interests

The authors declare no competing or financial interests.

### Author contributions

Conceptualization: M.H., O.F., A.I., F.O.; Methodology: M.H., O.F., A.I., F.O.; Software: A.I.; Formal analysis: M.H., A.I.; Investigation: M.H.; Resources: O.F., F.O.; Data curation: M.H.; Writing - original draft: M.H.; Writing - review & editing: M.H., O.F., A.I., F.O.; Supervision: O.F., A.I., F.O.; Project administration: O.F., F.O.; Funding acquisition: O.F., F.O.

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### References

- Anttila, K., Couturier, C. S., Øverli, Ø., Johnsen, A., Marthinsen, G., Nilsson, G. E. and Farrell, A. P. (2014). Atlantic salmon show capability for cardiac acclimation to warm temperatures. *Nat. Commun.* **5**, 4252.
- Asbury, D. A. and Angilletta, M. J. Jr (2010). Thermodynamic effects on the evolution of performance curves. *Am. Nat.* **176**, E40–E49.
- Beamish, F. W. H. (1978). Swimming capacity. In *Fish Physiology* (ed. W.S. Hoar and D. J. Randall), pp. 101–187. New York: Academic Press.
- Beamish, F. W. H. (1981). Swimming performance and metabolic rate of three tropical fishes in relation to temperature. *Hydrobiologia* **83**, 245–254.
- Bell, W. H. and Terhune, L. D. B. (1970). *Water tunnel design for fisheries research*. Fisheries Research Board of Canada, Technical Report No. 195.
- Black, E. C. (1958). Hyperactivity as a lethal factor in fish. *J. Fish. Res. Bd Can.* **15**, 245–251.
- Bourret, V., Kent, M. P., Primmer, C. R., Vasemägi, A., Karlsson, S., Hindar, K., McGinnity, P., Verspoor, E., Bernatchez, L. and Lien, S. (2013). SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*). *Mol. Ecol.* **22**, 532–551.
- Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Can.* **21**, 1183–1226.
- Brett, J. (1965). The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). *Journal of the Fisheries Research Board of Canada* **22**, 1491–1501.
- 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.
- Claireaux, G. and Lefrançois, C. (2007). Linking environmental variability and fish performance: integration through the concept of scope for activity. *Phil. Trans. R. Soc. B Biol. Sci.* **362**, 2031–2041.
- Claireaux, G., Webber, D. M., Lagardère, J.-P. and Kerr, S. R. (2000). Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). *J. Sea Res.* **44**, 257–265.
- Claireaux, G., Couturier, C. and 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., Jeffries, K. M., Hinch, S. G. and Farrell, A. P. (2011). Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. *J. Exp. Biol.* **214**, 3074–3081.
- Clark, T. D., Sandblom, E. and Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J. Exp. Biol.* **216**, 2771–2782.
- Day, N. and Butler, P. J. (2005). The effects of acclimation to reversed seasonal temperatures on the swimming performance of adult brown trout *Salmo trutta*. *J. Exp. Biol.* **208**, 2683–2692.
- Deslauriers, D. and Kieffer, J. D. (2011). The influence of flume length and group size on swimming performance in shortnose sturgeon *Acipenser brevirostrum*. *J. Fish Biol.* **79**, 1146–1155.
- Eliason, E. J. and Farrell, A. P. (2016). Oxygen uptake in Pacific salmon *Oncorhynchus* spp.: when ecology and physiology meet. *J. Fish Biol.* **88**, 359–388.
- Elliott, J. M. (1991). Tolerance and resistance to thermal stress in juvenile Atlantic salmon, *Salmo salar*. *Freshw. Biol.* **25**, 61–70.
- Farrell, A. P. (2002). Cardiorespiratory performance in salmonids during exercise at high temperature: insights into cardiovascular design limitations in fishes. *Comp. Biochem. Physiol. A* **132**, 797–810.
- Farrell, A. P. (2007). Cardiorespiratory performance during prolonged swimming tests with salmonids: a perspective on temperature effects and potential analytical pitfalls. *Philos. Trans. R. Soc. B* **362**, 2017–2030.
- Farrell, A. P. (2016). Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *J. Fish Biol.* **88**, 322–343.
- Fry, F. E. J. (1947). Effect of the environment on animal activity. *Univ. Toronto Stud. Biol. Ser.* **55**, 1–62.

- Fry, F. E. J. (1958). Temperature compensation. *Annu. Rev. Physiol.* **20**, 207-224.
- Fry, F. and Hart, J. (1948). The relation of temperature to oxygen consumption in the goldfish. *Biol. Bull.* **94**, 66-77.
- Gräns, A., Jutfelt, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O. and Einarsdottir, I. (2014). Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO<sub>2</sub> in Atlantic halibut. *J. Exp. Biol.* **217**, 711-717.
- Handeland, S. O., Björnsson, B. Th., Arnesen, A. M. and 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. and Stefansson, S. O. (2008). The effect of temperature and fish size on growth, food 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. and Fjellidal, 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.
- Hazel, J. R. and Prosser, C. L. (1974). Molecular mechanism of temperature compensation in poikilotherms. *Physiol. Rev.* **54**, 620-677.
- Healy, T. M. and Schulte, P. M. (2012). Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (*Fundulus heteroclitus*). *Physiol. Biochem. Zool.* **85**, 107-119.
- Hevroy, E. M., Tipsmark, C. K., Remø, S. C., Hansen, T., Fukuda, M., Torgersen, T., Vikeså, V., Olsvik, P. A., Waagbø, R. and Shimizu, M. (2015). Role of the GH-IGF-1 system in Atlantic salmon and rainbow trout postsmolts at elevated water temperature. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **188**, 127-138.
- Hoar, W. S. and Randall, D. J. (ed.) (1978). *Fish Physiology. Volume VII, Locomotion*. New York: Academic Press.
- Horreo, J. L., Machado-Schiaffino, G., Griffiths, A. M., Bright, D., Stevens, J. R. and Garcia-Vazquez, E. (2011). Atlantic salmon at risk: apparent rapid declines in effective population size in southern European populations. *Trans. Am. Fish. Soc.* **140**, 605-610.
- Houston, A. H. and DeWilde, M. A. (1969). Environmental temperature and the body fluid system of the fresh-water teleost. Hematology and blood volume of thermally acclimated brook trout, *Salvelinus fontinalis*. *Comp. Biochem. Physiol.* **28**, 877-885.
- Houston, A. H. and DeWilde, M. A. (1968). Thermoacclimatory variations in the haematology of the common carp, *Cyprinus carpio*. *J. Exp. Biol.* **49**, 71-81.
- Jain, K. E. and Farrell, A. P. (2003). Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **206**, 3569-3579.
- Jensen, A. J., Karlsson, P. F., Hansen, L. P., Østborg, G. M. and Hindar, K. (2014). Origin and life history of Atlantic salmon (*Salmo salar*) near their northernmost oceanic limit. *Can. J. Fish. Aquat. Sci.* **71**, 1740-1746.
- Johansson, D., Ruohonen, K., Juell, J.-E. and 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.
- Khan, J. R., Pether, S., Bruce, M., Walker, S. P. and Herbert, N. A. (2014). Optimum temperatures for growth and feed conversion in cultured hapuku (*Polyprion oxygeneios*) – Is there a link to aerobic metabolic scope and final temperature preference? *Aquaculture* **430**, 107-113.
- 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.
- Lee, C. G., Farrell, A. P., Lotto, A., Hinch, S. G. Healey, M. C. (2003). Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical speed swimming. *J. Exp. Biol.* **206**, 3253-3260.
- 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<sub>2</sub> and their interaction. *Conserv. Physiol.* **4**, cow009.
- Mallek, R. and Lagardère, J. P. (2002). Effect of temperature and dissolved oxygen concentration on the metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. *J. Fish Biol.* **60**, 1105-1115.
- Morgan, I. J., McDonald, D. G. and Wood, C. M. (2001). The cost of living for freshwater fish in a warmer, more polluted world. *Glob. Chang. Biol.* **7**, 345-355.
- Nelson, J. S. (2016). *Fishes of the World*, 5th edn. Hoboken, NJ: John Wiley & Sons, Inc.
- Nilsson, G. E., Crawley, N., Lunde, I. G. and Munday, P. L. (2009). Elevated temperature reduces the respiratory scope of coral reef fishes. *Glob. Change Biol.* **15**, 1405-1412.
- Norin, T., Malte, H. and 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.
- Ohlberger, J., Staaks, G. and Hölker, F. (2006). Swimming efficiency and the influence of morphology on swimming costs in fishes. *J. Comp. Physiol. B* **176**, 17.
- Pagnotta, A., Brooks, L. and Milligan, L. (1994). The potential regulatory roles of cortisol in recovery from exhaustive exercise in rainbow trout. *Can. J. Zool.* **72**, 2136-2146.
- Plaut, I. (2001). Critical swimming speed: its ecological relevance. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **131**, 41-50.
- Pörtner, H. O. and Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**, 95-97.
- Pörtner, H. O. and Peck, M. A. (2010). Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *J. Fish Biol.* **77**, 1745-1779.
- Priede, I. G. (1985). Metabolic scope in fish. In *Fish Energetics: New Perspectives* (ed. P. Tyler and P. Calow), pp. 33-64. London: Croom Helm.
- Reddin, D. G. (1985). Atlantic salmon (*Salmo salar*) on and east of the Grand Bank. *J. Northw. Atl. Fish. Sci.* **6**, 157-164.
- Remen, M., Solstorm, F., Bui, S., Klebert, P., Vågseth, T., Solstorm, D., Hvas, M. and Oppedal, F. (2016). Critical swimming speed in groups of Atlantic salmon *Salmo salar*. *Aq. Env. Int.* In press.
- Rome, L. C. (1990). Influence of temperature on muscle recruitment and muscle function in vivo. *Am. J. Physiol.* **28**, R210-R222.
- Rome, L. C., Sosnicki, A. A. and Choi, I. (1992). The influence of temperature on muscle function in the fast swimming scup. I. Shortening velocity and muscle recruitment during swimming. *J. Exp. Biol.* **163**, 259-279.
- Roots, B. I. (1968). Phospholipids of goldfish (*Carassius auratus* L.) brain: the influence of environmental temperature. *Camp. Biochem. Physiol.* **25**, 457-466.
- Scholander, P. F., Flagg, W., Walters, V. and Irving, L. (1953). Climatic adaptation in arctic and tropical poikilotherms. *Physiol. Zool.* **26**, 67-92.
- Sidell, B. D. and Moerland, T. S. (1989). Effects of temperature on muscular function and locomotory performance in teleost fish. *Adv. Comp. Env. Physiol.* **5**, 115-155.
- Sisson, J. E. and Sidell, B. D. (1987). Effect of thermal acclimation on muscle fiber recruitment of swimming striped bass (*Morone saxatilis*). *Physiol. Zool.* **60**, 310-320.
- Somero, G. N. and DeVries, A. L. (1967). Temperature tolerance of some Antarctic fishes. *Science* **156**, 257-258.
- Somero, G. N. and Hochachka, P. W. (1971). Biochemical adaptation to the environment. *Am. Zool.* **11**, 159-167.
- Taylor, S. E., Egginton, S. and Taylor, E. W. (1996). Seasonal temperature acclimatisation of rainbow trout: cardiovascular and morphometric influences on maximum sustainable exercise level. *J. Exp. Biol.* **199**, 835-845.
- Tudorache, C., Vianen, P., Blust, R. and De Boeck, G. (2007). Longer flumes increase critical swimming speeds by increasing burst-glide swimming duration in carp *Cyprinus carpio*. *J. Fish Biol.* **71**, 1630-1638.
- Valiente, A. G., Juanes, F. and Garcia-Vazquez, E. (2004). Effects of global warming on Atlantic salmon sea-run timing at the southern edge of the European distribution. ICES CM/S:06. Copenhagen: ICES.
- Videler, J. J. (1993). *Fish Swimming*. London: Chapman and Hall.
- Wang, T., Lefevre, S., Iversen, N. K., Findorf, I., Buchanan, R. and McKenzie, D. J. (2014). Anaemia only causes a small reduction in the upper critical temperature of sea bass: is oxygen delivery the limiting factor for tolerance of acute warming in fishes? *J. Exp. Biol.* **217**, 4275-4278.
- Wood, C. M., Turner, J. D. and Graham, M. S. (1983). Why do fish die after severe exercise? *J. Fish. Biol.* **22**, 189-201.