Vulnerability of Atlantic salmon to climate warming across the life cycle

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Abstract

Climate change is inducing more frequent and severe heat waves than organisms have encountered in recent evolutionary history. Predicting the consequences of extreme temperatures for biodiversity depends on understanding how thermal tolerance varies within species, yet this is limited by a lack of empirical data across all life stages, particularly those predicted to be most vulnerable: embryos and the reproductive stage. We measured acute upper thermal tolerance limits across all life stages (embryo to spawning adult) and both sexes of Atlantic salmon (*Salmo salar*) acclimated to a common temperature. Thermal tolerance was lowest in embryos, followed by spawning adults, and highest in intermediate life stages, with variation of ~6°C across the life cycle, and no sex differences for any life stage. However, comparing tolerance to freshwater habitat temperature records revealed that juveniles and adults on their spawning migration have the smallest thermal safety margins and therefore highest vulnerability to temperature extremes, while early life stages have the largest thermal safety margins. Our findings indicate that further warming of rivers poses a severe risk to river-bound juvenile and migrating spawner life stages of Atlantic salmon, suggesting targeted conservation actions may become necessary for the persistence of some populations.

Main Text

Introduction

Ongoing climatic warming is reshaping species distributions and the structure and function of ecological communities (1, 2). Heat waves are already leading to mass mortality in many aquatic animal species (e.g., 3, 4), and climate change is predicted to increase both their frequency and severity (5–7). Predicting the consequences of warming for a species requires an understanding of its thermal tolerance limits and the environmental temperatures that it encounters throughout its life cycle (8).

Migratory and anadromous species, such as Atlantic salmon (*Salmo salar*), can be particularly vulnerable to rising temperatures as they move between different habitats that differ in thermal stability (9). For example, salmon may face heightened thermal stress during critical life stages such as juvenile development, migration, and spawning, which occur in rivers, while temperatures during growth at sea tend to be more stable, and cooler deep water is more accessible. Further, Atlantic salmon inhabit high latitudes, which are experiencing the fastest rates of warming (6, 10), and in parts of their range high river temperatures are already inducing thermoregulatory behavior and mortality (11, 12). Understanding the limits of thermal performance in this species is increasingly a priority due to the diminishing of stocks (e.g., 13) and its importance for cultural traditions, ecosystem services, and for recreational and commercial fisheries (14, 15).

Thermal tolerance limits, which provide boundaries outside of which organisms are unable to survive (16), may vary dramatically across the life cycle (17–20). In Atlantic salmon, empirical data on thermal tolerance covering the full life cycle are critically lacking. Embryos and spawning adults are predicted to be most vulnerable to warming, with reported upper temperature thresholds (defined using a variety of methods) of 16.0°C and 7.6°C (the latter imputed from phylogenetic modelling), respectively, compared to 28.0°C for larvae and 32.0°C for immature adults (17). Yet the imputed upper temperature threshold for spawners is likely unrealistic, as Atlantic salmon on their spawning migration typically ascend rivers during spring and summer while maturation is ongoing (21, 22). During this migration, maturing salmon experience a wide range of water temperatures often exceeding the suggested 7.6°C limit (23).

In related salmonid species, knowledge of upper thermal tolerance limits for alevins (yolk-sac larvae), smolts (migrating juveniles) and marine life stages is also extremely limited, with a complete absence of data for fully mature adults. Available data (consisting of different endpoints of tolerance) suggests that thermal tolerance may increase from embryos to fry (9), as predicted by Dahlke et al. (17). However, with the transition from fry to sexually maturing adults, the trend may differ, with thermal tolerance decreasing, increasing, or showing minimal change (9). Interestingly, for Chilko sockeye salmon (*Oncorhynchus nerka*), the thermal tolerance window may be widest for

migrating adults (9). This variability complicates predictions of climate vulnerability and highlights the need for comprehensive, life-stage specific data.

Understanding the vulnerability of spawners and migrating adult salmonids is critical to predicting population persistence, particularly as climate change is exacerbating river heat waves during spawning migrations. Spawning females are expected to show greater vulnerability to warming than males, given the higher oxygen and energetic costs of maintaining a larger gonadal mass (20, 24). Yet data on sex-specific thermal tolerance in fishes is variable. For example, no differences in acute upper thermal tolerance were observed between mature female and male zebrafish (*Danio rerio*) (25). Indirect evidence nevertheless suggests that female salmonids can be more vulnerable than males—mortality in some migrating Pacific salmon, such as sockeye, is higher in females, although this pattern is not observed in pink salmon (24, 26). Whether thermal tolerance differs between the sexes in Atlantic salmon, however, remains unknown for most life stages.

To (i) investigate how thermal tolerance varies across the life cycle of Atlantic salmon and (ii) identify life stages that may be particularly vulnerable to heat waves, we measured the acute upper thermal tolerance limits from embryo to spawning stage, using a consistent acclimation temperature and critical thermal maxima (CT_{max}) methodology. Life stages we compared were: embryos; larvae (hereafter alevins); juveniles at different ages and thermal conditions, including fry after first feeding, first-summer old and 1-year old (hereafter 0+ and 1+ parr); migratory juveniles (hereafter smolts); as well as individuals phenotypically similar to salmon at sea (post-smolts); individuals maturing the same year as they smoltified (hereafter pre-grilse); and mature spawners. For post-smolts, pre-grilse and spawners, we applied two different warming rates, and also measured their internal temperature lag (thermal inertia) to avoid overestimation of CT_{max} due to thermal inertia of large-sized individuals (27). Salmon from every life stage were also sexed, allowing the most comprehensive assessment of the influence of sex on thermal tolerance across the life cycle of a fish. Finally, CT_{max} measurements were compared with temperatures measured over six decades in the river system with the largest salmon population in the Baltic Sea, River Tornionjoki, to gain insight into the thermal safety margins (the difference between a species' thermal tolerance and the temperature it experiences in nature) for all measured life stages. Based on the literature, we predicted that early (embryo) and late (spawning) life stages would have lower CT_{max} values due to physiological differences between the life stages (17, 20). Additionally, we predicted that intermediate (fry, parr, smolt) and late (spawner) life stages would be the most vulnerable to climate change-driven acute warming events as these life stages are most likely to occur in environments that may already be reaching temperatures close to their thermal limits.

Results

Thermal inertia of post-smolt and spawner salmon

At fast (0.3°C min^{-1}; 18°C h^{-1}) and medium (3.6°C h^{-1}) warming rates, there was a lag between tissue temperature and water temperature for both post-smolts (Figs. 1A, 1B) and spawners (Figs. 1C, 1D, 1E). At the fast warming rate, there was a 10–15 min lag before the internal body temperature of post-smolts reached the same rate of warming as the water, producing approximately a 1–2°C temperature differential between the dorsal muscle and the water (Figs. 1B, S2A, S2B) and approximately a 1–1.4°C temperature differential between the distal intestine and the water (Figs. 1A, S2A, S2B). For spawners, however, the rate of warming of the body was still increasing, with a temperature differential >7°C for some individuals at the fast warming rate (Figs. 1C, 1D, S2E, S2F), and a 1–2.6°C temperature differential after 1.5 h at the medium warming rate (Figs. 1C, 1D, S2D). Similarly, the biologger implanted in the intraperitoneal cavity indicated that at the fast warming rate (18°C h^{-1}), there was a delay of approximately 30 min before the core temperature and water temperature were warming at the same rate, generating a ~5°C temperature differential (Fig. 1C). At the slow warming rate (1°C h^{-1}), the dorsal muscle temperature and water temperature were in equilibrium (Figs. 1D, S2C), however, there was approximately a 0.4°C temperature differential between the intraperitoneal cavity and water temperatures (accounting for a 0.2°C difference at the start of the trial) after 1.5 h (Fig. 1E).

Ontogenetic variation in thermal tolerance

CT_{max} varied significantly across the life cycle of salmon, with a difference of ~6°C between the least and most thermally tolerant life stages. The CT_{max} of embryos (22.47 ± 0.22°C) was significantly lower than for all other life stages (all $p<0.001$; Fig. 2, Table S3, S5, S6). The highest thermal tolerance was observed for 0+ parr (27.90 ± 0.04°C), post-smolt (27.89 ± 0.06°C [1°C h^{-1}] and 28.47 ± 0.08°C [18°C h^{-1}]) and pre-grilse (27.43 ± 0.16°C [1°C h^{-1}] and 28.68 ± 0.15°C [18°C h^{-1}]) salmon (Fig. 2; Table S3, S5, S6); although at the fast warming rate there was no significant difference between the CT_{max} of 0+ parr and spawners (27.24 ± 0.13°C; $p=0.295$; Fig. 2, Table S5).

CT_{max} measured at the fast (18°C h^{-1}) compared to the slow (1°C h^{-1}) warming rate was slightly higher for all three life stages that were tested at both warming rates. CT_{max} of post-smolts warmed at 18°C h^{-1} was 28.47 ± 0.08°C compared to 27.89 ± 0.06°C at 1°C h^{-1} ($p<0.001$); for pre-grilse, CT_{max} was 28.68 ± 0.15°C when warmed at 18°C h^{-1} compared to 27.43 ± 0.16°C at 1°C h^{-1} ($p=0.029$); and for spawners CT_{max} was 27.24 ± 0.13°C at 18°C h^{-1} compared to 25.86 ± 0.13°C at 1°C h^{-1} ($p<0.001$) (mean ± S.E.; Fig. 2; Table S3, S4).

Sex differences in thermal tolerance

There was no significant effect of sex on CT_{max} for any life stage (all $p>0.05$) (Fig. 3; Table S7).

Effect of fluctuating thermal conditions on CT_{max}

Three life stages (fry, 1+ parr and smolts) experienced two natural temperature fluctuations of +6.5°C (over ~16 h) and +9.5°C (over ~18 h) (Fig. S3) in the week prior to testing. Fry (28.03 ± 0.02°C) and 1+ parr (28.57 ± 0.06°C) were observed to have amongst the highest CT_{max} values, however these were not significantly different from similar life stages that did not experience temperature fluctuations (0+ parr, post-smolt, pre-grilse, all $p>0.05$; Table S3, S9, S10; Fig. S3). The CT_{max} of smolts (28.2 ± 0.05°C) also was not significantly different from fry ($p=0.998$), 0+ parr ($p=0.952$), post-smolts (at 18°C h^{-1} [$p=0.983$] or 1°C h^{-1} [$p=0.977$]) or pre-grilse (at 18°C h^{-1} [$p=0.743$] or 1°C h^{-1} [$p=0.065$]), but was significantly lower than for 1+ parr ($p<0.001$), which experienced the same heat wave (Table S8, S9; Fig. S3).

Thermal safety margins

To demonstrate how thermal tolerance measured at different life stages may translate to thermal safety margins for Atlantic salmon, we used a publicly available six-decade time series of measured river surface temperatures of River Tornionjoki. Between May to November, the surface temperature of River Tornionjoki varies by ~20°C (Fig. 4A). Temperatures of the warmest months have increased since the 1960s, with the 2020s so far being the warmest decade on record (Fig. 4A). As the CT_{max} of spawners at 1°C h^{-1} warming (25.86°C) was the lowest out of the life stages that occur in the river during the summer months (Fig. 2, 4C), we contrasted this temperature to the observed river temperatures. We found that for months when spawners may be found in the river prior to spawning (May–Oct; Fig. 4C), a total of 69 days came within a 3°C safety margin (water temperature ≥22.86°C), 49 of which (71%) occurred since 2000, while 236 days came within a 5°C margin (water temperature ≥20.86°C), 156 of which (66%) occurred since 2000 (Fig. 4B). Similarly for 0+ parr, 3 days came within a 3°C safety margin (water temperature ≥24.90°C), while 69 days came within a 5°C margin (water temperature ≥22.90°C), 49 of which (71%) occurred since 2000 (Fig. 4B). In comparison, embryos do not encounter days when the margin is <5°C, as their development occurs entirely at cold temperatures (Fig. 4).

Discussion

Predicting ontogenetic vulnerability to warming remains challenging, largely due to a lack of empirical data across the entire life cycle of most species (29, 30), leading to heavy reliance on data collected using different methodologies, or imputation techniques to fill substantial data gaps (17, 31). To address this problem, we assessed thermal tolerance across all life stages in Atlantic salmon, using consistent methodology and acclimation temperature. While the ontogenetic pattern in thermal tolerance broadly followed predictions, the magnitude of variation across the full life cycle was smaller than previously suggested (17). Rather than embryos and spawners representing major physiological bottlenecks that define the vulnerability of this species to climate warming, we find that juvenile and adult stages that occur in rivers during summer heat waves are more likely to represent bottlenecks under climate change.

Thermal tolerance increased from embryos to the intermediate life stages (0+ parr, post-smolt and pre-grilse), and then decreased slightly again in spawners (Fig. 2). However, the difference between the most and least thermally tolerant life stages was just 6°C, in contrast to the 24.4°C previously reported for Atlantic salmon (calculated from the life stage thresholds reported by Dahlke et al. [17]). In our study, spawners showed surprising resilience, with an upper thermal tolerance limit that was not significantly different from alevins or 0+ parr, and was just 1–2°C lower than for immature post-smolts (Fig. 2). This discrepancy between our results and previous estimates of life stage sensitivity likely reflects methodological differences. While we consistently used the CT_{max} method across all life stages, previous work combined various, potentially incomparable thermal tolerance metrics and relied on imputed values for life stages lacking empirical data (see: 31).

Given the complex life histories of salmonids, comprising different stages that exploit a variety of habitats, integration of both physiological tolerance and thermal exposure across the life cycle is needed to understand the vulnerability of populations to climate warming. While the marine environment is generally more thermally stable, river systems typically lack comparable buffering capacity and thermal refugia are more sparsely available (28, 29). River-bound life stages may therefore be particularly vulnerable to temperature extremes. The concept of thermal safety margins can be used to indicate vulnerability to heat stress (30). When comparing the life-stage-specific CT_{max} data from this study to daily river temperatures collected over six decades in River Tornionjoki, it is notable that a relatively narrow margin of <5°C and <3°C for parr, smolts, and migrating spawners is becoming increasingly common (Fig. 4). Therefore, life stages that are found in rivers are likely more vulnerable to heat waves than marine life stages, not because of lower thermal tolerance, but because river temperatures already approach, or even exceed, their upper thermal limits. For post-smolt Atlantic salmon (~300 g), the incremental thermal maximum (a chronic measure of upper thermal tolerance using 1°C day^{-1} increase from 8 to 16°C, then 0.3°C day^{-1} until morbidity or death) was reported to be ~23°C (31), with poor appetite, lower condition factor (32), and acute thermal stress responses (33) also observed to occur at this temperature in both laboratory and wild fish. Adverse effects may occur at lower temperatures, however: reduced growth efficiency is reported in post-smolts following chronic (up to one month) exposure to 20°C (34). With climate change intensifying heat waves at high latitudes (6), summer river temperatures are likely to rise further, increasing the frequency of acute stress and mortality events in wild Atlantic salmon populations. It should be noted that because our experiment was designed to test for life-stage differences and therefore used a common acclimation temperature, the estimates of thermal safety margins do not account for acclimation to the typical summer temperatures (as suggested by Desforges et al. [35]), which may lead to an underestimation of the acute thermal limits. Further, while some of the variation between spawners and other life stages studied here could be partly due to genetic differences between populations (36, 37), this has little bearing on our conclusions at the species level. All salmon in our study had experienced very similar thermal histories through their development from the embryo stage in the same hatchery, and all originated from Baltic Sea populations.

To a varying extent, ectothermic animals can increase thermal tolerance at benign temperatures by, for example, adjusting membrane fluidity and protein expression (38–40). However, as the organism reaches the limit of

acclimation capacity ('concrete ceiling'), thermal tolerance is not increased by further warm acclimation, but may decrease with the accumulation of thermal injury (39). Atlantic salmon appear to have a limited acclimation capacity. For example, fish acclimated at up to 20.9°C for two months, exhibit a CT_{max} merely ~3.5°C higher than that of fish acclimated to 6°C in our study (41), and in another study the CT_{max} of salmon was only 2.2°C higher (and below 30°C) after acclimation to 15°C compared to 8°C (42). From these studies, the acclimation response in Atlantic salmon is approximately 0.2–0.3°C per 1°C acclimation, indicating a modest acclimation capacity in this species. Likewise, high temperatures substantially reduce stress tolerance, particularly in large Atlantic salmon, which can exhibit substantial post-stress mortality even at 19°C (43). Overall, this relatively poor acclimation capacity and rigid ceiling of thermal tolerance in salmon indicates that conservation efforts should include the protection/maintenance of thermal refugia in rivers where thermal safety margins of salmon risk being exceeded with climate warming.

Thermal limits in many fishes decline with increasing exposure duration (44–46), thus CT_{max}, particularly measured under fast warming rates may overestimate tolerance to prolonged heat stress (27, 38). However, Elliot & Elliot (47), found no difference in CT_{max} of parr tested under various warming rates >1°C h^{-1}. In this study, we found significant, yet small, reductions in CT_{max} measured at a slow compared to a fast warming rate in post-smolt (0.6°C difference), pre-grilse and spawner life stages (1.3°C difference for both), where trials lasted for more than 20 versus 1.5 hours, respectively. This pattern is consistent with greater accumulation of thermal injury during the prolonged heat exposures (39). However, longer exposure to elevated temperature also increases the time available to thermally acclimate, which may be detectable within hours (40, 48) and acts in the opposite direction, thus increasing thermal tolerance (38, 49). In zebrafish, a slow ramping rate can increase CT_{max} after prior acclimation at low temperature, when individuals have a high capacity for acclimation, but the opposite occurs in warm acclimated individuals where thermal injury accumulation is higher than the capacity for further warm acclimation (49). As salmon generally have a moderate to low acclimation capacity, we do not expect acclimation to play a major role in differences detected between ramping rates.

We found large differences between water and body temperatures during warming due to substantial thermal inertia in larger fish. Similarly, Sandrelli & Gamperl (50) observed a large difference in core versus water temperature in ~800 g salmon measured at a heating rate of 10°C h^{-1} versus no difference at 2°C h^{-1}. However, the difference we observed in CT_{max} between heating rates was smaller than predicted by the lag of core body temperature, which may offer clues to the mechanisms driving CT_{max}. Assuming little acclimation occurs, if core temperature, or tissues with high inertia (muscle or inner organs) were triggering loss of equilibrium (LOE) at CT_{max}, then we would expect CT_{max} of spawners measured at the slow warming rate to be >5°C lower than when measured at the fast warming rate; however, we observed a difference of just 1.3°C in CT_{max}. In addition, post-smolt salmon had a much lower thermal inertia than spawners, but their CT_{max} was also reduced under the slow versus the fast warming rate, albeit by just 0.6°C. This suggests that LOE at CT_{max} may be primarily triggered by tissues with lower inertia than deep muscle tissue. A plausible mechanism for eliciting LOE is neural function in the brain, e.g., via limitation of oxygen supply to locomotory neurons (51–53), most likely in connection to several other mechanisms. To further understand the

limits of thermal tolerance of Atlantic salmon under ecologically relevant scenarios, more studies are needed to assess CT_{max} at slow warming rates following acclimation to warm temperatures, as well as to evaluate the physiological mechanisms underlying thermal tolerance across different warming rates.

The influence of sex on the thermal physiology of ectotherms has been largely overlooked (54). In wild ectothermic animals, there may be a weak sex effect, whereby females shift their upper thermal limits by 0.02°C more than males for each degree increase in acclimation temperature (54); however, there is mixed evidence on whether thermal tolerance differs between the sexes for fish. For example, studies on zebrafish (25), cichlids, cyprinids (55), and Atlantic salmon (31) found no effect of sex on CT_{max}, while differences have been observed in other species, such as poeciliids (55). In adult brook trout (*Salvelinus fontinalis*), differences in warm acclimation response between sexes have also been observed, with females exhibiting a higher CT_{max} than males after repeat sampling (56). In this study, we did not observe any differences between sexes in salmon for any life stage. Previous studies on Pacific salmonids have suggested higher sensitivity to environmental stressors in spawning females than males based on higher blood cortisol levels and lower maximum oxygen consumption rate (57, 58), and higher mortality has been found in female than male Sockeye salmon when migrating at higher temperatures (59). Notably, most Pacific salmonids are semelparous, in contrast to the iteroparous Atlantic salmon. It is therefore possible that performance at high temperatures is not as sex-specific in this species, which is also suggested by a similar energy loss due to migration and spawning in male and female Atlantic salmon (60). However, further studies would need to evaluate how variation in sea age, which is particularly strong in Atlantic salmon, ranging from 1 to 5 years at sea before first maturation (61), as well as repeated spawning, may affect thermal tolerance in Atlantic salmon spawners.

Conclusions

This study represents the most comprehensive and methodologically-consistent assessment of variation in thermal tolerance across the life cycle of Atlantic salmon. Our results reveal substantially less variation in acute upper thermal tolerance across the full life cycle than previously predicted. We did not find evidence that early or late life stages represent the major physiological bottlenecks previously proposed for this species. Instead, when considering that salmon rivers are already reaching lethal temperatures (>23°C), it becomes clear that the river-bound juvenile and migrating spawner life stages are most vulnerable to climate warming, with the ongoing rapid warming of northern salmon rivers posing a severe risk. Appropriately planned conservation actions, such as establishing thermal refugia, may become necessary for the persistence of some Atlantic salmon populations.

Materials and Methods

Ethical approval

The experiments were conducted under a permit from the Project Authorisation Board of Finland (ESAVI/3373/2024).

Fish acquisition and maintenance

In this study we refer to Atlantic salmon life stages using nomenclature suggested by Allan & Ritter , adapted for captive individuals reared in freshwater. The following life stages were tested: embryo; alevin (yolk-sac fry); fry (independently-feeding juvenile stage, in the first few weeks of free-swimming); 0+ parr (juveniles that have developed vertical, camouflaging stripes); 1+ parr (juvenile salmon over 1 but less than 2 years old); smolt (identified from partially silvered coloration and elongated shape in May); post-smolt (identified from silvered coloration in November after smoltifying in the spring); pre-grilse (individuals that reached maturity in the same year as smoltifying); spawner (reproductively mature adults, age 4+).

Embryo, alevin, fry and 0+ parr

For the early life stages, we used individuals from the same four families. The families were produced from crosses of a broodstock of Atlantic salmon from River Iijoki (Finland) on 12 October 2023 at the Natural Resources Institute Finland (Luke) Taivalkoski hatchery. The broodstock had hatched in 2017 and consisted of individuals, whose parents (i.e., the grandparents of the studied individuals) were hatchery-reared until smolts, then released, and later captured during their spawning migration to the river, and had therefore completed a partly natural life cycle. The eggs were incubated in family-specific trays according to standard hatchery procedures in a flow-through system at 2.4 ± 0.6°C (mean ± S.D.), using natural river water (Ohtaoja River) mixed with groundwater. On 5 February 2024, after reaching the eyed stage (approximately 285 degree-days), embryos were shocked (by shaking) and dead individuals removed, before being transferred to Luke Laukaa fish research facilities on 8 February 2024. Approximately 500 individuals from each of the four families were placed into their own family-specific tray with flow-through water from Lake Peurunkajärvi at 1.8 ± 0.1°C (9–15.02.24), and incubated in the dark. Embryos were staged on 9 February 2024 and were determined to be in the first stage of the "Formation of caudal rays" subperiod, as defined by Gorodilov (62, 63), based on the completion of vascularization of the yolk sac, blood circulating through four pairs of gill arteries, blood in the caudal artery reaching the terminal segments and extending towards the posterior end of the notochord, appearance of the first melanophores on the dorsal surface of the head in some, but not all, individuals, and absence of caudal fin rays. Starting on 15 February 2024, the holding temperature of the embryos was incrementally increased (approximately 0.5°C per day) to 6°C (22 February 2024) and maintained at this temperature until 15 May 2024 (6.12 ± 0.44°C; Table 1). Two natural temperature fluctuations occurred due to water mixing in Lake Peurunkajärvi, which raised water temperature by +6.5°C over ~16 h on 15 May and +9.5°C over ~18 h on 19 May; water temperature between these two fluctuations returned to 7.1 ± 0.6°C (range = 6.0–8.5°C).

After the yolk sac had been absorbed (12 April), the fry were moved from the trays into family-specific rearing tanks (80 cm diameter) and feeding was initiated using automatic belt feeders, which provided small amounts of food (BioMar, Aarhus, Denmark) constantly for approximately 12 h each day. Feed rations were calculated assuming feed conversion efficiency of 0.8, using growth predictions by Elliot & Hurley (64), and adjusted so a small amount of uneaten food always remained in the tank, indicating satiation. Tanks were cleaned five days a week, removing dead individuals and uneaten food. Fish were reared under light regime natural to the site of experiments, and under constant light (in line with the naturally-long daylight period) from first feeding until the trials on fry (22 May). Fish were then maintained over summer following standard hatchery procedures with natural fluctuation in water temperature until 5 Nov, when water temperature was again maintained at 6°C.

1+ parr, smolt, post-smolt and pre-grilse

The 1+ parr, smolts and post-smolts used in this study originated from a single family crossed in 2022 using similar methodology and the same population of origin to the early life-stages (described above), apart from the age of parental broodstock, which had hatched in 2013. The offspring were reared in similar conditions to the early life stages with natural fluctuation in water temperature. On 9 Jan 2024, they were moved to a 7 m^2 tank, where they were reared under a natural light cycle and fed a mixture of 1.2 mm (Alltech Fennoaqua, Raisio, Finland) and 1.5 mm (BioMar) feed. Individuals were then moved from the large tank to be acclimated to 6°C in the same rearing tanks used for the fry on 13 March 2024, 69 d before the CT_{max} trials in the spring (1+ parr and smolts), and on 15 October 2024, 30 d before trials in the autumn (post-smolts). As these acclimations in the spring were done at the same time as the acclimations of fry, the acclimation temperatures and durations are similar across these life stages (Table 1).

Spawners

Given that salmon take several years to reach maturation, spawners from the same cohorts or population of other life stages could not be used in this study. Instead, spawners in this study came from a broodstock, whose parents were captured from River Kymijoki, Finland, in 2019. Gametes were collected from the parents (23 females and 28 males) at Luke Keminmaa hatchery on 1 Nov 2019 following standard practices for broodstocks, maximizing the number of full-sib families in the cohort. Individuals were reared with standard hatchery practices using flow-through river/lake water first at Luke Keminmaa until the eyed egg stage, and then transported to Luke Laukaa, where they were reared until adulthood, and PIT-tagged for individual identification. On 3 Nov 2024, at spawning time, the adults were weighed, measured, and mature individuals were sexed (by appearance). A total of 40 mature individuals (20 females and 20 males) were selected for CT_{max} trials and separated from the other fish with fences. The temperature in the rearing tank naturally decreased during the 10 days prior to the CT_{max} trials (range = 4.9–7.1°C; 30/10/24–08/11/24; Table 1). It was not feasible to maintain a steady temperature of exactly 6°C for the spawners due to the high demand of water in the flowthrough system, but the natural decrease in temperature provided the opportunity to test if the spawners showed the hypothesized decrease in CT_{max} compared to other life stages at a similar acclimation temperature.

Thermal inertia of post-smolt and spawner salmon

To measure the difference between the water and the internal body temperature of larger fish (thermal inertia), temperature sensors (thermocouples) were implanted into the dorsal muscle and distal intestine of anaesthetized post-smolts and spawners (Table S1). Two thermocouples were also placed in the water. Thermal ramping was then conducted at a rate of 18°C h^{-1} (post-smolts and spawners), 3.6°C h^{-1} (spawners), or 1°C h^{-1} (spawners) and temperature was recorded at 2 sec intervals for the duration of the trials. These estimates of thermal inertia were used to select a slow ramping rate (1°C h^{-1}), at which it was predicted that the core tissues would remain in thermal equilibrium with the water temperature (27). All fish were fully anaesthetized with 100 mg L^{-1} sodium bicarbonate-buffered tricaine methanesulfonate (MS-222) and were maintained on forced ventilation using 5 L min^{-1} submersible pumps (Eheim) for the duration of the implantation surgery and trials. Immediately following the trial all individuals were euthanized (i.e., they were not recovered from anesthesia).

Thermal inertia of one spawner was also tested without anesthesia using a micro-ACT biologger (Star-Oddi, Gardabaer, Iceland) surgically inserted into the intraperitoneal cavity of a female salmon (weight = 1718 g, length = 579 mm; Table S2). Fish remained under anesthesia for the duration of the implantation (initially 150 mg L^{-1}, then 75 mg L^{-1} during surgery). The biologger was inserted through a 2 cm wound which was closed with two sutures. The biologger was programmed to record temperature at 1 min intervals. The individual was then released in a tank that was warmed at a rate of 1°C h^{-1} for 4 h before being returned to holding temperature, then, the following day, warmed at 18°C h^{-1} for 1h. Both trials were conducted within one day from the implantation. The fish was euthanized with an overdose of anesthetic and the biologger recovered immediately after the trial.

CT_{max} methods

Acute upper thermal tolerance was measured using critical thermal maximum (CT_{max}) methodology. All CT_{max} trials were conducted in normoxic water (approximately 100% air saturation) and began close to the holding temperature. All life stages were tested at a heating rate of 0.3°C min^{-1}, with additional trials using different individuals conducted at a rate of 1°C h^{-1} for post-smolts, pre-grilse and spawners (Table S3). Temperature and water oxygen content in the test arena were continuously recorded using a robust fiber-optic oxygen sensor (OXROB-10) and a temperature sensor (PT100) connected to an optical oxygen and temperature meter (FireSting-O2, PyroScience GmbH, Aachen, Germany). Upon reaching CT_{max}, individuals (with the exception of embryos, which were not removed from the test arena until all individuals had reached CT_{max} - described below) were removed from the test arena and placed into water at holding temperature to recover. Recovery and survival were recorded after 1 h (all life stages), then all individuals were euthanized with an overdose of MS-222 (250 mg L^{-1}) followed by insertion of a scalpel through the brain.

Embryos and alevins

Each trial of embryos and alevins consisted of individuals from the same family (i.e., siblings) of the same life stage. The CT_{max} setup consisted of a glass dish (12 cm diameter; the test arena) filled with 150 (embryos) or 200 (alevins) mL water, and supplied with air bubbling through a blunted hypodermic needle (embryos) or thin plastic tube (alevins) (Fig S1). A grid was fixed to the bottom of the glass dish during embryo trials. The test arena was placed in a larger glass bowl (17 cm diameter), which formed a flow-through heating mantle, with water pumped in (via an Eheim Universal 600 L h^{-1}) from a water bath (27 L) on one side of the bowl, and a lip allowing the water to flow out from the bowl on the other side into a surrounding tray (23 cm diameter), which had an outflow back into the water bath. The test arena walls were elevated above the glass bowl so that no water from the heating mantle entered the test arena. Water temperature in the test arena was adjusted by turning on or off heaters in the water bath.

CT_{max} was measured in groups of embryos (15 per group; total=105), following the method described by Cowan et al. (65), where CT_{max} was defined as the temperature of the last movement for each embryo. Individual embryos were placed in wells in the test arena (Fig. S1) and allowed to habituate for 5 min at holding temperature, after which thermal ramping began. Trials were recorded via Samsung Galaxy XCover6 Pro and embryos were additionally continuously visually monitored. The trial ended when no movements were observed in any individual within the trial for 5 min, however final movements were extracted from the video recordings. At the end of the trial, all embryos were removed from the test arena and placed into recovery for 1 h at holding temperature; recovery was 100% after 1 h. Finally, all individuals were euthanized and a small sample (approximately the posterior third of the embryo) was frozen at -70ºC for later genotyping to determine sex.

CT_{max} was measured in groups of alevins (10 per group; 120 total) that were approximately 14 days post-hatching. Groups of alevins were added to the test arena (Fig. S1C,E) at holding temperature and allowed to habituate for 5 min, after which the trial began. Individuals were continuously monitored and CT_{max} was defined as the temperature at which individuals did not respond to 3 consecutive touches that were 3 sec apart (51, 65). Touches were applied to the trunk of the alevin using the tip of a small zip tie. Recovery was 100% after 1 h. Following euthanasia, a caudal sample was frozen at -70ºC for later genotyping to determine sex.

Fry, parr, smolts, post-smolts, pre-grilse and spawners

CT_{max} of fry, 1+ parr and smolts was tested in the spring on 21–22 May. However, two natural temperature fluctuations occurred due to water mixing in Lake Peurunkajärvi, which raised water temperature by +6.5°C (over ~16 h) and +9.5°C (over ~18 h) in the week prior to testing. This may have increased the CT_{max} in the subsequent trials, as it is known that heat shocks can increase CT_{max} (66). We therefore ran further trials in the autumn (after water temperature in the tanks had naturally declined to 6°C; Table 1) on 0+ parr (the same cohort as fry), post-smolts and pre-grilse (both the same cohort as 1+ parr and smolts). Two additional CT_{max} trials were conducted on 0+ parr that followed the naturally declining water temperature, similar to the spawners. These trials were conducted on the same day as the spawners to assess if the slight differences in timing and acclimation temperature affected CT_{max}. The CT_{max} of these

0+ parr measured after stable versus naturally declining acclimation temperature did not differ, and thus they were combined as one life stage in the analysis.

For fry, parr, smolts, post-smolts, pre-grilse and spawners, CT_{max} was measured in groups following an established protocol (27), where CT_{max} was defined as the temperature at which individuals exhibited loss of equilibrium (LOE) for 3 sec. The groups consisted of 6–16 individuals, depending on life-stage and test arena size: 15–16 per group, total = 120 for fry; 10–12 per group for 1+ parr and smolts, tested together in mixed groups in May, total=19 parr and 13 smolts; 12 per group for 0+ parr tested in November, total=60; 6–9 per group for post-smolts and pre-grilse, tested together in mixed groups, total=35 post-smolts and 4 pre-grilse; 8 per group for spawners, total=40). Groups were added to the test arena at holding temperature and allowed to habituate (10 min for fry, 0+ parr and smolts; 30 min for 1+ parr, post-smolts and pre-grilse; overnight [14–19 h] for spawners), after which thermal ramping began. A longer habituation period was used for the spawners due to the unavoidably longer duration of stress associated with capture and transport to the test tank (approx. 30-60 min), which would likely elicit a stronger stress response than the comparatively quick dip-netting for the other life stages. Recovery was 100% for fry, 100% for parr, 100% for smolts, 97% for post-smolts, 75% for pre-grilse and 95% for spawners. Following euthanasia, a sample (caudal fin clip) was collected from fry, which was frozen at -70ºC for later genotyping to determine sex. Parr, smolts and post-smolts were dissected and gonads visually inspected to determine sex.

For fry, the CT_{max} setup consisted of a Styrofoam water bath filled with 12 L water, connected to a Styrofoam test arena filled with 12 L water (total volume of 24 L in system). For parr and smolts, the CT_{max} setup consisted of the same Styrofoam water bath filled with 15 L water, connected to a Styrofoam test arena filled with 15 L water (total volume of 30 L in system). Two water pumps were placed inside the water bath (Eheim Universal 300 L h^{-1} for mixing and Eheim Universal 600 L h^{-1} for pumping water to the test arena). Oxygenation was maintained via two airstones in the water bath. Heating was via 300 W heaters in the water bath (four for fry, five for parr and smolts), which were turned on or off to maintain a steady heating rate.

The volume of the test arena for post-smolts and pre-grilse was 200 L, and for spawners was 300 L. To achieve the fast heating rate (0.3°C min^{-1}), water from the test arena was pumped (Eheim Universal 600 L h^{-1}) through heating coils in a 1000 L water bath, which was pre-heated via two 2.5 kW heaters, and back into the test arena. The slow heating rate (1°C h^{-1}) was achieved by 2–4 300W heaters placed directly in the test arena, which were manually switched on and off. Four water pumps (Eheim Universal 300 L h^{-1}) ensured mixing in the test arena and oxygenation was maintained via 2–4 airstones and one air stone connected to an oxygen tank that was switched on or off depending on water oxygenation (maintained at 96.95 ± 9.16% AS [1°C h^{-1}] and 102.05 ± 10.88% AS [0.3°C min^{-1}], mean ± S.D.).

Sex determination

DNA from samples of early life stages (embryos, alevins, and fry) was extracted using QuickExtract DNA extraction solution (Lucigen, Middleton, WI, U.S.A) and diluted 20x in water. Individuals were sexed based on a genetic marker (SDY), as in Sinclair-Waters et al. (67), using BioRad CFX qPCR instrument at Luke Viikki laboratory, Helsinki, Finland. Genotypic sex was determined by the visual clustering of fluorescence signals. Parr, smolts and post-smolts were dissected and gonads visually inspected to determine sex. Spawners' sex and sexual maturity was verified based on presence of eggs or milt during length and weight measurements.

River temperatures

Data on surface temperatures of River Tornionjoki, which has the largest salmon stock in the Baltic sea area (36), were downloaded from open environmental information systems of the Finnish Environmental Institute (hydrological observations, Syke & Economic Development Center). The data were collected from Kukkolankoski (65°58'40.428"N, 24°03'20.834"E [WGS84]) during non-winter seasons (when there was no ice cover) between August 1960–September 2025. The dataset consisted of once daily measurements of water temperature, taken at approximately 20 cm depth near the shore in a location where water is well mixed. Time of day for the measurements is unknown. Daily variation in river temperature is generally not strong in this system (e.g., in 2023, daily variation during summer months was 1.3 ± 0.8°C (mean ± S.D.) and maximum variation was 3.5°C [J. Prokkola, unpublished observation]). To illustrate trends in river temperature through time, we plotted daily surface temperature for each year, with averages taken across decades (1960–1969, 1970–1979, 1980–1989, 1990–1999, 2000–2009, 2010–2019, 2020–2025). To estimate thermal vulnerability of salmon through time, we counted the number of days within each of these decades that were within 3°C and 5°C of the CT_{max} of each life stage (3°C and 5°C thermal safety margins), for months where each life stage is present in the river.

Data analysis

Microsoft 365 Copilot (M365 Copilot), powered by GPT-5, was used to support data analysis through troubleshooting and code development. All resulting code was reviewed, tested, and verified before final implementation in the reported analyses.

Statistical analyses were performed using RStudio, version 2024.12.1.563 (68) (R, version 4.4.3 (69)). The significance of effects was considered at the significance level $\alpha = 0.05$. Model assumptions were visually assessed using residual plots, as well as tested using the *DHARMa* package (70).

The effect of warming rate (1°C h^{-1} or 18°C h^{-1}) on CT_{max} in post-smolt, pre-grilse, and spawner salmon was assessed using linear models. A separate model was run for each life stage (post-smolt, pre-grilse and spawner), with CT_{max} as the response variable and ramping rate as a fixed effect.

Ontogenetic differences in CT_{max} were assessed using linear mixed-effects models (*nlme* package (71)). The models included CT_{max} as the response variable, life stage as a fixed effect, a unique trial identifier as a random intercept, and a variance structure allowing residual variances to differ among life stages. Two models were fitted: (1) model one compared CT_{max} across all life stages (embryo, alevin, 0+ parr, post-smolt, pre-grilse, spawner) measured at a warming rate of 18°C h^{-1}; and (2) model two compared CT_{max} across the same life stages with embryo, alevin and 0+ parr measured at a warming rate of 18°C h^{-1} and post-smolt, pre-grilse and spawner life stages measured at a warming rate of 1°C h^{-1}. The significance of the random and fixed effects was assessed using a Likelihood Ratio Test. Post-hoc pairwise comparisons of the estimated marginal means of the treatment groups were conducted using Tukey's HSD test to adjust for multiple comparisons (*emmeans* package (72)).

The effect of sex on CT_{max} was assessed using linear mixed-effects models, which included CT_{max} as the response variable, sex, life stage, and their interaction as fixed effects, and a unique trial identifier as a random intercept. A separate model was run for each heating rate and was fitted with the packages *lme4* (73) and *lmerTest* (74). Marginal and conditional R^2 values were calculated with the *MuMIn* package (75). Post-hoc pairwise comparisons of sex within each life stage were conducted using estimated marginal means using Tukey's HSD to adjust for multiple comparisons (72).

Linear mixed-effects models (*nlme* package (71)) were used to assess how exposure of three life stages (fry, 1+ parr, and smolts) to two natural temperature fluctuations in the week prior to CT_{max} testing influenced acute upper thermal tolerance. The models included CT_{max} as the response variable, life stage as a fixed effect, a unique trial identifier as a random intercept, and a variance structure allowing residual variances to differ among life stages. Two models were fitted: (1) model one compared CT_{max} across all life stages (embryo, alevin, fry, 0+ parr, 1+ parr, smolt, post-smolt, pre-grilse, spawner) measured at a warming rate of 18°C h^{-1}; and (2) model two compared CT_{max} across the same life stages with embryo, alevin, 0+ parr, 1+ parr, and smolt measured at a warming rate of 18°C h^{-1} and post-smolt, pre-grilse, and spawner life stages measured at a warming rate of 1°C h^{-1}. Post-hoc pairwise comparisons of estimated marginal means were performed using Tukey's HSD to adjust for multiple comparisons (72).

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Figures and Tables

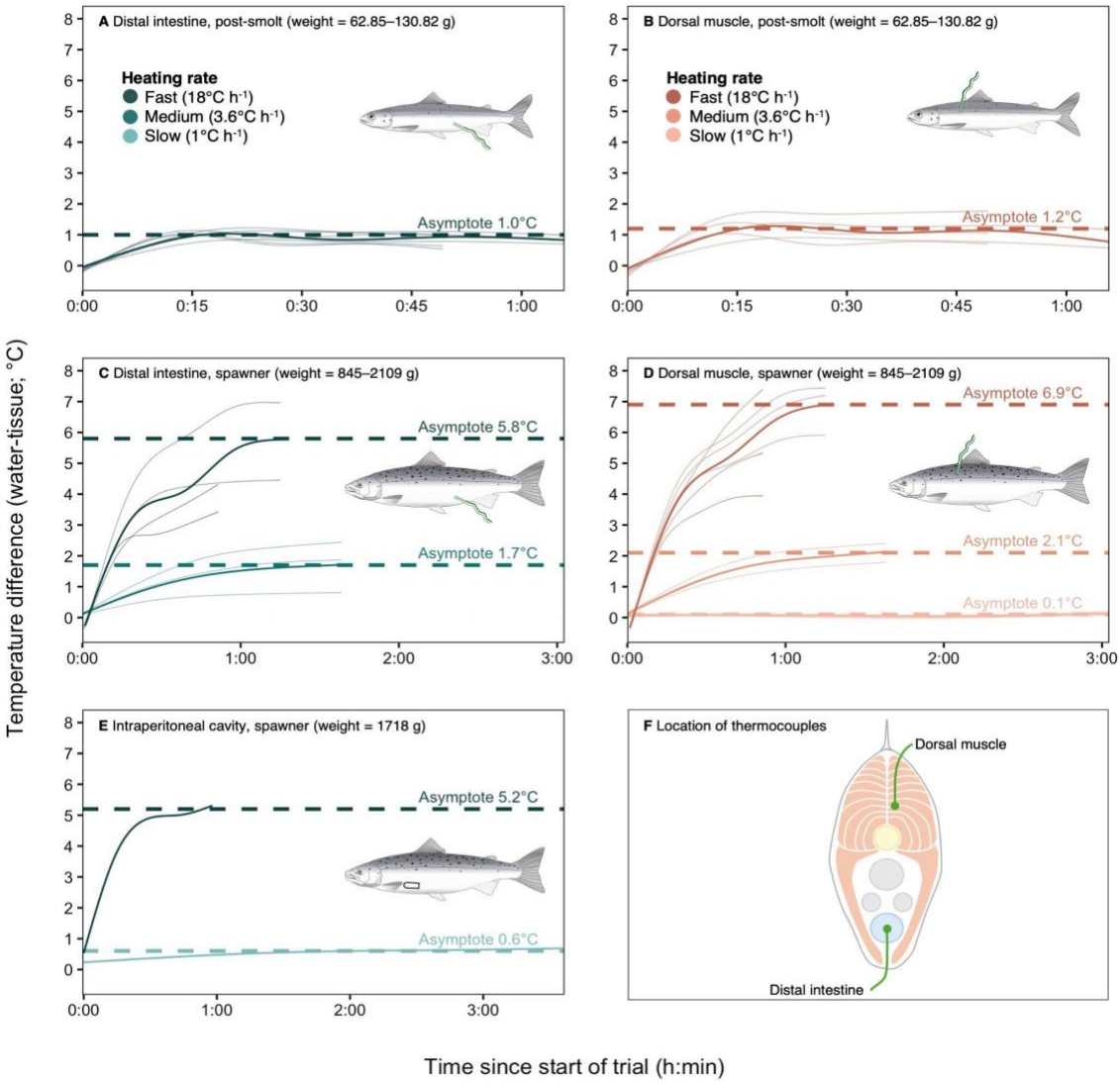
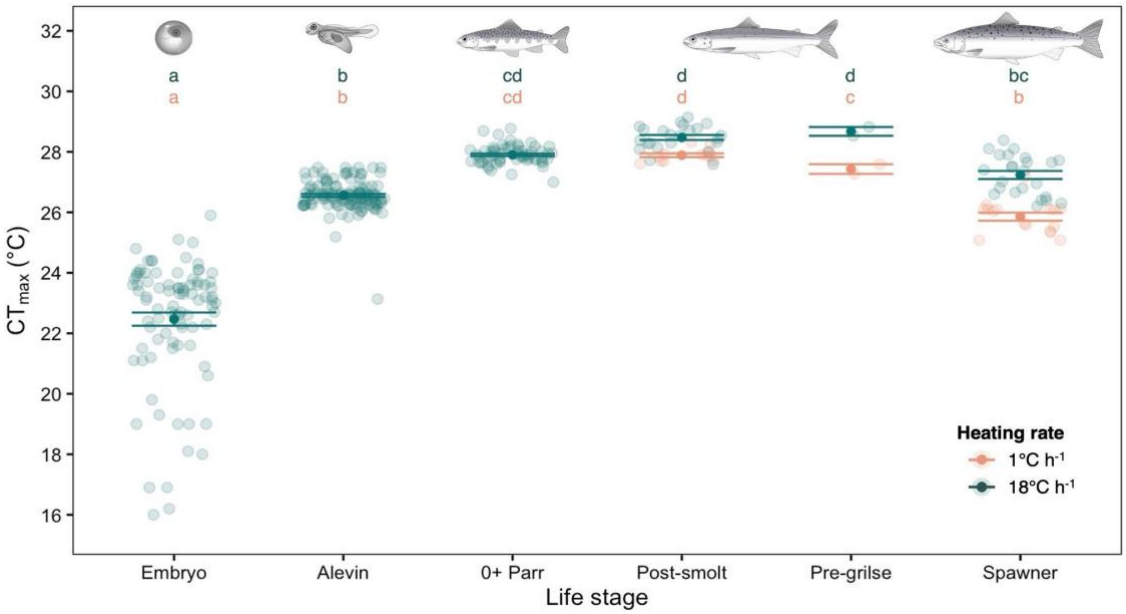


Figure 1. Thermal inertia of post-smolt and spawning stage Atlantic salmon. **A–D** Tissue temperature was measured using thermocouples implanted in the **A, C** distal intestine or **B, D** dorsal muscle of fully anaesthetized salmon. **E** Tissue temperature was measured using a micro-ACT biologger (Star-Oddi, Gardabaer, Iceland) implanted in the intraperitoneal cavity of a female salmon that was moving freely during the trial. **F** shows the location of the thermocouples inside the fish. Data on fish size and thermocouple implant depth are in Tables S2 and S3. For **A–D**, thinner lines represent generalized additive model (GAM) -smoothed measurements from individual fish and thicker lines represent the GAM-smoothed line through all measurements at each ramping rate within a life stage. Approximate asymptote lines (horizontal dashed lines) added for visualization of the plateauing temperature lag.



639

640 **Figure 2.** Acute upper thermal tolerance (CT_{max}) across life stages in Atlantic salmon (*Salmo salar*), acclimated to
641 6°C. Larger points represent CT_{max} of an individual fish; solid points and error bars show the mean CT_{max} ± S.E. for
642 each life stage. Letters indicate significant differences based on post-hoc comparisons from linear mixed models using
643 Tukey's HSD test to adjust for multiple comparisons: letters in teal (top row) at the fast ramping rate (Table S5),
644 letters in orange (bottom row) at the slow ramping rate for embryo to 0+ parr and slow ramping rate for post-smolt to
645 spawner (Table S6).

646

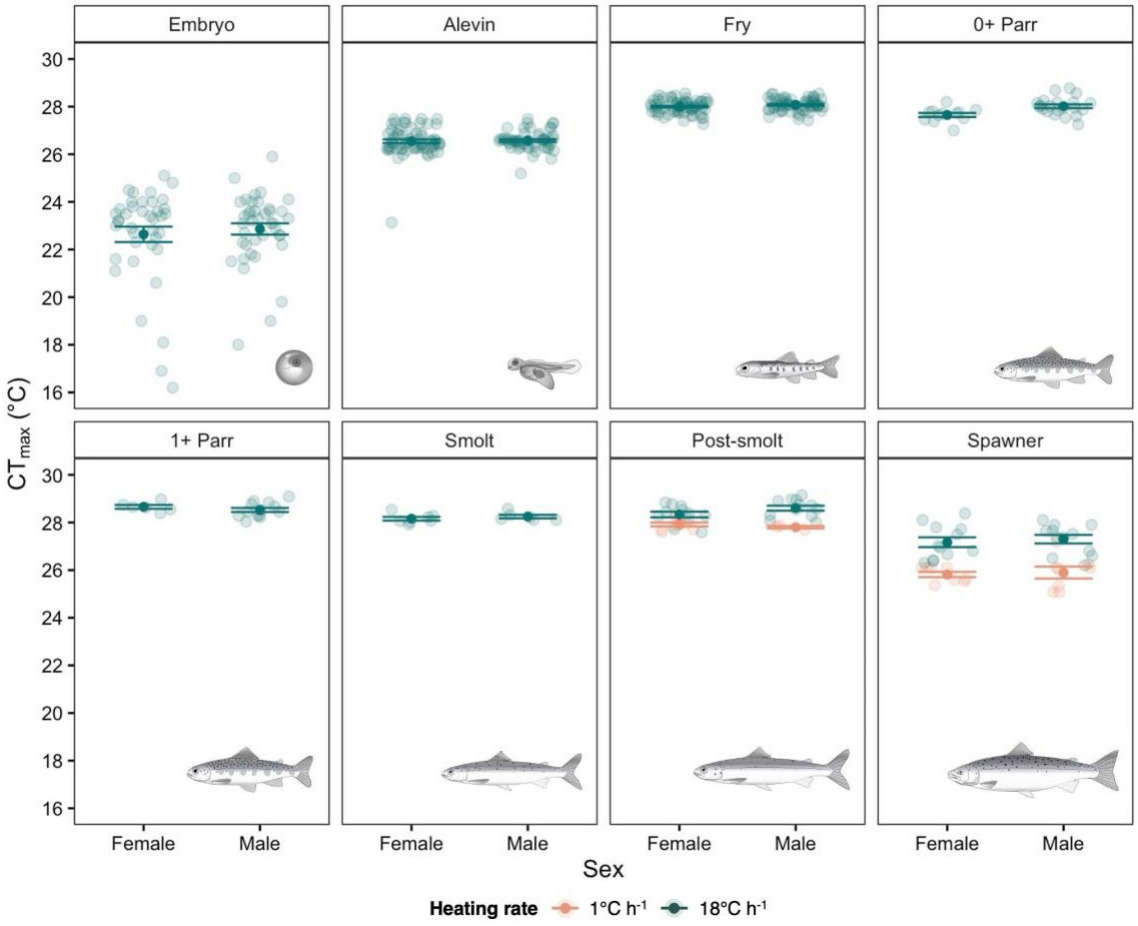
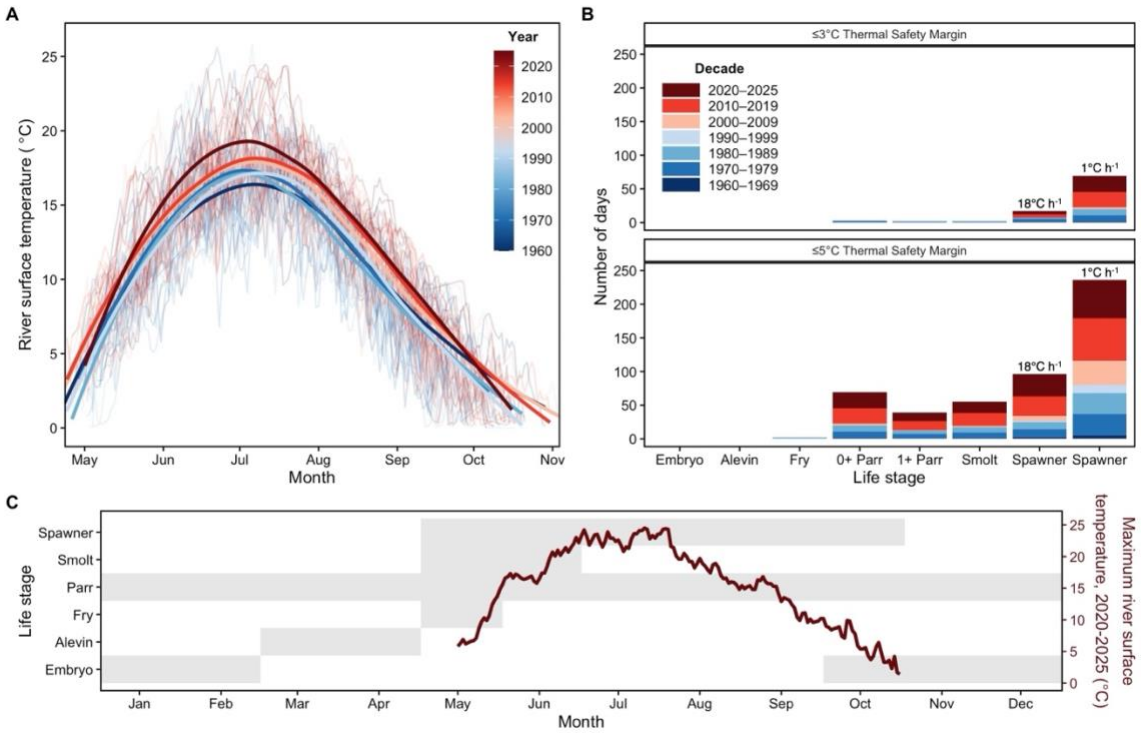


Figure 3. No effect of sex on CT_{max}. Individuals for which sex was not determined, and pre-grilse, which are only males, were excluded. Larger points represent CT_{max} of an individual fish; smaller points and error bars show the mean CT_{max} ± S.E. for each sex and life stage.



652

653 **Figure 4. A** Once daily measurements of surface temperatures of River Tornionjoki, which has the largest salmon
654 stock in the Baltic sea area (36), measured from Kukkolankoski (65°58'40.428"N, 24°03'20.834"E [WGS84]) during
655 non-winter seasons (when there was no ice cover) between August 1960–September 2025 (time of day not known;
656 source: hydrological observations, Syke & Economic Development Center, Finland). Fine lines represent daily surface
657 temperature for each year; thicker lines are plotted with loess smoothing and represent daily surface temperature,
658 averaged across decades (1960–1969, 1970–1979, 1980–1989, 1990–1999, 2000–2009, 2010–2019, 2020–2025). **B**
659 Number of days, by decade, where river temperature is within a 3 or 5°C thermal safety margin for each life stage, for
660 months when that life stage is present in the river. Note that the most recent decade contains data for just 2020–2025.
661 **C** Months during which life stages of Atlantic salmon may be present in River Tornionjoki; red line represents highest
662 of (non-winter) once daily surface temperature measurements on a given calendar day between 2020–2025. Note that
663 temperatures during the remaining months are colder than the summer temperature (i.e., 0–10°C).

664

Table 1. Acclimation conditions, warming rates, morphometric measurements, and family/age details for critical thermal maximum (CT_{max}) trials across Atlantic salmon (*Salmo salar*) life stages. Calculations of length/weight were filtered to remove NA values. Acclimation temperatures of fry, 1+ parr and smolt were impacted by two natural heatwaves, which occurred on 15.05.2024 and 19.05.2024. Weight of spawners includes gonad mass. After the trial, female spawners were stripped of eggs; stripped weight was 1180 ± 190 g for fish measured at 1°C h^{-1} and 1373 ± 281 g for fish measured at 18°C h^{-1}.

Life stage	Acclimation temperature, °C (mean ± S.D.)	Ramping rate, °C h^{-1}	Date tested	Length, mm (mean ± S.D.)	Weight, g (mean ± S.D.)	Note
Embryo	5.72±0.38[a]	18	11–12.03.24			[†] Same cohort, four families.
Alevin	5.77±0.32[b]	18	27–28.03.24	23.18 ± 1.26		[†] Same cohort, four families.
Fry	6.28±0.91[c]	18	22.05.24	29.57 ± 2.05		[†] Same cohort, four families.
0+ Parr	5.83±0.48[d]; 5.82±0.35[e]	18	7&15.11.24	96.25 ± 14.85	7.93 ± 3.35	[†] Same cohort, four families.
1+ Parr	6.27±0.91[f]	18	21.05.24	84.47 ± 7.96	4.45 ± 1.32	[‡] Same cohort, one family. Age 1+
Smolt	6.27±0.91[f]	18	21.05.24	123.85 ± 8.07	14.38 ± 2.71	[‡] Same cohort, one family. Age 1+
Post-smolt	6.15±0.62[g]	1	14.11.24	205.50 ± 25.49	74.06 ± 23.85	[‡] Same cohort, one family. Age 1+
	6.15±0.62[g]	18	14.11.24	210.35 ± 26.93	80.60 ± 29.45	[‡] Same cohort, one family. Age 1+
Pre-grilse	6.15±0.62[g]	1	14.11.24	191.50 ± 6.36	67.36 ± 7.33	[‡] Same cohort, one family. Age 1+
	6.15±0.62[g]	18	14.11.24	189.50 ± 0.71	68.34 ± 1.89	[‡] Same cohort, one family. Age 1+
Spawner	6.02±0.95[h]	1	6–7.11.24	549.75 ± 26.39	1417.69 ± 187.11	[§] Same cohort, multiple families. Age 4+
	5.90±0.96[i]	18	6–8.11.24	565.09 ± 25.55	1554.05 ± 278.32	[§] Same cohort, multiple families. Age 4+

Acclimation temperatures were recorded as follows (acclimation duration in brackets): [a] continuous measurements every 10 min with temp logger (22.02.2024–12.03.2024); [b] continuous measurements every 10 min with temp logger (22.02.2024–28.03.2024); [c] weighted average from continuous measurements every 10 min with temp logger (22.02.2024–20.05.2024) then manual readings at 19:00 on 20.05, 07:30 on 21.05 and 07:45 on 22.05; [d] holding temperature of individuals tested on 7.11.24 followed the natural seasonal decline in water temperature, with a weighted average and S.D. calculated from continuous measurements from a temperature logger every 30 min (01–05.11.24) then every hour (05–7.11.24); [e] continuous measurements from a temperature logger once per hour (05–15.11.24) for individuals tested on 15.11.24; [f] weighted average from continuous measurements every 10 min with temp logger (22.02.2024–20.05.2024) then manual readings at 17:00 and 19:00 on 20.05, and 07:30 on 21.05; [g] daily temperature recordings (30.10.24–12.11.24; no recording from 5/6/13.11.24); [h] daily temperature recordings (30.10–7.11.2024); [i] daily temperature recordings (30.10–8.11.2024).

Supplementary information

Figures

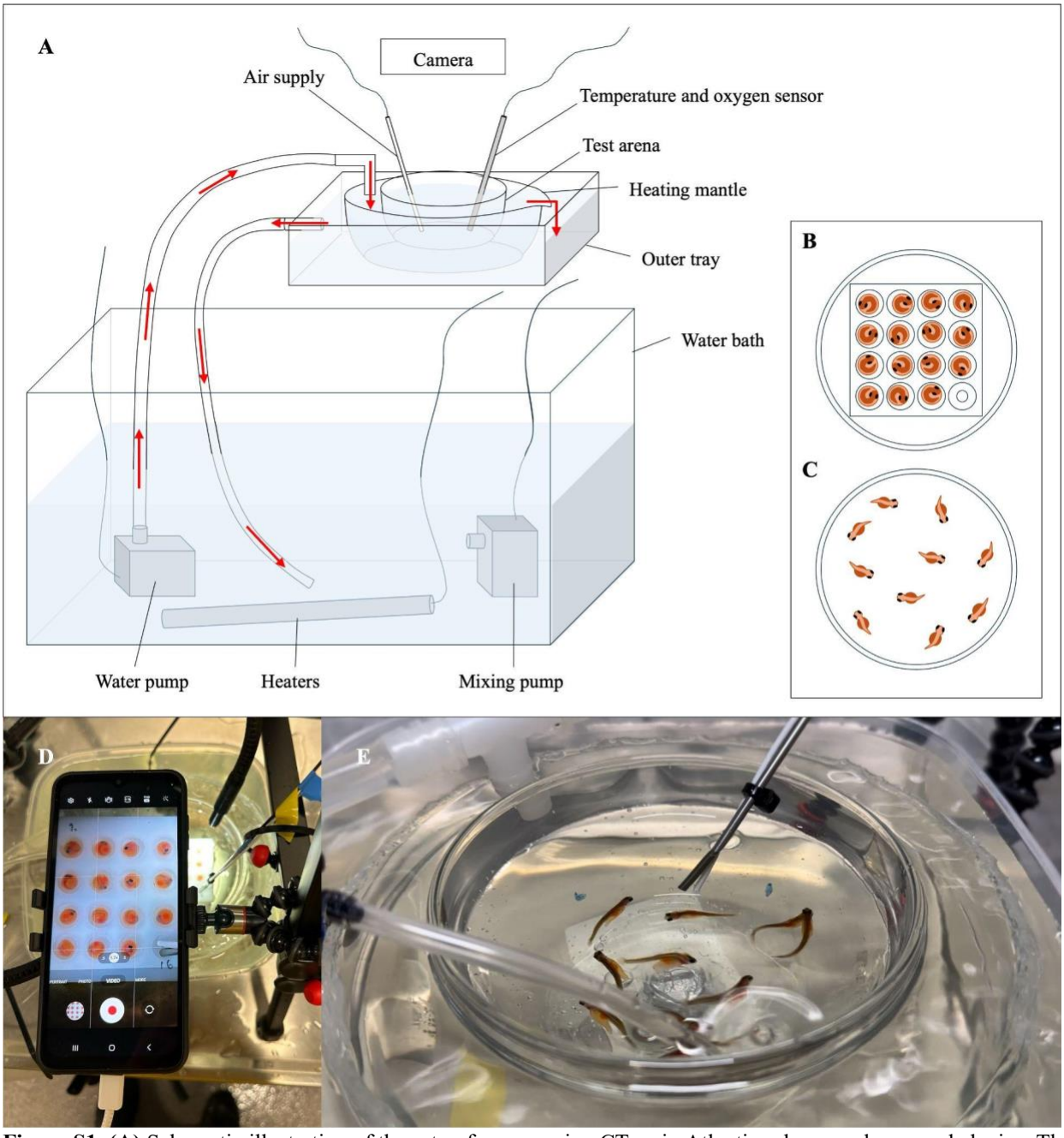
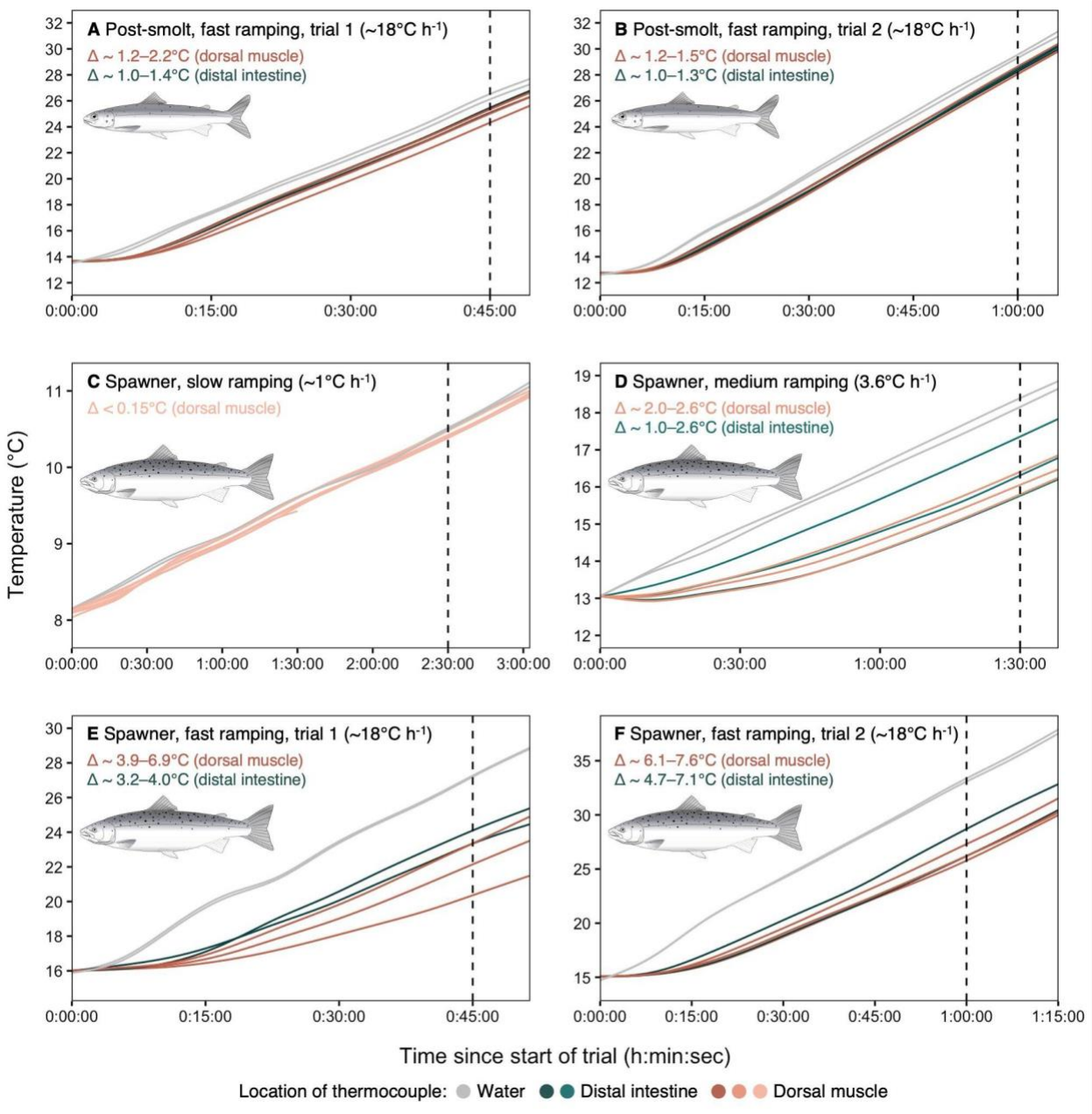


Figure S1. (A) Schematic illustration of the setup for measuring CT_{max} in Atlantic salmon embryos and alevins. The design follows Cowan et al. (2023) and is modified to accommodate the larger salmon life stages tested herein. **(B, D)** Embryos (n=15) were contained within individual, numbered wells of a tray within the test arena; a hole in the bottom of each well aided heat exchange. The temperature and oxygen sensor was positioned in one well, as shown in **(D). (C, E)** Alevins (n=10) were able to move freely within the test arena.



689

690 **Figure S2.** Thermal inertia of large (post-smolt and spawner) Atlantic salmon (*Salmon salar*). Trials **(A–F)** were
691 conducted on fully anaesthetized individuals, which were maintained during the trial on forced ventilation. **(A, B, D,**
692 **E, F)** Thermocouples were implanted in the dorsal muscle and distal intestine of 2–3 individuals; trials **(D)** and **(F)**
693 used the same 3 individuals. **(C)** Thermocouples were implanted in the dorsal muscle of 6 individuals (one was
694 dislodged at approximately 1 h 30 min). Data on fish size and thermocouple implant depth are provided in Table S2.
695 Δ = difference between internal temperature of salmon and water temperature, measured at the point marked by the
696 vertical dashed line.

697

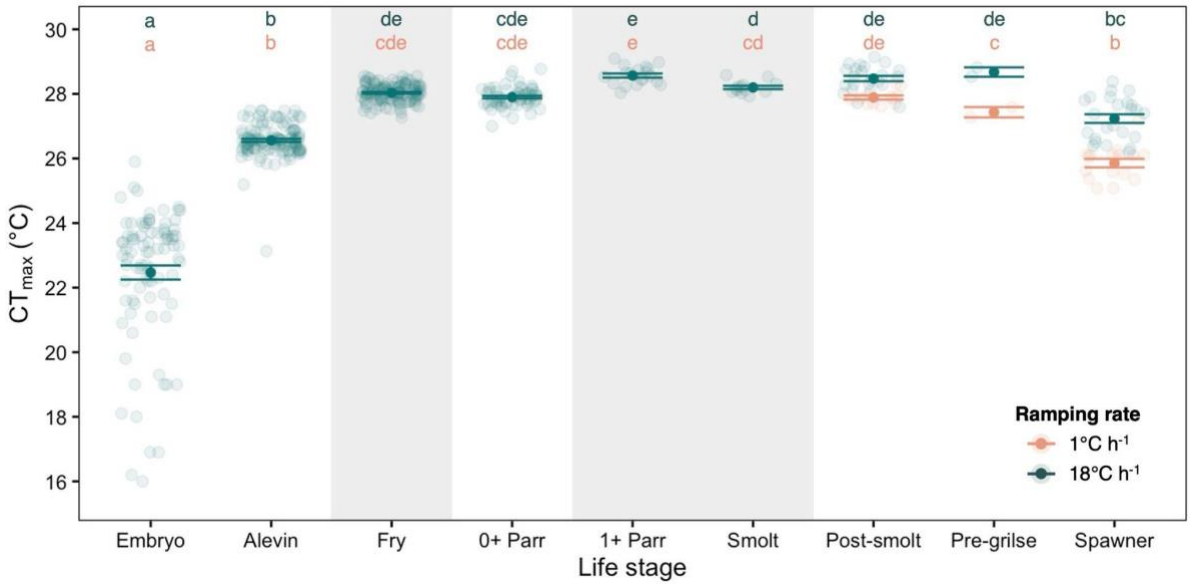


Figure S3. Acute upper thermal tolerance (CT_{max}) across life stages in Atlantic salmon (*Salmon salar*). All life stages were acclimated to 6°C, however three life stages (indicated by grey shading) were exposed to two natural temperature fluctuations of +6.5°C (over ~16 h) and +9.5°C (over ~18 h) (figure in supplementary), which occurred due to water mixing in Lake Peurunkajärvi where the flow-through water is drawn from, in the week prior to CT_{max} measurements. Larger points represent CT_{max} of an individual fish; solid points and error bars show the mean CT_{max} ± S.E. for each life stage. Letters in teal (top row) indicate significant differences at the fast ramping rate (Table S8), letters in orange (bottom row) indicate significant differences at the fast ramping rate for embryo to smolt and slow ramping rate for post-smolt to spawner (Table S9).

Tables

Table S1. Life-stage-specific characteristics and thermocouple placement details for thermal inertia measurements. † = these two trials were conducted on the same individuals; Sex = F (Female), M (Male); post-smolts were not sexed, except for one individual (*pre-grilse) that was observed to have reached sexual maturity.

Life stage	Warming rate (°C h^{-1})	Trial number	Fish ID	Sex	Total length (mm)	Weight (g)	Location of thermocouple	Thermocouple implant depth (mm)
Post-smolt	18	1	1		245	130.82	Distal intestine	75
							Dorsal muscle	15
			2		195	62.85	Distal intestine	75
							Dorsal muscle	15
			3		218	91.52	Distal intestine	70
							Dorsal muscle	20
Post-smolt	18	2	1		192	70.02	Distal intestine	46
							Dorsal muscle	15
			2		193	80.84	Distal intestine	45
							Dorsal muscle	35
*Pre-grilse			3	M	195	82.09	Distal intestine	35
Spawner	1	1	1	F	593	2109	Dorsal muscle	50
			2	F	598	1911	Dorsal muscle	46
			3	M	550	1402	Dorsal muscle	59
			4	F	533	1210	Dorsal muscle	50
			5	M	562	1578	Dorsal muscle	58
			6	M	543	1423	Dorsal muscle	70
Spawner†	3.6	1	1	M	545	1551	Distal intestine	82
							Dorsal muscle	35
			2	F	550	1979	Distal intestine	70
							Dorsal muscle	40
			3	F	530	1730	Distal intestine	100
							Dorsal muscle	18
Spawner	18	1	1	M	530	1430	Distal intestine	120
							Dorsal muscle	50
			2	F	525	1677	Distal intestine	90
							Dorsal muscle	40
			3	M	442	845	Distal intestine	60
							Dorsal muscle	50
Spawner†	18	2	1	M	545	1551	Distal intestine	82
							Dorsal muscle	35
			2	F	550	1979	Distal intestine	70
							Dorsal muscle	40
			3	F	530	1730	Distal intestine	100
							Dorsal muscle	18

Table S2. Characteristics of the fish implanted with a micro-ACT biologger (Star-Oddi, Gardabaer, Iceland) for thermal inertia measurements. After the trial, the fish was stripped of eggs and the egg mass estimated by calculating weight (below) minus the stripped weight (1334 g). The same fish was tested at the slow (1°C h^{-1}) then the fast (18°C h^{-1}) warming rate.

Life stage	Sex	Total length (mm)	Weight (g)	Egg mass (g)
Spawner	Female	579	1718	384

Table S3. Critical thermal maximum (CT_{max}) across life stages of Atlantic salmon (*Salmon salar*). All life stages were measured at a warming rate of 18°C h^{-1}; post-smolts, pre-grilse and spawners were additionally measured at a warming rate of 1°C h^{-1}. n is the number of individuals from each life stage tested at a particular warming rate. Life stages that were affected by two natural temperature fluctuations in the week prior to testing are indicated by grey background.

Life stage	***Warming rate (°C h^{-1})***	***n***	***CT_{max}***	***S.D.***	***S.E.***
Embryo	18	88	22.47	2.05	0.22
Alevin	18	120	26.56	0.52	0.05
Fry	18	120	28.03	0.27	0.02
0+ Parr	18	60	27.90	0.31	0.04
1+ Parr	18	19	28.57	0.28	0.06
Smolt	18	13	28.20	0.19	0.05
Post-smolt	1	12	27.89	0.22	0.06
Post-smolt	18	23	28.47	0.40	0.08
Pre-grilse	1	2	27.43	0.23	0.16
Pre-grilse	18	2	28.68	0.21	0.15
Spawner	1	16	25.86	0.53	0.13
Spawner	18	24	27.24	0.66	0.13

Table S4. Effect of warming rate (1°C h^{-1} or 18°C h^{-1}) on critical thermal maximum (CT_{max}) in (i) post-smolt, (ii) pre-grilse, and (iii) spawner Atlantic salmon (*Salmon salar*), estimated using linear models. CT_{max} was modelled as a function of warming rate separately for each life stage.

lm(CTmax ~ Ramping_rate)				
(i) Post-smolt				
Predictor	***Estimate, β***	***S.E.***	***t-value***	***p-value***
Intercept (1°C h^{-1})	27.887	0.101	277.16	**<0.001**
18°C h^{-1}	0.586	0.124	4.72	**<0.001**
Residual S.E.	0.349 on 33 d.f.			
Multiple R^2/Adjusted R^2	0.403/0.385			
F-statistic	22.321 on 1 and 33 d.f.			
p value	<0.001			
(ii) Pre-grilse				
Predictor	***Estimate, β***	***S.E.***	***t-value***	***p-value***
Intercept (1°C h^{-1})	27.430	0.153	179.65	**<0.001**
18°C h^{-1}	1.245	0.216	5.77	**0.029**
Residual S.E.	0.216 on 2 d.f.			
Multiple R^2/Adjusted R^2	0.943/0.915			
F-statistic	33.245 on 1 and 2 d.f.			
p value	0.029			
(iii) Spawner				
Predictor	***Estimate, β***	***S.E.***	***t-value***	***p-value***
Intercept (1°C h^{-1})	25.856	0.153	169	**<0.001**
18°C h^{-1}	1.379	0.197	7	**<0.001**
Residual S.E.	0.611 on 38 d.f.			
Multiple R^2/Adjusted R^2	0.563/0.552			
F-statistic	48.973 on 1 and 38 d.f.			
p value	<0.001			

Table S5. Differences in critical thermal maximum (CT_{max}) across Atlantic salmon (*Salmon salar*) life stages measured under a fast warming rate (18°C h^{-1}), estimated using a linear mixed effects model. The model included CT_{max} as the response variable, life stage as a fixed effect, trial ID as a random intercept, and a variance structure allowing different residual variances across life stages. All life stages were acclimated to 6°C. Post-hoc pairwise comparisons of the estimated marginal means of the treatment groups were conducted using Tukey's HSD test to adjust for multiple comparisons.

lme(fixed = CTmax ~ Life_stage, random = ~ 1 | UniqueTrialID, weights = varIdent(form = ~ 1 | Life_stage)

Parameter	***Value***	***S.E.***	***D.F.***	***t-value***	***p-value***
Intercept (Embryo)	22.478	0.257	285	87.445	**<0.001**
Alevin	4.084	0.285	26	14.335	**<0.001**
0+ Parr	5.423	0.318	26	17.037	**<0.001**
Post-smolt	5.999	0.360	285	16.671	**<0.001**
Pre-grilse	6.182	0.355	285	17.393	**<0.001**
Spawner	4.757	0.356	26	13.359	**<0.001**
Random effect (UniqueTrialID)	***S.D.***				
UniqueTrialID	0.410				
Residual	0.366				
Variance function	***Parameter estimates***				
Embryo	5.250				
Alevin	1.000				
0+ Parr	0.867				
Post-smolt	1.125				
Pre-grilse	0.136				
Spawner	0.926				
Model fit					
AIC	628.666				
BIC	677.283				
Log-likelihood	-301.333				
N(Observations)	317				
N(Groups)	30				
Marginal R2 / Conditional R2	0.941/ 0.974				
Post-hoc pairwise comparisons with Tukey adjustment					
Contrast	***Estimate***	***S.E.***	***df***	***t-ratio***	***p-value***
Embryo - Alevin	-4.084	0.285	26	-14.335	**<0.001**
Embryo - 0+ Parr	-5.423	0.318	26	-17.037	**<0.001**
Embryo - Post-smolt	-5.999	0.360	285	-16.671	**<0.001**
Embryo - Pre-grilse	-6.182	0.355	285	-17.393	**<0.001**
Embryo - Spawner	-4.757	0.356	26	-13.359	**<0.001**
Alevin - 0+ Parr	-1.339	0.224	26	-5.966	**<0.001**
Alevin - (Post-smolt)	-1.914	0.280	26	-6.833	**<0.001**
Alevin - (Pre-grilse)	-2.097	0.274	26	-7.641	**<0.001**
Alevin - Spawner	-0.673	0.275	26	-2.443	0.179
0+ Parr - Post-smolt	-0.576	0.314	26	-1.833	0.463
0+ Parr - Pre-grilse	-0.759	0.309	26	-2.455	0.175
0+ Parr - Spawner	0.666	0.310	26	2.149	0.295
Post-smolt - Pre-grilse	-0.183	0.113	285	-1.614	0.590
Post-smolt - Spawner	1.242	0.352	26	3.524	**0.018**
Pre-grilse - Spawner	1.425	0.348	26	4.096	**0.004**

Table S6. Differences in critical thermal maximum (CT_{max}) across Atlantic salmon (*Salmon salar*) life stages, estimated using a linear mixed effects model. The model compared embryos, alevins, and 0+ parr measured at a warming rate of 18°C h^{-1}, and post-smolts, pre-grilse, and spawners measured at a warming rate of 1°C h^{-1}. The model included CT_{max} as the response variable, life stage as a fixed effect, trial ID as a random intercept, and a variance structure allowing different residual variances across life stages. All life stages were acclimated to 6°C. Post-hoc pairwise comparisons of the estimated marginal means of the treatment groups were conducted using Tukey's HSD test to adjust for multiple comparisons.

lme(fixed = CTmax ~ Life_stage, random = ~ 1 | UniqueTrialID, weights = varIdent(form = ~ 1 | Life_stage)

Parameter	***Value***	***S.E.***	***D.F.***	***t-value***	***p-value***
Intercept (Embryo)	22.476	0.247	268	90.897	**<0.001**
Alevin	4.086	0.270	24	15.132	**<0.001**
0+ Parr	5.424	0.297	24	18.246	**<0.001**
Post-smolt	5.438	0.358	268	15.201	**<0.001**
Pre-grilse	4.954	0.354	268	14.008	**<0.001**
Spawner	3.379	0.378	24	8.939	**<0.001**
Random effect (UniqueTrialID)	***S.D.***				
UniqueTrialID	0.358				
Residual	0.367				
Variance function	***Parameter estimates***				
Embryo	5.293				
Alevin	1.000				
0+ Parr	0.866				
Post-smolt	0.510				
Pre-grilse	<0.001				
Spawner	1.459				
Model fit					
AIC	598.131				
BIC	645.929				
Log-likelihood	-286.065				
N(Observations)	298				
N(Groups)	28				
Marginal R2 / Conditional R2	0.946/ 0.972				
Post-hoc pairwise comparisons with Tukey adjustment					
Contrast	***Estimate***	***S.E.***	***df***	***t-ratio***	***p-value***
Embryo - Alevin	-4.086	0.270	24	-15.132	**<0.001**
Embryo - 0+ Parr	-5.424	0.297	24	-18.246	**<0.001**
Embryo - Post-smolt	-5.438	0.358	268	-15.201	**<0.001**
Embryo - Pre-grilse	-4.954	0.354	268	-14.008	**<0.001**
Embryo - Spawner	-3.379	0.378	24	-8.939	**<0.001**
Alevin - 0+ Parr	-1.339	0.198	24	-6.777	**<0.001**
Alevin - (Post-smolt)	-1.352	0.280	24	-4.822	**0.001**
Alevin - (Pre-grilse)	-0.868	0.275	24	-3.154	**0.044**
Alevin - Spawner	0.707	0.306	24	2.311	0.229
0+ Parr - Post-smolt	-0.013	0.307	24	-0.043	1.000
0+ Parr - Pre-grilse	0.471	0.302	24	1.560	0.631
0+ Parr - Spawner	2.045	0.330	24	6.194	**<0.001**
Post-smolt - Pre-grilse	0.484	0.054	268	8.962	**<0.001**
Post-smolt - Spawner	2.059	0.386	24	5.340	**<0.001**
Pre-grilse - Spawner	1.574	0.382	24	4.125	**0.005**

744 **Table S7.** Differences in critical thermal maximum (CT_{max}) between sexes within each life stage of Atlantic salmon
745 (*Salmo salar*). Separate models were run for each heating rate and included CT_{max} as a response variable, sex, life
746 stage, and their interaction as fixed effects, and a unique trial identifier as a random intercept. All individuals were
747 acclimated to 6°C.

*SalmonSex.mod <- lmer(CTmax ~ Sex * Life_stage (1|UniqueTrialID)*
emmeans(SalmonSex.mod, pairwise ~ Sex | Life_stage, adjust="tukey")

(i) Heating rate = 18°C h^{-1}

Life stage	***Contrast***	***Estimate, β***	***S.E.***	***df***	***t-value***	***p-value***
Embryo	Female–Male	-0.207	0.185	393	-1.124	0.262
Alevin	Female–Male	-0.054	0.154	404	-0.349	0.727
Fry	Female–Male	-0.060	0.151	396	-0.399	0.690
0+ Parr	Female–Male	-0.364	0.303	405	-1.201	0.230
1+ Parr	Female–Male	0.131	0.400	384	0.329	0.743
Smolt	Female–Male	-0.038	0.469	408	-0.082	0.935
Post-smolt	Female–Male	-0.299	0.349	409	-0.856	0.393
Spawner	Female–Male	-0.130	0.330	382	-0.394	0.694

(ii) Heating rate = 1°C h^{-1}

Life stage	***Contrast***	***Estimate, β***	***S.E.***	***df***	***t-value***	***p-value***
Post-smolt	Female–Male	0.083	0.354	20.7	0.234	0.817
Spawner	Female–Male	-0.137	0.286	18.8	-0.481	0.636

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Table S8. Differences in acute upper thermal tolerance (CT_{max}) across Atlantic salmon (*Salmon salar*) life stages measured under a fast warming rate (18°C h^{-1}), estimated using a linear mixed effects model. The model included CT_{max} as the response variable, life stage as a fixed effect, a unique trial identifier as a random intercept, and a variance structure allowing different residual variances across life stages. All life stages were acclimated to 6°C, however three life stages (fry, 1+ parr, smolts) were exposed to two natural temperature fluctuations of +6.5°C (over ~16 h) and +9.5°C (over ~18 h) in the week prior to CT_{max} measurements. Post-hoc pairwise comparisons of the estimated marginal means of the treatment groups were conducted using Tukey's HSD test to adjust for multiple comparisons.

lme(fixed = CTmax ~ Life_stage, random = ~ 1 | UniqueTrialID, weights = varIdent(form = ~ 1 | Life_stage)

Parameter	***Value***	***S.E.***	***D.F.***	***t-value***	***p-value***
Intercept (Embryo)	22.475	0.241	424	93.208	**0**
Alevin	4.087	0.260	36	15.697	**0**
Fry	5.557	0.267	36	20.788	**0**
0+ Parr	5.426	0.283	36	19.148	**0**
1+ Parr	6.090	0.310	424	19.630	**0**
Smolt	5.715	0.307	424	18.595	**0**
Post-smolt	6.001	0.316	424	19.009	**0**
Pre-grilse	6.185	0.313	424	19.780	**0**
Spawner	4.760	0.312	36	15.267	**0**
Random effect (UniqueTrialID)	***S.D.***				
UniqueTrialID (Intercept)	0.32				
Residual	0.37				
Variance function	***Parameter estimates***				
Embryo	5.324				
Alevin	1.000				
Fry	0.680				
0+ Parr	0.864				
1+ Parr	0.744				
Smolt	0.459				
Post-smolt	1.117				
Pre-grilse	0.199				
Spawner	0.935				
Model fit					
AIC	677.871				
BIC	756.364				
Log-likelihood	-319.936				
N(Observations)	469				
N(Groups)	41				
Marginal R2 / Conditional R2	0.949/ 0.971				
Post-hoc pairwise comparisons with Tukey adjustment					
Contrast	***Estimate***	***S.E.***	***df***	***t-ratio***	***p-value***
Embryo - Alevin	-4.087	0.260	36	-15.697	**<0.001**
Embryo - Fry	-5.557	0.267	36	-20.788	**<0.001**
Embryo - 0+ Parr	-5.426	0.283	36	-19.148	**<0.001**
Embryo - 1+ Parr	-6.089	0.310	424	-19.630	**<0.001**
Embryo - Smolt	-5.715	0.307	424	-18.595	**<0.001**
Embryo - Post-smolt	-6.001	0.316	424	-19.009	**<0.001**
Embryo - Pre-grilse	-6.185	0.313	424	-19.780	**<0.001**
Embryo - Spawner	-4.760	0.312	36	-15.269	**<0.001**
Alevin - Fry	-1.470	0.152	36	-9.697	**<0.001**

Alevin - 0+ Parr	-1.338	0.178	36	-7.505	**<0.001**
Alevin - 1+ Parr	-2.002	0.218	36	-9.164	**<0.001**
Alevin - Smolt	-1.628	0.214	36	-7.593	**<0.001**
Alevin - Post-smolt	-1.914	0.226	36	-8.461	**<0.001**
Alevin - Pre-grilse	-2.098	0.222	36	-9.450	**<0.001**
Alevin - Spawner	-0.673	0.221	36	-3.049	0.089
Fry - 0+ Parr	0.131	0.188	36	0.696	0.999
Fry - 1+ Parr	-0.533	0.227	36	-2.349	0.341
Fry - Smolt	-0.158	0.223	36	-0.711	0.998
Fry - Post-smolt	-0.444	0.234	36	-1.898	0.620
Fry - Pre-grilse	-0.629	0.230	36	-2.731	0.173
Fry - Spawner	0.797	0.229	36	3.483	**0.032**
0+ Parr - 1+ Parr	-0.664	0.245	36	-2.704	0.182
0+ Parr - Smolt	-0.289	0.242	36	-1.197	0.952
0+ Parr - Post-smolt	-0.576	0.252	36	-2.281	0.379
0+ Parr - Pre-grilse	-0.760	0.249	36	-3.056	0.087
0+ Parr - Spawner	0.666	0.247	36	2.692	0.187
1+ Parr - Smolt	0.374	0.078	424	4.774	**<0.001**
1+ Parr - Post-smolt	0.088	0.282	424	0.312	1.000
1+ Parr - Pre-grilse	-0.096	0.279	424	-0.344	1.000
1+ Parr - Spawner	1.330	0.278	36	4.788	**<0.001**
Smolt - Post-smolt	-0.286	0.279	424	-1.026	0.983
Smolt - Pre-grilse	-0.470	0.276	424	-1.706	0.743
Smolt - Spawner	0.955	0.274	36	3.480	**0.032**
Post-smolt - Pre-grilse	-0.184	0.118	424	-1.560	0.826
Post-smolt - Spawner	1.241	0.284	36	4.374	**0.003**
Pre-grilse - Spawner	1.426	0.280	36	5.082	**<0.001**

Table S9. Differences in acute upper thermal tolerance (CT_{max}) across Atlantic salmon (*Salmon salar*) life stages, estimated using a linear mixed effects model. The model compared embryos, alevins, fry, 0+ parr, 1+ parr, and smolts measured at a warming rate of 18°C h^{-1}, and post-smolts, pre-grilse, and spawners measured at a warming rate of 1°C h^{-1}. The model included CT_{max} as the response variable, life stage as a fixed effect, a unique trial identifier as a random intercept, and a variance structure allowing different residual variances across life stages. All life stages were acclimated to 6°C, however three life stages (fry, 1+ parr, smolts) were exposed to two natural temperature fluctuations of +6.5°C (over ~16 h) and +9.5°C (over ~18 h) in the week prior to CT_{max} measurements. Post-hoc pairwise comparisons of the estimated marginal means of the treatment groups were conducted using Tukey's HSD test to adjust for multiple comparisons.

lme(fixed = CTmax ~ Life_stage, random = ~ 1 | UniqueTrialID, weights = varIdent(form = ~ 1 | Life_stage)

Parameter	***Value***	***S.E.***	***D.F.***	***t-value***	***p-value***
Intercept (Embryo)	22.473	0.234	407	95.947	**0**
Alevin	4.089	0.249	34	16.411	**0**
Fry	5.559	0.254	34	21.885	**0**
0+ Parr	5.427	0.267	34	20.349	**0**
1+ Parr	6.091	0.288	407	21.123	**0**
Smolt	5.717	0.285	407	20.038	**0**
Post-smolt	5.441	0.307	407	17.719	**0**
Pre-grilse	4.957	0.302	407	16.398	**0**
Spawner	3.382	0.330	34	10.237	**0**
Random effect (UniqueTrialID)	***S.D.***				
UniqueTrialID (Intercept)	0.270				
Residual	0.369				
Variance function	***Parameter estimates***				
Embryo	5.355				
Alevin	1.000				
Fry	0.677				
0+ Parr	0.859				
1+ Parr	0.741				
Smolt	0.457				
Post-smolt	0.507				
Pre-grilse	0.001				
Spawner	1.446				
Model fit					
AIC	645.019				
BIC	722.711				
Log-likelihood	-303.510				
N(Observations)	450				
N(Groups)	39				
Marginal R2 / Conditional R2	0.955/ 0.970				
Post-hoc pairwise comparisons with Tukey adjustment					
Contrast	***Estimate***	***S.E.***	***df***	***t-ratio***	***p-value***
Embryo - Alevin	-4.089	0.249	34	-16.411	**<0.001**
Embryo - Fry	-5.559	0.254	34	-21.885	**<0.001**
Embryo - 0+ Parr	-5.427	0.267	34	-20.349	**<0.001**
Embryo - 1+ Parr	-6.091	0.288	407	-21.123	**<0.001**
Embryo - Smolt	-5.717	0.285	407	-20.038	**<0.001**
Embryo - Post-smolt	-5.441	0.307	407	-17.719	**<0.001**
Embryo - Pre-grilse	-4.957	0.302	407	-16.398	**<0.001**

Embryo - Spawner	-3.382	0.330	34	-10.237	**<0.001**
Alevin - Fry	-1.470	0.130	34	-11.317	**<0.001**
Alevin - 0+ Parr	-1.339	0.153	34	-8.732	**<0.001**
Alevin - 1+ Parr	-2.002	0.188	34	-10.625	**<0.001**
Alevin - Smolt	-1.628	0.184	34	-8.862	**<0.001**
Alevin - Post-smolt	-1.352	0.216	34	-6.260	**<0.001**
Alevin - Pre-grilse	-0.868	0.209	34	-4.150	**0.006**
Alevin - Spawner	0.707	0.248	34	2.849	0.138
Fry - 0+ Parr	0.131	0.161	34	0.815	0.996
Fry - 1+ Parr	-0.533	0.195	34	-2.734	0.174
Fry - Smolt	-0.158	0.190	34	-0.833	0.995
Fry - Post-smolt	0.118	0.222	34	0.532	1.000
Fry - Pre-grilse	0.602	0.215	34	2.802	0.152
Fry - Spawner	2.176	0.253	34	8.607	**<0.001**
0+ Parr - 1+ Parr	-0.664	0.211	34	-3.144	0.073
0+ Parr - Smolt	-0.290	0.207	34	-1.400	0.890
0+ Parr - Post-smolt	-0.013	0.236	34	-0.056	1.000
0+ Parr - Pre-grilse	0.471	0.230	34	2.049	0.522
0+ Parr - Spawner	2.045	0.266	34	7.699	**<0.001**
1+ Parr - Smolt	0.374	0.078	407	4.775	**<0.001**
1+ Parr - Post-smolt	0.650	0.260	407	2.500	0.235
1+ Parr - Pre-grilse	1.135	0.255	407	4.457	**<0.001**
1+ Parr - Spawner	2.709	0.287	34	9.426	**<0.001**
Smolt - Post-smolt	0.276	0.257	407	1.076	0.977
Smolt - Pre-grilse	0.760	0.251	407	3.029	0.065
Smolt - Spawner	2.335	0.284	34	8.212	**<0.001**
Post-smolt - Pre-grilse	0.484	0.054	407	8.962	**<0.001**
Post-smolt - Spawner	2.059	0.306	34	6.724	**<0.001**
Pre-grilse - Spawner	1.574	0.301	34	5.225	**<0.001**

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