Physiological effects of environmentally relevant, multi-day thermal stress on wild juvenile Atlantic salmon (Salmo salar)

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The frequency of extreme thermal events in temperate freshwater systems is expected to increase alongside global surface temperature. The Miramichi River, located in eastern Canada, is a prominent Atlantic salmon (Salmo salar) river where water temperatures can exceed the proposed upper thermal limit for the species (~27°C). Current legislation closes the river to recreational angling when water temperatures exceed 20°C for two consecutive nights. We aimed to examine how natural thermal variation, representative of extreme high thermal events, affected the thermal tolerance and physiology of wild, juvenile Atlantic salmon. We acclimated fish to four thermal cycles, characteristic of real-world thermal conditions while varying daily thermal minima (16°C, 18°C, 20°C or 22°C) and diel thermal fluctuation (e.g. Δ5°C–Δ9°C). In each cycling condition, we assessed the role that thermal minima played on the acute thermal tolerance (critical thermal maximum, (CTMax)), physiological (e.g. heat shock protein 70 (HSP70), ubiquitin) and energetic (e.g. hepatic glycogen, blood glucose and lactate) status of juvenile Atlantic salmon throughout repeated thermal cycles. Exposure to 16–21°C significantly increased CTMax (+0.9°C) compared to a stable acclimation temperature (16°C), as did exposure to diel thermal fluctuations of 18–27°C, 20–27°C and 22–27°C, yet repeated exposure provided no further increases in acute thermal tolerance. In comparison to the reference condition (16–21°C), consecutive days of high temperature cycling with different thermal minima resulted in significant increases in HSP70 and ubiquitin, a significant decrease in liver glycogen, and no significant cumulative effect on either blood glucose or lactate. However, comparison between thermally taxed treatments suggested the diel thermal minima had little influence on the physiological or energetic response of juvenile salmon, despite the variable thermal cycling condition. Our results suggest that relatively cooler night temperatures in the summer months may play a limited role in mitigating physiological stress throughout warm diel cycle events.

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Introduction
Climate warming is expected to alter distribution patterns of fishes, with temperatures in some systems already approaching the upper thermal tolerance of endemic species (Caissie, 2006). In salmonids, warming water temperatures affects migration (Crossin et al., 2008), smoltification (McCormick et al., 1999), growth and survival (Swansburg et al., 2002).
The Miramichi River, located in eastern Canada, is a prominent Atlantic salmon (Salmo salar) river (DFO, 2013) where maximum summer temperatures can reach 27–30°C (Caissie et al., 2014), well beyond the range for optimal growth in juveniles (15–20°C; Jobling, 1981; Elliott and Hurley, 1997; Jonsson et al., 2001; Elliott and Elliott, 2010). The upper threshold for normal feeding behavior (i.e., 22–24°C; Breau et al., 2011) can be surpassed for as many as 62 days during the spring/summer (Caissie et al., 2012). Peak water temperatures typically occur in conjunction with diel fluctuations of ≤9°C in summer months (our unpublished work). Thus, the Miramichi River is an ideal system for understanding the effects of climate warming on salmon physiology. An appreciation of a fish’s physiological capacity to cope with climate-driven environmental stress is emerging as a powerful approach in conservation and management as has been demonstrated for Pacific salmon (Cooke et al., 2012, Muñoz et al., 2014).

Natural diel thermal cycles impact thermal tolerance (Wehrly et al., 2007), bioenergetics (Beauregard et al., 2013; Eldridge et al., 2015), and metabolic status (Oligny-Hébert et al., 2015) of fishes. However, largely due to practical issues of temperature control in the laboratory, comparatively few studies investigate how temperature cycling, compared to stable acclimation temperatures, affect fish biology (see Threader and Houston, 1983; Houston and Gingras-Bedard, 1994; Mesa et al., 2002; Narum et al., 2013; Tumah et al., 2017 as examples). Given that water temperatures in traditionally productive Atlantic salmon habitat, such as the Miramichi River, are increasing beyond the presumed thermal limit for this species (Elliott and Elliott, 1995), we were interested in whether the nature of the thermal cycle differentially influenced physiology. Specific to current federal (DFO) regulations whereby river closures occur when water temperature is ≥20°C for two consecutive nights (DFO, 2012), we investigated the effects of warming temperature cycles with distinct thermal minimums on the physiological response (i.e., thermal tolerance) of juvenile salmon. Although federal guidelines are designed primarily with mature life stages in mind, juveniles were used as a readily accessible and abundant proxy for their adult counterpart. The link between adult and juvenile fish physiology has been defined in many fishes (see Rodnick et al., 2004; Porpner et al., 2008; Fowler et al., 2009; Morita et al., 2010), and although a more thermally tolerant life stage, juveniles provide insight into the physiological capacity of salmonids to withstand extreme events.

Our main objective was to determine how distinct, natural thermal variation, representative of summer high temperature events, affected the thermal tolerance and physiology of wild juvenile Atlantic salmon, with the goal of informing conservation and management efforts. To this end, we acclimated fish to four thermal cycles, representative of real-world conditions but with differing minimum nighttime temperatures (16°C, 18°C, 20°C, 22°C) and diel temperature fluctuations (e.g., ΔT = 5°C–9°C). In each cycling condition, we measured critical thermal maximum (CTMax) as a proxy for acute thermal tolerance (Beitinger et al., 2000), recognizing that CTMax is influenced by acclimation temperature (e.g., Fangue et al., 2006). We also measured indicators of physiological (HSP70, ubiquitin) and energetic (lactate, glycogen) stress. We hypothesized that nighttime temperature throughout a thermal event dictates physiological limits. If supported, then we predicted that fish exposed to conditions with the highest nighttime temperature will have reduced acute thermal tolerance, and will experience enhanced metabolic and cellular stress compared to those exposed to cooler nighttime temperatures.

Methods

Fish collection & maintenance

Wild Atlantic salmon parr (N = 320; fork length = 6.1–10.4 cm; weight = 1.9–12 g) were collected on 16 June 2013, using a Smith-Root LR-24 backpack electrofisher from the Cains River (N46°25′59″; W066°01′29″, 1—N46°26′05″; W066°01′10″. 8 ± 15 m), a tributary of the Southwest Miramichi River. Fish were transported to the Harold Crabtree Aquatic Facility at Mount Allison University, NB, Canada and placed in a 750 L (circular fiberglass) recirculation holding tank at 15.00 ± 0.03°C. Fish were fed a mixture of dehydrated krill and commercial pellet feed (Corey Nutrition Company) twice daily until satiation. Water temperature was maintained until parr were weaned to feed exclusively on pellets (~10 days). Fish were subsequently fed once daily to satiation and exposed to a fluctuating ‘acclimation regime’ with nighttime minimum and daytime maximum temperatures (Tmin and Tmax) of 16 and 21 ± 0.2°C, respectively (mean ± SD; 12 h warming: 12 h cooling) with a natural photoperiod (~16 h light:8 h dark). Water temperature was monitored using an iBcod temperature logger (±1°C, 15 min interval, Alpha Mach Inc.) and dissolved oxygen (DO) was measured daily (7.5–10 mg L−1; YSI Pro 20, Xylem Inc.).

Experimental design

A significant heat event occurred in the Little Southwest Miramichi River (LSWM) in early July 2010 (Fig. 1A). Maximum daytime water temperatures during the July 6–8 event ranged from 28.0°C to 30.7°C (mean: 29.3 ± 0.5°C); nighttime temperatures ranged from 20.7°C to 24.5°C (mean: 22.8 ± 0.9°C). Such temperatures are known to alter metabolism in juvenile Atlantic salmon (Breau et al., 2011). To address our objectives, we chose to model this particular heat event for our experimental treatments. We established an acclimation regime based on the mean diel Tmax and Tmin of the 2 weeks prior to the event (16–21°C). To minimize thermal stress mortality, Tmax was set at 27°C for all treatments, just below the upper incipient lethal limit (7-day survival: 27.8°C; Elliott, 1991). Three temperature treatments were established within the limits of ΔT measured in the
Diel thermal cycles and sampling

LSWM River in 2010 (Fig. 1B). We established minimum (i.e. nighttime) water temperatures as: (i) above the current legislated diel minimum temperature that determines river closure (≥20°C; DFO, 2012), 22–27°C; (ii) at the threshold, 20–27°C; and (iii) within the optimum range for growth and survival, 18–27°C. In order to subject parr to 27°C without exceeding the maximum natural diel ΔT (9°C) measured in the LSWM, an intermediate (‘ramp’) day was placed between the 16–21°C-acclimation regime and the temperature treatments. Temperatures on this day reached a maximum of 23°C and minimum of 18°C (Fig. 1B).

Series 1: Thermal physiology

Diel thermal cycles and sampling

Once in experimental tanks, fish remained at 16–21°C for a 5-day acclimation period. All fish were then subjected to a 1-day intermediate exposure (Day-6; 18–23°C) prior to exposure to 3 days of heat cycling (Day 7–9; T_{\text{max}} = 27°C in all treatments) with treatment-specific thermal minima. A fourth treatment group was maintained at 16–21°C throughout the experimental procedure. We ran two experimental trials, each lasting 9.5 days (Trial 1: 16–21°C and 22–27°C treatments, July 13–22; Trial 2: 18–27°C and 20–27°C treatments, July 22–31). Each thermal treatment was assigned a bank of three 300 L circular fiberglass tanks with each bank connected to separate recirculation systems. Parr were randomly selected from the holding tank (at 16–21°C) and transferred to one of the two tanks (i.e. total N = 6 tanks). Fish were distributed equally among all experimental tanks (i.e. 108 fish per trial; ~36 fish per tank). For each sampling event, nine fish were sampled from a single tank within a bank. Sampling regimens were scheduled such that no tank was subject to consecutive samplings in order to eliminate recurring stress from repeated sampling within short time periods (minimum time between sampling a particular tank = 48 h). Fish were not fed 24 h prior to sampling.

Fish were sampled throughout the diel temperature cycle according to the following schedule: (i) T_{\text{min}} following the ‘intermediate’ temperature increase (t = 48 h); (ii) T_{\text{max}} the first day of heat cycling (t = 60 h); (iii) the first T_{\text{min}} throughout heat cycle (16°C, 18°C, 20°C or 22°C, depending on treatment, t = 72 h); (iv) T_{\text{max}}, the third day of heat cycling (t = 108 h); and (v) T_{\text{min}}, the third day of heat cycling (t = 120 h, Fig. 1B). These sampling time points were chosen to establish the physiological condition prior to heat exposure, the implications of 1 day of heat cycling, and the potential cumulative effective of 3 days of cycling. This 5-timepoint sampling regime was performed on all treatment groups. However, to address the potential effect of ‘trial’ on our results, two additional sampling time points (t = 12 h and t = 24 h) were established in one treatment group in each of the two trials (16–21°C for Trial 1 and 18–27°C for Trial 2), prior to the heat cycle. This additional sampling allowed us to further address the incipient, ‘pre-thermal stress’ condition.

Fish were anaesthetized with a buffered ethyl 3-aminobenzoate methanesulfonate (MS-222; Sigma-Aldrich) solution with supplemental aeration. Once anesthetized, mass and fork length were measured, and blood extracted from the caudal artery. Whole blood (WB) lactate and glucose were measured using a Lactate Pro™ Portable Blood Lactate Analyzer and a OneTouch® Ultra 2 Meter (Gallant et al., 2017). Environmental stressors are known to alter the blood chemistry of fish. Blood glucose levels rise to supply ATP and fuel activity if the situation becomes critical. Similarly, blood lactate is a byproduct of anaerobic metabolism, and an increase in this metabolite indicates a switch to anaerobic ATP production suggesting aerobic energy stores are diminished. Parr were terminated by a swift blow to the head followed by severance of the spinal cord. Liver was dissected, immediately frozen in liquid nitrogen and stored at −80°C until processing. Blood samples were centrifuged at 5000 rpm for 3 min and 13°C to separate blood constituents. White blood cells and plasma were discarded while red blood cells (RBC) were flash frozen in liquid nitrogen and stored at −80°C.
Series 2: Critical thermal maximum

CTMax tests were performed on 9–11 salmon parr at each of three $T_{min}$ time points (between 04:00 and 09:00); to establish an initial pre-thermal stress value (24 h), the effect of a singular thermal cycle (60 h), and of multiple heat cycles (120 h; Fig. 1B). Prior to testing, fish were transported to a plastic CTMax experimental chamber (40 × 13.5 × 11 cm) with a clear Plexiglas® lid. Chambers contained an aerated water flow of 4 L min$^{-1}$ and had an initial water temperature matching that of the experimental procedure. Once the fish was in the chamber, water temperature was increased acutely at a rate 0.32 ± 0.002°C min$^{-1}$ (mean ± SEM; Becker and Genoway, 1979) until fish lost equilibrium. DO and temperature were measured at 1-min intervals; DO saturation remained >72% in all trials.

In addition to protocol described above, a separate group of 10 parr were isolated at the time of field collection and maintained at a constant 16°C for 1 week prior to being measured for fork length (±1 mm), wet weight (±0.1 g) and individually tagged with visible implant elastomer (Northwest Marine Technology Inc.). Fish recovered for 60 h prior to CTMax testing on 4 July 2013 at 06:00 h. After testing, fish recovered at 16°C for 5 days prior to acclimation to 16–21°C for 36 days. The test was then repeated under the same conditions to evaluate the in vivo thermal stress for Atlantic salmon (Lund et al., 2002; Chadwick et al., 2015; Tunnah et al., 2017). Soluble protein in liver and RBC were extracted as in Tunnah et al. (2017) and LeBlanc et al. (2011), respectively, and assayed using the Lowry-based DC protein assay kit (Bio-Rad, Mississauga, ON, Canada). Standards (bovine serum albumin; Bio-Rad) and samples were diluted in protein extraction buffer or salmon saline, respectively, and absorbance read at 750 nm using a SpectraMax M5 plate reader and SoftMax Pro software. Samples were prepared using 15 µg soluble protein and western blots performed as in Kolhatkar et al. (2014). We used a rabbit anti-salmonid HSP70 primary antibody (AgriSera, AS05061; 1:50 000 dilution in blocking buffer) and a goat anti-rabbit secondary (Enzo Life Sciences; SAB-300 1:50 000) for immunodetection. This antibody is specific for the inducible form of HSP70 and does not cross-react with the constitutive isoform in salmonids (Rendell et al., 2006; Fowler et al., 2009; Tunnah et al., 2017). Protein bands were visualized using an ECL Advance Chemiluminescent Western Blotting Detection kit (Amersham Pharmacia Biotech) imaged using a Versadoc Imaging System and Quantity One software (Bio-Rad).

Analyses

Glycogen

Protocols for the liver glycogen extraction (Clow et al., 2004) and assay were modified from Bergmeyer et al. (1974). Hydrolysates were frozen at −80°C until analyzed for glucose content. The glucose assay was modified to use 25 µl of sample, diluted 1:7 in assay media (Clow et al., 2004) and added to each well of a microtiter plate. Twenty-five microlitres of 100 µml G-6-PDH was added to each well to eliminate any endogenous G-6-P that remained in solution. The plate was read on a VERSAmax Tunable Microplate Reader (Molecular Devices Corporation) at 340 nm until absorbance stabilized. Hexokinase (25 µl) was then added and the absorbance was read after 15–25 min.

Heat shock protein 70

The induction of heat shock protein 70 (HSP70) may be considered an ecologically relevant indicator of thermal stress for Atlantic salmon (Lund et al., 2002; Chadwick et al., 2015; Tunnah et al., 2017). Soluble protein in liver and RBC were extracted as in Tunnah et al. (2017) and LeBlanc et al. (2011), respectively, and assayed using the Lowry-based DC protein assay kit (Bio-Rad, Mississauga, ON, Canada). Standards (bovine serum albumin; Bio-Rad) and samples were diluted in protein extraction buffer or salmon saline, respectively, and absorbance read at 750 nm using a SpectraMax M5 plate reader and SoftMax Pro software. Samples were prepared using 15 µg soluble protein and western blots performed as in Kolhatkar et al. (2014). We used a rabbit anti-salmonid HSP70 primary antibody (AgriSera, AS05061; 1:50 000 dilution in blocking buffer) and a goat anti-rabbit secondary (Enzo Life Sciences; SAB-300 1:50 000) for immunodetection. This antibody is specific for the inducible form of HSP70 and does not cross-react with the constitutive isoform in salmonids (Rendell et al., 2006; Fowler et al., 2009; Tunnah et al., 2017). Protein bands were visualized using an ECL Advance Chemiluminescent Western Blotting Detection kit (Amersham Pharmacia Biotech) imaged using a Versadoc Imaging System and Quantity One software (Bio-Rad).

Table 1: Two-way ANOVA performed on physiological variables of juvenile Atlantic salmon exposed to pre-thermal stress sampling conditions (16–21°C)

| Endpoint      | Time | Treatment | Interaction |
|---------------|------|-----------|-------------|
|               | df   | F-statistic | P-value | df | F-statistic | P-value | df | F-statistic | P-value |
| CTMax**       | –    | –         | –         | 1  | 4.07       | 0.073     | –  | –         | –       |
| L glycogen    | 1    | 1.26      | 0.272     | 1  | 0.99       | 0.330     | 1  | 0.57      | 0.458    |
| WB glucose    | 1    | 8.45      | 0.006*    | 1  | 6.70       | 0.014*    | 1  | 1.23      | 0.276    |
| WB lactate    | 1    | 0.07      | 0.790     | 1  | 0.15       | 0.702     | 1  | 2.79      | 0.106    |
| L HSP70       | 1    | 27.62     | <0.001*   | 1  | 8.28       | 0.008*    | 1  | 0.28      | 0.601    |
| L Ub          | 1    | 0.23      | 0.633     | 1  | 0.22       | 0.640     | 1  | 0.30      | 0.588    |
| RBC HSP70     | 1    | 16.88     | <0.001*   | 1  | 182.09     | <0.001*   | 1  | 2.67      | 0.113    |
| RBC Ub        | 1    | 0.12      | 0.737     | 1  | 0.28       | 0.598     | 1  | 0.35      | 0.558    |

CTMax = critical thermal maximum; L = liver; M = muscle; WB = whole blood; RBC = red blood cell; HSP70 = heat shock protein 70; Ub = ubiquitin. Asterisks indicate significance of two-way ANOVA with $\alpha = 0.05$ and $P < 0.05$.

**One-way ANOVA performed.
Relative band density was quantified using Image Lab software (Bio-Rad) and calculated from the standard curve on each blot.

**Ubiquitin**

We used ubiquitin (Ub) as an indirect measure of protein damage/turnover. Soluble protein samples of known concentration were diluted and 5 µg of protein was dotted on a nitrocellulose membrane as in MacLellan et al. (2015). To ensure equal protein loading, a Ponceau-S stain (Sigma-Aldrich) was applied to each membrane after imaging, according to manufacturer’s instructions.

**Calculations and statistical analyses**

Data were analyzed using R statistical software (R Development Core Team, 2016). Prior to analyses, data were divided into two sections: t = 12 h and t = 24 h to assess the effect of ‘trial’ (i.e. temperature regime shared by all experimental groups), and t = 48 h through t = 120 h to test experimental effects of temperature and time. In the case of glycogen, WB glucose, WB lactate, HSP70 (liver & RBC) and liver Ub, data were log-transformed to meet the assumptions of normality and homoscedasticity. A linear model two-way ANOVA was conducted to compare the main effects of independent variables (thermal cycle and time point) and the inherent interaction effect on indicators of physiological and energetic stress. In case of a significant interaction between sampling time point and thermal cycle, a one-way ANOVA was used to discern differences between temperature treatments at individual time points. Pre-thermal stress sampling time points were treated in a similar fashion with the exception of the CTMax data, as only one pre-thermal stress time point occurred. In this instance, a linear one-way ANOVA was used to test for the effect of trial. When appropriate, a subsequent Tukey post-hoc test was used. In all cases, α = 0.05 and values were expressed as mean ± SEM. Although our study does not use a repeated measures design, line graphs are presented for a clearer view of trends within the data.

**Results**

**Pre-thermal stress sampling**

Pre-thermal stress sampling time points differed in three of the variables analyzed (Table 1). There was a significant effect of sampling time \((F_{1,1} = 8.45, P = 0.006)\) and thermal cycle \((F_{1,1} = 6.70, P = 0.014)\) with no significant interaction \((F_{1,1} = 1.23, P = 0.276)\) in WB glucose. Significant differences occurred in incipient pre-exposure values for liver HSP70, where there was an effect of sampling time \((F_{1,1} = 27.62, P = 0.001)\) and thermal cycle \((F_{1,1} = 8.28, P = 0.008)\), but not the interaction \((F_{1,1} = 0.28, P = 0.601)\). Significant differences were also observed in RBC HSP70 where there was an effect of sampling time \((F_{1,1} = 16.88, P = 0.001)\) and thermal cycle \((F_{1,1} = 182.09, P = <0.001)\), but not the interaction \((F_{1,1} = 0.06, P = 0.11; \text{Table 1})\). No significant effects of sampling time or thermal cycle were observed in CTMax, WB lactate, liver Ub or RBC Ub (see Table 1).

**Critical thermal maximum**

Fish exposed to 16–21°C lost equilibrium at 32.5 ± 0.09°C, a temperature significantly higher than those maintained at a stable acclimation temperature of 16°C (31.4 ± 0.09°C; \(F_{2,48} = 43.0, P < 0.001\)). Overall, CTMax increased in all thermally cycled groups compared to the 16–21°C-control group \((F_{1,64} = 31.92, P < 0.001)\). Significant increases in CTMax were observed in all temperature treatments after one

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**Figure 2**: CTMax of wild juvenile salmon exposed to diel cycles: (A) 16–21°C (control group); or multi-day thermal stress (closed symbols; 60–120 h) of (B) 18–27°C; (C) 20–27°C; (D) 22–27°C. Data are presented as mean ± SEM \((n = 5–10)\). Open symbols (t = 24 h) represent pre-thermal stress sampling time points and were secondarily used to assess the effect of ‘trial’. Asterisks indicate significant differences among treatments \(P < 0.05\).
day of cycling ($t = 72\,\text{h}; P < 0.001$), with no significant differences observed between groups (Fig. 2B–D; $P = 0.11–0.77$). No significant differences were observed between $t = 72\,\text{h}$ and $t = 120\,\text{h}$ of heat cycling within or between temperature treatments (pooled mean of all heat exposed fish = $33.1 \pm 0.08^\circ\text{C}; P = 0.96–0.99$; Table 2; Fig. 2).

**Metabolites**

Liver glycogen levels in the 16–21°C group remained relatively stable at a mean of $67.4 \pm 4.8\,\text{mg}\,\text{g}^{-1}$ throughout the experimental sampling period (Fig. 3). Although liver glycogen decreased over time in all but the 16–21°C group, a significant interaction was observed between time and temperature treatment ($F_{12, 160} = 2.63, P < 0.003$; Table 2) indicating that the pattern of decline was different in each thermally cycled group. No significant differences in glycogen were observed prior to the first heat cycle in any temperature treatment ($t = 48\,\text{h}; F_{3, 23} = 1.08, P = 0.37$; Fig. 3). Differences among thermally cycled groups were apparent at the peak of the first heat cycle ($t = 60\,\text{h}$). The 20–27°C and 22–27°C groups experienced the most pronounced decline in liver glycogen, but by the end of the experiment ($t = 120\,\text{h}$) only the 22–27°C group was significantly different than the 16–21°C group (19.1 ± 4.0 mg g$^{-1}$, $P = 0.006$). One noteworthy exception to the overall pattern is the observed spike in liver glycogen at $t = 72\,\text{h}$ in the 20–27°C group (Fig. 3C) that was significantly greater than the 16–21°C and 22–27°C groups ($P = 0.04$ and $P < 0.001$, respectively).

WB glucose did not change with temperature treatment ($F_{3, 160} = 2.56, P = 0.057$) or time ($F_{4, 160} = 1.72, P = 0.15$; Table 3). However, as noted above, significant differences were observed between WB glucose pre-thermal stress time points ($P = 0.007$; Table 1).

We did not observe any differences in blood lactate among temperature treatments ($F_{3, 160} = 2.33, P = 0.08$; Table 3); however, we did note differences in blood lactate over time ($F_{4, 160} = 5.65, P < 0.001$). Blood lactate at $T_{\text{min}}$ throughout the elevated thermal cycles ($t = 72–120\,\text{h}$) did not differ from the pre-thermal stress intermediate $T_{\text{min}}$ ($t = 48; P = 0.96$ and $P = 0.87$, respectively), nor was there a significant difference at the peak of the first heat cycle ($t = 60\,\text{h}; P = 0.06$). However, blood lactate was significantly higher at the peak of the third heat cycle compared with the initial pre-temperature stress condition prior to thermal ramping ($t = 108\,\text{h}; P = 0.02$). Significant decreases in blood lactate were observed at $t = 120\,\text{h}$ compared with both thermal peaks ($t = 60\,\text{h}$ and $t = 108\,\text{h}; P = 0.004$ and $P = 0.001$, respectively).

**Cellular stress response**

Liver HSP70 was not induced under control conditions (Fig. 4A); however, a significant interaction was observed with time and temperature treatment ($F_{12, 160} = 9.68, P < 0.001$), indicating that the pattern of induction depended on thermal regime. Liver HSP70 was induced in the 18–27°C and 22–27°C groups at the pre-thermal stress sampling point after exposure to 23°C ($t = 48\,\text{h}; P < 0.001$ & $P = 0.02$, respectively). The peak of the first day of heat exposure induced a significant increase in liver HSP70 in all three high thermal cycles compared to the 16–21°C group ($t = 60\,\text{h}; P < 0.001$; Fig. 4); however, HSP70 was not significantly different among the temperature treatments. Although the specific pattern of liver HSP70 induction varied among temperature treatments, levels remained elevated throughout the duration of the 3-day heat event ($t = 60–120\,\text{h}; P < 0.001$ in all cases). After the onset of the event, treatments did not vary from one another statistically ($P = 0.07–0.99$), with the exceptions of the diel minima following the first and third heat cycles ($t = 72$ and 120 h) where HSP70 levels in the 18–27°C were significantly greater than in the 22–27°C group ($P = 0.018$ and 0.008, respectively; Fig. 4).

As was the case with HSP70, liver Ub was significantly increased over time ($F_{4, 160} = 4.29, P = 0.002$; Fig. 5), but we did not observe statistically significant differences among

**Table 2: Two-way ANOVA performed on physiological variables of juvenile Atlantic salmon exposed to multi-day thermal stress**

| Endpoint | Time | Treatment | Interaction |
|----------|------|-----------|-------------|
|          | df   | F-statistic | P-value | df | F-statistic | P-value | df | F-statistic | P-value |
| CTMax    | 1    | 1.14       | 0.290   | 3  | 31.93      | <0.001* | 3  | 0.16       | 0.920   |
| L glycogen | 4   | 6.93       | <0.001* | 3  | 6.73       | <0.001* | 12 | 2.63       | 0.003*  |
| WB glucose | 4   | 1.72       | 0.149   | 3  | 2.55       | 0.057   | 12 | 1.44       | 0.155   |
| WB lactate | 4   | 5.65       | <0.001* | 3  | 2.33       | 0.077   | 12 | 1.74       | 0.062   |
| L HSP70  | 4    | 100.61     | <0.001* | 3  | 177.05     | <0.001* | 12 | 9.68       | <0.001* |
| L Ub     | 4    | 4.29       | 0.002*  | 3  | 2.54       | 0.058   | 12 | 1.38       | 0.179   |
| RBC HSP70| 4    | 73.18      | <0.001* | 3  | 207.50     | <0.001* | 12 | 22.25      | <0.001* |
| RBC Ub   | 4    | 4.78       | 0.001*  | 3  | 15.03      | <0.001* | 12 | 1.07       | 0.388   |

Asterisks indicate significance of two-way ANOVA with $\alpha = 0.05$ and $P < 0.05$. 
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At the end of the experiment (t = 120 h), RBC HSP70 remained elevated in all thermal groups, but was significantly lower in the 20–27°C group compared to the 18–27°C and 22–27°C groups.

As was the case with HSP70, RBC Ub levels were comparatively lower than in liver (Fig. 7). RBC Ub changed over time and between temperature treatments (F(4, 160) = 4.78, P = 0.001; F(3, 160) = 15.03, P < 0.001; Table 2). Similar to liver Ub, all three thermal groups had significantly greater RBC Ub than the control at 16–21°C (P < 0.001), but did not differ significantly from one another (P = 0.47–0.99). RBC Ub significantly increased at t = 72 h, after the peak of the first heat cycle (P = 0.007). Unlike the case in liver where Ub levels were no longer significantly different from pre-thermal exposure at 120 h (P = 0.91), RBC Ub remained significantly higher than the pre-thermal exposure (t = 48 h) after the final thermal cycle (t = 120 h; P < 0.001).

Discussion

Environmental thermal patterning has been recognized as being intrinsically linked to fish physiology and performance (Hellman et al., 2009; Vasseur et al., 2014) and important for predicting the effects of climate change. We hypothesized that the nature of the thermal cycle, specifically nighttime temperature would dictate the physiological limits of juvenile Atlantic salmon. We found that our thermal cycles did initiate increases in CTMax and provided evidence of both cellular and metabolic stress. However, contrary to our expectation, we did not observe a correlation between the overall metabolic/cellular condition throughout a simulated heat event and the daily thermal minima. Thus, when fish are exposed to warming diel cycles approaching their critical temperature, we conclude that the diel thermal minima normally experienced by juvenile Atlantic salmon may not play a critical role in the ability of these fish to deal with extreme events. Notably, our experimental design, where we used a fixed maximum temperature, did not allow us to disentangle possible effects of the magnitude of diel thermal fluctuation from minimum nighttime temperatures. However, recent research from our group concluded that the nature of ecologically relevant diel thermal cycling (e.g. accumulated thermal exposure, magnitude, rate of change) did not significantly affect metabolism or the stress response in wild Atlantic salmon (Tunnah et al., 2017). Regardless, distinguishing between the importance of Tmin and ΔT is an important direction for future research.

Exposure to ecologically relevant thermal cycles (16–21°C) increased acute thermal tolerance (CTMax) compared to fish held at a stable acclimation temperature (16°C). Furthermore, we determined that exposure to a single elevated high temperature pulse (27°C) increased CTMax...
values by −1°C but neither diel $T_{\text{min}}$ nor exposure to repeated diel pulses further elevated CTMax. Acclimation temperature has long been known to influence thermal tolerance in ectotherms (Beitinger et al., 2000); however, few studies have examined the effects of thermal cycles on CTMax (see Bennett and Beitinger, 1997; Currie et al., 2004; Fangue et al., 2011). In support of our findings, diel cycling did not affect CTMax in killifish (Fundulus heteroclitus) (Healy and Schulte, 2012) or the northern two-lined salamander (Eurycea bislineata) (Rutledge et al., 1987). In both cases, CTMax increased only when overall acclimation temperature was increased. Furthermore, Healy and Schulte (2012) suggest a complex association between variation in acute thermal tolerance and physiological (i.e. altered membrane fluidity) and environmental (i.e. photoperiod, hypoxia and time of day) processes. In nature, maximum temperature tolerance is poorly understood and thought to be a function of time, diel mean and ΔT (Wehrly et al., 2007) with periods of repeated sublethal stress capable of delaying mortality (Selong et al., 2001).

Our thermal cycles induced significant changes in blood lactate and liver glycogen, with minimal differences among thermal regimes. Alterations to the initial metabolic condition when an organism is exposed to increasing (LeBlanc et al., 2011) and diel thermal stress are often short lived (<24 h; Tunnah et al., 2017). In our study, transitory cyclic increases in both blood lactate and glucose occurred in the warm thermal cycles. The modest decreases in liver glycogen we observed with high temperature cycling may be indicative of rapid mobilization of free glucose to surrounding metabolically active tissues resulting in a loss of metabolic capacity (Viant et al., 2003). After 3 days of thermal cycling, no difference in liver glycogen was apparent among treatments suggesting a comparably high energetic demand in all our diel thermal cycles. Our results therefore suggest that salmon metabolically manage diel cycles in an equivalent manner, regardless of nighttime temperature, refuting the notion that warmer thermal minima are most strenuous (see DFO, 2012). Wilkie et al. (1997) exposed exercised Atlantic salmon to stable recovery temperatures and determined fish were able to recover to a pre-stress metabolic status faster at warmer temperatures (23°C vs. 12 and 18°C). There is some field evidence for the preference of warm recovery temperatures during periods of thermal stress. For example, juvenile salmonids have been observed dispersing from aggregations formed at cool water sources at nightfall despite temperatures remaining above the species’ thermal optima (Belchik et al., 2004; Corey et al., unpublished data). Although it is implied that this behavior is driven by a thermal cue, our results indicate that this response may not be directly associated with a metabolic or cellular advantage to a particular $T_{\text{min}}$, but may be an effort to minimize the magnitude of temperature change experienced by the fish.

We know that temperatures >22°C will induce HSP70 in wild juvenile Atlantic salmon in the Miramichi River (Lund et al., 2002). Diel temperature stress induced an upregulation of six heat shock genes in redband trout (Oncorhynchus mykiss gairdneri) (Narum et al., 2013) and HSP70 in several tissues of Atlantic salmon (Tunnah et al., 2017). Given this information, the current temperature conditions of the Miramichi River, and regulations regarding recreational angling (closure when $T \geq 20$°C for two consecutive nights.

Table 3: Mean whole blood lactate and glucose (±SEM) in juvenile Atlantic salmon exposed to 16–21°C (control group) for 5 days; or multi-day thermal stress (18–27°C, 20–27°C or 22–27°C)

| Treatment (°C) | Time point (h) | 12°C | 24° | 48° | 60° | 72° | 108° | 120° |
|---------------|----------------|------|-----|-----|-----|-----|------|------|
| Whole blood glucose | 16-21 | 1.9 ± 0.29 | 3.2 ± 0.37 | 3.1 ± 0.32 | 2.1 ± 0.39 | 2.1 ± 0.37 | 2.0 ± 0.27 | 2.0 ± 0.19 |
| | 18-27 | 1.5 ± 0.15 | 2.0 ± 0.26 | 2.9 ± 0.29 | 2.7 ± 0.56 | 2.0 ± 0.26 | 2.7 ± 0.36 | 1.8 ± 0.18 |
| | 20-27 | – – | 1.8 ± 0.26 | 3.2 ± 0.42 | 2.7 ± 0.15 | 3.0 ± 0.42 | 1.9 ± 0.31 | 1.9 ± 0.31 |
| | 22-27 | – – | 2.9 ± 0.28 | 2.8 ± 0.64 | 2.1 ± 0.36 | 1.9 ± 0.30 | 2.3 ± 0.28 | 2.3 ± 0.28 |
| Whole blood lactate | 12°C | 2.6 ± 0.28 | 3.3 ± 0.42 | 3.7 ± 0.44 | 3.4 ± 0.60 | 4.6 ± 0.71 | 4.8 ± 0.85 | 2.1 ± 0.22 |
| | 16-21 | 3.7 ± 0.83 | 2.8 ± 0.34 | 3.1 ± 0.39 | 4.5 ± 0.88 | 2.6 ± 0.25 | 3.8 ± 0.30 | 1.9 ± 0.39 |
| | 18-27 | – – | 2.5 ± 0.37 | 4.3 ± 0.42 | 2.9 ± 0.37 | 4.5 ± 0.50 | 4.0 ± 0.73 | 4.0 ± 0.73 |
| | 20-27 | – – | 2.6 ± 0.79 | 5.2 ± 0.50 | 3.2 ± 0.36 | 4.8 ± 0.92 | 3.2 ± 0.16 | 3.2 ± 0.16 |
| | 22-27 | – – | 3.6 ± 0.79 | 5.2 ± 0.50 | 3.2 ± 0.36 | 4.8 ± 0.92 | 3.2 ± 0.16 | 3.2 ± 0.16 |

Stress occurred from 60 to 120 h. Pre-thermal stress sampling (12 and 24 h) assessed the effect of ‘trial’ and ‘bank’ and was analyzed separately. Letters are indicative of significance between sampling time points during thermal ramping ($t = 48$ h–120 h; $P < 0.05$). Asterisks indicate a complex association between variation in thermal stress and biological or environmental conditions.
DFO, 2012), our prediction was that juveniles experiencing higher nighttime temperatures would display a higher magnitude heat shock response (HSR) than those experiencing a lower diel \(T_{\text{min}}\). Instead, our data indicated that exposure to diel, environmentally relevant, sub-lethal heat stress, had cellular level consequences but the nature of high temperature cycling (i.e. nighttime temperature) had minor effects on the HSR. Thus, the magnitude of the thermal stress, regardless of diel cycle, was consistent with the magnitude of the HSR.

Assuming that thermally induced protein denaturation/damage triggers the HSR (Ananthan et al., 1986), our data suggest that protein damage is similar among our different diel cycles. In support of this, induction of Ub occurred at the peak of the first heat cycle and remained elevated throughout the heating event, regardless of thermal cycle, as was the case with HSP70. Tunnah et al. (2017) also demonstrated consistency of the HSR with Ub induction in thermally cycled Atlantic salmon.

Our measured physiological responses to thermal cycling were strikingly similar amongst the treatments and could be indicative of partial acclimation to increased temperatures caused by a slow ramping rate, or could suggest phenotypic plasticity to cope with thermal variability (Schulte et al., 2011; McBryan et al., 2013; Anttila et al., 2014). Metabolic stress in Atlantic salmon occurs at temperatures between 22°C and 24°C (Breau et al., 2011) demonstrating a limited ability to tolerate temperatures >28°C (Garside, 1973;
enduring 33°C only in acute circumstances (Elliott and Elliott, 1995). It is possible that genetic makeup and thermal history, specifically the frequency that these upper thermal thresholds are surpassed, may play a larger role in the metabolic and cellular status of juvenile salmon, while nighttime temperature may have little influence on a fish’s ability to re-establish basal cellular and metabolic conditions. Wehrly et al. (2007) determined maximum temperature tolerance to be a function of time in two trout species, Salvelinus fontinalis and Salmo trutta, where prolonged warm periods negatively influenced upper thermal tolerance limits. Furthermore, in chronically warmed European perch (Perca fluviatilis), Sandblom et al. (2016) determined that, despite cardiorespiratory plasticity to deal with a warmer resting condition, the upper thermal limit remained relatively rigid in both cold and warm adapted fish, consequently reducing the available ‘thermal buffer’.

Understanding how animals cope with large thermal fluctuations is critical to safeguard species in a changing climate. It has been suggested that if the rate of evolutionary or plastic responses lag behind the rate of climate change, there could be local extinction due to limitations in physiological capacities compared to the environmental variation (Chown et al., 2010). Here, we investigated key markers of the stress response in Atlantic salmon to determine how fish physiology responded to distinct warming scenarios with different nighttime temperatures. If high nighttime temperatures...

Figure 6: RBC HSP70 in juvenile salmon exposed to diel cycles: (A) 16–21°C only; or multi-day thermal stress (closed symbols; 60–120 h) of (B) 18–27°C; (C) 20–27°C; (D) 22–27°C. Data are presented as mean ± SEM (n = 5–9). Pre-thermal stress sampling (open symbols; t = 12 and 24 h) assessed the effect of ‘trial’ and was not included in the analysis. Letters indicate significant differences among treatments (P < 0.05) within sampling time points.

Figure 7: RBC Ub in juvenile salmon exposed to the following diel cycles: (A) 16–21°C only; or multi-day thermal stress (closed symbols; 60–120 h) of (B) 18–27°C; (C) 20–27°C; (D) 22–27°C. Points represent mean ± SEM (n = 4–9). Pre-thermal stress sampling (open symbols; t = 12 and 24 h) assessed the effect of ‘trial’ and was not included in the analysis. Asterisks indicate significant differences between treatments. Lettered shaded bars indicate significant differences between time points (P < 0.05).
resulted in physiological stress, we would expect that the warmest (22–27°C) treatment would elicit the most obvious signs of cellular and metabolic disturbance. However, this was not the case as different $T_{\text{min}}$ thermal scenarios had little effect on our dependent variables. These findings lead us to reject the hypothesis that environmentally relevant nighttime temperature is a principal driver in the ability of Atlantic salmon to tolerate multi-day thermal events at, or near, critical temperatures. With future climate change scenarios predicting an increase in thermal extremes and increases in diel thermal minima, it is likely that physiologically important thresholds will be surpassed more frequently. Current regulations regarding recreational angling close the Miramichi River when $T \geq 20^\circ\text{C}$ for two consecutive nights (DFO, 2012). Our results suggest that these regulations be revisited given that the overall effects of ‘warm nights’, at least within the temperature ranges tested here, do not appear to be a critical factor influencing Atlantic salmon. We do show that environmentally relevant diel thermal cycles up to 27°C are stressful for these fish; thus, management decisions should focus on ecologically grounded and relevant simulations of thermal stress and pay attention to maximum temperatures and $\Delta T$. While turning down the temperature of the planet is not an option, an understanding of such biological responses to warming water temperatures will inform the design of effective habitat management strategies to ensure the availability of cool water refugia, protecting Atlantic salmon during these inevitable thermal challenges.

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References

Ananthan J, Goldberg, A, Voellmy R (1986) Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. Science 232: 522–524.

Anttila K, Couturier CS, Överli Ø, Johnsen A, Marthinsen G, Nilsson GE, Farrell AP (2014) Atlantic salmon show capability of cardiac acclimation to warm temperatures. Nat Commun 5: 4252. doi:10.1038/ncomms5252.

Beauregard D, Enders E, Boisclair D (2013) Consequences of circadian fluctuations in water temperatures on the standard metabolic rate of Atlantic salmon parr (Salmo salar). Can J Fish Aquat Sci 70: 1072–1081.

Becker CD, Genoway RG (1979) Evaluation of the critical thermal maximum for determining thermal tolerance in freshwater fish. Environ Biol Fishes 4: 245–256.

Beitinger TL, Bennett WA, McCauley RW (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environ Biol Fishes 58: 237–275.

Belchik M, Hillemeier D, Pierce RM (2004) The Klamath River fish kill of 2002; analysis of contributing factors. Yurok Tribal Fisheries Program, 42 pp.

Bennett WA, Beitinger TL (1997) Temperature tolerance of the sheepshead minnow, Cyprinodon variegatus. Copeia: 77–88.

Bergmeyer HU, Gawehn K, Grassi M (1974) Enzymes as biochemical reagents. In HU Bergmeyer, ed, Methods of Enzymatic Analysis. Academic Press, New York, pp 423–522.

Breau C, Cunjak RA, Peake SJ (2011) Behaviour during elevated water temperatures: can physiology explain movement of juvenile Atlantic salmon to cool water? J Anim Ecol 80: 844–853.

Caisse D (2006) The thermal regime of rivers: a review. Freshwater Biol 51:1389–1406.

Caisse D, Breau C, Hayward J, Cameron P (2012) Water temperature characteristics within the Miramichi and Restigouche rivers. Can Sci Advis Sec Res Doc 2012/nmn. vi + xx p.

Caisse D, El-Jabi N, Turkkan N (2014) Stream water temperature modeling under climate change scenarios B1 & B2. Can Tech Rep Fish Aquat Sci 3106: ix + 51p.

Chadwick JG Jr, Nislow KH, McCormick SD (2015) Thermal onset of cellular and endocrine stress responses correspond to ecological limits in brook trout, an iconic cold-water fish. Conserv Physiol 3: 1–12. doi:10.1093/conphys/cov017.

Chown SL, Hoffmann AA, Kristensen TN, Angilletta MJ, Stenseth NC, Pertoldi C (2010) Adapting to climate change: a perspective from evolutionary physiology. Clim Res 43(3): 3–15.

Clow KA, Rodnick KJ, MacCormack TJ, Drydick WR (2004) The regulation and importance of glucose uptake in the isolated Atlantic cod
heart: rate-limiting steps and effects of hypoxia. J Exp Biol 207: 1865–1874.

Cooke SJ, Donaldson MR, Clark TD, Eliason EJ, Crossin GT, Raby GD, Jeffries KM, Lapointe M, Miller K, Patteson DA, et al. (2012) Conservation physiology in practice: how physiological knowledge has improved our ability to sustainably manage Pacific salmon during up-river migration. Phil Trans R Soc B 367: 1757–1769.

Crossin GT, Hinch SG, Cooke SJ, Welsh DW, Patteson DA, Jones SRM, Lotto AG, Leggatt RA, Mathes MT, Shrimpton JM, et al. (2008) Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migration. Can J Zool 86: 127–140.

Currie RJ, Bennett WA, Beitinger TL, Cherry DL (2004) Upper and lower temperature tolerances of juvenile freshwater game-fish species exposed to 32 days of cycling temperatures. Hydrobiologia 523: 127–136.

DFO (2012) Temperature threshold to define management strategies for Atlantic salmon (Salmo salar) fisheries under environmentally stressful conditions. DFO Can Sci Advis Sec Sci Rep 2012/019.

DFO (2013) Atlantic salmon (Salmo salar) returns to the Miramichi River (NB) for 2012. DFO Can Sci Advis Sec Sci Rep 2013/009.

Eldridge WH, Sweeney BW, Law JM (2015) Fish growth, physiological stress, and tissue condition in response to rate of temperature change during cool or warm diel thermal cycles. Can J Fish Aquat Sci 72: 1527–1537.

Elliott JM (1991) Tolerance and resistance to thermal stress in juvenile Atlantic salmon, Salmo salar. Freshwater Biol 25: 61–70.

Elliott JM, Elliott JA (1995) The effect of the rate of temperature increase on the critical thermal maximum for parr of Atlantic salmon and brown trout. J Fish Biol 47: 917–919.

Elliott JM, Elliott JA (2010) Temperature requirements of Atlantic salmon Salmo salar, brown trout Salmo trutta and Arctic char Salvelinus alpinus: predicting the effects of climate change. J Fish Biol 77: 1793–1817.

Elliott JM, Hurley MA (1997) A functional model for maximum growth of Atlantic salmon parr, Salmo salar, from two populations in northwest England. Funct Ecol 11: 592–603.

Fangue NA, Osbourne EJ, Todgham AE, Schulte PM (2011) The onset temperature of the heat-shock response and whole-organism thermal tolerance are tightly correlated in both laboratory-acclimated and field-acclimated tidepool sculpins (Oligocottus maculosus). Physiol Biochem Zool 84: 341–352.

Fangue NA, Hofmeister M, Schulte PM (2006) Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, Fundulus heteroclitus. J Exp Biol 209: 2859–2872.

Fowler SL, Hamilton D, Currie S (2009) A comparison of the heat shock response in juvenile and adult rainbow trout (Oncorhynchus mykiss)-implications for increased thermal sensitivity with age. Can J Fish Aquat Sci 66: 91–100.

Gallant MJ, LeBlanc S, MacCormack TJ, Currie S (2017) Physiological responses to a short-term, environmentally realistic acute heat stress in Atlantic salmon, Salmo salar. Facets. in press. doi: 10.1139/facets-2016-0053.

Garside ET (1973) Ultimate upper lethal temperature of Atlantic salmon Salmo salar L. Can J Zool 51: 898–900.

Healy TM, Schulte PM (2012) Factors affecting plasticity in whole-organism thermal tolerance in common killifish (Fundulus heteroclitus). J Comp Physiol B 182: 49–62.

Helfman G, Collette BB, Facey DE, Bowen BW (2009) The diversity of fishes: biology, evolution, and ecology. John Wiley & Sons, Hoboken, pp 720.

Houston AH, Gingras-Bedard JH (1994) Variable versus constant temperature acclimation regimes: effects on hemoglobin isomorph profile in goldfish, Carassius auratus. Fish Physiol Biochem 13: 445–450.

Jobling M (1981) Temperature tolerance and the final preferendum-rapid methods for assessment of optimum growth temperatures. J Fish Biol 19: 439–455.

Jonsson B, Forseth T, Jensen AJ, Naessje TF (2001) Thermal performance of juvenile Atlantic salmon, Salmo salar L. Funct Ecol 15: 701–711.

Kolhatkar A, Robertson CE, Thistle ME, Gampfer AK, Currie S (2014) Coordination of chemical (trimethylamine oxide) and molecular (heat shock protein 70) chaperone responses to heat stress in elasmobranch red blood cells. Physiol Biochem Zool 87: 652–662.

LeBlanc S, Middleton S, Gilmour KM, Currie S (2011) Chronic social stress impairs thermal tolerance in the rainbow trout (Oncorhynchus mykiss). J Exp Biol 214: 1721–1731.

Lund SG, Caissie D, Cunjak RA, Vijayan MM, Tufts BL (2002) The effects of environmental heat stress on heat-shock mRNA and protein expression in Miramichi Atlantic salmon (Salmo salar) parr. Can J Fish Aquat Sci 59: 1553–1562.

MacLellan RJ, Tunnah L, Barnett D, Wright PA, MacCormack T, Currie S (2015) Chaperone role for TMAO and HSP70 during hyposmotic stress in the spiny dogfish shark (Squalus acanthias). J Comp Physiol B 185: 729–740.

McBryant TL, Antilla K, Healy TM, Schulte PM (2013) Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. Inter Comp Biol 53: 648–659.

McCormick SD, Cunjak RA, Dempson B, O’Dea MF, Carey JB (1999) Temperature-related loss of smolt characteristics in Atlantic salmon (Salmo salar) in the wild. Can J Fish Aquat Sci 56: 1649–1658.

Mesa MG, Weiland LK, Wagner P (2002) Effects of acute thermal stress on the survival, predator avoidance, and physiology of juvenile fall Chinook salmon. Northwest Sci 76: 118–128.
Moritz K, Fukuwaka M, Tanimata N (2010) Size-dependent thermal preferences in a pelagic fish. Oikos 119: 1265–1272.

Muñoz NJ, Farrell AP, Heath JW, Neff BD (2014) Adaptive potential of a Pacific salmon challenged by climate change. Nat Clim Change. doi:10.1038/nclimate2473.

Narum SR, Campbell NR, Meyer KA, Miller MR, Hardy RW (2013) Thermal adaptation and acclimation of ectotherms from differing aquatic climates. Mol Ecol 22: 3090–3097.

Oligny-Hébert H, Senay C, Enders EC, Boisclair D (2015) Effects of diel temperature fluctuation on the standard metabolic rate of juvenile Atlantic salmon (Salmo salar): influence of acclimation temperature and provenience. Can J Fish Aquat Sci 72: 1306–1315.

Pörtner H-O, Bock C, Knust R, Lannig G, Mark FC, Sartoris FJ (2008) Cod and climate in a latitudinal cline: physiological analyses of climate effects in marine fishes. Clim Res 37: 253–270.

R Development Core Team. 2016 R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rendell JL, Fowler SL, Cockshutt A, Currie S (2006) Development-dependent differences in intracellular localization of stress proteins (hsps) in rainbow trout, Oncorhynchus mykiss, following heat shock. Comp Biochem Phys D 1: 238–252.

Rodnick KJ, Gamprel AK, Lizards KR, Bennett MT, Rausch RN, Keely ER (2004) Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. J Fish Biol 64: 310–335.

Rutledge PS, Spotila JR, Easton DP (1987) Heat hardening in response to two types of heat shock in the lungless salamanders Eurycea bislineata and Desmognathus ochrophaeus. J Therm Biol 12: 235–241.

Sandblom E, Clark TD, Gräns A, Ekström A, Brijs J, Sundström LF, Odelström A, Adili A, Aho T, Jutfelt F (2016) Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. Nat Commun 7: 11447. doi:10.1038/ncomms11447.

Schulte PM, Healy TM, Fangue NA (2011) Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integr Comp Biol 51: 691–702.

Selong JH, McMahon TE, Zale AV, Barrows FT (2001) Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerance in fishes. Trans Am Fish Soc 130: 1026–1037.

Swansburg E, Chaput G, Moore D, Caissie D, El-Jabi N (2002) Size variability of juvenile Atlantic salmon: links to environmental conditions. J Fish Biol 61: 661–683.

Threader RW, Houston AH (1983) Heat tolerance and resistance in juvenile rainbow trout acclimated to diurnally cycling temperatures. Comp Biochem Physiol 75: 153–155.

Tunnah L, Currie S, MacCormack T (2017) Do prior diel thermal cycles influence the physiological response of Atlantic salmon (Salmo salar) to subsequent heat stress? Can J Fish Aquat Sci 74:127–139.

Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CDG, McCann KS, Savage V, Tunney TD, O’Connor MI (2014) Increased temperature variation poses a greater risk to species than climate warming. Proc R Soc B 281: 20132612. http://dx.doi.org/10.1098/rspb.2013.2612.

Viant MR, Werner I, Rosenblum ES, Gantner AS, Tjeerdema RS, Johnson ML (2003) Correlation between heat-shock protein induction and reduced metabolic condition in juvenile steelhead trout (Oncorhynchus mykiss) chronically exposed to elevated temperature. Fish Physiol Biochem 29:159–171.

Wehrly KE, Wang L, Mitro M (2007) Field-based estimates of thermal tolerance limits for trout: incorporating exposure time and temperature fluctuation. Trans Am Fish Soc 136: 365–374.

Wilkie MP, Brobbel MA, Davidson K, Forsyth L, Tufts BL (1997) Influences of temperature upon the postexercise physiology of Atlantic salmon (Salmo salar). Can J Fish Aquat Sci 54: 503–511.