Intraspecific Variation in Thermal Tolerance and Acclimation Capacity in Brook Trout (*Salvelinus fontinalis*): Physiological Implications for Climate Change*

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Accepted 9/18/2013; Electronically Published 12/30/2013

ABSTRACT

Cold-water fishes are becoming increasingly vulnerable as changing thermal conditions threaten their future sustainability. Thermal stress and habitat loss from increasing water temperatures are expected to impact population viability, particularly for inland populations with limited adaptive resources. Although the long-term persistence of cold-adapted species will depend on their ability to cope with and adapt to changing thermal conditions, very little is known about the scope and variation of thermal tolerance within and among conspecific populations and evolutionary lineages. We studied the upper thermal tolerance and capacity for acclimation in three captive populations of brook trout (*Salvelinus fontinalis*) from different ancestral thermal environments. Populations differed in their upper thermal tolerance and capacity for acclimation, consistent with their ancestry: the northernmost strain (Lake Nipigon) had the lowest thermal tolerance, while the strain with the most southern ancestry (Hill's Lake) had the highest thermal tolerance. Standard metabolic rate increased following acclimation to warm temperatures, but the response to acclimation varied among strains, suggesting that climatic warming may have differential effects across populations. Swimming performance varied among strains and among acclimation temperatures, but strains responded in a similar way to temperature acclimation. To explore potential physiological mechanisms underlying intraspecific differences in thermal tolerance, we quantified inducible and constitutive heat shock proteins (HSP70 and HSC70, respectively). HSPs were associated with variation in thermal tolerance among strains and acclimation temperatures; HSP70 in cardiac and white muscle tissues exhibited similar patterns, whereas expression in hepatic tissue varied among acclimation temperatures but not strains. Taken together, these results suggest that populations of brook trout will vary in their ability to cope with a changing climate.

Introduction

Environmental temperature is a major factor limiting the habitat preferences and biogeographical distributions of ectotherms (Pörtner 2002; Somero 2011). As temperatures rise, rates of metabolic reactions increase until an optimum temperature is reached, followed by a corresponding decrease in reaction rate. Within this thermal optimum, metabolism, scope for activity, and other key attributes of organismal performance function at maximum efficiency (Brett 1971; Ficke et al. 2007); outside this optimum, organismal performance declines (e.g., Ficke et al. 2007; Schulte et al. 2011).

Climate change is predicted to have large impacts on many species and ecosystems (Ficke et al. 2007; Brander 2010; Somero 2010; Tomanek 2010). Globally, near-surface air temperatures are expected to rise on average by 3.5°–4.2°C over the next 50 yr (IPCC 2007) and possibly as much as 7°C over the next 100 yr (Prowse et al. 2006). In freshwater lakes, climate change is predicted to affect water temperature profiles (Snucins and Gunn 2000), which will be devastating for cold-water fishes. For example, an increase of 3°C in water temperature is predicted to result in a 20% decrease in the range and abundance of cold-water salmonid populations (Casselman 2002). Without options for migration or dispersal, lacustrine populations of cold-water fish species will be forced to endure local environmental changes.

The ability to adjust physiological performance to prevailing environmental conditions (acclimation) may allow individuals

* This paper was submitted in response to a call for papers for a Focused Issue on "Conservation Physiology."

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Physiological and Biochemical Zoology 87(1):15–29. 2014. 1522-2152/2014/8701-2153$15.00. DOI: 10.1086/675259

to maximize fitness in the face of environmental change. Recent studies suggest, however, that members of different populations may differ in their capacity to acclimate, implying that such plasticity may be genetically constrained (Seebacher et al. 2012). Understanding intraspecific variation in performance and capacity for acclimation can provide insights into population- and species-level adaptive potential for coping with climate change (Seebacher and Franklin 2012; Seebacher et al. 2012).

One possible mechanism underpinning whole-organism thermal tolerance is the heat shock response (HSR; Mosser and Bols 1988; Feder et al. 1996; Krebs and Bettencourt 1999). In response to a thermal insult, the HSR produces a family of proteins that act to prevent protein aggregation and denaturation and provide molecular chaperoning (Feder and Hofmann 1999). Two highly conserved and expressed proteins from the HSR are the 70 kDa heat shock proteins (HSPs), heat shock protein 70 (HSP70) and heat shock cognate 70 (HSC70). HSP70 expression is elevated under stressful conditions to facilitate cellular protection and repair. It acts to prevent protein aggregation, aids in folding of nascent polypeptides, assists in repair and degradation of denatured proteins, and acts as a molecular chaperone (Currie 2011). In contrast, HSC70 is constitutively expressed at the cellular level to provide and facilitate similar protection and chaperoning (reviewed in Lindquist and Craig 1988; Feder and Hofmann 1999). Despite the putative link between thermal tolerance and the HSR across a variety of terrestrial and aquatic species (Tomanek and Somero 2002; Garbuz et al. 2003; Sortre and Hoffman 2005), the exact nature of this relationship is unclear, particularly in fish (LeBlanc et al. 2011; Healy and Schulte 2012).

Although salmonid fishes have been extensively used as vertebrate models for studying thermal performance and optima in aquatic systems (Brett 1944, 1971; Elliott 1981), previous research has yielded conflicting evidence for the ability of salmonid fishes to respond and adapt to variations in temperature. Redband trout (*Oncorhynchus mykiss gairdneri*) have shown localized adaptation to warmer temperatures (Narum et al. 2010), and sockeye salmon (*Oncorhynchus nerka*) populations exhibit differential thermal adaptive capacity linked to cardiorespiratory physiology in response to varying thermal conditions along migratory routes (Eliason et al. 2011). By contrast, intraspecific differences in thermal tolerance were not found between two populations of rainbow trout (*Oncorhynchus mykiss*; Myrick and Cech 2000). Other studies have also suggested that cold-water fishes have limited adaptive potential with regard to changing climates (Larsson et al. 2005; Elliott and Elliott 2010).

In this study, we compared the thermal tolerance of three strains of hatchery-reared brook trout (*Salvelinus fontinalis*), predicted to differ in upper thermal tolerance limits based on their ancestral geographic origins. As a broadly distributed species with a complex phylogeographic history, brook trout are an ideal candidate for examining intraspecific variation in thermal tolerance. Brook trout are considered stenothermal, with a thermal zone of preference that spans from 14° to 18°C, and avoid temperatures in excess of 20°C (Cherry et al. 1975; Power 1980). Their native range spans both formerly glaciated and nonglaciated regions of eastern North America (Scott and Crossman 1973), with geographic structuring among six phylogeographic lineages (Danzmann et al. 1998). Climate change is impacting much of their geographic range, suggesting that populations from the species range limits may be adversely affected (Meisner 1990*a*, 1990*b*; Gunn and Snucins 2010). However, differences in growing degree-days could lead to local adaptation in thermal tolerance and differences in the ability of wild populations to acclimatize (Conover et al. 2005). Although intraspecific variation in thermal tolerance of the genus *Salvelinus* has been investigated previously (McCauley 1958; Sale 1962; Wehrly et al. 2007), there is no information regarding acclimation temperatures as proxy for climate change or consideration of the HSR following acclimation.

We tested the hypothesis that differences among strains of brook trout in thermal tolerance and performance traits could be attributed to biogeographic and climatic differences among ancestral source populations. The effects of population origin and acclimation on upper thermal tolerance, standard metabolic rate (SMR), and swimming performance were tested using a factorial experimental design. We also sought to identify potential mechanisms underlying thermal tolerance by assessing expression levels of the highly stress-inducible HSP70 and its constitutive isoform, HSC70, across several tissues. The observed differences at organismal and molecular levels provide compelling evidence for substantial intraspecific variation in thermal tolerance and adaptive potential.

Material and Methods

Brook Trout Strains

We studied three hatchery strains of brook trout (Dickson Lake, Lake Nipigon, and Hill's Lake) used by the Ontario Ministry of Natural Resources (OMNR) for stocking purposes. These strains differ in their geographic origin, climate, and ecological traits (OMNR 2005; Kerr 2006). The Dickson Lake strain was recently established in the hatchery system from wild spawn collections from Dickson Lake in south-central Ontario (Algonquin Provincial Park; 45°47′N, 78°12′W). This population had been in the hatchery system for one full generation at the initiation of the experiment. The Lake Nipigon strain originated from wild spawn collections from Lake Nipigon (49°50′N, 88°30′E) in northwestern Ontario and has been maintained in the provincial hatchery system for eight generations (OMNR 2005). The Hill's Lake strain originated from multiple sources more than 80 yr ago (25+ generations) and was originally founded from fish collected in Pennsylvania (OMNR 2005). Despite periodic infusion of genes from wild Ontario populations, the Hill's Lake strain retains substantial Pennsylvanian ancestry and is considered a domesticated strain due to its many hatchery generations (OMNR 2005; Kerr 2006). Broodstocks for all strains are held at the OMNR Hill's Lake Fish Culture Station (47°44′N, 80°2′W) in northeastern Ontario under the same environmental conditions at seasonally ambient light and temperature regimes. The facility has a mean annual water

temperature of 5.8°C, with a seasonal range of 2.0°–9.0°C (OMNR 2005).

Growing degree-days (GDD) represents the time integral of the daily temperature above 5°C. It is calculated by subtracting 5°C from the mean daily temperature: if the value is <5°C, then the day receives a 0; if it is ≥5°C, then the mean daily temperatures are added together for the year. The threshold value of 5°C is a physiologically scaled temperature since fish are ectotherms and temperature dictates physiological processes (reviewed in Neuheimer and Taggart 2007). Brook trout across the species range experience 800–2,700 GDD over 5°C (Watson and MacIver 2000). Climate conditions for the local source strains range from Lake Nipigon, with 1,200–1,300 GDD and a mean July air temperature of 16.5°C, to Dickson Lake, with 1,600–17,00 GDD and 18.1°C, to Hill's Lake Fish Culture Station, with 2,600–2,700 GDD and 19.6°C (Watson and MacIver 2000; IPCC 2005).

Experimental Subjects and Husbandry

Adults from each strain were spawned in fall 2008 at the OMNR Hill's Lake Fish Culture Station using one 5 × 5 cross for each strain (mating five females with each of five males). Families of fertilized eggs were kept separate and transported to the OMNR Codrington Fisheries Research facility (44°9′N, 77°48′W), where they were raised to hatching in identical partitioned Heath trays. Upon hatching, fry were transferred from Heath trays to replicate tanks and maintained in family-specific lots. All families were kept separate but reared under common controlled conditions, through all life stages, prior to the beginning of experiments. All families were reared under seasonally ambient temperature and light conditions, with exposure to natural light. In May 2010, all yearling brook trout received population-specific tags using visible implant elastomers (VIE; Northwest Marine Technologies, Shaw Island, WA) implanted subcutaneously behind their left eye. As the elastomer markings were used to distinguish between strains being reared at the same acclimation temperatures, each strain received only one mark. Although tracking family-specific information would have been desirable, this was not possible due to logistical constraints.

Experimental Design

Equal numbers of fish (200 individuals per strain, 150 total individuals per acclimation treatment) were randomly and evenly divided (25 fish strain^{-1} tank^{-1}) into eight 200-L thermal acclimation tanks (Frigid Units, Toledo, OH). In this way, fish from all three strains were held under identical conditions for each rearing temperature but were distinguishable based on VIE markings. All acclimation tanks were at ambient temperature (ca. 8°C) when the fish were first introduced to the tanks. Tanks were then randomly assigned to one of four target temperatures (8°, 12°, 16°, and 20° ± 1°C); two temperatures (8° and 20°C) were outside the thermal preference zone for brook trout, and two (12° and 16°C) were within this range (summarized in Power 1980). There were two replicate tanks for each temperature. The water temperature of each tank was gradually raised at a rate of 1°C d^{-1} until the desired temperatures were achieved and then maintained (±1.0°C) for a minimum of 4 wk to allow fish to fully acclimate. Dissolved oxygen levels in acclimation tanks were maintained above 6 mg L^{-1} using a combination of air stones and compressed air. Fish were fed to satiation twice daily using 1.5 mm Optimum Salmonid feed (COREY Nutrition, Fredericton, NB). Prior to any experimental challenge, food was restricted for the previous 24 h. Fish were reared under ambient lighting. All experimental procedures were approved by the Trent University and OMNR Animal Care Committees (protocols 10038 and 09-85, respectively).

Upper Thermal Tolerance

To assess intraspecific variation in thermal tolerance, we performed a critical thermal maximum challenge, whereby the temperature at which individual fish lost equilibrium following a 0.33°C min^{-1} increase in water temperature was measured (e.g., Beitinger et al 2000). Using the temperature at which equilibrium was first lost (27.5°C), we then measured Elliott's (1981) hybrid critical thermal maximum. This is the length of time individuals can remain at the minimum endpoint temperature (determined above) before losing equilibrium (defined as an inability to maintain dorsoventral orientation).

Groups of randomly selected fish from each strain (minimum 12 individuals per strain) were introduced into a 172-L thermal challenge tank at their acclimation temperature (8°, 12°, 16°, or 20°C) and allowed to acclimate overnight. The temperature of each thermal challenge tank was then increased to 27.5°C over a 1-h period through adjustment of both the flow of hot water (ca. 60°C) via four aluminum plated heating elements at the base of the tank and the inflow of fresh ambient water (ca. 8°C) into the center of the tank. To ensure normoxia, dissolved oxygen levels were monitored using a YSI Pro dissolved oxygen probe (±0.2 mg L^{-1}). Water temperature of each tank was maintained at 27.5°C, and fish were kept under continuous observation. Individuals were removed from the test tank as soon as they exhibited any sign of loss of equilibrium and time at removal was recorded. This time represented Elliott's (1981) hybrid critical thermal maximum and was used as a proxy for upper thermal tolerance. Fish were then immediately anesthetized using a $NaHCO_3$-buffered solution (Sigma-Aldrich, St. Louis, MO) in order to obtain length and mass measurements.

Standard Metabolic Rate

The rate of resting oxygen consumption was determined for 120 individuals (10 individuals per strain, 3 strains per acclimation temperature, 4 acclimation temperatures) using a single-channel, intermittent, open-flow respirometry system (QUBIT Systems, Kingston, ON). Fish were individually introduced into a glass respiration chamber (800 mL) at the fish's

acclimation temperature (8°, 12°, 16°, or 20°C) for a 2-h orientation period. Following this orientation period, resting oxygen consumption rates were determined over a 60-min period. The individual was then removed from the chamber, anesthetized with MS-222 (Sigma-Aldrich), weighed and measured, and then returned to its original holding tank for recovery.

Resting oxygen consumption was determined for each individual by obtaining three regression lines ($\Delta O_2/\Delta T$) using intermittent-flow respirometry. These regression values were obtained following the 2-h acclimation period and were multiplied by the total volume of the respirometer. These values were averaged to obtain resting oxygen consumption, as an estimate of SMR.

Critical Swimming Performance

We measured critical swimming performance (U_{crit}) using a 90-L Brett-type swim tunnel respirometer (Brett 1964). Randomly selected fish from the same acclimation temperature were introduced to the recirculating swim flume in the swim tunnel in groups of five at their acclimation temperature, such that fish from the different strains at a shared acclimation temperature were tested together. In total, 120 fish had their swimming performance measured (2 trials of 5 individuals per trial, 10 individuals per strain, 3 strains per acclimation temperature, 4 acclimation temperatures). Trials were run on groups rather than individuals, as salmonids can be challenged in schools without significantly affecting swimming performance (Gregory and Wood 1998). Fish were allowed to acclimate to the swim flume for 1 h at a low water velocity (ca. 0.5 body length [BL] s^{-1}) to become oriented with the flume. Following the 1-h orientation, water flow was increased to 1 BL s^{-1}. Every 5 min water flow was progressively increased by 1 BL s^{-1} (following McClelland et al. 2006) until the fish exhibited any sign of loss of equilibrium, at which point the individual was immediately removed from the tank. The time and water velocity at removal were recorded. Fish were then placed in a recovery tank, contained within the original acclimation tank (following Fangue et al. 2008).

The U_{crit} was calculated following Brett (1964). The final swimming speed achieved prior to fatigue was defined as U_{crit} and calculated as follows:

$$U_{crit} = V_{1s} + \left(\frac{t_s}{t_i}\right) \times V_i,$$

where t_i is the time interval between water velocity increases, t_s is the time swam during final time interval, V_i is the velocity increment of increase, and V_{1s} is the final velocity increment achieved.

Heat Shock Challenge

Groups of fish from each strain (5 individuals from each strain per trial, 2 heat shock trials per acclimation temperature, 3 strains per acclimation temperature; $n = 120$ total) were introduced to the 172-L thermal challenge tank ($n = 15$ fish per trial total) at their acclimation temperature and allowed to acclimate overnight. Tanks received continuous aeration to ensure a dissolved oxygen concentration of greater than 6 mg L^{-1} using a dissolved oxygen probe (YSI Pro 20; ± 0.2 mg L^{-1}), and food was restricted for 24 h prior to experimentation.

The heat shock challenge followed Currie et al. (2010). Briefly, initial water temperature was set at the fish's acclimation temperature and then gradually increased at a rate of 3°C h^{-1} to a maximum of 25°C. It should be noted that fish from each acclimation temperature experienced a different magnitude of heat shock, with the 20°C-acclimated fish reaching 25°C in less than 2 h, while it took over 5 h for the 8°C-acclimated fish to begin the heat shock. Fish remained at 25°C for 1 h, after which water temperature was cooled to the initial acclimation temperature over a 1-h period. Fish remained in the thermal challenge tank for 24 h of recovery and were then killed. Measurements of mass and length were made, and white muscle, liver, and cardiac tissues were rapidly collected and immediately frozen in liquid nitrogen and then stored at −80°C until analyzed for HSPs. To determine protein levels prior to heat shock, we removed fish from each strain (10 individuals from each strain, 3 strains per acclimation temperature, 120 individuals total) from the acclimation tank and immediately killed, measured, and dissected them as above.

Heat Shock Protein Quantification

Soluble protein was extracted from tissues using methods described in LeBlanc et al. (2011), and protein concentrations for each sample were quantified using a Bio-Rad (Hercules, CA) DC protein assay, with bovine serum albumin as a standard. Microplates were read at 750 nm using a 96-well tunable microplate reader (Versa Max, Sunnyvale, CA). HSPs from cardiac, hepatic, and white muscle tissues (15 μg of soluble protein) were quantified through Western blotting using a Novex Midi gel system (Invitrogen, Carlsbad CA) as in Currie et al. (2010). For both HSP70 and HSC70, a sample from a heat-shocked fish was loaded onto every gel to act as a control and allow direct comparison between gels. Rabbit antisalmonid–inducible HSP70 polyclonal antibodies or rabbit antisalmonid constitutive–expressed HSC70 polyclonal antibodies (Agrisera, Vannas, SE) were used for immunodetection. Goat antirabbit IgG (SAB-300) was used as the secondary antibody. The goat antirabbit was then detected using the ECL Advance Chemiluminescent Western Blotting Detection Kit (GE Healthcare, Baie d'Urfe, QC), and protein bands were quantified using a VersaDoc MP 4000 (Bio-Rad) molecular imager and Image Lab software (Bio-Rad). Protein levels were divided by the control sample band density to give relative band density for each individual.

Statistical Analysis

All fish were treated as individual data points in all statistical analyses. Data met assumptions of normality, and, where required, data were $\log_e$ transformed to meet the assumption of

homogeneity of variance (e.g., mass, knockdown time, oxygen consumption, absolute U_{crit}, and heat shock). A two-way ANOVA was used to test for significant differences in body mass and fork length among strains in each treatment, testing for effects of population origin, acclimation temperature, and their interaction. To examine the effects of population and acclimation temperature on Elliott's (1981) hybrid critical thermal maximum (knockdown time), SMR, and U_{crit}, we used an ANCOVA with body mass as a covariate or fork length in the case of U_{crit}. Condition factor was also used as a covariate and showed results similar to body mass (data not shown). All ANCOVA data met the homogeneity of slopes assumption. To avoid the use of ratios, statistics were conducted on absolute U_{crit} values (m s^{-1}); however, figures are expressed in relative U_{crit} values (relative to fork length − body lengths s^{-1}), to allow for comparisons with the published literature. For upper thermal tolerance, SMR, and U_{crit}, we calculated the proportion of variance explained by the strain and acclimation temperature effects in this model using ω^2 (Quinn and Keough 2002).

To analyze inducible HSP70 levels prior to heat shock (for white muscle, the only tissue showing a response at this time point) and after heat shock (for cardiac, hepatic, and white muscle), we performed separate two-way ANOVA with population origin and acclimation temperature as main effects and the interaction between population origin and acclimation temperature. A two-way ANOVA was performed on expression of HSC70 in cardiac, hepatic, and white muscle tissues. Effect time period (before and after heat shock) was tested and analyses were subsequently split by time period to test for the effects of population origin, acclimation temperature, and their interaction on HSC70 following heat shock. When significant effects were detected, Tukey's HSD was used to explore significant differences. Statistical significance was claimed at $P < 0.05$. All analyses were performed using JMP v 8.0.2 (SAS Institute, Cary, NC).

Results

Physical Characteristics of Brook Trout Strains

Following thermal acclimation, the three strains of brook trout differed significantly in body mass and fork length ($F_{2,553} = 141.64$, $P < 0.0001$ and $F_{2,553} = 272.66$, $P < 0.0001$, respectively; table 1). The Hill's Lake strain was significantly heavier (21.00 ± 0.49 g; $P < 0.001$) and longer (12.30 ± 0.09 cm; $P < 0.001$) than either the Lake Nipigon (mass = 13.11 ± 0.29 g, length = 11.15 ± 0.07 cm) or Dickson Lake (mass = 13.32 ± 0.29 g, length = 11.12 ± 0.07 cm) strains. The Dickson Lake and Lake Nipigon strains did not differ significantly from each other in length or mass ($P = 0.97$ and $P = 0.92$, respectively). Because fish within each strain had been randomly assigned to the different treatments, mass and length did not differ among acclimation temperature ($F_{3,553} = 1.29$, $P = 0.27$ and $F_{3,553} = 2.16$, $P = 0.09$, respectively). However, there was a significant interaction between population origin and acclimation temperature for mass and length ($F_{6,553} = 3.35$, $P = 0.0029$ and $F_{6,553} = 3.75$, $P = 0.0011$, respectively), suggesting that the strains differed in their growth responses to the different acclimation temperatures. The Hill's Lake strain was significantly larger and heavier at each acclimation temperature, while the other two strains did not differ from each other (table 1).

Table 1: Fork length (cm) and body mass (g) of three brook trout strains maintained for at least 30 d at one of four acclimation temperatures

Acclimation temperature (°C) and strain	*N*	Fork length (cm)	Mass (g)
8:			
Lake Nipigon	44	11.00 ± .13^{A}	12.52 ± .51^{A}
Dickson Lake	44	11.36 ± .16^{AB}	13.97 ± .66^{A}
Hill's Lake	41	12.16 ± .16^{B}	20.14 ± .92^{B}
12:			
Lake Nipigon	46	11.16 ± .16^{A}	12.78 ± .65^{A}
Dickson Lake	51	11.42 ± .16^{A}	14.60 ± .79^{A}
Hill's Lake	51	12.43 ± .20^{B}	21.54 ± 1.09^{B}
16:			
Lake Nipigon	52	11.45 ± .20^{A}	14.26 ± .89^{A}
Dickson Lake	42	10.85 ± .19^{A}	12.14 ± .81^{A}
Hill's Lake	44	13.07 ± .22^{B}	24.75 ± 1.40^{B}
20:			
Lake Nipigon	43	10.86 ± .17^{A}	12.45 ± .82^{A}
Dickson Lake	48	10.72 ± .16^{A}	12.51 ± .71^{A}
Hill's Lake	47	12.74 ± .21^{B}	24.29 ± 1.25^{B}

Note. Significant differences within acclimation temperatures are denoted by different superscript letters for both fork length and mass. Values are mean ± SEM; *N* = number of fish.

Upper Thermal Tolerance

Upper thermal tolerance (time until loss of equilibrium) differed significantly among brook trout strains ($F_{2,190} = 49.57$, $P < 0.001$) and among acclimation temperatures ($F_{3,190} = 630.78$, $P < 0.001$). Importantly, there was a significant interaction between population origin and acclimation temperature ($F_{6,190} = 10.21$, $P < 0.001$). There were no significant differences among strains at 20°C. Strains that had superior thermal tolerance at lower acclimation temperatures did not necessarily have superior tolerance at higher acclimation temperatures. Body mass was a significant covariate ($F_{1,190} = 4.68$, $P = 0.032$). Thermal tolerance varied significantly among all brook trout strains ($P < 0.001$ for all ordered difference comparisons among brook trout strains) and among all acclimation temperatures. On average, the Hill's Lake strain had the highest upper thermal tolerance, the Lake Nipigon strain had the lowest upper thermal tolerance, and the Dickson Lake strain was intermediate. Thermal tolerance increased significantly with increasing acclimation temperature ($P < 0.001$ for all comparisons among acclimation treatments; fig. 1). Lake Nipigon fish had the lowest thermal tolerance within each temperature, with the exception of 20°C, where there was no significant difference

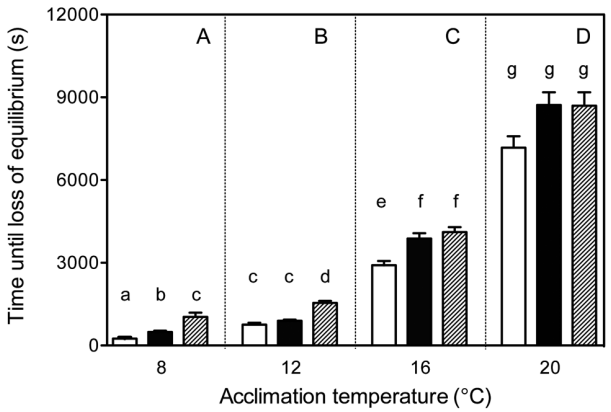

Figure 1. Upper thermal tolerance of three brook trout strains acclimated to four temperatures. Thermal tolerance was estimated as the time until loss of equilibrium at 27.5°C. Brook trout strains: Lake Nipigon = white; Dickson Lake = black; Hill's Lake = hatched. Significant differences between strains are indicated by different lowercase letters, and significant differences between acclimation temperatures are indicated by different uppercase letters. Values are means ± SEM.

among strains. Acclimation temperature had a much stronger effect than population of origin on upper thermal tolerance, with 81.2% of the observed variation in thermal tolerance attributed to acclimation temperature and 4.2% attributed to strain.

Standard Metabolic Rate

All strains increased their SMR with increasing acclimation temperatures up to 16°C, above which SMR began to decrease ($F_{3, 119} = 39.04$, $P < 0.001$; fig. 2). The SMRs of individuals acclimated to 8° and 12°C were significantly lower than those held at 16° and 20°C (all $P < 0.01$), while fish held at the 16°C acclimation temperature exhibited significantly higher SMRs than those acclimated and held at 20°C ($P < 0.001$). SMR did not differ among individuals acclimated to 8° and 12°C ($P > 0.05$). On average, SMR did not vary significantly among brook trout strains ($F_{2, 119} = 2.12$, $P = 0.13$; body mass covariate: $F_{1, 119} = 87.23$, $P < 0.001$). However, there was a significant population origin–by–acclimation temperature interaction ($F_{6, 119} = 4.29$, $P < 0.001$), indicating that the pattern by which energy consumption varied with environmental temperature differed among the strains (fig. 2). Within an acclimation temperature, a significant difference among strains was detected only at 8°C, with fish from Lake Nipigon having a lower SMR than fish from Dickson Lake (fig. 2). Some of the observed variance in SMR was associated with strain and acclimation temperature; however, neither accounted for a large portion of the variance in this model (strain: $\omega^2 = 0.0004$, acclimation temperature: $\omega^2 = 0.034$).

Critical Swimming Performance

The U_{crit} differed significantly among the brook trout strains ($F_{2, 116} = 14.98$, $P < 0.001$) and among acclimation temperatures ($F_{3, 116} = 4.37$, $P = 0.006$). The Dickson Lake strain had significantly higher U_{crit} than either the Lake Nipigon or Hill's Lake strains ($P < 0.001$ for both), which did not differ from each other ($P = 0.95$; fig. 3). Brook trout from the 20°C acclimation temperature had significantly lower U_{crit} than brook trout acclimated to 12°C ($P = 0.005$). There was no significant interaction between brook trout population origin and acclimation temperature ($F_{6, 116} = 1.57$, $P = 0.16$), suggesting that populations responded similarly to acclimation. Fork length was included as a covariate but did not significantly predict U_{crit} within the size range of our fish ($F_{1, 116} = 2.57$, $P = 0.11$). Little of the variance in our model was attributable to either strain or acclimation temperature (strain: $\omega^2 = 0.0028$, acclimation temperature: $\omega^2 = 0.0003$).

HSP70 White Muscle

Prior to heat shock, HSP70 was detected only in white muscle and only at 20°C (fig. 4). Prior to heat shock in white muscle, there was a significant effect of brook trout strain ($F_{2, 22} = 3.69$, $P = 0.043$). The Lake Nipigon strain, the strain from the coldest thermal origin, exhibited significantly higher expression of HSP70 than the Hill's Lake strain ($P = 0.022$) and the Dickson Lake strain ($P = 0.035$). The expression in the Dickson Lake strain was not significantly different from that in the Hill's Lake strain ($P = 0.83$).

Following an acute heat shock, levels of inducible HSP70 in white muscle tissue (fig. 5*a*) decreased significantly as accli-

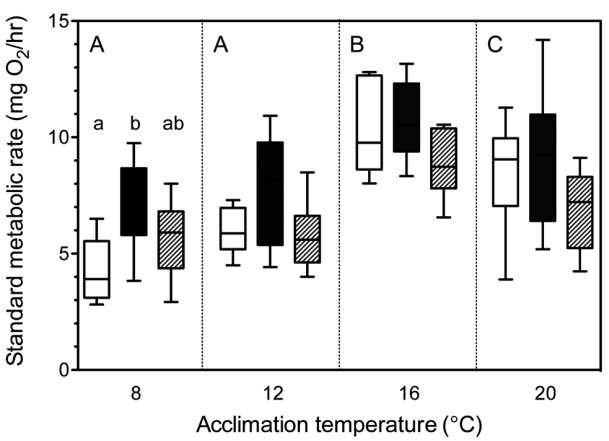

Figure 2. Standard metabolic rate (mg oxygen consumed h^{-1}) of three brook trout strains acclimated to four temperatures. Brook trout strains: Lake Nipigon = white; Dickson Lake = black; Hill's Lake = hatched. Significant differences between strains are indicated by different lowercase letters, and significant differences between acclimation temperatures are indicated by different uppercase letters. Boxes represent the first and third quartiles, whiskers represent the fifth and ninety-fifth percentiles, and the horizontal lines represent the median.

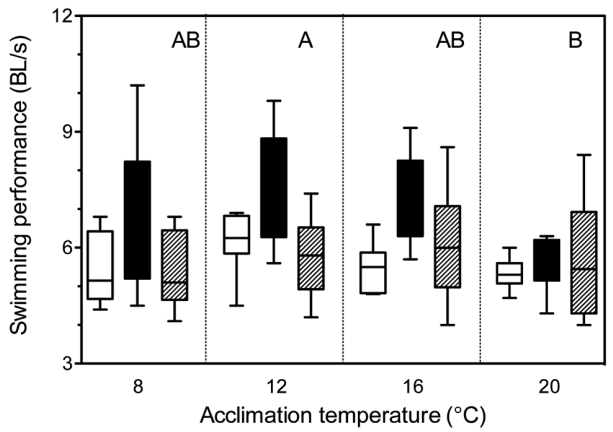

Figure 3. Critical swimming performance (U_{crit}) of three brook trout strains acclimated to four temperatures, measured as body lengths per second (BL s^{-1}). Brook trout strains: Lake Nipigon = white; Dickson Lake = black; Hill's Lake = hatched. Significant differences between acclimation temperatures are indicated by different uppercase letters. Boxes represent the first and third quartiles, whiskers represent the fifth and ninety-fifth percentiles, and the horizontal lines represent the median. On the figure, swimming performance is depicted as BL s^{-1} to allow for comparisons with the literature. In the statistical analysis, performance was measured as velocity (m s^{-1}), with fork length included as a statistical covariate.

mation temperature increased ($F_{3, 95} = 18.96$, $P < 0.001$) but did not differ between strains ($F_{2, 95} = 2.98$, $P = 0.056$). There was no significant interaction effect between acclimation temperature and strain ($F_{6, 95} = 1.33$, $P = 0.25$). Individuals acclimated to 20°C had significantly lower HSP70 levels following heat shock than individuals acclimated to the other temperatures (8°, 12°, and 16°C; all $P < 0.001$), but these animals experienced a lower-magnitude heat shock. HSP70 levels following heat shock in the 8°C-acclimated fish, where the magnitude of the heat shock was greatest, were significantly greater than expression in the 16°C- and 20°C-acclimated fish ($P < 0.05$).

HSP70 Cardiac Muscle

HSP70 was not detected in cardiac muscle prior to heat shock (data not shown). Twenty-four hours following a 1-h acute heat shock, HSP70 was induced, and levels in cardiac tissue (fig. 5*b*) varied significantly between strains ($F_{2, 88} = 10.5$, $P < 0.001$) and acclimation temperatures ($F_{3, 88} = 12.56$, $P < 0.001$). There were no significant interaction effects between acclimation temperature and strain ($F_{6, 88} = 1.15$, $P = 0.339$). On average, the Lake Nipigon strain had significantly higher cardiac HSP70 levels 24 h following an acute heat shock than either the Dickson Lake or Hill's Lake strains ($P = 0.016$ and $P < 0.001$, respectively), suggesting this strain was under greater stress when exposed to the same conditions. The Dickson Lake strain was not significantly different from the Hill's Lake strain ($P = 0.138$). Heat-induced expression of the inducible HSP70 in cardiac muscle was higher in 12°- and 16°C-acclimated fish than either 8° or 20°C ($P < 0.01$ for all ordered comparisons), but levels expressed at 12° and 16°C did not differ from each other ($P = 0.972$). Inducible HSP70 expression in cardiac tissue did not differ between 8°- and 20°C-acclimated groups ($P = 0.66$), suggesting that at least in this tissue temperatures outside of the preferred thermal range confer a reduced capacity for the HSR to deal with thermal stress, regardless of the magnitude of the heat shock (fig. 5*b*).

HSP70 Liver

Similar to cardiac muscle, HSP70 was not detectable prior to heat shock in liver (data not shown). HSP70 was induced with acute heat shock (fig. 5*c*), and 24 h following this thermal challenge, liver HSP70 levels varied with acclimation temperature ($F_{3, 95} = 4.37$, $P = 0.007$) but not strain ($F_{2, 95} = 1.65$, $P = 0.199$). There was no significant interaction between acclimation temperature and strain ($F_{6, 95} = 0.73$, $P = 0.632$). Individuals acclimated to 16°C had significantly greater heat-induced HSP70 levels than individuals acclimated to 12°C ($P = 0.003$); there were no other significant differences among acclimation temperatures (fig. 5*c*).

HSC70

Following acute heat shock, HSC70 levels were significantly greater in white muscle ($F_{1, 188} = 15.25$, $P < 0.001$), cardiac ($F_{1, 180} = 41.21$, $P < 0.001$), and liver ($F_{1, 185} = 21.84$, $P < 0.001$) tissue than before heat shock. Thus, data were split by time period (before and 24 h after heat shock) and analyzed for variation in HSC70 levels using population origin, acclimation temperature, and their interaction as parameters.

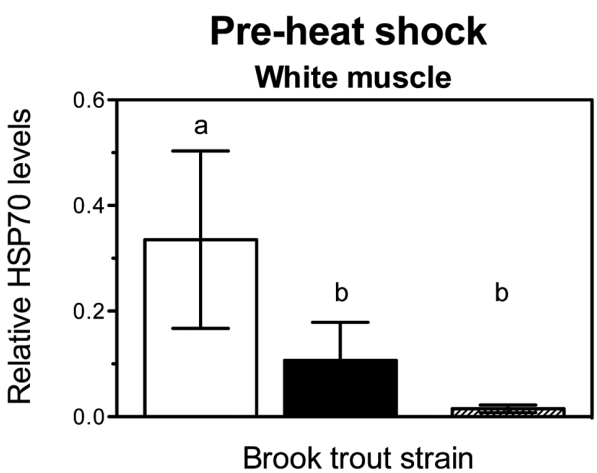

Figure 4. Pre-HSP70 levels in white muscle of brook trout strains acclimated to 20°C. Levels are expressed relative to a control (HSP70 sample). Brook trout strains: Lake Nipigon = white; Dickson Lake = black; Hill's Lake = hatched. Significant differences among strains at each temperature are indicated by different lowercase letters. Values are least squares means ± SEM.

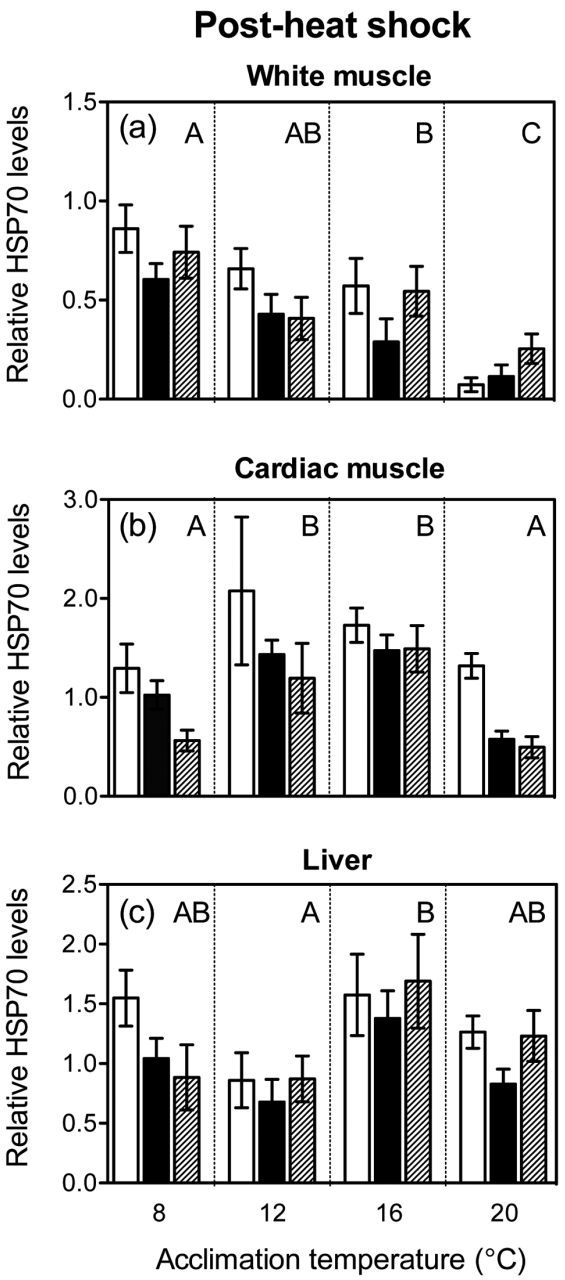

Figure 5. Inducible HSP70 response following heat shock for three brook trout strains, measured relative to a control (HSP70 sample). *a*, White muscle; *b*, cardiac muscle; *c*, liver. Brook trout strains: Lake Nipigon = white; Dickson Lake = black; Hill's Lake = hatched. Significant differences between acclimation temperatures are indicated by different uppercase letters. Values are least squares means ± SEM.

HSC70 White Muscle

Overall, white muscle HSC70 did not differ significantly among strains prior to heat shock ($F_{2,91} = 0.18$, $P = 0.838$; fig. 6*a*), although significant differences were observed among acclimation temperatures ($F_{3,91} = 5.18$, $P = 0.003$). There was also a significant interaction between strain and acclimation temperature ($F_{6,91} = 2.72$, $P = 0.019$), indicating that strains responded differently to increasing acclimation temperatures. Within the 8° and 12°C acclimation treatments, there were significant differences among strains, although there were no strain effects at 16° or 20°C. Individuals acclimated to 20°C had significantly greater expression of white muscle HSC70 prior to heat shock than individuals at either the 8°C ($P = 0.003$) or 16°C ($P = 0.047$) acclimation temperatures, suggesting that the acclimation to 20°C elicited more stress than the other temperatures. There were no other significant differences noted. Twenty-four hours following an acute heat shock (fig. 6*b*), white muscle HSC70 levels did not vary significantly among strains ($F_{2,95} = 1.89$, $P = 0.156$) or acclimation temperatures ($F_{3,95} = 1.54$, $P = 0.209$). There was also no interaction between strain and acclimation temperature ($F_{6,95} = 0.55$, $P = 0.769$). These data suggest that white muscle HSC70 levels were relatively insensitive to increasing thermal stress.

HSC70 Cardiac Muscle

Before heat shock, cardiac HSC70 (fig. 6*c*) varied with acclimation temperature ($F_{3,91} = 3.68$, $P = 0.015$) but not strain ($F_{2,91} = 2.08$, $P = 0.132$); there was no interaction between strain and acclimation temperature ($F_{6,91} = 0.54$, $P = 0.776$). Individuals acclimated to 20°C had significantly greater HSC70 expression than the 16°C ($P = 0.02$) acclimation treatment. There were no other significant differences. Twenty-four hours following acute heat shock, cardiac HSC70 also varied with acclimation temperature ($F_{3,88} = 11.28$, $P < 0.001$; fig. 6*d*) but not with strain ($F_{2,88} = 1.88$, $P = 0.159$); there was no significant interaction between strain and acclimation temperature ($F_{6,88} = 1.49$, $P = 0.192$). Individuals acclimated to 8°C had significantly lower HSC70 levels 24 h following an acute heat shock than all other acclimation temperatures (all $P < 0.05$).

HSC70 Liver

Liver HSC70 levels differed significantly among strains ($F_{2,89} = 4.03$, $P = 0.022$) and acclimation temperature ($F_{3,89} = 7.49$, $P < 0.001$) prior to an acute heat shock (fig. 6*e*). Fish from each strain behaved similarly, irrespective of acclimation temperature (strain × acclimation temperature: $F_{6,89} = 0.73$, $P = 0.626$). Before heat shock, the Dickson Lake strain had significantly lower expression of HSC70 than the Hill's Lake strain ($P = 0.017$). The Lake Nipigon strain was not significantly different from either of the other two strains (both $P > 0.20$). HSC70 levels were lower in fish acclimated to 16°C than in fish acclimated to either 8° or 20°C ($P < 0.01$). Following heat shock (fig. 6*f*), liver HSC70 levels did not differ among strains ($F_{2,95} = 0.52$, $P = 0.595$) or acclimation temperatures ($F_{3,95} = 2.31$, $P = 0.082$). Populations responded in a similar way, irrespective of acclimation temperature (population × acclimation temperature: $F_{6,95} = 1.05$, $P = 0.398$).

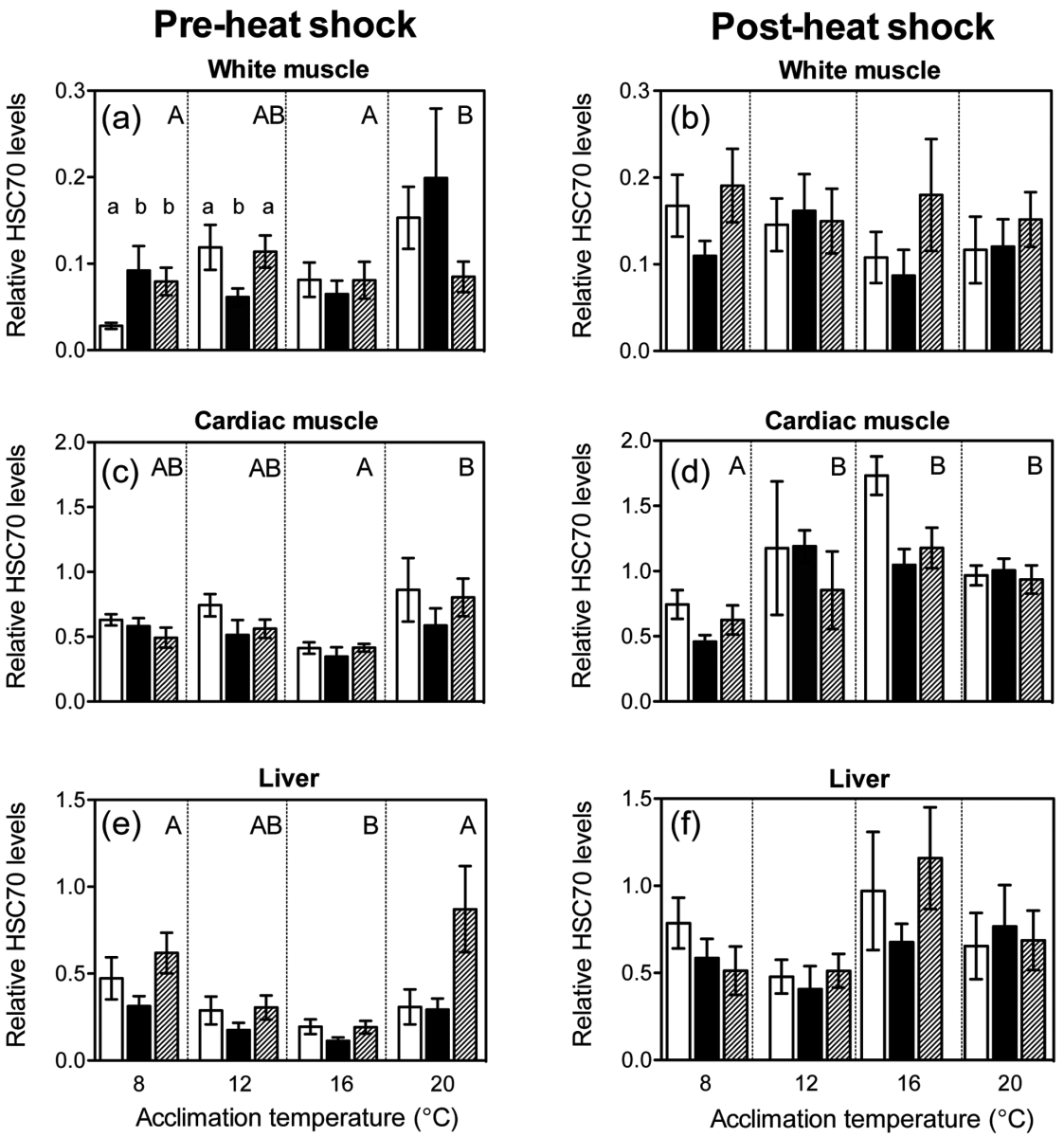

Figure 6. Constitutive HSC70 response for three brook trout strains across four acclimation temperatures, measured relative to a control (HSC70 sample). White muscle is shown for pre–heat shock (*a*) and post–heat shock (*b*). Cardiac muscle is shown for pre–heat shock (*c*) and post–heat shock (*d*). Liver is shown for pre–heat shock (*e*) and post–heat shock (*f*). Significant differences between strains are indicated by different lowercase letters, and significant differences between acclimation temperatures are indicated by different uppercase letters. Values are least squares means ± SEM.

Discussion

The strains of brook trout tested here exhibited differences in both whole-organism thermal physiology and the magnitude of their HSR. Across acclimation temperatures, there was a general trend for the northernmost strain (Lake Nipigon) to exhibit the lowest thermal tolerance and for the strain with the most southern origin (Hill's Lake) to exhibit the greatest thermal tolerance. There was also a strong effect of acclimation temperature on thermal tolerance within each strain. Together, our data provide evidence that both heritable (genetic) and phenotypically plastic components are important in the physiological response of brook trout to variation in environmental temperature. This combination of responses presumably helps wild brook trout populations to cope with thermal challenges over ecological and evolutionary timescales.

Thermal tolerance is closely linked to the biogeographical distribution of a species (Pörtner 2002). Strains of brook trout from widely separated portions of the species range differed

from each other in thermal tolerance, suggesting evolutionary adaptation to local environmental conditions. However, in addition to biogeography, phylogeography has likely influenced geographic variation in intraspecific thermal tolerance. Throughout the native range of brook trout, one lineage is widespread throughout the northern portion of their distribution (Danzmann et al. 1991, 1998). Experimental evidence from manipulative field experiments also suggests that there was strong selection for cold hardiness in brook trout during migration following glaciation (Ashford and Danzmann 2001). The generally lower thermal tolerance exhibited in our study by individuals from the northernmost strain (Lake Nipigon) may be due to natural selection favoring a more cold-adapted population.

Acclimating individuals to various environmental temperatures showed that phenotypic plasticity can play a prominent role in coping with thermal stress in brook trout. Thermal tolerance to warm temperatures increased as acclimation temperatures increased, particularly for temperatures exceeding the species' thermal optimum. Similar results have been reported previously for other salmonids (e.g., Elliott and Elliott 1995; Currie et al. 1998). Although there was population-level variation in thermal tolerance at lower acclimations temperatures, at the highest acclimation temperature (20°C) thermal tolerance was similar among populations. This may have ecological implications. For example, the thermal optima for aerobic scope of populations of sockeye salmon (*Oncorhynchus nerka*) correlate with historic river temperatures, and, thus, increased water temperatures resulting from climate change may impact salmon migratory performance (Eliason et al. 2011). To demonstrate population-specific variation in adaptive potential at the uppermost acclimation temperature in our study, variation in thermal tolerance within populations must be investigated (McDermid et al. 2012); however, this is beyond the scope of this study.

Metabolism and Swimming Performance

On average, SMR did not vary among strains but varied with acclimation temperature. Importantly, strains varied in their capacity for thermal acclimation of SMR (as indicated by a significant strain by acclimation temperature interaction). With exposure to increasing water temperatures, the northernmost strain (Lake Nipigon) had the largest increase in SMR while the southernmost strain (Hill's Lake) had the smallest increase. This suggests that at intermediate temperatures the Lake Nipigon strain may be at a disadvantage when compared with other strains. At the highest acclimation temperatures (20°C) SMR decreased in all strains. Although it could be argued that at 20°C fish had passed their *pejus* temperature (Pörtner et al. 2001, 2006) and were allocating resources elsewhere to cope, this seems unlikely. The SMR of 20°C-acclimated fish was still higher than that of individuals acclimated to 12°C, a temperature within their preferred range. The reasons for the decline in SMR at 20°C remain unknown.

Although swimming performance differed among populations and acclimation temperatures, the similar capacity for acclimation of swimming performance among the strains suggests that any long-term increases in water temperature resulting from climate warming would affect the performance of the three strains similarly. Differences in U_{crit} among geographically separated populations have been reported previously (e.g., killifish; Fangue et al. 2008) but not always (Atlantic cod *Gadus morhua* [Hanna et al. 2008] and rainbow trout [Myrick and Cech 2000]). The selective factors favoring population divergence in performance can be expected to vary among species but presumably include metabolic efficiency in relation to local environmental conditions, foraging ability, and predation avoidance, as well as those related to thermal guild and geographic influences on local environmental and thermal regimes.

The similar capacity for thermal acclimation of swimming performance among the three strains, despite differing capacities for thermal acclimation of SMR, has possible energetic implications for population-level responses to climatic warming; U_{crit} has been correlated with metabolic rates (e.g., Plaut 2000) and is thought to reflect maximum oxygen consumption rates (Farrell and Steffensen 1987). As such, our data suggest that strains may differ both in how changing temperatures impact their scope for activity and in their ability to cope with intra- and interspecific competition.

To measure swimming performance, we swam fish in schools rather than individually. There is some evidence that schooling offers an energetic advantage (e.g., Svendsen et al 2003), which may have had an impact if there were a difference in the relative proportion of strains tested. However, in most cases the proportion of each strain was generally equal, and there was always representation from each strain, despite random sampling. As such, we do not think the presence of schooling contributed significantly to the patterns we detected.

Heat Shock Proteins

The HSP70/HSC70 expression profiles are complex and vary in their dependence on tissue type, acclimation temperature, and strain. In some cases, the HSR mirrored patterns of whole-animal thermal tolerance, with the northernmost strain (Lake Nipigon) having the lowest thermal tolerance and greatest induction of cardiac HSP70 while strains from warmer sites were similar to each other. However, given that our data do not indicate that the more thermally tolerant strain (Hill's Lake) has lower levels of HSP70, we cannot directly and universally link HSP70 levels with thermal tolerance. Acclimation to different rearing temperatures resulted in tissue-specific effects on the HSR for both HSP70 and its constitutive isoform (HSC70). As such, expression of HSPs showed evidence of heritability and phenotypic plasticity among the strains, supporting the utility of these physiological markers for assessing thermal stress.

Ancestral and contemporary thermal histories have been shown to affect the induction of HSPs (Dietz and Somero 1993). For example, DiIorio et al. (1996) showed that ancestral thermal history affected expression of inducible HSP70 in *Poe-*

ciliopsis species and hybrids. Similarly, Fangue et al. (2006) showed that members of northern populations of *Fundulus heteroclitis* expressed higher *hsp*70 mRNA levels than members of southern populations at the same temperature, suggesting that capacity for HSP expression may have allowed the southern population to live in warmer waters. In our study, the different brook trout strains tested exhibited differences in thermal performance consistent with their differing ancestries, despite two of the strains having been displaced from their ancestral (wild) environments for multiple generations.

Contemporary thermal history has also been shown to influence the HSR. For example, Buckley et al. (2001) showed seasonal variation in induction temperatures of HSP70 in *Mytilus trossulus*, with greater HSP70 expression in warm-acclimatized individuals. The brook trout strains used in our study had spent a minimum of two generations in a common thermal environment. If contemporary thermal history were solely responsible for thermal tolerance, induction of HSP70 would have been more similar among the experimental strains. In contrast, molecular responses (HSP70) among brook trout strains were predicted by temperatures of their historical source water bodies, as found similarly by Hofmann et al. (2000) for notothenoids and Fangue et al. (2006) for killifish.

Several studies have suggested that HSC70 is primarily implicated in thermal tolerance, while HSP70 plays a secondary role (DiIorio et al. 1996; Hofmann et al. 2000; Place and Hofmann 2005). For example, DiIorio et al. (1996) showed that both HSP70 and HSC70 help to govern thermal tolerance in *Poeciliopsis* species and hybrids but that HSC70 is more important than the inducible isoforms of HSP70. On the other hand, Healy and Schulte (2012) suggest that the inducible HSP70 (and not HSC70) may have a role in thermal tolerance in killifish (*F. heteroclitus*). As was the case for our HSP70 data, we did not observe any consistent or convincing patterns in HSC70 expression that would support a clear involvement of this stress protein in thermotolerance in this fish. Our data are correlative, however, and given that a specific role for HSPs in the thermal tolerance of fish is likely complex (Healy and Schulte 2012), further experiments aimed at pinpointing a direct role for HSPs in this process are necessary.

Although fish induce HSC70 levels with increasing acclimation temperature (Deane and Woo 2005), tissue-specific expression suggests a complex response at the organismal level. In our study, acclimation temperature had a significant effect on HSC70 levels in each tissue prior to heat shock, although patterns across tissues were not consistent. After heat shock, only cardiac tissue showed any clear pattern, with an increase in HSC70 levels at temperatures above 8°C. It has been shown previously that vital organs require elevated basal levels of HSC70 to provide molecular chaperoning, protection, and repair and are protected by the HSR (cardiac function [Kim et al. 1997], nervous system [Latchman 1998], cardiac and hepatic function [Mizushima et al. 2000]) whereas frequently the nonvisceral tissues are not.

Alternative Explanations for Physiological Patterns

Differences in thermal performance among the three brook trout strains could arguably be attributed to factors other than local adaptation to ancestral thermal regimes. For example, thermal performance may have been influenced by differences in body size (allometric effects/thermal inertia; Robinson et al. 2008), growth rates (differential allocation of resources), and generations of domestication (preexperimental hatchery effects), although each of these also has genetic components (Carline and Machung 2001; Devlin et al. 2005). However, where differences in body size existed between strains, they were factored into statistical analyses. Thus, we argue that size was unlikely to have played a significant role in the patterns we detected.

Considerable research has shown differences in survival and performance between wild-type and domesticated strains of fish (e.g., Webster and Flick 1981; Lachance and Magnan 1990*a*, 1990*b*; McDonald et al. 1998; Rhodes and Quinn 1999). Although the three brook trout strains in our study had been housed in the same hatchery for a minimum of two generations (one generation at Hill's Lake Fish Culture Station and one generation at the Codrington facility), the Hill's Lake strain has been in captivity for more than 25 generations (OMNR 2005), likely leading to a high level of domestication (Fraser 1981; Kerr 2006). Domestication has been shown to decrease upper thermal tolerance in brook trout (e.g., Vincent 1960), presumably as a result of adaptation to benign hatchery conditions (Carline and Machung 2001). Contrary to what would be predicted based on level of domestication, though (Carline and Machung 2001), the Hill's Lake strain had the highest temperature tolerance. In contrast, the Lake Nipigon strain, which originated from close to the northern limit of the species range (Scott and Crossman 1973; OMNR 2005) and has the lowest mean July air temperatures of any of the wild sources (McKenney et al. 2010), exhibited the lowest thermal tolerance of all experimental populations. Their adaptation for lower temperatures and reduced thermal tolerance appears to have persisted despite the strain having been maintained for the past eight generations at the Hill's Lake Fish Culture Station alongside the Hill's Lake strain (OMNR 2005). We suggest that the thermal tolerances observed in the Hill's Lake and Lake Nipigon strains reflect historical adaptations to conditions in their ancestral sources and that these adaptations have been retained despite the strains having been maintained for generations in the hatchery. At present, we cannot determine whether the degree of domestication dampened, increased, or had no effect on the thermal tolerance of the Hill's Lake strain. Although Carline and Machung (2001) showed that domestication in brook trout can reduce upper thermal tolerance, their study did not include strains with different degrees of domestication.

Variation among strains in thermal tolerance was unlikely a result of differential domestication; however, demonstrating no effect of domestication on swimming performance is more challenging. Rates of selection for captivity (domestication) can be rapid, often within one to eight generations (Fraser et al.

2011). For example, F_1 hatchery-reared brown trout (*Salmo trutta*) and Atlantic salmon had a significantly lower maximum velocity (U_{burst}) than their wild-reared counterparts (Pedersen et al. 2008). Although U_{burst} utilizes different metabolic substrates than does U_{crit}, the decline in U_{burst} as a result of domestication does highlight potential impacts on exercise performance. The Dickson Lake brook trout strain had the greatest U_{crit} but was also most recently removed from the wild (i.e., least domesticated). As such, it seems plausible that domestication may have negatively impacted swimming performance in the Nipigon and Hill's Lake brook trout strains, which have been in captivity for at least eight generations.

Common hatchery-related stressors might be expected to confound molecular differences we detected among the tested strains. However, such stressors have been shown to have little or no effect on HSP70 expression in salmonids (e.g., Washburn et al. 2002; Zarate and Bradley 2003). Nonetheless, rearing conditions or domestication can influence the cellular response to thermal stress. For example, hatchery-reared rainbow trout (*Oncorhynchus mykiss*) have lower HSP levels in muscle than their wild-type counterparts (Werner et al. 2006). As the Hill's Lake strain of brook trout had been in the hatchery system for the greatest amount of time, domestication may have depressed the cellular response. Nonetheless, a lack of difference in inducible HSP70 expression between the Hill's Lake strain and the Dickson Lake strain (with the least amount of time in the hatchery system) suggests that ancestral thermal history had a greater influence on the HSR than domestication.

Conclusions and Implications

Effective conservation of cold-adapted species in changing climates will require inclusion of geographic and physiological perspectives, as well as an improved understanding of the mechanistic responses to environmental stressors. Building on the pioneering studies of thermal performance in cold-water fishes (e.g., Fry et al. 1946; Brett 1952; Brett et al. 1958; McCauley 1958), our research demonstrates that populations can possess substantial thermal acclimation capacity, as well as heritable variation in thermal tolerance among populations (e.g., Danzmann et al. 1998; Timusk et al. 2011). Whole-organism thermal performance was augmented greatly through acclimation, suggesting that with changing climatic conditions, populations of brook trout may have some degree of plasticity to cope with acute and chronic thermal stressors. However, localized populations and strains will vary in their ability to cope with these thermal stressors, depending on the historical thermal regimes that they have experienced over ancestral (evolutionary) and contemporary (ecological) timescales.

Future research should examine within-population (family-scale) variation in thermal tolerance and performance, as well as the underlying mechanisms and their heritability. Additionally, elucidating the effects of temperature acclimation at different life stages (embryonic, larval, and parental) on heritable thermal tolerances would identify the potential to adapt to thermal stress, as well as investigating potential epigenetic mechanisms for rapid adaptive responses. As populations that are unable to adapt may become extirpated, information on physiological and thermal performance will assist in determining how best to reestablish extirpated populations, in hopes that the species may retain its current biogeographic distribution.

Acknowledgments

Financial support for this work was provided by the National Science and Engineering Research Council (NSERC), the Ontario Ministry of Natural Resources (OMNR), Wildlife Conservation Society Canada (WCS), the Canadian Foundation for Innovation, and the Ontario Innovation Trust. Pablo Peres-Neto (Université du Québec à Montréal) generously loaned the swim mill. We thank Bill Sloan, Scott Ferguson, Erin Scharf, and Graham Branscombe at the OMNR Codrington Fisheries Research Facility for fish rearing and maintenance as well as logistic support; Sacha LeBlanc, Kavish Chandra, and Maria Thistle, Mount Allison University, for help with data collection; and Michael Fox, Mark Ridgway, and three anonymous reviewers for constructive feedback on earlier drafts of the manuscript.

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