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ARTICLE

Cold tolerance performance of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*Oncorhynchus mykiss*) and its potential role in influencing interspecific hybridization

M.M. Yau and E.B. Taylor

Abstract: Hybridization between rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) and westslope cutthroat trout (*Oncorhynchus clarkii lewisi* (Girard, 1856)) occurs commonly when rainbow trout are introduced into the range of westslope cutthroat trout. Typically, hybridization is most common in warmer, lower elevation habitats, but much less common in colder, higher elevation habitats. We assessed the tolerance to cold water temperature (i.e., critical thermal minimum, CTMin) in juvenile rainbow trout and westslope cutthroat trout to test the hypothesis that westslope cutthroat trout better tolerate low water temperature, which may explain the lower prevalence of rainbow trout and interspecific hybrids in higher elevation, cold-water habitats (i.e., the "elevation refuge hypothesis"). All fish had significantly lower CTMin values (i.e., were better able to tolerate low temperatures) when they were acclimated to 15 °C (mean CTMin = 1.37 °C) versus 18 °C (mean CTMin = 1.91 °C; $p < 0.001$). Westslope cutthroat trout tended to have lower CTMin than rainbow trout from two populations, second–generation (F_2) hybrids between two rainbow trout populations, and backcrossed rainbow trout at 15 °C (cross type × acclimation temperature interaction; $p = 0.018$). Differential adaptation to cold water temperatures may play a role in influencing the spatial distribution of hybridization between sympatric species of trout.

Key words: westslope cutthroat trout, *Oncorhynchus clarkii lewisi*, rainbow trout, *Oncorhynchus mykiss*, hybridization, critical thermal minimum, water temperature.

Résumé : L'hybridation de truites arc-en-ciel (*Oncorhynchus mykiss* (Walbaum, 1792)) et de truites fardées de Yellowstone (*Oncorhynchus clarkii lewisi* (Girard, 1856)) se produit couramment quand des truites arc-en-ciel sont introduites dans l'aire de répartition de la truite fardée de Yellowstone. En général, l'hybridation est plus répandue dans les habitats plus chauds et de plus faible altitude et beaucoup moins répandue dans les habitats plus froids de plus haute altitude. Nous avons évalué la tolérance aux faibles températures de l'eau (c.-à-d. température minimum critique, CTMin) de truites arc-en-ciel et de truites fardées de Yellowstone juvéniles pour vérifier l'hypothèse voulant que les truites fardées de Yellowstone tolèrent mieux l'eau froide, ce qui pourrait expliquer la plus faible prévalence de truites arc-en-ciel et d'hybrides interspécifiques dans les habitats d'eau plus froide de plus haute altitude (c.-à-d. l'hypothèse de l'altitude comme refuge). Tous les poissons présentaient des CTMin significativement plus faibles (c.-à-d. toléraient mieux de faibles températures) quand ils étaient acclimatés à 15 °C (CTMin moyenne = 1,37 °C) qu'à 18 °C (CTMin moyenne = 1,91 °C; $p < 0{,}001$). Les truites fardées de Yellowstone avaient tendance à présenter de plus faibles CTMin que les truites arc-en-ciel de deux populations, des hybrides de deuxième génération de deux populations de truites arc-en-ciel et des truites arc-en-ciel rétrocroisées à 15 °C (interaction type de croisement × température d'acclimatation; $p = 0{,}018$). L'adaptation différentielle à de faibles températures de l'eau pourrait avoir une influence sur la répartition spatiale de l'hybridation d'espèces sympatriques de truites. [Traduit par la Rédaction]

Mots-clés : truite fardée de Yellowstone, *Oncorhynchus clarkii lewisi*, truite arc-en-ciel, *Oncorhynchus mykiss*, hybridation, température minimum critique, température de l'eau.

Introduction

Biotic interactions that are mediated by spatially variable abiotic factors may play an important role in defining a species' distribution in nature (Baltz et al. 1982; Rahel 1984; Dunson and Travis 1991; Warner et al. 1993; De Staso and Rahel 1994). For example, in freshwater fishes spatial patterns of species presence or abundance are often observed along an elevational gradient in rivers and streams (e.g., Paul and Post 2001). Factors that vary with elevation such as water temperature, water velocity, and substrate may favour one species over another (Vannote et al. 1980; Rahel 1984; Fausch et al. 1994; Taniguchi et al. 1998). In particular, water temperature appears to figure prominently in shaping the distribution of salmonid fishes (salmon, trout, and their relatives), where small changes of only 1 °C have been linked to the presence or absence of certain species (Fausch et al. 1994). Water temperature alters metabolism and behaviour in ectotherms, which may influence relative competitive abilities between species and help explain these distributional patterns (Taniguchi et al. 1998; Selong et al. 2001; Bear et al. 2007; Culumber et al. 2012).

A common distribution pattern in habitats that have been stocked with non-native salmonids (i.e., salmon, trout, and their

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relatives) is the predominance of the native species in high-elevation tributaries, followed by interspecific hybrids in intermediate-elevation areas, and predominance of the introduced species in downstream areas (Rahel and Hubert 1991; Fausch et al. 1994; Hitt et al. 2003; Weigel et al. 2003; Rubidge and Taylor 2005; Rasmussen et al. 2010). In such cases, cold waters at high elevations have been suggested to provide a refuge for the native species allowing them to persist despite the absence of physical barriers that would otherwise stop the movement of introduced trout and hybrids upstream—the so-called "elevation refuge hypothesis" (Paul and Post 2001; Weigel et al. 2003; Rubidge and Taylor 2005; Rasmussen et al. 2010). According to this hypothesis, trout whose native distribution includes high-elevation areas enjoy a competitive advantage at cold temperatures and the reverse is true at low elevations where introduced taxa typically predominate (Paul and Post 2001). Hybrids, when present, exhibit intermediate behaviour and temperature-related performance, which may explain their predominance at intermediate elevations (Rasmussen et al. 2010). In fact, for two naturally sympatric and hybridizing poecilid fishes (swordtail genus *Xiphophorus* Heckel, 1848), Culumber et al. (2012) demonstrated that critical thermal minima (CTMin) varied between parental species, with hybrids showing intermediate performance, and was inversely related to the water temperatures where wild fish were captured; fish captured at higher elevations at lower water temperatures had greater tolerance to cold water. Laboratory trials confirmed that at least some differences in thermal tolerance had a genetic component (Culumber et al. 2012).

Laboratory and controlled field studies have, however, yielded mixed results in support of the elevation refuge hypothesis in salmonids (see review in McHugh and Budy 2005). De Staso and Rahel's (1994) study on native Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus* (Cope, 1872)) and introduced brook trout (*Salvelinus fontinalis* (Mitchill, 1814)) supported a shift in competitive ability as water temperature changed. By contrast, McMahon et al. (2007) failed to detect a reversal in competitive advantage favouring native bull trout (*Salvelinus confluentus* (Suckley, 1859)) over introduced brook trout at cold temperatures. Similarly, native Bonneville cutthroat trout (*Oncorhynchus clarkii utah* (Suckley, 1874)) were not any more successful than exotic brown trout (*Salmo trutta* L., 1778) in high-elevation habitats (McHugh and Budy 2005). When a reversal in competitive ability was not observed, McHugh and Budy (2005) argued that physiological limitations of non-native species exerted a stronger influence at cold temperatures such that interspecific competition was likely driving species dominance only at warmer, lower low-elevation sites and that non-native fishes would displace indigenous taxa only to a point upstream where their metabolic needs could no longer be met (McHugh and Budy 2005).

The westslope cutthroat trout (*Oncorhynchus clarkii lewisi* (Girard, 1856); WSCT) is 1 of up to 14 subspecies of cutthroat trout (*Oncorhynchus clarkii* (Richardson, 1836)) in western North America (Behnke 1992). Across most of its native range, WSCT are threatened by hybridization with introduced rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792); RT) (Rubidge et al. 2001; US Federal Register 2003; Shepard et al. 2005; Trotter 2008; Rasmussen et al. 2010). The two species have largely allopatric distributions in western North America, but RT have been widely introduced into the range of WSCT to supplement recreational fisheries (Behnke 1992; Shepard et al. 2005). In western Alberta, Canada, in particular, WSCT inhabit only approximately 20% of their historical range and hybridization with non-native RT is widespread (COSEWIC 2006; Mayhood and Taylor 2011; Yau and Taylor 2013). In most areas of western North America where hybridization between these species has been investigated, there is a persistent spatial pattern of hybridization; RT tend to predominate in low elevation, warmer streams, while WSCT tend to predominate in higher elevation, colder streams, and the level of hybridization tends to be positively associated with water temperature and negatively associated with elevation (e.g., Hitt et al. 2003; Rubidge and Taylor 2005; Muhlfeld et al. 2009; Rasmussen et al. 2010; Yau and Taylor 2013). Although the two parental taxa have virtually identical optimal growth temperatures (WSCT = 13.6 °C and RT = 13.1 °C; Bear et al. 2007), various studies have reported that RT tend to be found at lower elevations and warmer waters regardless of the elevation at which they were introduced (e.g., Paul and Post 2001; Hitt et al. 2003; Yau and Taylor 2013). Bear et al. (2007) also demonstrated that RT can grow over a broader range of temperatures and exhibit an upper lethal temperature that is 4.7 °C higher than WSCT, suggesting that the two species occupy different, but overlapping, thermal regimes (Bear et al. 2007). These observations suggest that the elevation refuge hypothesis may be applicable to interactions of WSCT with introduced salmonids, particularly RT (Paul and Post 2001; Rasmussen et al. 2010). Rasmussen et al. (2010) proposed a potential mechanism to explain the spatial gradient in species occurrence and hybridization in WSCT and RT. Westslope cutthroat trout with some level of admixture of RT alleles grow more quickly and mature faster than nonadmixed WSCT, and a trade-off exists between metabolic scope (RT) and growth efficiency (WSCT) such that RT are favoured at high temperatures by their increased metabolic scope and WSCT are favoured at low water temperatures by increased growth efficiency (Rasmussen et al. 2010). Subsequently, Rasmussen et al. (2012) documented interspecific differences in metabolism in terms of rates of oxygen consumption, and the activity of metabolically relevant enzymes, lactate dehydrogenase and citrate synthase, and concluded that fish with RT alleles tended to have higher metabolic demands than fish comprised primarily of a WSCT genomic background. Hybrids generally had intermediate metabolic traits and were better able to balance the trade-off between energetic scope and growth efficiency, allowing them to be successful in cooler, more upstream locations than RT (Rasmussen et al. 2012). Consequently, nonadmixed WSCT may be observed to persist predominately in high-elevation tributaries as a function of the physiological and metabolic limitations of RT and interspecific hybrids in these habitats (Rasmussen et al. 2010, 2012).

Despite the potential differences in performance between species at high versus low water temperatures, most studies on freshwater fishes have focused on thermal maxima, the upper threshold of temperature tolerance (see review in Beitinger et al. 2000). Typically, these studies assay the critical maximum temperature (CTMax) defined as the maximum temperature at which a fish can maintain a normal, upright swimming position ("equilibrium"). The minimum temperature at which a fish can maintain such equilibrium is termed CTMin and has been much less commonly studied for a variety of reasons (but see Barrett et al. 2011; Darveau et al. 2012). In fact, McHugh and Budy (2005) highlighted a need to better understand the physiological performance of interacting species and their hybrids at low thermal limits.

In this study, we tested the CTMin of WSCT and RT to evaluate one of the predictions of the "elevation refuge hypothesis"—that WSCT should perform better at low water temperatures, i.e., have a lower CTMin, and that such physiological differences may help explain the predominance of genetically nonadmixed WSCT at higher elevations. Our study is a natural next step in trying to understand the processes behind the consistent spatial patterns in hybridization between these two species in nature (e.g., Rasmussen et al. 2012; Yau and Taylor 2013).

Materials and methods

Trout populations

All procedures in our study were conducted under approval of The University of British Columbia (UBC) Animal Care Committee (permit No. A27-2956). Our choice of study populations was impacted by the desire to test fish collected from the wild, under as similar environmental conditions as possible, and to sample as wide an array of genotypes as possible. Consequently, we focused on sampling adults for gametes and making artificial crosses and raising the offspring under controlled conditions. Spawning adult WSCT, however, can only be obtained from the Freshwater Fisheries Society of British Columbia every second year, and were collected from a source population in Connor Lakes, two interconnected high elevation (1850 m) lakes in the east Kootenay region of southeastern British Columbia (B.C.; 50°08′50″N, 115°05′05″W). The WSCT used in our experiments were derived from adult fish collected in 2012 and represent random samples of juveniles resulting from crossing 21 males and 34 females. Fry were reared at the Kootenay Hatchery in Cranbrook, B.C., until about 1.5 g in size before being transferred to the UBC Aquatics Facility in the autumn of 2012. Fish from this brood stock have been used in all WSCT stocking events in B.C. over the last three decades and represent a population with no known history of hybridization with rainbow trout (Taylor et al. 2003).

Rainbow trout gametes were obtained from fish collected from the Blackwater River (BW; 53°16′47″N, 122°14′58″W; elevation 800–840 m) and Tzenzaicut Lake (TZ; 52°32′09″N, 122°50′52″W; elevation 1139 m), in the Chilcotin area of central B.C. during the spring of 2011. The number of adults used in making these crosses ranged from a single male and female to three males and females (Supplementary Table S1).[1] These wild populations were chosen based on ease of access and divergence in specific characters. For example, BW rainbow trout are characterized by fast growth and recommended for stocking in competitive habitats, while TZ rainbow trout are suited to colder habitats with low productivity and survival after stocking is significantly higher in TZ fish compared with BW fish (Clarke et al. 2008[2]; Northrup and Godin 2009[3]). Based on these characteristics, the TZ fish appear to be more similar to WSCT (i.e., slow-growing, use habitats often of relatively low productivity), while BW fish possess attributes more typically associated with RT (i.e., fast growth and highly competitive) (Rasmussen et al. 2010). We tested these two RT populations to try and obtain some measure of intraspecific differences in thermal tolerance and because no WSCT other than the Connor Lakes population was readily available. The availability of sexually mature first-generation (F_1) hybrids between BW and TZ presented an opportunity to further assess intraspecific variation by looking at interpopulation hybrids in RT. In particular, by testing cold tolerance in the second generation (F_2) and backcrosses to each parental RT population (Blackwater backcross, BWB; Tzenzaicut backcross, TZB), we could assess how later-generation hybrids between a WSCT-like trout (TZ) and an "average" RT (BW) perform in CTMin while assessing thermal performance in a wide array of RT genotypes. Unfortunately, logistical constraints prevented the generation of WSCT × RT hybrids (i.e., Connor Lakes WSCT are only collected for hatchery production only every other year).

Fish husbandry

Fertilized eggs were placed into baskets constructed from 5 cm PVC (polyvinyl chloride) pipe and mesh netting sealed with aquarium-grade silicone in an environmental chamber (manufactured by Environmental Growth Chambers) in the Biological Sciences Building at UBC at a water temperature of 10 °C. The baskets were housed in a vertical incubator, with a continuous flow of fresh water. Individual baskets were labeled by family. After approximately 30 days after fertilization, the eggs "eyed-up", and following another 20 days, alevins (larvae) began to hatch. One week after hatch, the alevins were pooled across families and transferred to plastic containers submerged in a fiberglass rearing trough with a 12 h light : 12 h dark photo cycle. Holes, 2.5 cm × 4 cm, were drilled in the sides of the container, covered in mesh netting, and sealed with aquarium-grade silicone to allow for water flow. The alevins remained in these containers until their yolk sacs absorbed and they were fed crushed trout chow (approximately 3 weeks after hatch). Fry were then transferred to wooden troughs that had been painted and sealed with fiberglass coating. The troughs were housed in a three-level shelving unit and each trough had a 1 inch (1 inch = 2.54 cm) PVC pipe that supplied a continuous flow of fresh water. The troughs drained to a common 4 cm pipe that flowed to a floor drain. Trout were fed 1.2 mm Biovita trout chow.

Acclimation

Each test group was acclimated for 2 weeks at 15 or 18 °C. Fish were housed in four 195 L fiberglass tanks that were connected to a common sump. Both the acclimation tanks and the CTMin apparatus were set up in the same environmental chamber. This chamber was held at 5 °C to control water cooling and eliminate fluctuations during the temperature trial. To achieve the appropriate acclimation temperature, Odyssey Heatpro aquarium heaters were placed in the common sump, which pumped water heated to 25 °C into each tank. A nozzle controlled the flow of heated water entering the tank at any given time. Fine control of the inflow of warm water, in combination with tank water being cooled by the ambient 5 °C air, allowed a constant acclimation temperature of 15 or 18 °C. A header tank was added so that cool, clean water could continuously be added to the flow-through system. During acclimation, fish were fed 1.2 mm Biovita trout chow every day. Fish acclimated to 18 °C were first acclimated to 15 °C for 2 weeks before the water temperature was raised to 18 °C for a 2-week acclimation. During the 18 °C acclimation, the WSCT developed a fungal infection. To eliminate the infection, affected fish were removed, the water temperature was lowered to 10 °C, and the fish were allowed to stabilize for 1 week after which the water temperature was raised back to 18 °C for the 2-week acclimation period.

CTMin determination

The experimental apparatus consisted of a single, large antifreeze bath in which 12 individual floating containers were held in place by a large Styrofoam sheet that was fitted with holes to hold each container. A large 90 L Rubbermaid container was placed on a table and elevated with cement blocks above the antifreeze bath. A flap was cut into the lid of this container to facilitate the addition of dry ice to the system, allow CO_2 to escape, and to prevent uncontrolled bubbling. The Rubbermaid container was fitted with a brass nozzle to control the flow of cooled antifreeze entering the antifreeze bath. Below the antifreeze bath, an identical Rubbermaid container collected the antifreeze that had already flowed through the apparatus. A Little Giant, 115 V and 60 Hz, pump in this container pumped the used antifreeze back up to the elevated Rubbermaid container to be cooled again and recirculated through the system during the trial. Before the trial, 25 °C water from the acclimation sump was added to the 12 containers in the antifreeze bath to allow the water temperature in each container to reach either 15 or 18 °C. Twelve fish were collected from the acclimation tanks and transferred into a bucket. A single fish was placed into each of the 12 containers once

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[1] Supplementary Tables S1 and S2 are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2014-0047.

[2] A. Clarke, T. Godin, T. Yesaki, and C. Cena. 2008. Assessment of 2N and 3N Fraser Valley rainbow trout strains for stocking coastal oligotrophic lakes. Unpublished data. Available from Freshwater Fisheries Society of British Columbia, Vancouver.

[3] S. Northrup and T.I. Godin. 2009. A review of rainbow trout strain performance: historical and contemporary data from British Columbia Lakes. Unpublished data. Available from the Freshwater Fisheries Society of British Columbia, Vancouver.

Table 1. Critical thermal minima (CTMin), fork length, mass, and raw CTMin and size-adjusted CTMin (corrected for differences in length and mass) of trout test groups acclimated to 15 and 18 °C.

				CTMin (°C)		
Test group	Fork length (cm)	Mass (g)	Condition factor	Mean	Size-adjusted	*n*
			15 °C acclimation			
RT	9.5±1.3	9.2±3.6	1.01±0.12	1.4±1.0	1.5	179
WSCT	7.3±0.8	4.0±1.7	0.99±0.09	1.0±0.8	0.8	36
BW	8.6±1.4	7.1±3.3	1.05±0.08	1.6±0.9	1.5	36
BWB	9.3±1.3	8.9±3.7	1.02±0.09	1.5±1.2	1.6	35
F_2	9.9±1.2	9.7±3.3	0.98±0.10	1.0±1.2	1.1	36
TZ	10.2±2.2	10.5±3.2	0.91±0.08	1.3±0.7	1.5	36
TZB	9.6±1.0	10.0±3.4	1.09±0.15	1.7±0.7	1.8	36
			18 °C acclimation			
RT	9.6±1.3	8.4±3.1	0.99±0.10	1.9±0.9	1.9	179
WSCT	7.4±0.8	4.7±1.9	1.09±0.11	1.9±0.7	2.0	36
BW	9.5±1.3	8.5±3.3	0.96±0.10	1.9±1.0	1.8	36
BWB	9.3±0.8	8.5±2.4	1.03±0.11	2.1±0.8	2.1	35
F_2	9.8±0.9	9.5±2.8	0.99±0.07	2.0±1.0	2.0	36
TZ	10.0±1.0	9.5±3.0	0.91±0.05	1.8±0.8	1.7	36
TZB	9.6±0.8	9.6±2.2	1.07±0.09	1.9±0.9	1.9	36

Note: Values are mean ± SD and *n* is the number of fish. Rainbow trout (*Oncorhynchus mykiss* (RT); all populations); westslope cutthroat trout (*Oncorhynchus clarkii lewisi* (WSCT)); RT populations—Blackwater River rainbow trout (BW), Blackwater River backcross (BWB), Blackwater River × Tzenzaicut Lake F_1 hybrid × Blackwater River × Tzenzaicut Lake F_1 hybrid (F_2), Tzenzaicut Lake rainbow trout (TZ), and Tzenzaicut Lake backcross (TZB). RT refers to the values averaged for all BW, BWB, F_2, TZ, and TZB fish and adjusted values are adjusted to a common mean fork length and mass of 9.2 cm and 8.3 g, respectively.

the water cooled to the acclimation temperature. Each container was equipped with an airstone to maintain oxygen levels and to ensure uniform cooling and an individual digital thermometer to ensure consistency of water temperatures during each trial. The fish were given 15 min to acclimate to the test container, during which dry ice was added to the elevated Rubbermaid container. After the 15 min acclimation, the nozzle of the Rubbermaid container was opened and the cooled antifreeze began to flow into the large bath. The pump in the collection container was turned on to circulate the antifreeze through the set-up. Each of the 12 containers within the antifreeze bath was connected to a digital thermometer and had an airstone to ensure the water was saturated with oxygen and to allow for homogenous cooling. The rate of cooling was monitored using the digital thermometers and kept to a decline rate of 0.3 °C/min by the addition of dry ice to the system (cf. Darveau et al. 2012). The 15 °C temperature with a cooling rate of 0.3 °C/min was used in our experiments so that a direct comparison could be made with previous work on RT (Becker et al 1977; Currie et al. 1998). A second acclimation temperature of 18 °C with the same rate of decline was chosen in an attempt to represent common summer temperatures for the populations studied and a temperature that is nearer the upper physiological limits of both species. An acclimation temperature below 15 °C was not employed because RT held at 10 °C in previous studies did not exhibit loss of equilibrium (LOE) when the water began to freeze at 0 °C (Becker et al. 1977; Currie et al. 1998). The temperature at which a fish lost equilibrium was recorded as its CTMin. Once all 12 fish lost equilibrium, the trial ended and the fish were euthanized in MS-222 (tricaine methanesulfonate), weighed, and measured. The fish were then labeled and preserved in 95% ethanol.

Statistical analysis

Cold tolerance data were analyzed first using simple linear regression (SLR) to test for effects of length (cm), mass (g), and condition factor on CTMin at both acclimation temperatures. Condition factor was calculated as $100 \times [\text{mass}/(\text{fork length})^3]$ after Ricker (1975). In some cases, these tests indicated significant influences of fish size on CTMin (see Results). Consequently, differences in means of the test groups were analyzed using two-way fixed-effects analysis of covariance (ANCOVA) using fork length and mass as covariates and we reported both the raw CTMin and the "size-adjusted" values that have been corrected for differences in both length and mass. Levene's test was used to verify homogeneity of variances (minimum p = 0.113). Statistical tests were carried out in R release 2.15.2 (R Development Core Team 2012).

Results

Effect of body size and condition factor on thermal performance

There were significant differences in fork length and mass among test groups (ANOVA; length: $p < 0.001$; mass: $p < 0.001$), and RT were generally larger than WSCT (Table 1). Simple linear regressions revealed that there was a significantly positive relationship between fork length and CTMin at an acclimation temperature of 15 °C in BW, BWB, and TZB (maximum p = 0.047), and mass showed a significantly positive effect in BWB and TZB (maximum p = 0.03). At 18 °C acclimation, both length and mass were significantly positively associated with CTMin in BWB (maximum p = 0.047). Consequently, although size-adjusted CTMin mean values did not differ from the actual values by more than 0.2 °C at 15 °C or 0.1 °C in 18 °C acclimated fish (Table 1), we performed statistical analyses on the size-adjusted values given the significant effects of size on CTMin within some populations.

There was also significant variation in condition factor among test groups (ANOVA, $p < 0.001$), but these differences were largely driven by the generally low condition factor of TZ rainbow trout (Table 1). In contrast to results for fork length and mass, relationships between condition factor and CTMin were inconsistent; in most groups, there was a negative (but largely nonsignificant) relationship between condition factor and CTMin, whereas in other groups the relationship was positive (e.g., BWB at 15 °C; WSCT at 18 °C). Given the few pairwise differences in condition factor and the inconsistent and largely nonsignificant relationships between condition factor and CTMin, we did not analyze CTMin values adjusted for the minor differences in condition factor (cf. Fangue et al. 2006).

Table 2. Summary statistics for analysis of covariance (ANCOVA, type III sum of squares) of critical thermal minimum (CTMin) of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*Oncorhynchus mykiss*) in response to fork length, mass, and cross type ("test group" in Table 1) when acclimated to 15 and 18 °C.

Source	Sum of squares	df	Mean square	*F*	Significance
Model	57.39	13	4.41	5.23	0.000
Intercept	1.27	1	1.27	1.51	0.220
Length	0.99	1	0.99	1.18	0.278
Mass	3.10	1	3.10	3.68	0.056
Type	10.23	5	2.05	2.43	0.035
Temperature	30.85	1	30.85	36.64	0.000
Type × temperature	11.63	5	2.33	2.76	0.018
Error	351.12	417	0.84		
Total	1573.17	431			

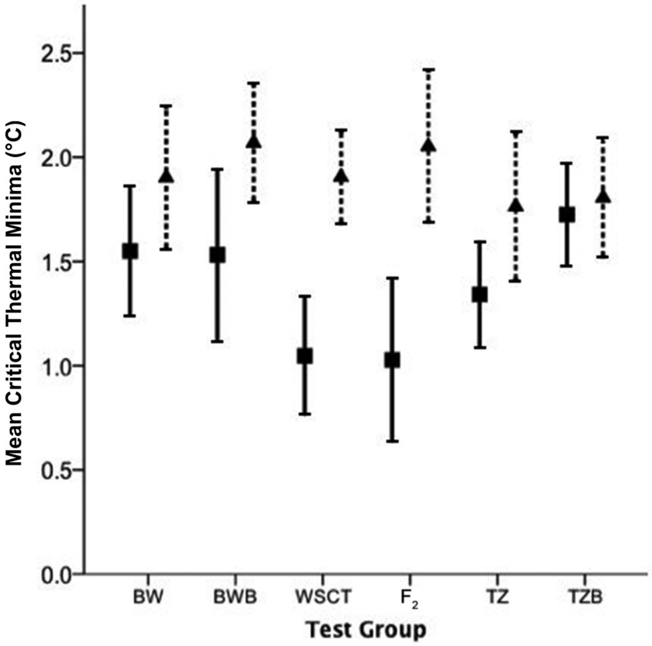
Note: $R^2 = 0.14$; adjusted $R^2 = 0.114$.

Effects of acclimation temperature and cross type on thermal performance

When all RT cross types were considered together, WSCT had a lower raw and size-adjusted CTMin than RT, but only at the 15 °C acclimation temperature (Tables 1, 2; Fig. 1). On average, RT experienced a 0.48 °C increase in CTMin when acclimated at 18 °C compared with 15 °C. The difference, however, was nearly double in WSCT; a 0.87 °C increase in CTMin was observed at 18 °C compared with acclimation to 15 °C (Table 1, Supplementary Table S2[1]). Also and as expected, TZ rainbow trout had a lower raw CTMin than BW rainbow trout, but again, the difference was only apparent at 15 °C. When adjusted for differences in size, however, the two populations of RT had identical CTmin at 15 °C (Table 1). The F_2 and TZ rainbow trout had the lowest raw and size-adjusted CTMin values among all RT crosses, but this difference was largely only observed at 15 °C. The RT from TZ had among the lowest CTMin at both acclimation temperatures (Table 1, Fig. 1).

The two-way ANCOVA revealed significant effects of acclimation temperature ($p < 0.001$) and cross type ($p = 0.035$) on size-adjusted CTMin, as well as a significant interaction between acclimation temperature and cross type ($p = 0.018$; Table 2). CTMin increased with acclimation temperature in all test groups (i.e., the temperature at which fish lost equilibrium increased with increasing acclimation temperature; Fig. 1).

Fig. 1. Mean critical thermal minima (CTMin) values for each test group at 15 °C (solid lines) and 18 °C (broken lines) acclimation. Error bars are 95% confidence intervals. Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*, WSCT); rainbow trout (*Oncorhynchus mykiss*) populations—Blackwater River (BW), Blackwater River backcross (BWB), Blackwater River × Tzenzaicut Lake F_1 hybrid × Blackwater River × Tzenzaicut Lake F_1 hybrid (F_2), Tzenzaicut Lake (TZ), and Tzenzaicut Lake backcross (TZB), where F_1 is first generation and F_2 is second generation.



Discussion

The results of our CTMin experiments provide some support for the elevation refuge hypothesis in that WSCT performed better at colder water temperatures than RT. On average, WSCT were better able to tolerate cold temperatures when both species were acclimated to 15 °C. When acclimation temperature was increased to 18 °C, there were no significant interspecific differences observed. Furthermore, notwithstanding the differences between species observed at 15 °C, when all RT were considered together, there was significant intraspecific variation in CTMin. In addition, we were unable to test more than one population of WSCT so it remains to be assessed whether or not the two species show consistent differences in CTMin after accounting for intraspecific variation in WSCT.

Despite sharing similar thermal optima, WSCT and RT appear to have significant differences in other aspects of their respective thermal regimes (Bear et al. 2007). Field studies have repeatedly described a gradient of species dominance that follows a gradient in elevation and water temperature when WSCT and RT exist in sympatry (Hitt et al. 2003; Weigel et al. 2003; Rubidge and Taylor 2005; Muhlfeld et al. 2009; Rasmussen et al. 2010; Yau and Taylor 2013). In laboratory studies, interspecific differences exist when both species were tested at the upper extremes of their thermal scope (Bear et al. 2007). Rainbow trout grow over a broader range of water temperatures and continue to grow at temperatures beyond 20 °C, which are typically lethal to juvenile WSCT. When tested for upper lethal limits, RT can survive temperatures 4.7 °C above WSCT thresholds (Bear et al. 2007). Our work, however, is the first to evaluate differences between WSCT and RT at the lower limits of their thermal ranges.

Acclimation temperature

To date, two studies have evaluated CTMin in RT acclimated to 10, 15, and 20 °C and there are no reported CTMin values for WSCT (Becker et al. 1977; Currie et al. 1998). The acclimation temperatures used in our study were chosen such that a quantifiable CTMin could be obtained for both species (cf. Becker et al. 1977; Currie et al. 1998). In our experiments, there was a significant effect of acclimation temperature on cold tolerance performance in WSCT and RT. Assuming a linear increase in CTMin with acclimation temperature, our data suggest an increase of 0.29 °C in

CTMin with every 1 °C increase in acclimation temperature for WSCT. This value is similar to those reported in mummichog (*Fundulus heteroclitus heteroclitus* (L., 1766)) (Fangue et al. 2006), sheepshead minnow (*Cyprinodon variegatus variegatus* Lacepède, 1803) (Bennett and Beitinger 1997), and other studies of RT (Becker et al. 1977; Currie et al. 1998). The RT tested in our work, however, only exhibited an estimated 0.16 °C increase in CTMin with every 1 °C increase in acclimation temperature. This is considerably lower than the value of 0.36 °C per 1 °C rise in acclimation reported by Becker et al. (1977) and Currie et al. (1998). Differences between these values could be the result of testing two acclimation temperatures that differed by only 3 °C. Becker et al. (1977) and Currie et al. (1998) evaluated three acclimation temperatures at 5 °C increments. Thus, the values drawn from our study are applicable to a smaller range of temperatures and thus may not have elicited as large as response as in these previous studies. Furthermore, the RT used in our work represent different populations and crosses, which increases the potential genetic variability in cold tolerance and may translate to higher variability in cold performance. Acclimation temperature generally explains a significant portion of the variation observed in CTMin experiments (Becker et al. 1977; Becker and Genoway 1979; Bennett and Beitinger 1997; Currie et al. 1998; Fangue et al. 2006).

Inter- and intra-specific differences

Our results revealed a significant difference in cold tolerance (CTMin) between RT and WSCT acclimated to 15 °C. There were high levels of variation in cold performance between different populations of RT; however, a substantial difference was only observed between two RT test groups (F_2 and TZB). On average, some F_2 hybrids between what are thought to be “warm” (BW) and “cold” (TZ) water adapted RT were able to perform just as well as WSCT when acclimated to 15 °C and had the highest degree of variation among all the groups tested. Assuming additive genetic variability for any trait, the F_2-hybrid offspring should be characterized by high levels of genetic variation, as genotypic combinations of both parental genotypes and hybrid combinations between the two populations are represented in this group. By contrast, the low CTMin obtained for this group acclimated to 15 °C is difficult to explain, as simple additive heritability would also predict a CTMin performance intermediate to the parental populations (BW, TZ). Unfortunately, the genetic architecture of cold tolerance in fishes is poorly understood (e.g., Moen et al. 2004). Without such understanding, it is difficult to know why the backcross groups (BWB, TZB) and the F_2 hybrids performed the way they did. The patterns observed, however, suggest that the genes controlling cold tolerance perhaps do not behave in a simple, additive manner. Alternatively, our sample sizes in terms of the numbers of parents used in these crosses (especially the single-parent crosses for BWB) are likely just too small to accurately assess CTMin among the different cross types in RT.

Tzenzaicut Lake rainbow trout had a lower mean CTMin than BW fish at both acclimation temperatures (i.e., TZ fish exhibited higher cold tolerance). This result is consistent with reports by Clarke et al. (2008)[2] and Northrup and Godin (2009)[3] that TZ fish perform better in growth and survival than BW fish when stocked in colder water habitats. Furthermore, it lends support to the rationale used in our study; i.e., wild fish populations that evolved in colder water perform better in cold tolerance trials. This result agrees with our predictions and suggests that the results of our study have relevance in nature.

Altogether, the CTMin values for RT reported in our study are higher than previous work (Becker et al. 1977; Currie et al. 1998). At an acclimation temperature of 15 °C and a rate of temperature decline of 0.3 °C/min, Currie et al. (1998) reported a CTMin of only 0.2 °C. Becker et al. (1977) reported a CTMin of 0.7 °C under similar conditions. In contrast, the mean CTMin for RT in our study was 1.4 °C tested under comparable conditions. This discrepancy among individuals of the same species could be the result of minor variation in methodology, population, age, and size of fish, etc. For instance, RT used by Becker et al. (1977) were collected from the Columbia River, while fish used in the study by Currie et al. (1998) had been obtained from a hatchery in Missouri. Our fish originated from experimental crosses using fish collected in the wild and subsequently raised under laboratory conditions. Currie et al. (1998) also used a CTMin endpoint that deviated from our methods. The temperature they reported was taken 1 min after the fish exhibited an initial LOE, but we recorded temperatures at the initial onset of LOE and did not wait 1 min to record temperatures. Furthermore, both Becker et al. (1977) and Currie et al. (1998) introduced cold water directly into their system, while we used an antifreeze bath to gradually cool the test water. The fish used by Currie et al. (1998) were only 4 cm long and relatively young at 6 weeks of age. Our fish, by contrast, were considerably larger and older and we demonstrated that fork length and mass can be associated with cold performance at least for some acclimation conditions.

At an acclimation temperature of 18 °C, there were no significant intra- and inter-specific differences in cold tolerance, and body length and mass were better predictors of CTMin. This suggests that any physiological differences that exist when fish are held at temperatures in the mid-range of their tolerance thresholds (15 °C) are no longer apparent at 18 °C (Bear et al. 2007). A temperature of 18 °C probably represents above average summer temperatures for WSCT and approaches the upper extreme at which juvenile fish can survive (survival drops dramatically above 20 °C; Beitinger et al. 2000; Bear et al. 2007). Interestingly, Culumber et al. (2012) showed that differences in CTMin between species of *Xiphophorus* collected from the wild can vary between seasons when the fish are exposed to natural variation in acclimation temperatures. The lack of differences between species at 18 °C, however, should be interpreted with some caution. Our ability to detect species-level differences at 18 °C could have been compromised as a result of the differences in the acclimation history of WSCT and RT at 18 °C given the fungal outbreak during the WSCT acclimation.

Our data suggest that there is considerable variability within RT in cold tolerance. Given that our RT were all raised under common environmental conditions, the persistence of differences in thermal tolerance suggests that such variation may be in part heritable. The possible existence of heritable variation in thermal tolerance, in addition to any role that physiological plasticity may play, may increase the likelihood of eventual invasion success of RT at higher elevations because high genetic diversity and plasticity are often associated with invasion success in other taxa (e.g., Sexton et al. 2002; Lindholm et al. 2005). Notwithstanding such intraspecific variation, when all RT were considered, the CTMin of WSCT was lower than that of RT, which provides some support for the elevation refuge hypothesis. Within each RT test group, however, there were individuals who performed just as well or exceeded the cold thermal scope of an average WSCT. The F_2 (RT) crosses in particular had a mean CTMin value equivalent to WSCT at 15 °C acclimation. These results suggest that it is possible for RT to displace WSCT even at higher elevations if particular genotypes of RT are present. It may simply be a matter of time, lack of competitive pressure at lower elevations, or elevational variation in other unknown factors that currently limits RT or hybrid genotype colonization farther upstream (e.g., gradients in flow, food distribution, genotype-specific metabolic rates; Rassmusen et al. 2010; Yau and Taylor 2013). Further tests are needed to determine whether and how variation in cold tolerance of RT might influence their competitive ability relative to WSCT in low- and high-elevation habitats.

Conclusions

With the exception of two test groups (F_2 and TZB), trends in cold tolerance performance met our predictions. The WSCT performed better than pure RT genotypes and RT individuals from BW fish had a higher CTMin than TZ fish. We did not resolve differences at 18 °C acclimation, which may have resulted from 18 °C being closer to the upper thermal tolerance of WSCT compared with RT or owing to the difference in acclimation history for the species at this temperature. Overall, we were able to show that there are measurable differences in cold tolerance between WSCT and RT, which is consistent with the hypothesis that physiological limitations may be constraining the colonization of cooler, higher elevation habitats by RT. This, in turn, may play a role in the species distribution and hybridization gradient reported in numerous field studies in trout (Rahel and Hubert 1991; Fausch et al. 1994; Hitt et al. 2003; Weigel et al. 2003; Rubidge and Taylor 2005; Rasmussen et al. 2010; Yau and Taylor 2013) and as proposed for other stream fishes (e.g., Culumber et al. 2012). The observation that some RT individuals exhibited cold tolerance comparable with WSCT, however, suggests that caution needs to be invoked when trying to offer simple explanations for these spatial patterns. A greater diversity of genotypes of both species and interspecific hybrids will need to be tested before definitive conclusions can be made about the extent to which interspecies differences in thermal tolerance may contribute to spatial variation of interspecific hybridization in nature (cf. Culumber et al. 2012).

The Alberta westslope cutthroat trout recovery plan has outlined efforts to restock native WSCT in habitats across their historical range (The Alberta Westslope Cutthroat Trout Recovery Team 2013). Conservation efforts for WSCT might be best focused on rehabilitating populations at high elevations that appear to provide natural refugia from RT invasion via cold water and low productivity as suggested by our study and previous work (McHugh and Budy 2005; Rasmussen et al. 2010, 2012). A better understanding of the genetics that underlies cold tolerance may also be valuable, particularly if certain populations of WSCT show a heightened tolerance to cold water temperature. If such populations could be identified, they could be useful in efforts to restock high-elevation locales, as their cold tolerance could provide an extra means of resistance to invasion by RT and subsequent hybridization. Conversely, where stocking with RT is considered necessary in drainages that also contain WSCT, RT populations with low tolerance to cold water temperatures may be the best candidates for stocking. Finally, projections of future climate warming across the range of WSCT suggest that the thermal niche and geographic range of the species will become compressed relative to that of RT (Wenger et al. 2011). Furthermore, as water temperatures increase between the range of 15 and about 20 °C, conditions are predicted to be more favourable for RT especially if winter flows also increase as predicted (Wenger et al. 2011). Although there is no one relationship between water temperature and hybridization, an increase in water temperatures across a range between 10 and 20 °C has been associated with increases in hybridization between WSCT and RT (e.g., Rasmussen et al. 2010; Yau and Taylor 2013). Consequently, projections of climate warming may have serious consequences for the persistence of WSCT both from reductions in their fundamental niche space and from increased threat of hybridization with RT.

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