The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2012, **105**, 56–72. With 7 figures

Metabolic traits of westslope cutthroat trout, introduced rainbow trout and their hybrids in an ecotonal hybrid zone along an elevation gradient

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Received 31 March 2011; revised 7 July 2011; accepted for publication 8 July 2011

In the Upper Oldman River, Alberta, introduced non-native hatchery rainbow trout (*Oncorhynchus mykiss*) hybridize with native westslope cutthroat trout (*O. clarkii*), resulting in a hybrid swarm. Rainbow trout dominate at low elevations (< 1250 m) in the river mainstem, cutthroat in high-elevation tributaries (> 1400 m), and hybrids are numerically dominant in the mid-elevation range. We hypothesized that metabolism of rainbow trout would exceed that of cutthroat trout, and that the elevation gradient in genetic makeup would be mirrored by a gradient in metabolic traits, with intermediate traits in the hybrid-dominated ecotone. Metabolic traits were measured and regressed against the genetic makeup of individuals and elevation. Rainbow trout had higher oxygen consumption rates (OCRs), higher white muscle lactate dehydrogenase (LDH), and citrate synthase (CS) activity, and higher plasma acetylcholinesterase (AchE) than cutthroat trout. Hybrids had intermediate OCRs and AchE, but LDH activity as high as rainbow trout. While hybrid zones are usually modelled as a balance between cross species mating and selection against hybrids, ecotonal hybrid zones, where hybrids proliferate in intermediate habitats and have traits that appear well suited to ecotonal conditions, have been proposed for some plants and animals, and may have important implications for resource management and conservation. *Biological Journal of the Linnean Society*, 2012, **105**, 56–72.

ADDITIONAL KEYWORDS: acetylcholinesterase – citrate synthase – ecotone – elevation – hybrid zone – introgression – lactate dehydrogenase – oxygen consumption.

INTRODUCTION

Hybrid zones pose a paradox for taxonomy, evolutionary biology and ecology in that they appear to challenge the 'separateness' of species, and the efficacy of isolating mechanisms. Endangered or important resource species often hybridize with others, posing vexing management problems (Allendorf *et al*., 2004). Although hybridization can threaten species, introgression can also be a bridge for the exchange of functional traits among species (Rieseberg & Wendel, 1993; Grant & Grant, 1996). Hybridization occurs widely among salmonids, but little is known about the ecological aspects of such introgression and the functional traits involved (Williams *et al*., 2008).

Hybrid zones are usually modelled as zones of tension between interspecific mating and factors that limit hybrid fitness (Fig. 1A), either through genomic incompatibility (Barton & Hewitt, 1985), ecological selection (Hatfield & Schluter, 1999), or both (Sperling & Spence, 1991; Springer & Heath, 2007). Alternatively, contact between populations that are segregated by habitat can produce an ecotonal hybrid zone, with fertile and successful hybrids exhibiting

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Archived data: data deposited at Dryad: doi:10.5061/dryad.rr388.

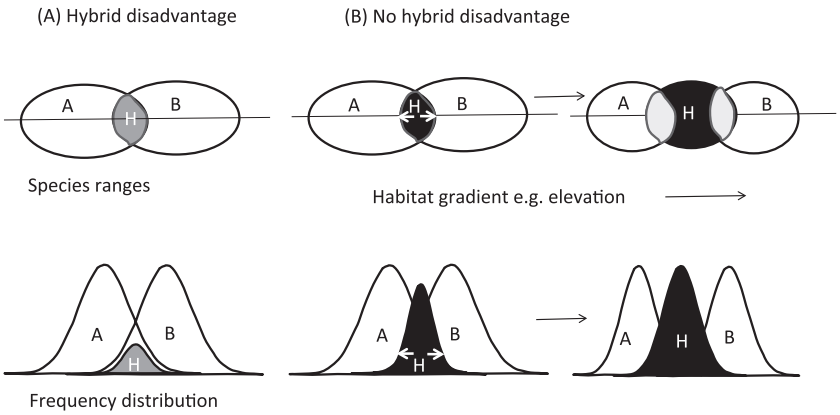

Figure 1. Schematic representation of hybrid zone dynamics. A, a classic 'tension' hybrid zone (dark grey) between two species (A, B) that produce disadvantaged hybrids – equilibrium between hybrid formation (H) and fitness. B, an ecotonal hybrid zone formed when A and B produce high-fertility hybrids (black) sufficiently successful to proliferate in the intermediate habitat along a gradient. H × H mating and backcrossing expands the hybrid zone, separating A and B, and reducing F_1 formation.

intermediate traits well suited to the ecotonal habitat (Moore, 1977; Arnold, 1997); the breakdown of species barriers in the hybrid swarm thus allows unrestricted introgression (Fig. 1B). Ecotonal hybrid zones have been described for a number of plants and animals; however, whether success of hybrid traits in ecotonal habitats contributes to breakdown of species boundaries is unknown.

Hybridization between rainbow trout (RT, *Oncorhynchus mykiss*) and cutthroat trout (CT, *O. clarkii*) occurs naturally along North America's west coast (Johnson *et al*., 1999; Behnke, 2002). In addition, introduction of hatchery RT inland has resulted in hybridization with previously allopatric populations of westslope cutthroat trout (WCT, *O. c. lewisii*) (Hitt *et al*., 2003; Weigel, Peterson & Spruell, 2003; Rubidge & Taylor, 2005), and other CT subspecies. Although hybrids might be expected to have low fertility, given differences in chromosome numbers (58–60 for RT, Thorgaard, 1983, vs. 64–68 for CT, Loudenslager & Thorgaard, 1979; Hartley & Horne, 1986), most species have 104 chromosomal arms allowing chromosome pairing to proceed as though karyotypes were the same. Thus RT × CT hybrids are often highly fertile (Behnke, 2002; Weigel *et al*., 2003). Fertility and success of such hybrids contributes to fragmentation of CT populations, and is a major risk factor for many CT subspecies (Shepard, May & Urie, 2005; COSEWIC, 2007, http://www.cosewic.gc.ca).

Where RT and CT hybridize naturally, e.g. along the west coast where coastal CT (*O. c. clarkii*) and coastal steelhead trout (*O. m. irideus*) hybridize, or in the lower Columbia River, where WCT and redband RT (*O. m. gairdneri*) hybridize, species boundaries usually do not break down. Although hybrids have usually not been clearly demonstrated to be at a disadvantage, and in fact, back-crosses and even hybrid swarms have been shown in some cases, usually associated with human disturbance, such as logging or hatchery RT introductions (e.g. Bettles *et al*., 2005; Heath, Bettles & Roff, 2010), their failure to proliferate widely implies that the hybrid zones are generally of the tension zone type (Ostberg, Slatton & Rodriguez, 2004; Kozfkay *et al*., 2007; Williams *et al*., 2008).

In the Upper Columbia River or adjacent drainages where RT have been introduced into the native interior range of WCT, regardless of the elevation at which RT were introduced, they tend to establish themselves at low elevation (Paul & Post, 2001; Hitt *et al*., 2003) by competitively displacing native WCT. Hybrids that proliferate upstream form a gradient over a broad elevational range in the river mainstems, confining pure CT populations to smaller high-elevation tributaries (Weigel *et al*., 2003; Rubidge & Taylor, 2004). The extensive hybrid zones that develop in the elevation mid-range often separate WCT from RT, resulting in few F_1s (Rubidge & Taylor, 2005; Boyer, Muhlfeld & Allendorf, 2008; Rasmussen, Robinson & Heath, 2010) and appear to fit the ecotonal model. The elevation gradient of RT alleles is associated with a life-history gradient, and fish with more RT alleles grow faster and have shorter lifespans (Rasmussen *et al*., 2010). This suite of traits is probably associated with the fundamental metabolic character of the fish, and we hypothesized that metabolism of RT would exceed that of CT, and that the elevation gradient in genetic makeup would be mirrored by a gradient in metabolic traits, with intermediate traits in the hybrid-dominated ecotone.

Laboratory physiological studies on hybrids of wild species are difficult, as hybrids are often not morphologically identifiable or available from hatcheries or suppliers. Field studies are also challenging as genotypes can only be scored a posteriori, and metabolic traits, being phenotypic, depend on uncontrolled variables such as body size and environment, limiting statistical precision. Metabolic traits are also difficult to measure in field studies. Thus it was necessary to employ a simple method of measuring oxygen consumption rate (OCR) suitable for field use (Cech, 1990).

Respiratory enzymes such as lactate dehydrogenase (LDH) in white muscle have been used to study metabolism in field studies on fish (e.g. Sherwood *et al*., 2002a, b). LDH is a cytoplasmic enzyme that catalyses the reversible conversion of lactate to pyruvate allowing quick bursts of anaerobic energy supported by muscle glycogen reserves (Somero & Childress, 1980; Garenc *et al*., 1999). Such anaerobic demands generally increase with body size in fishes (Childress & Somero, 1990). LDH clears lactate and acidosis allowing such activities as pursuing prey, chasing competitors, escaping predators, and overcoming currents to be sustained, and has been linked to the activity budget (Sherwood *et al*., 2002a; Rasmussen *et al*., 2008). Aerobic pathways complement anaerobic power production, by allowing sustained moderate activity, and quick recovery following exertion, making more effective and efficient use of muscle glycogen reserves. Citrate synthase (CS) is a mitochondrial enzyme whose activity has been used to reflect the aerobic pathway in fish muscle (Guderley & Gawlicka, 1992; Gamperl *et al*., 2002; Rodnick *et al*., 2004). Acetylcholinesterase (AchE) is a key neuro-enzyme that cleaves and clears acetylcholine from cholinergic synapses and myoneural junctions, allowing junctions to 'reload' with a very short latency period (Baslow & Nigrelli, 1961). AchE has also been implicated in a broad range of cellular communication functions including stress and immune responses (Kawashima & Fujii, 2008). In this study, we aimed to enhance our understanding of metabolic trade-offs and functional ecology in hybrid trout, through the application of physiological and biochemical assays within a field setting.

Objectives

To test the hypothesis that a suite of physiological traits is associated with the genotypic status, we measured OCR, LDH, CS, and AchE in trout collected at various sites along the elevation gradient, and statistically analysed relationships between these traits, elevation, and fish genotype. Genotyping was based upon three bi-parentally inherited and co-dominant nuclear genetic markers, and a mitochondrial marker, all of which were independently re-validated against samples of WCT and RT obtained from Alberta Fish and Wildlife hatchery stock.

MATERIAL AND METHODS

Study area

This study was conducted in the Upper Oldman River basin of south-western Alberta, Canada, and included the Oldman and Crowsnest rivers upstream of the Oldman River reservoir (Fig. 2). RT (~1.5 million) were stocked into the study area from the 1920s to the 1970s (D. Wig, Alberta Fish and Wildlife, Blairmore, AB T0K 0E0, unpublished stocking records) representing the Shasta, Arlee, Montana, and Donaldson stocks (J. Underwood, Allison Creek Trout Hatchery, Box 394 Coleman, AB T0K 0 M0, personal communication). Ten streams (2nd to 5th order) were sampled, with sites ranging in elevation from 1100 to 1700 m measured with a handheld GPS. (Fig. 2).

Fish and tissue collection and genetic analysis

In July–August 2005 and 2006, 60 fish per site were collected by single-pass backpack electrofishing (LR-24 Smith-Root Inc., Vancouver, WA, Canada). Genetic analysis was done on fin clips stored in 95% ethanol for DNA extraction. Ten fish from each site were killed with MS-222 (0.1 g L^{-1}) and measured (fork length, mm), weighed (±0.1 g), and otoliths and a scale sample were removed. Fin clips were digested overnight (37 °C) by rocking gently in 150 mL lysis buffer (50 mM Tris-HCl, pH 8.0, 1.0% SDS, and 25 mM EDTA) with 70 mg proteinase K. DNA was extracted using a modified column-based technique (Elphinstone *et al*., 2003). Three diagnostic, bi-parentally inherited, co-dominant nuclear species markers were employed, including OCC16 (Ostberg & Rodriguez, 2002; Ostberg *et al*., 2004), OCC36 (Ostberg & Rodriguez, 2004), and IKAROS (Baker, Bentzen & Moran, 2002), together with ND3, a mitochondrial marker (Docker, Dale & Heath, 2003). Markers were validated by testing on samples of WCT and RT from the Alberta Fish & Wildlife hatchery stock. Polymerase chain reactions (PCRs) were performed using 25-mL reactions that contained 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 200 mM dNTPs, 0.05 mg of each primer, 0.5 units of DNA *Taq* polymerase, and approximately 100 ng of genomic DNA template. The optimized thermocycler profile consisted of a 'hot start' and 2 min of initial denaturation (94 °C), followed by 35–40 cycles of a 1-min denaturation cycle (94 °C), 1 min annealing

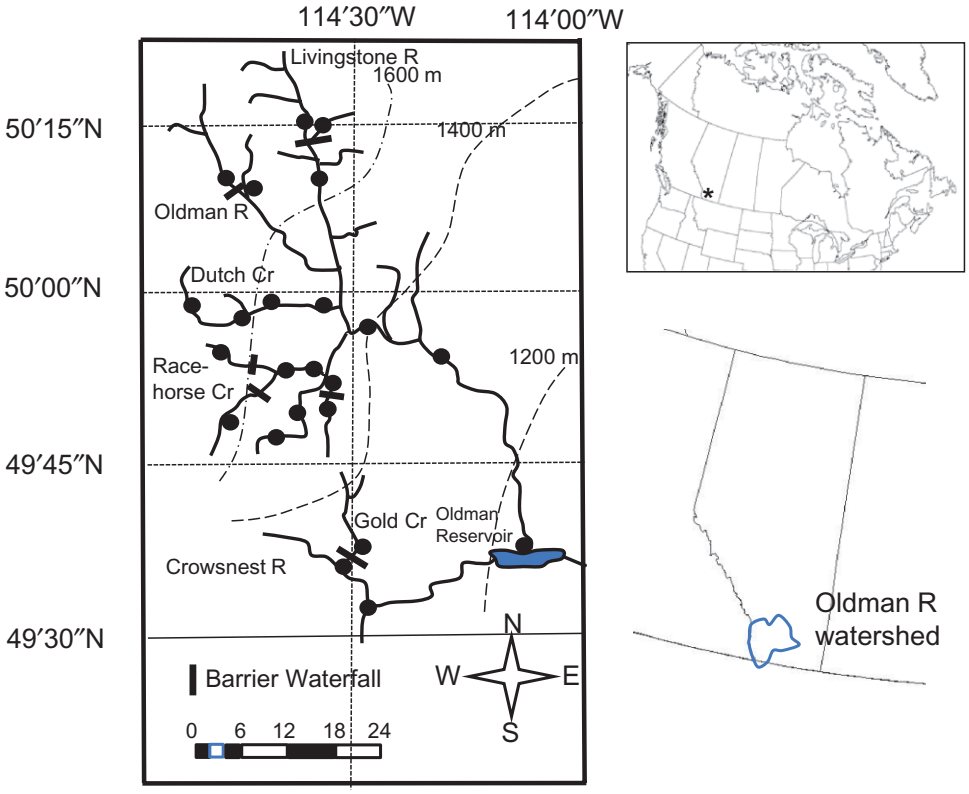

Figure 2. Sampling sites (closed circles) on the Oldman, Livingstone, and Crowsnest rivers within the Upper Oldman River system upstream of the Oldman River Reservoir. Insets: the location of the Oldman River basin in south-western Alberta, Canada. Dashed lines represent elevation contours, and solid bars represent waterfalls that were considered to be impassable for trout.

(variable annealing temperatures), 1.5 min extension (72 °C), and ending with a final 5-min extension cycle (72 °C). The IKAROS and ND3 species assays included post-PCR restriction enzyme treatment to generate species-specific restriction fragment length polymorphisms (RFLPs): ND3 is cut with *DdeI*, whereas IKAROS is cut with *HinfI* restriction enzymes following the manufacturer's instructions. PCR products, size polymorphisms, and RFLPs were separated by gel electrophoresis at 80–90 V through a 1.8% agarose gel. All fragments were visualized using ethidium bromide staining and UV transillumination; ambiguous gel images were repeated. For each fish, and for the population at each site, we calculated pRT, the frequency of RT alleles, which equals the number of RT markers, divided by seven (two times the number of nuclear markers plus one mitochondrial marker).

Oxygen consumption measurements

Measurements of OCR were carried out on 98 fish from 14 sites (5–10 fish each) throughout the study area (1234–1722 m) ranging from 2nd- to 5th-order streams, 5–40 m in width, during July–August 2005. Fish were caught by electrofishing, held instream in a covered screen-sided tub for 30–60 min prior to testing, then placed individually in a sealed Erlenmeyer flask of stream water with a Yellow Springs Instruments 85 oxygen electrode inserted, and allowed to acclimate for 30 min. Fish of 20–60 g were tested in a 1-litre flask, and larger fish in a 2-litre flask. The probe was calibrated to water temperature and elevation for each measurement, and the flask was incubated in flowing stream water during the test. OCR (mg O_2) was monitored for 30 min and was reported as an hourly rate. The test was terminated early for some individuals that consumed > 50% of the available oxygen before 30 min. After the test was completed, fish were fin clipped for genetic analysis, and placed in the instream container and monitored for 15 min prior to release.

Enzyme activities

A sample of fish (10–20 from each site) were killed for enzyme analysis (July–August 2005). White muscle was dissected from the caudal peduncle, immediately

frozen in liquid nitrogen, and transferred into a –80 °C freezer until assays were performed. Samples (~0.5 g) were pulverized in liquid nitrogen and homogenized for 10 s in a buffer solution [0.07 M, KPO_4, pH 7.0, 1 mM EDTA, 0.2% (v/v) Triton X-100] at a dilution of 50 volumes using a VWR Power Max drill with a Troemner 7 × 95 mm bit (Sherwood *et al.*, 2002b); 1 mL of supernatant (50×) was aliquoted and re-frozen at –80 °C for the CS assay. A second dilution (1000×) was performed for LDH and protein analysis and then refrozen. Both assays were run in duplicate at 23 °C using an Ultrospec 3100pro UV/visible spectrophotometer with a 1-cm path length. Samples with duplicates that differed by > 5% were rerun. Activities were expressed as International Units (IU, amount of enzyme needed to produce 1 μmol of product) standardized to 1 mg of protein. Protein was measured with the Bradford colorimetric assay (595 nm) standardized to bovine serum albumin. LDH activity was measured using a colorimetric assay, which follows the reduction of pyruvate to lactate using NADH. CS activity was measured on 84 of these tissue samples using a colorimetric assay which follows the production of citric acid from oxaloacetate and acetyl coenzyme A with the formation of 5-thio-2-nitrobenzoic acid (Pelletier, Guderley & Dutil, 1993). Blood samples from ten fish were taken from four sites along the elevational gradient [a Crowsnest R tributary 1234 m, two Racehorse Creek tributaries (1478 and 1539 m), and the Oldman River mainstem (1722 m)] in July 2006. Plasma AchE activity [(μmol min) ml^{-1}], liver glycogen, plasma glucose and cortisol, and gill Na^+/K^+ ATPase activity were measured in tissue and blood samples from these fish (Chuiko, 2000; Levesque *et al.*, 2002, 2003; Quinn, Rasmussen & Hontela, 2010).

Statistical analyses

Physiological variables, including OCR, LDH, CS, and AchE, were individually regressed against body weight, elevation, and pRT using standard multiple regression in JMP IN (SAS Institute Inc., Cary, NC, USA). ANCOVA analyses based on genotype, employed the categorical variable GEN [RT, WCT, and hybrid (HYB)] together with body weight as the co-variate. Although the physiological parameters tested are, of course, not statistically independent of each other, a multivariate response variable (i.e. MANOVA) could not be tested as the measures were generally carried out on different fish. Our analyses were therefore 'side by side' allometric comparisons treating traits as functions of body size as a covariate. This approach was a convenient framework for comparison among genotypic categories; however, as it did not provide multivariate residuals, it did not allow us to examine correlations between trait expressions at the individual level.

The frequencies of occurrence of each of the four markers and their pairwise and three-way combinations were compared using a χ^2 contingency test to test for any significant departures from Hardy–Weinberg proportions. Analyses based on three nuclear and one mitochondrial marker, and a sample of 60 fish per site, allows pRT values as low as 1% to be detected with 95% probability, and will thus provide a robust picture of the geographical gradient in pRT (Rasmussen *et al.*, 2010). Scores for individual fish will, however, be fairly crude, and more markers would be expected to detect more hybrids. For example, for a site with a pRT of 0.05, with three nuclear markers 16 hybrids will be expected from a sample of 60 fish at Hardy–Weinberg equilibrium, whereas if four nuclear markers were used 20 hybrids would be expected from the same sample. Thus if more markers had been employed more hybrids would be detected along the entire gradient, but neither the geographical pattern of hybrid distribution nor the frequency distribution of genotypes would be expected to change significantly. Fish with no detectable RT markers will be termed WCT, although they may not be actually 'pure', and fish with no detectable WCT markers will be termed RT.

RESULTS

A total of 1235 fish from 23 sampling sites ranging from 1100–1700 m elevation were genetically analysed; 177 fish from 14 sites (1230–1700 m) were scored as hybrids, with the hybrid zone covering ~300 km of the Upper Oldman River system (Fig. 2). Hybrids were the dominant genotype at three mainstem sites (1250–1400 m, 4th order, 20–40 m), whereas at lower elevations most fish were RT, and at higher elevations most were WCT (Fig. 3). Although hybrids were numerically dominant at these sites, genotypes did not deviate significantly from Hardy–Weinberg proportions anywhere along the elevation gradient (Fisher's exact test, and χ^2 contingency tests, $P < 0.05$). The upper reaches where WCT dominated were mostly lower order (≤ 3rd order, < 10 m wide) tributaries, and the downstream reaches dominated by RT were mainstem sites on Oldman and Crowsnest Rivers (4th order, 15–30 m wide). Only three of the 177 hybrids were heterozygous for all three nuclear markers, and thus possible F_1 hybrids, and most appeared to be backcrosses to WCT and RT. The overall RT scores for all markers were not significantly different, and among the hybrids, the frequency of occurrence of RT alleles on the ND3 (mitochondrial) marker was not significantly different from the three nuclear markers, indicating symmetric hybridization. Addi-

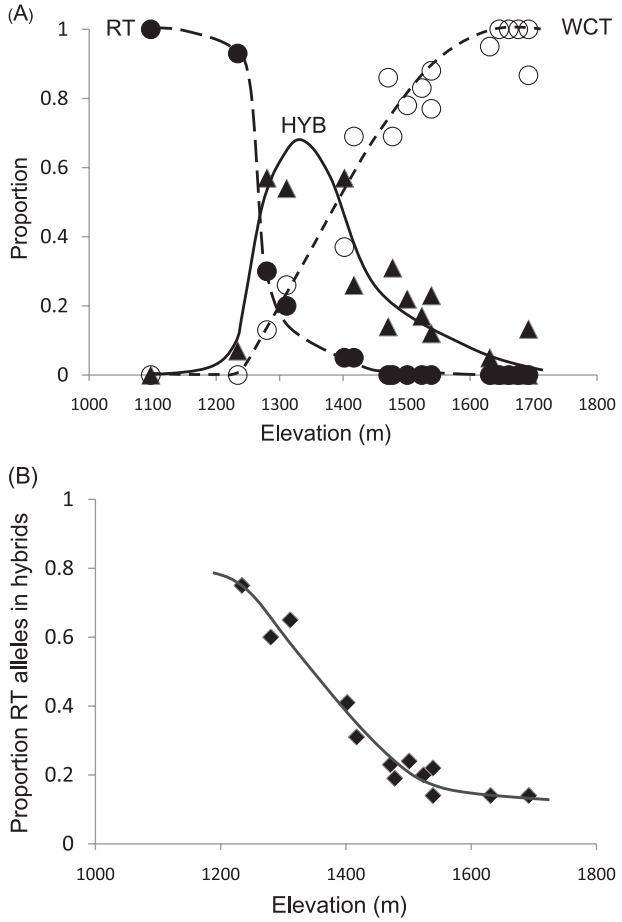

Figure 3. A, relative contribution of hybrids (HYB), rainbow trout (RT), and westlope cutthroat trout (WCT) and B, the genetic makeup of hybrids in relation to elevation, along the elevation gradient of the Upper Oldman River system. Estimates obtained for stations upstream of impassable barriers were not included on these graphs.

tional details pertaining to the genetic analyses and results from these sampling sites were reported by Rasmussen *et al*. (2010).

Oxygen consumption

OCR ranged from 4.7 to 19.4 mg O_2 h^{-1}, increased with body weight (23.4–132 g), and decreased with the elevation (1230–1722 m) (Table 1, eqn 1); differences among genotypes revealed by ANCOVA on log-log regressions were statistically significant with RT > WCT, and hybrids intermediate (Fig. 4; eqn 3). Relationships between OCR and elevation were much stronger among than within genotypic categories. Equation 2 shows the relationship obtained for WCT, which was the genotype with the largest sample size and broadest elevation range (1300–1722 m). As the genetic identity of the fish tested was unknown at the time OCR measurements were taken, and WCT were much more abundant overall than the other two genotypic categories, fewer hybrids and RT than WCT were tested.

Although the categorical genotype effect was highly significant (eqn 3), a multiple regression using pRT in place of genotypes was also highly significant (eqn 4). Elevation effects, when tested together with genotype effects, were not statistically significant. Thus genetic information on the fish for which OCR was measured explains most of the variability in OCR that had been explained by elevation. The temperature at which OCR measurements were made ranged from 9.5 to 11 °C, and made no significant contributions to models predicting OCR.

Metabolic enzymes

LDH activity ranged from 0.76 to 4.11 IU (mg protein)$^{-1}$, increased with body weight (20–1335 g), and decreased with elevation (1230–1722 m) (Table 1 eqns 5–8); differences among genotypes obtained using ANCOVA in a log-log regression were statistically significant with RT, hybrids > WCT (Fig. 5, eqn 7). Just as with OCR, the effect of elevation was much weaker within the genotypic categories than across the whole data set. For WCT, which had the largest sample size and the broadest elevation range (1300–1722 m), the elevation effect was weak and not significant (eqn 6). Although the categorical genotype effect was highly significant (eqn 7), the multiple regression using pRT in place of genotypes was also significant (eqn 8). Although LDH was not nearly as well explained by the predictors as OCR, effects of the elevation gradient were again displaced by the genetic variables, and were not statistically significant when either of these was included in the model.

CS, which ranged from 0.011 to 0.067 IU (mg protein)$^{-1}$, exhibited the opposite pattern to that seen for LDH. It decreased, though only weakly, with body weight (20–1335 g) but increased strongly with elevation (1230–1722 m) (eqn 9); differences among genotypes were statistically significant with RT > hybrids and WCT (Fig. 6; eqn 15). Whereas the effects of elevation on OCR and LDH were weaker within genetic categories, the effect of elevation on CS was even stronger within the WCT than across the whole data set; eqn 10 shows the regression obtained for WCT, which had the largest sample size spanning the broadest elevation range (1300–1722 m). The body size term, which had been weakly significant in the general model, was not significant in any within-genotype models. Significant genotype effects were found both in the ANCOVA model (eqn 11) and in the

Table 1. Multiple regression models predicting oxygen consumption rates (OCRs), lactate dehydrogenase (LDH), citrate synthase (CS), and plasma acetylcholinesterase (AchE) activity from body weight together with elevation (ELEV) and genotype

Dependent variable	Intercept (±SE)	+a_1 Log_{10}Wt (g)	+a_2 ELEV(km)	+a3 GEN or pRT	F	R^2	RMS	N	Eqn
Log_{10}OCR All genotypes	0.14 (±0.13)	+0.69 (±0.06) $P < 0.0001$	−0.24 (±0.06) $P < 0.0001$		$F_{2,92} = 76$	0.60	0.09	95	1
Log_{10}OCR WCT	0.08 (±0.20)	+0.71 (±0.07) $P < 0.0001$	−0.13 (±0.08) $P = 0.15$		$F_{2,63} = 58$	0.65	0.09	66	2
Log_{10}OCR All genotypes	−0.24 (±0.10)	+0.72 (±0.06) $P < 0.0001$	n.s.	0.06 RT 0.00 HYB −0.06 WCT $P < 0.0001$	$F_{3,91} = 52$	0.63	0.09	95	3
Log_{10}OCR All genotypes	−0.27 (±0.10)	0.70 (±0.06) $P < 0.0001$	n.s.	0.11(±0.02) *pRT, $P < 0.0001$	$F_{2,92} = 76$	0.63	0.09	95	4
Log_{10}LDH All genotypes	0.18 (±0.09)	+0.16 (±0.02) $P < 0.0001$	−0.14 (±0.05) $P = 0.007$		$F_{2,206} = 37$	0.26	0.11	209	5
Log_{10}LDH WCT	0.06 (±0.13)	+0.16 (±0.025) $P < 0.0001$	−0.08 (±0.07) $P = 0.21$		$F_{2,150} = 26$	0.25	0.11	153	6
Log_{10}LDH All genotypes	−0.02 (±0.16)	+0.16 (±0.02) $P < 0.0001$	n.s.	0.014 RT 0.016 HYB −0.030 WCT $P = 0.03$	$F_{3,205} = 24$	0.63	0.11	209	7
Log_{10}LDH All genotypes	−0.06 (±0.04)	+0.17 (±0.04) $P < 0.0001$	n.s.	0.06 (±0.026) pRT, $P = 0.0275$	$F_{2,206} = 35$	0.26	0.11	209	8
Log_{10}CS All genotypes	−2.22 (±0.19)	−0.09 (±0.04) $P = 0.03$	+0.58 (±0.04) $P < 0.0001$		$F_{2,81} = 13$	0.24	0.15	84	9
Log_{10}CS WCT	−3.06 (±0.30)	−0.02 (±0.07) $P = 0.83$	+0.98 (±0.20) $P < 0.0001$		$F_{2,32} = 13$	0.44	0.13	35	10
Log_{10}CS All genotypes	−2.65 (±0.22)	n.s.	+0.76 (±0.16) $P < 0.0001$	0.100 RT −0.057 HYB −0.047 WCT $P < 0.005$	$F_{3,80} = 11.2$	0.30	0.14	84	11
Log_{10}CS All genotypes	−2.91 (±0.25)	n.s.	+0.90 (±0.17) $P < 0.0001$	0.16 (±0.05) pRT, $P = 0.005$	$F_{2,81} = 15$	0.30	0.14	84	12
Log_{10}AchE All genotypes	2.84 (±0.17)	−0.19 (±0.04) $P = 0.0002$	−0.60 (±0.09) $P < 0.0001$		$F_{2,38} = 26$	0.58	0.11	41	13
Log_{10} AchE WCT	2.55 (±0.34)	+0.14 (±0.05) $P = 0.01$	−0.047 (±0.19) $P < 0.21$		$F_{2,22} = 5.6$	0.34	0.10	25	14
Log_{10} AchE All genotypes	2.80 (±0.30)	−0.19 (±0.05) $P = 0.0002$	n.s.	0.093 RT 0.037 HYB −0.130 WCT $P < 0.0001$	$F_{3,37} = 14$	0.53	0.12	41	15
Log_{10} AchE All genotypes	1.83 (±0.09)	−0.16 (±0.05) $P = 0.0018$	n.s.	+0.23 (±0.04) pRT, < 0.0001	$F_{2,38} = 21$	0.52	0.12	41	16

Empty cells represent variables not included in the models shown, and n.s. indicates that the variable would not have been statistically significant if added to the model.

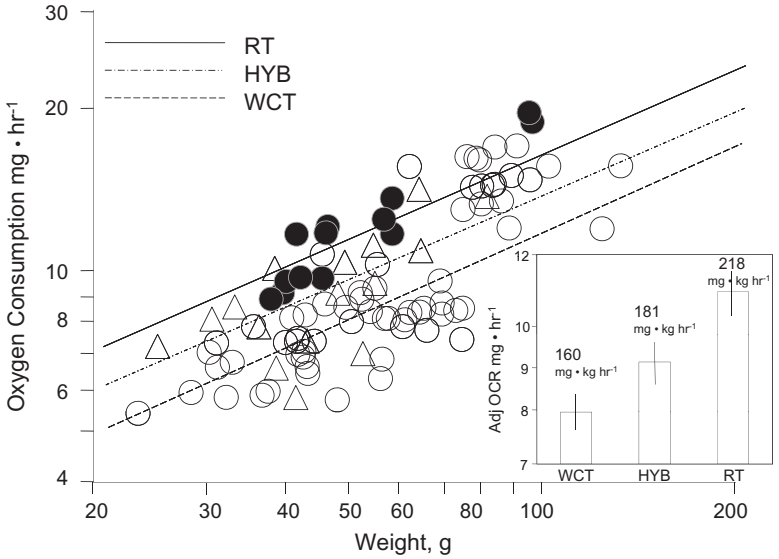

Figure 4. Oxygen consumption rate (OCR) against fish weight. Log-log regression lines (solid circles, RT; dot-dash triangles, hybrids; dash open circles, WCT) determined by ANCOVA (eqn 6). Inset: OCR comparison adjusted for 50-g fish, obtained from the *y*-intercepts (±SE) of eqn 3 with Log(Wt/50) as the covariate. WCT < HYB, RT, and RT, HYB > WCT ($P < 0.05$, Tukey test).

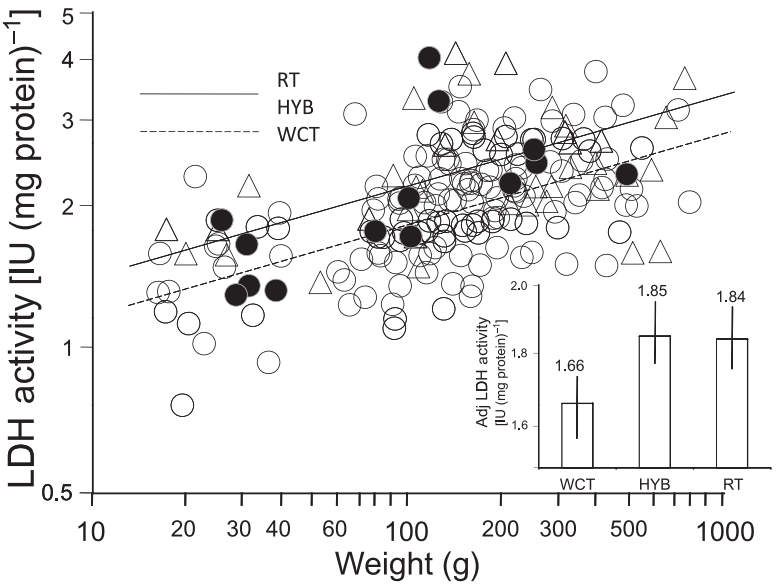

Figure 5. LDH activity against fish weight. Log-log regression lines (solid circles, RT and triangles, hybrids together; dash open circles, WCT) determined by ANCOVA (eqn 7). Inset: LDH comparison adjusted for 50-g fish, obtained from the *y*-intercepts (±SE) of eqn 11 with Log(Wt/50) as the covariate. WCT < HYB, RT ($P < 0.05$, Tukey test).

corresponding multiple regression model using pRT (eqn 12). Body weight was not statistically significant in either of these models. Whereas for OCR and LDH, the elevation explained little variation in models that contained genetic variables, for CS, elevation effects were slightly stronger in models containing genetic variables.

AchE activity in blood plasma ranged from 21.8 to 104.2 IU min^{-1} ml^{-1} and, like weight-specific metabolic rate, it decreased with body size (15–324 g) and elevation (1230–1722 m) (eqn 13); differences among genotypes were statistically significant with RT > WCT, and hybrids intermediate (Fig. 7; eqn 15). Within genotypic categories, the effect of elevation was much

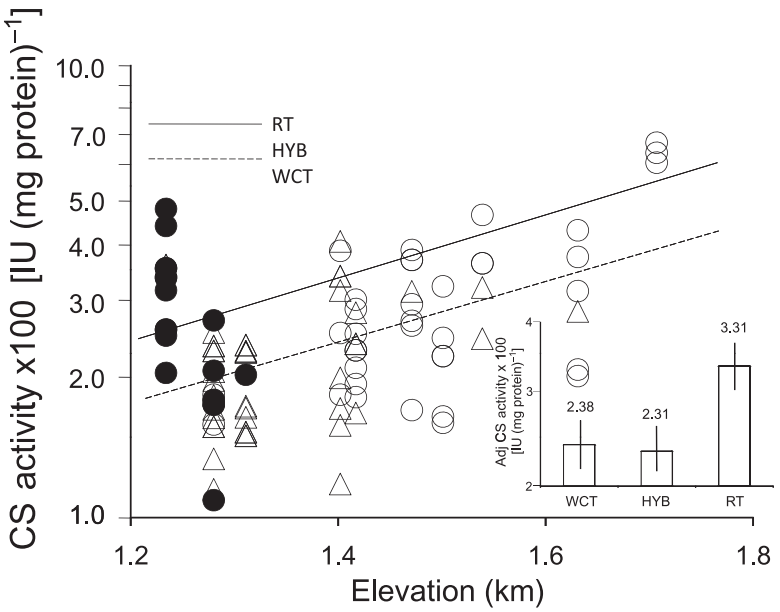

Figure 6. CS activity against elevation. Regression lines (solid circles, RT; dash open circles, WCT and triangles, HYB together) determined by ANCOVA (eqn 11). Inset: CS comparison adjusted for 1.4-km elevation, obtained from the *y*-intercepts (±SE) with ELEV-1.4 as the covariate. WCT, HYB < RT ($P < 0.05$, Tukey test).

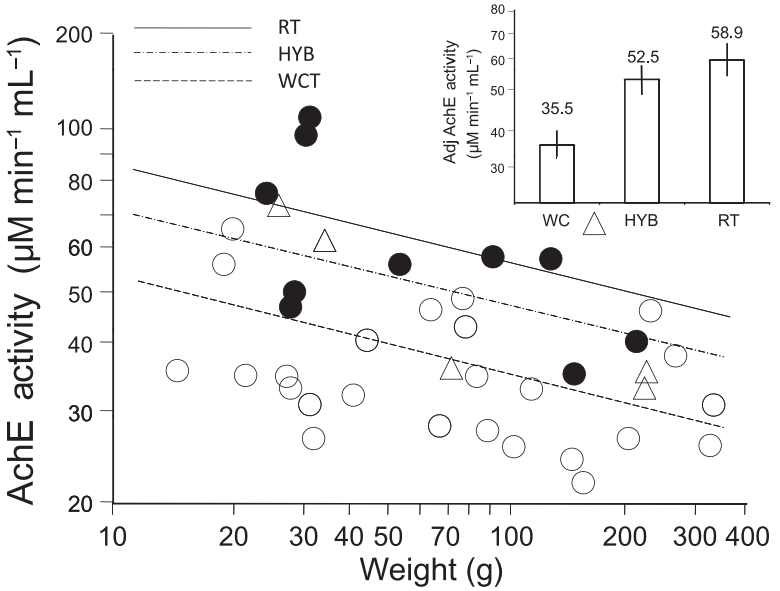

Figure 7. AchE activity in plasma against body weight. Log-log regression lines (solid circles, RT; dot-dash triangles, hybrids; dash open circles, WCT) determined by ANCOVA (eqn 15). Inset: AchE comparisons adjusted for body weight of 50 g, obtained from the *y*-intercepts (±SE) with log (Wt/50) as the covariate. WCT < HYB, RT, and RT > HYB, WCT ($P < 0.05$, Tukey test).

weaker, but still statistically significant. For WCT, which had the largest sample size spanning the broadest elevation range (1300–1722 m), the elevation effect was much weaker than that obtained for the whole data set (eqn 14). Significant genotype effects were found both in the ANCOVA model (eqn 15), and in the corresponding multiple regression model using pRT (eqn 16). No differences among genotypes were found for blood glucose or cortisol, liver glycogen, and gill Na/K ATPase activity, although both blood glucose and Na/K ATPase increased with body size.

Patterns of change along the elevation gradient among and within genotypic groups

Strong elevation gradients were detected in all four traits, and for three of these, OCR, LDH and AchE, these gradients were negative (decreased with elevation) and much weaker within the WCT population than they were across the genotypic gradient. Thus while all of these traits were enhanced at lower elevations, their expression was greater in fish that possessed RT alleles than in WCT, reinforcing the overall effect of elevation.

The comparison of elevation effects for metabolic traits within the WCT population and across genotypes can be demonstrated using absolute *t*-values for the elevation term in the multiple regressions (Table 2). For the whole population, i.e. with all genotypes represented, the elevation term had *t*-values of 4.28, 2.71, and 6.10 for OCR, LDH, and AchE respectively, whereas the corresponding *t*-values obtained for the WCT population were lower (1.25, 1.24, 2.48); thus, each of these *t*-values was lower (by 71, 54, and 59%) within the WCT population models than in the general population. The relationships to elevation for these traits were comparably weak within the RT population and the hybrids as well, although the numbers of fish sampled as well as their elevation range were much smaller than the WCT, making direct statistical comparisons of *t*-values unreliable.

The pattern for CS was notably different from that described for the previous three traits. Not only did CS increase with elevation, where OCR, LDH, and AchE had all decreased, but the strength of the relationship was even slightly greater within the WCT population ($t = 4.82$) than for the whole genotypic gradient ($t = 4.67$), despite smaller sample size. Thus the slight effect that RT alleles had on CS activity worked against the effect of the elevation gradient as pRT decreased with elevation.

Table 2. Strength of elevation effects on metabolic traits across genotypes vs. within WCT as measured by *t*-values from regression models

	Across genotype $\lvert t \rvert$-values		Within WCT $\lvert t \rvert$-values	
Trait	Elevation coefficient	Eqn	Elevation coefficient	Eqn
OCR	4.28	1	1.25	2
LDH	2.71	5	1.24	6
CS	4.67	9	4.82	10
AchE	6.10	13	1.48	14

OCR, oxygen consumption rate; LDH, lactate dehydrogenase; CS, citrate synthase; AchE, plasma acetylcholinesterase.

DISCUSSION

The genotypic gradient in RT alleles with elevation along the hybrid zone gradient of the Upper Oldman River was mirrored by a gradient in metabolic traits; however, the strong inter-relationship between the distribution of genotypes and elevation limits our ability to conclusively dissociate their effects on metabolic traits in a field study. The absence of statistically significant elevation effects on OCR, LDH, and AchE, within the WCT genotype, however, strongly suggests that for these three traits, the effects that we observed primarily reflect genetic ancestry, and that there exists a very strong relationship between RT alleles and metabolic traits.

While RT introductions have occurred across a broad elevation range, most of the feral populations that became established are at mid- to low elevations (Paul & Post, 2001). The hybrid zone forms in the mid-elevation range and expands upstream due to the success and proliferation of hybrid genotypes, tending to separate the CT and RT populations along the elevational gradient (Fig. 1B). Hybrid zones similar to that at Oldman River have also been demonstrated in rivers west of the continental divide (Hitt *et al*., 2003; Weigel *et al*., 2003; Rubidge & Taylor, 2005; Boyer *et al*., 2008). The breakdown of species boundaries to form a hybrid swarm in the mid-elevation range, where hybrids are numerically dominant but consist mostly of back-crosses with few F_1 individuals, and have traits well suited to intermediate habitats, are all attributes that fit the model of an ecotonal hybrid zone (Fig. 1B).

While this study concerns a hybridization event involving introduced RT, species breakdowns to form ecotonal hybrid swarms do occur in many natural populations, and the process is probably of considerable evolutionary importance. Ecotonal hybrid zones are well known in plants, and have been well described for temperate zone tree species, such as spruces, poplars, and willows (Sutton *et al*., 1994; Khasa & Dancik, 1996; Bennuah, Wang & Aitken, 2004). These hybrids often dominate at least a portion of the elevation or latitudinal gradients in the contact zone between the parental species. A similar example, involving animals, occurs among the swallowtail butterflies, *Papilio* (Sperling, 1989). Hybrids (*P. machaon*, a boreal and interior species × *P. zelicaon*, a coastal and mountain species) are the most abundant forms along the elevation gradient of the Alberta foothills. Although it has not been demonstrated that hybrids have superior fitness in the ecotone, their phenology may be better

adapted to the growing season in the mid-elevation range between 1000 and 2000 m than either of the parent species (Sperling, 1987). Ecotonal hybrid zones in the marine environment are also known, and often involve introduced species; the seagrass *Spartina alterniflora* native to the Atlantic coast has invaded the Pacific coast of North America, and the hybrids formed with native *Spartina* spp. are highly fertile and can dominate extensive tracts of coastline (Anttila *et al*., 2000; Ayres *et al*., 2004). In each of these cases cited, isolating mechanisms appear to have broken down, allowing hybrids and backcrosses to proliferate within the ecotonal environment; however, neither the extent to which hybrid traits contribute to hybrid success nor the extent to which the ecotonal habitat gradient contributes to the breakdown of species boundaries is clear.

Although hybrids were numerically dominant in the Oldman River mainstem between 1250 and 1400 m, their genotypic frequencies did not depart from Hardy–Weinberg expectations; nor did hybrid frequencies increase with age (Rasmussen *et al*., 2010). Thus while the predominantly intermediate character of hybrid metabolic traits are probably well suited to conditions in the upper mainstem mid-elevation reaches of the river, there is no evidence that they are at an advantage over parental genotypes. Muhlfeld *et al*. (2009) argued that RT × WCT hybrids were less fit than WCT on the grounds that they produced fewer offspring with increasing RT admixture; however, it must be noted that RT produce fewer eggs than CT of similar size (Schill *et al*., 2010). RT reared in the Allison Creek Brood station in Alberta (age 3) produce ~40% fewer eggs relative to body weight than WCT, although their eggs have ~40% greater volume (L. Ripley, Alberta Fish & Wildlife, pers. comm.). Such an egg size vs. number trade-off has also been shown for steelhead trout, coastal cutthroat trout, and their hybrids (Hawkins & Foote, 1998), and trade-offs of this kind are common in many teleost groups (Elgar, 1990). Thus offspring from females with greater RT admixture, although less numerous, may be more competitive. While laboratory competition studies have never been conducted on WCT and hybrids, Seiler & Keeley (2007, 2009) have shown that Yellowstone CT (YCT; *O. c. bouveri*) × RT hybrids were demonstrably superior to YCT in feeding and swimming ability.

Relationship between OCR, metabolic enzymes and fish genotype

Our results generally supported our hypothesis that metabolism of RT exceeded that of WCT, and that the genetic gradient with elevation would be mirrored by a gradient in metabolic traits. Thus we observed an overall positive relationship between metabolic traits (OCR, LDH, CS and AchE) and RT alleles, with hybrids, which dominate at mid-elevations, exhibiting intermediate expression for OCR, and for AchE.

The OCR estimates obtained using our field method correspond reasonably well to previously published measurements on routinely active fish, and produced allometric relationships very similar to previously published values for salmonids (Table 3). Such OCRs are well below estimates of active metabolism, where fish are forced to swim at speeds near their critical limits, but at the same time, more than double estimates of resting metabolism, which are obtained as the zero activity intercept of the relationship between OCR and activity (Fry, 1957; Dwyer & Kramer, 1975). Published measurements are available for several strains of hatchery RT, including the Shasta and Arlee strains, which are the main sources of the RT introduced to the Upper Oldman River (Kindschi, Smith & Koby, 1991; Miller, Wagner & Bosakowski, 1995; Myrick & Cech, 2000). These values are quite similar to the OCRs that we obtained for RT and hybrids in this study, despite the methodological differences. No previously published OCR estimates were found for WCT, and in fact, data were available only for YCT from the Snake River, Idaho (Kindschi & Koby, 1994). These data tend to support the pattern observed in this study, that RT have higher metabolic rates than CT. In a somewhat related finding, Hawkins & Quinn (1996) showed that steelhead (*O. mykiss*) were superior swimmers (U_{crit}) to coastal cutthroat trout, and that hybrids were intermediate. Although the authors relate this to morphological differences, OCR and U_{crit} are strongly related (Brett, 1995; Claireaux *et al*., 2005).

The increase in LDH activity with body size shown here is consistent with the expected allometric pattern (Childress & Somero, 1990), and after adjusting for the expected relationship to body size, LDH activity levels of both RT and hybrids were, as expected, significantly higher than those of WCT. This is consistent with the observed level of activity and aggressiveness of RT (Hawkins & Quinn, 1996; Seiler & Keeley, 2007, 2009) and indicates that hybrids are likely to have similar levels of anaerobic capacity. No significant difference was found between the LDH levels of hybrids and RT. While as expected the CS activity was highest in RT, hybrids and WCT were not different, and in both of these, CS increased strongly with elevation. Aerobic pathways are responsible for supporting most of a fish's sustained swimming capacity and much of its capacity to recover from bursts (Webb, 1971; Hudson, 1973). Thus, it is possible that sustained activity may pose a disproportionate challenge under the short growing season and long winter regime characteristic of high-elevation

Table 3. Published estimates of weight-specific oxygen consumption for RT and CT; corrections for body size were made using the following eqn 1

Species, strain	Technique	$OCR_{size,\ T°C}$ (mg kg^{-1})	$OCR_{50g,\ 9°C}$ (mg kg^{-1})	Reference
RT	Routine resting metabolism	$232_{50g,\ 10.5°C}$	221	This study
RT × WCT		$192_{50g,\ 10°C}$	187	
WCT		$168_{50g,\ 10°C}$	164	
YCT, Bar BC	Flow through	$167_{40g,\ 9°C}$	163	Kindschi and Koby (1994)
RT, Eagle Lake wild trout	Flow through	$213_{27g,\ 9°C}$	193	Kindschi *et al*. (1991)
RT, Arlee	Flow through	$233_{36g,\ 9°C}$	222	Kindschi *et al*. (1991)
RT, Shasta	Flow through	$216_{30g,\ 10°C}$	181	Myrick and Cech (2000)
RT, Sand Creek	Flow through	$245_{62g,\ 10°C}$	215	Miller *et al*. (1995)
Cutthroat trout, Utah strain	Flow through, active	$490_{90g,\ 10°C}$	498	Dwyer and Kramer (1975)
	Flow through, standard	$74_{90g,\ 10°C}$	108	
RT, unknown hatchery strain	Flow through, standard	$58.5_{100g,\ 5°C}$ $116_{100g,\ 15°C}$	102	Rao (1968, 1971)
RT, unknown hatchery strain	Flow through, active	$360_{100g,\ 5°C}$ $586_{100g,\ 15°C}$	472	Rao (1968, 1971)
RT, unknown hatchery strain	Flow through, standard	$110_{264g,\ 15°C}$	105	Rao (1968, 1971)
RT, unknown hatchery strain	Flow through, active	$658_{264g,\ 15°C}$	485	Webb (1971)

Temperature corrections were based on Dwyer and Kramer (1975): OCR = 19.6 + 5.7TC, 5–20 r^2 = 98. Eqn 1, 5.6/°C, 5–20.

streams, requiring aerobic pathways to be up-regulated for moderate levels of activity to be sustainable. Such a compensatory shift might help to explain why metabolism for WCT varies so little over the broad range of elevation that these fish occupy. The association of RT alleles with plasma AchE activity supports the overall link between RT alleles and high performance.

The ecological significance of metabolic trade-offs along the elevational gradient

The elevational separation of RT and WCT together with their metabolic differences suggest that RT and WCT trade off energetic scope against growth efficiency, allowing RT to competitively displace WCT from the lower portion of its elevation range where productivity, temperature and growing season can support the higher metabolic requirements of RT. Direct comparisons of swimming ability between RT and WCT have not been carried out, although RT (steelhead) are known to have higher sustained swimming ability than coastal CT (Hawkins & Quinn, 1996) and hatchery RT have superior swimming ability, higher foraging rates and faster growth than YCT (Seiler & Keeley, 2007, 2009). Metabolism has been shown to be positively correlated with aggression in several salmonid species (Lahtai *et al*., 2001, 2002; McCarthy, 2001; Metcalfe, Taylor & Thorpe, 2004), and Finstad *et al*. (2011) argue that differences in aggression and trade-offs against growth efficiency can explain salmonid species replacements along climatic gradients in streams. Aggressive behaviour requires frequent bursts of high power, and should thus depend on both anaerobic and aerobic capacity. The fact that both OCR and LDH are higher in RT and hybrids is therefore consistent with the idea that such fish are more aggressive competitors than WCT, and that their food requirements are probably higher as well (Morinville & Rasmussen, 2003).

RT are most dominant in the Crowsnest River, a low-elevation tributary of the Oldman River, where anthropogenic enrichment mainly from domestic sewage leads to high primary productivity (Rasmussen *et al*., 2010). WCT, on the other hand, dominate in the cold and unproductive headwater reaches, which are conducive to a low-output lifestyle, and where the high food requirements of RT cannot be met. Several studies have shown that trade-offs between metabolic rate and growth efficiency can contribute to habitat partitioning in stream salmonids (Morinville & Rasmussen, 2006; Van Leeuwen, Rosenfeld & Richards, 2011). The suggestion that RT and WCT partition the elevation gradient via metabolic trade-offs is not meant to imply a direct causal relationship to elevation per se, but rather the indirect effects of a suite of inter-related factors such as growing season length, the partial pressure of oxygen, stream size (order, depth, width), hydraulic habitat features (discharge, velocity), and productivity. All of these can potentially exert effects on the productivity of food resources and the energy budget of fish.

Although no competition studies have been carried out on WCT × RT hybrids, YCT × RT hybrids have been shown to outcompete YCT (Seiler & Keeley, 2007, 2009) by virtue of superior swimming speeds and feeding rates. As hybrid growth rate (Rasmussen *et al.*, 2010) and metabolism (this study) have been shown to be intermediate, hybrids should be less dependent on high productivity, allowing them to be successful further upstream than RT, as this and previous studies have shown. Thus the upstream propagation of RT alleles by hybrids may be facilitated by an efficient trade-off between metabolic efficiency and competitive ability. However, the fact that WCT populations persist in the high-elevation tributary streams (even when no physical barriers are present) probably reflects the metabolic limitations of hybrids in these streams.

Implications for conservation and management

Introgression poses special problems for managers, especially when hybrids of threatened species are involved. The US Fish and Wildlife Service applies a phenotypic criterion to the definition of WCT, which corresponds to an introgression threshold of ≤ ~20% (US Fish and Wildlife Service, 2003; Campton & Keading, 2005). Only the relatively small fraction of populations introgressed beyond this threshold are considered a threat to the integrity of pure WCT populations, and as such, the US Fish and Wildlife Service (2003) asserted that the threat level to the WCT was not sufficient to consider them threatened under the Endangered Species Act. Applying this phenotypic criterion (20%) to the Oldman River would include all portions of the drainage ≥ 1400 m, and thus the better part of the river system upstream of the Oldman Dam (Fig. 2), limiting the introgressed portion to the mainstem, plus its highly impacted Crowsnest River tributary.

On the other hand, Allendorf *et al.* (2004, 2005) suggest that a species should be defined by its genome, and thus only genetically pure populations (< 1% introgressed) should be considered WCT. Although no empirical evidence was provided, they considered all populations > 1% introgressed to pose a threat to the integrity of the WCT genome. Populations with this level of purity exist, for the most part in small, isolated fragments, and in the Oldman River this criterion would include only the small headwater streams above 1700 m (Rasmussen *et al.*, 2010), and classify the majority of the system as introgressed. Such small isolated fragments have poor prospects for long-term viability (Shepard *et al.*, 1997; Hilderbrand & Kershner, 2000). Thus, depending on which of these two extreme threshold values are chosen, the 'problem' is either highly intractable or insignificant. By comparison, an intermediate 5% criterion sets the limit at 1500 m, and includes large sections of Racehorse and Dutch Creeks and tributaries, all of the Livingstone River, and much of the Upper Oldman mainstem as WCT waters, and yet considers almost half of the Upper Oldman system as introgressed. Thus a 5% threshold leaves a large and manageable upstream unit as 'in need of protection' from a similarly large downstream introgressed population, and thus would lead to a very different picture from the both the US Fish and Wildlife Service conclusion, that introgression was not a threat, or from the 1% criterion which regards the WCT population as highly fragmented and on the verge of extinction.

The physiological traits examined here, and the life-history traits of Rasmussen *et al.* (2010), are also phenotypic traits, although arguably more relevant to salmonid ecology than the taxonomic characters emphasized by the US Fish and Wildlife Service. Our study, while demonstrating that metabolic traits are very relevant to the problem, does not have sufficient precision to pinpoint the introgression threshold at which hybrids are demonstrably different from parental genotypes. This would probably require a carefully controlled laboratory study wherein WCT and hatchery RT are crossed, and hybrids progressively backcrossed to WCT over several generations. If accurate introgression thresholds could be determined for functional traits of this kind, it is possible that an ecologically meaningful approach to managing introgressed populations could be developed.

Another highly controversial management debate centres on how to best protect upstream WCT populations from downstream introgressed populations. Land-use practices that minimize clear-cutting in the headwaters and protect riparian vegetation can help protect spawning and rearing habitat in small streams, and prevent both warming and nutrient enrichment (Likens & Bormann, 1974), which are factors that appear to facilitate the upstream movement of fish with RT traits, although climate change effects may ultimately undermine such efforts (Keleher & Rahel, 1996). Management practices aimed at ameliorating the risk that introgressed populations pose to 'pure' CT populations are highly controversial, and include artificial barriers, selective recreational fishing limits, and even aggressive measures aimed at direct elimination of introgressed populations. While it is beyond our scope to assess the feasibility and desirability of such management options, it is important to realize that in many systems ecological competition from other introduced salmonids such as brook trout *Salvelinus fontinalis* and brown trout *Salmo trutta* pose another major threat to WCT populations (Peterson & Fausch, 2003; Peterson, Fausch & White, 2004). Introgressed

populations with their RT alleles and associated competitive traits would be expected to be more resistant to such invaders. Thus management efforts to reduce or remove introgressed fish, whether by means of selective limits, habitat manipulations, changes in land-use practices, nutrient reduction, or more aggressive interventions (e.g. piscicides), to the extent that they are successful at all, may exacerbate the risk of upstream invasions by exotic species (Peterson *et al*., 2008). In the Oldman River, introduced brook trout *Salvelinus fontinalis* and brown trout *Salmo trutta* have had little success in invading the headwaters, despite having been introduced at many locations over many years, and this may be partially linked to the widespread occurrence of RT and WCT × RT hybrids in the river mainstem.

ACKNOWLEDGEMENTS

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant to J.B.R and to A.H., by the Alberta Conservation Association (ACA) and an NSERC, IPS (II) scholarship through a partnership with Trout Unlimited, Canada, to M. D. R. Ryan MacDonald, Corinna Robinson, Caitlin Friesen, and Jennifer Van der Lee provided excellent assistance in the field.

REFERENCES

Allendorf FW, Leary RF, Hitt NP, Knudsen KL, Boyer MC, Spruell P. 2005. Cutthroat trout hybridization and the U.S. Endangered Species Act: one species, two policies. *Conservation Biology* **19:** 1326–1328.

Allendorf FW, Leary RF, Hitt NP, Knudsen KL, Lundquist LL, Spruell P. 2004. Intercrosses and the U.S. Endangered Species Act: should hybridized populations be included as westslope cutthroat trout? *Conservation Biology* **18:** 1203–1213.

Anttila CK, King RA, Ferris C, Ayres DR, Strong DR. 2000. Reciprocal hybrid formation of *Spartina* in San Francisco Bay. *Molecular Ecology* **9:** 765–770.

Arnold ML. 1997. *Natural hybridization and evolution*. Oxford: University Press UK.

Ayres DR, Smith DL, Zaremba K, Klorh S, Strong DR. 2004. Spread of exotic cordgrasses and hybrids (*Spartina* sp.) in the tidal marshes of San Francisco Bay, California, USA. *Biological Invasions* **6:** 221–231.

Baker J, Bentzen P, Moran P. 2002. Molecular markers distinguish coastal cutthroat trout from coastal rainbow trout/steelhead and their hybrids. *Transactions of the American Fisheries Society* **131:** 404–417.

Barton NH, Hewitt GM. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* **16:** 113–148.

Baslow MH, Nigrelli RF. 1961. Muscle acetylcholinesterase levels as an index of general activity in fishes. *Copeia* **1961:** 8–11.

Behnke RJ. 2002. *Trout and salmon of North America*. New York: The Free Press.

Bennuah SY, Wang TL, Aitken SN. 2004. Genetic analysis of the *Picea* sitchensis × *glauca* introgression zone in British Columbia. *Forest Ecology and Management* **197:** 65–77.

Bettles CM, Docker MF, Dufour B, Heath DD. 2005. Hybridization dynamics between sympatric species of trout: loss of reproductive isolation. *Journal of Evolutionary Biology* **18:** 1220–1233.

Boyer MC, Muhlfeld CC, Allendorf FW. 2008. Rainbow trout (*Oncorhynchus mykiss*) invasion and the spread of hybridization with native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). *Canadian Journal of Fisheries and Aquatic Sciences* **65:** 658–669.

Brett JR. 1995. Some principles in the thermal requirements of fishes. *The Quarterly Review of Biology* **32:** 75–87.

Campton DE, Keading LR. 2005. Westslope cutthroat trout, hybridization, and the U.S. Endangered Species Act. *Conservation Biology* **19:** 1323–1325.

Cech JJ Jr. 1990. Respirometry. In: Schreck CB, Moyle PB, eds. *Methods for fish biology*. Bethesda: American Fisheries Society, 335–362.

Childress JJ, Somero GN. 1990. Metabolic scaling: a new perspective based on scaling of glycolytic enzyme activities. *American Zoologist* **30:** 161–173.

Chuiko GM. 2000. Comparative study of acetylcholinesterase and butyrylcholinesterase in brain and serum of several freshwater fish: specific activities and in vitro inhibition by DDVP, an organophosphorus pesticide. *Comparative Biochemistry and Physiology Part C* **127:** 233–242.

Claireaux G, McKenzie DJ, Genge AG, Chatelier A, Aubin J, Farrell AP. 2005. Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. *The Journal of Experimental Biology* **208:** 1775–1784.

COSEWIC. 2007. Committee on the status of endangered wildlife in Canada. http://www.cosewic.gc.ca Accessed 23 January 2007.

Docker MF, Dale A, Heath DD. 2003. Erosion of interspecific reproductive barriers resulting from hatchery supplementation of rainbow trout sympatric with cutthroat trout. *Molecular Ecology* **12:** 3515–3521.

Dwyer WP, Kramer RH. 1975. Influence of temperature on scope for activity in Cutthroat trout, *Salmo clarki*. *Transactions of the American Fisheries Society* **104:** 552–554.

Elgar MA. 1990. Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. *Oikos* **59:** 283–287.

Elphinstone MS, Hinten GN, Anderson MJ, Nock CJ. 2003. An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. *Molecular Ecology Notes* **3:** 317–320.

Finstad AG, Forseth T, Jonsson B, Bellier E, Hesthagen T, Jensen AJ, Hessen DO, Foldvik A. 2011. Competition

exclusion along climate gradients: energy efficiency influences the distribution of two salmonid fishes. *Global Change Biology* **17:** 1703–1711.

Fry FEJ. 1957. The aquatic respiration of fish. In: Brown ME, ed. *The physiology of fishes, volume 1. Metabolism*. New York: Academic Press, 1–63.

Gamperl AK, Rodnick KJ, Faust HA, Venn EC, Bennett MT, Crawshaw LI, Keeley ER, Powell MS, Li HW. 2002. Metabolism, swimming performance, and tissue biochemistry of high desert redband trout (*Oncorhynchus mykiss* ssp.): evidence for phenotypic differences in physiological function. *Physiological and Biochemical Zoology* **75:** 413–431.

Garenc C, Couture P, Laflamme MA, Guderley H. 1999. Metabolic correlates of burst swimming capacity of juvenile and adult threespine stickleback (*Gasterosteus aculeatus*). *Journal of Comparative Physiology B* **169:** 113–122.

Grant BR, Grant PR. 1996. High survival of Darwin's finch hybrids: effects of beak morphology and diets. *Ecology* **77:** 500–509.

Guderley H, Gawlicka A. 1992. Qualitative modification of muscle metabolic organization with thermal acclimation of rainbow trout, *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry* **10:** 123–132.

Hartley SE, Horne MT. 1986. Chromosome relationships in the genus *Salmo*. *Chromosoma* **90:** 229–237.

Hatfield T, Schluter D. 1999. Ecological speciation in sticklebacks: environment dependent hybrid fitness. *Evolution* **53:** 866–873.

Hawkins DK, Foote CJ. 1998. Early survival and development of coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead trout (*Oncorhynchus mykiss*), and reciprocal hybrids. *Canadian Journal of Fisheries and Aquatic Sciences* **55:** 2097–2104.

Hawkins DK, Quinn TP. 1996. Critical swimming velocity and associated morphology of juvenile coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead trout (*Oncorhynchus mykiss*), and their hybrids. *Canadian Journal of Fisheries and Aquatic Sciences* **53:** 1487–1496.

Heath D, Bettles CM, Roff D. 2010. Environmental factors associated with reproductive barrier breakdown in sympatric trout populations on Vancouver Island. *Evolutionary Applications* **3:** 77–90.

Hilderbrand RH, Kershner JL. 2000. Conserving inland cutthroat trout in small streams: how much stream is enough. *North American Journal of Fisheries Management* **20:** 513–520.

Hitt NP, Frissell CA, Muhlfeld CC, Allendorf FW. 2003. Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences* **60:** 1440–1451.

Hudson RCL. 1973. On the function of the white muscles in teleosts at intermediate swimming speeds. *Journal of Experimental Biology* **58:** 509–522.

Johnson OW, Ruckelshaus MH, Grant WS, Waknitz FW, Garrett AM, Bryant GJ, Neely K, Hard JJ. 1999. Status review of coastal cutthroat trout from Washington, Oregon and California. NOAA Technical Memorandum NMFS-MWFSC-37.

Kawashima K, Fujii T. 2008. Basic and clinical aspects of non-neuronal acetylcholine: overview of non-neuronal cholinergic systems and their biological significance. *Journal of Pharmacological Sciences* **106:** 167–173.

Keleher CJ, Rahel FJ. 1996. Thermal limits to salmonid distributions in the Rocky Mountain region and potential habitat loss due to global warming: a geographic information system (GIS) approach. *Transactions of the American Fisheries Society* **125:** 1–13.

Khasa PD, Dancik BP. 1996. Rapid identification of white-Engelmann spruce species by RAPD markers. *Theoretical and Applied Genetics* **92:** 46–52.

Kindschi GA, Koby RF. 1994. Performance and oxygen consumption of Snake River Cutthroat Trout reared at four densities with supplemental oxygen. *Progressive Fish Culturist* **56:** 13–18.

Kindschi GA, Smith CE, Koby RF. 1991. Oxygen consumption of two strains of rainbow trout reared at four densities with supplemental oxygen. *Progressive Fish Culturist* **53:** 210–215.

Kozfkay CC, Campbell MR, Yundt SP, Peterson MP, Powell MS. 2007. Incidence of hybridization between naturally sympatric westslope cutthroat trout and rainbow trout in the middle fork salmon river drainage, Idaho. *Transactions of the American Fisheries Society* **136:** 624–638.

Lahtai K, Huuskonen H, Laurila A, Piironen J. 2002. Metabolic rate and aggressiveness between Brown Trout populations. *Functional Ecology* **16:** 167–174.

Lahtai K, Laurila A, Enberg K, Piironen J. 2001. Variation in aggressive behaviour and growth rate between populations and migratory forms in the brown trout, *Salmo trutta*. *Animal Behaviour* **62:** 935–944.

Levesque HM, Dorval J, Hontela A, Van Der Kraak GJ, Campbell PGC. 2003. Hormonal, morphological and physiological responses of yellow perch (*Perca flavescens*) to chronic environmental metal exposures. *Journal of Toxicology and Environmental Health, Part A* **66:** 657–676.

Levesque HM, Moon TW, Campbell PGC, Hontela A. 2002. Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. *Aquatic Toxicology* **60:** 257–267.

Likens GE, Bormann FH. 1974. Linkages between terrestrial and aquatic ecosystems. *BioScience* **24:** 447–456.

Loudenslager EJ, Thorgaard GH. 1979. Karyotypic and evolutionary relationships of the Yellowstone (*Salmo clarki bouvieri*) and west slope (*Salmo clarki lewisi*) cutthroat trout. *Journal of the Fisheries Research Board of Canada* **36:** 630–635.

McCarthy ID. 2001. Competitive ability is related to metabolic asymmetry in juvenile rainbow trout. *Journal of Fish Biology* **59:** 1002–1014.

Metcalfe NB, Taylor AC, Thorpe JE. 2004. Metabolic rate, social status and life-history strategies in Atlantic salmon. *Animal Behavior* **49:** 431–436.

Miller SA, Wagner EJ, Bosakowski T. 1995. Performance and oxygen consumption of rainbow trout reared at two

densities in raceways with oxygen supplementation. *Progressive Fish Culturist* **57:** 206–212.

Moore WS. 1977. Evaluation of narrow hybrid zones in vertebrates. *Quarterly Review of Biology* **52:** 263–277.

Morinville GR, Rasmussen JB. 2003. Early juvenile bioenergetic differences between anadromous and resident brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* **60:** 401–410.

Morinville GR, Rasmussen JB. 2006. Does life-history variability in salmonids affect habitat use by juveniles? A comparison among streams open and closed to anadromy. *Journal of Animal Ecology* **75:** 693–704.

Muhlfeld CC, Kalinowski ST, McMahon TE, Taper ML, Painter S, Leary RF, Allendorf FW. 2009. Hybridization rapidly reduces fitness of native trout in the wild. *Biology Letters* **5:** 328–331.

Myrick CA, Cech JJ. 2000. Temperature influences on California rainbow trout physiological performance. *Fish Physiology and Biochemistry* **22:** 245–254.

Ostberg CO, Rodriguez RJ. 2002. Novel molecular markers differentiate *Oncorhynchus mykiss* (rainbow trout and steelhead) and the *O. clarki* (cutthroat trout) subspecies. *Molecular Ecology Notes* **2:** 197–202.

Ostberg CO, Rodriguez RJ. 2004. Bi-parentally inherited species-specific markers identify hybridization between rainbow and cutthroat trout subspecies. *Molecular Ecology Notes* **4:** 26–29.

Ostberg CO, Slatton SL, Rodriguez RJ. 2004. Spatial partitioning and asymmetric hybridization among sympatric coastal steelhead trout (*Oncorhynchus mykiss irideus*), coastal cutthroat trout (*O. clarki clarki*) and interspecific hybrids. *Molecular Ecology* **13:** 2773–2788.

Paul AJ, Post JR. 2001. Spatial distribution of native and nonnative salmonids in streams of the eastern slopes of the Canadian Rocky Mountains. *Transactions of the American Fisheries* **130:** 417–430.

Pelletier D, Guderley H, Dutil JD. 1993. Does the aerobic capacity of fish muscle change with growth rates? *Fish Physiology and Biochemistry* **12:** 83–93.

Peterson DP, Fausch KD. 2003. Upstream movement by nonnative brook trout (*Salvelinus fontinalis*) promotes invasion of native cutthroat trout (*Oncorhynchus clarki*) habitat. *Canadian Journal of Fisheries and Aquatic Sciences* **60:** 1502–1516.

Peterson DP, Fausch KD, White GC. 2004. Population ecology of an invasion: effects of brook trout on native cutthroat trout. *Ecological Applications* **14:** 754–772.

Peterson DP, Rieman BE, Dunham JB, Fausch KD, Young MK. 2008. Analysis of trade-offs between threats of invasion by nonnative brook trout (*Salvelinus fontinalis*) and intentional isolation for native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). *Canadian Journal of Fisheries and Aquatic Sciences* **65:** 557–573.

Quinn AL, Rasmussen JB, Hontela A. 2010. Physiological stress response of mountain whitefish (*Prosopium williamsoni*) and white sucker (*Catstomus commersoni*) sampled along a gradient of temperature and agrichemicals in the Oldman River, Alberta. *Environmental Biology of Fishes* **88:** 119–131.

Rao GMM. 1968. Oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. *Canadian Journal of Zoology* **46:** 781–786.

Rao GMM. 1971. Influence of activity and salinity on the weight-dependent oxygen consumption of the rainbow trout *Salmo gairdneri*. *Marine Biology* **8:** 205–212.

Rasmussen JB, Gunn JM, Sherwood GD, Iles A, Gagnon A, Campbell PGC, Hontela A. 2008. Direct and indirect (foodweb mediated) effects of metal exposure on the growth of yellow perch (*Perca flavescens*): implications for ecological risk assessment. *Human and Ecological Risk Assessment* **14:** 317–350.

Rasmussen JB, Robinson MD, Heath DD. 2010. Ecological consequences of hybridization between native westslope cutthroat (*Oncorhynchus clarkii lewisi*) and introduced rainbow (*Oncorhynchus mykiss*) trout: effects on life history and habitat use. *Canadian Journal of Fisheries and Aquatic Sciences* **67:** 357–370.

Rieseberg LH, Wendel JF. 1993. Introgression and its consequences in plants. In: Harrison RG, ed. *Hybrid zones and the evolutionary process*. Oxford: Oxford University Press, 70–114.

Rodnick KJ, Gamperl AK, Lizars KR, Bennett MT, Rausch RN, Keeley ER. 2004. Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. *Journal of Fish Biology* **64:** 310–335.

Rubidge EM, Taylor EB. 2004. Hybrid zone structure and the potential role of selection in hybridizing populations of native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*). *Molecular Ecology* **13:** 3735–3749.

Rubidge EM, Taylor EB. 2005. An analysis of spatial and environmental factors influencing hybridization between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*O. mykiss*) in the upper Kootenay River drainage, British Columbia. *Conservation Genetics* **6:** 369–384.

Schill DJ, LaBar GW, Mamer ERJM, Meyer KA. 2010. Sex ratio, fecundity, and models predicting length at sexual maturity of redband trout in Idaho desert streams. *North American Journal of Fisheries Management* **30:** 1352–1363.

Seiler SM, Keeley ER. 2007. Morphological and swimming stamina differences between Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*), rainbow trout (*Oncorhynchus mykiss*), and their hybrids. *Canadian Journal of Fisheries and Aquatic Sciences* **64:** 127–135.

Seiler SM, Keeley ER. 2009. Competition between native and introduced salmonid fishes: cutthroat trout have lower growth rate in the presence of cutthroat–rainbow trout hybrids. *Canadian Journal of Fisheries and Aquatic Sciences* **66:** 133–141.

Shepard BB, May BE, Urie W. 2005. Status and conservation of westslope cutthroat trout within the western United States. *North American Journal of Fisheries Management* **4:** 1426–1440.

Shepard BB, Sanborn B, Ulmer L, Lee DC. 1997. Status and risk of extinction for westslope cutthroat trout in the upper Missouri River Basin, Montana. *North American Journal of Fisheries Management* **17:** 1158–1172.

Sherwood GD, Kovecses J, Hontela A, Rasmussen JB. 2002a. Simplified food webs lead to energetic bottlenecks in polluted lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **59:** 1–5.

Sherwood GD, Pazzia I, Moeser A, Hontela A, Rasmussen JB. 2002b. Shifting gears: enzymatic evidence for the energetic advantage of switching diet in wild-living fish. *Canadian Journal of Fisheries and Aquatic Sciences* **59:** 229–241.

Somero GN, Childress JJ. 1980. A violation of the metabolism–size scaling paradigm: activities of glycolytic enzymes in muscle increase in larger-size fish. *Physiological Zoology* **53:** 322–337.

Sperling FAH. 1987. Evolution of the *Papilio machaon* species group in western Canada (Lepidoptera: Papilionidae). *Quaestiones Entomologicae* **23:** 198–315.

Sperling FAH. 1989. Natural hybrids of *Papilio* (Insecta: Lepidoptera): poor taxonomy or interesting evolutionary problem. *Canadian Journal of Zoology* **68:** 1790–1799.

Sperling FAH, Spence JR. 1991. Structure of an asymmetric hybrid zone between two water strider species (Hemiptera: Gerridae: *Limnoporus*). *Evolution* **45:** 1370–1383.

Springer SA, Heath DD. 2007. Environment-specific heterozygote deficiency and developmental instability in hybrid *Mytilus*. *Marine Biology Research* **3:** 182–187.

Sutton BCS, Pritchard SC, Gawley JR, Newton CH. 1994. Analysis of Sitka spruce–interior spruce introgression zone in British Columbia using cytoplasmic and nuclear DNA probes. *Canadian Journal of Forest Research* **24:** 278–285.

Thorgaard GH. 1983. Chromosomal differences among rainbow trout populations. *Copeia* **1983:** 650–662.

US Fish and Wildlife Service. 2003. Endangered and threatened wildlife and plants: reconsidered finding for an amended petition to list westslope cutthroat trout as threatened throughout its range. *Federal Register* **68:** 46989–47009.

Van Leeuwen TE, Rosenfeld JS, Richards JG. 2011. Variation in metabolic rate between juvenile coho salmon and steelhead trout: insight into the physiological basis of habitat partitioning and adaptive tradeoffs among juvenile salmonids. *Journal of Animal Ecology* **80:** 1012–1023.

Webb PW. 1971. The swimming energetics of trout II. Oxygen consumption and swimming efficiency. *Experimental Biology* **55:** 521–540.

Weigel DE, Peterson JT, Spruell P. 2003. Introgressive hybridization between native cutthroat trout and introduced rainbow trout. *Ecological Applications* **13:** 38–50.

Williams I, Reeves GH, Graziano SL, Nielsen JL. 2008. Genetic investigation of natural hybridization between rainbow and coastal cutthroat trout in the copper river Delta, Alaska. *Transactions of the American Fisheries Society* **136:** 926–942.