Metabolism, Swimming Performance, and Tissue Biochemistry of High Desert Redband Trout (*Oncorhynchus mykiss* ssp.): Evidence for Phenotypic Differences in Physiological Function

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ABSTRACT

Redband trout (*Oncorhynchus mykiss* ssp.) in southeastern Oregon inhabit high-elevation streams that exhibit extreme variability in seasonal flow and diel water temperature. Given the strong influence and potential limitations exerted by temperature on fish physiology, we were interested in how acute temperature change and thermal history influenced the physiological capabilities and biochemical characteristics of these trout. To this end, we studied wild redband trout inhabiting two streams with different thermal profiles by measuring (1) critical swimming speed (U_{crit}) and oxygen consumption in the field at 12° and 24°C; (2) biochemical indices of energy metabolism in the heart, axial white skeletal muscle, and blood; and (3) temperature preference in a laboratory thermal gradient. Further, we also examined genetic and morphological characteristics of fish from these two streams. At 12°C, maximum metabolic rate ($Mo_{2\ max}$) and metabolic power were greater in Little Blitzen redband trout as compared with those from Bridge Creek (by 37% and 32%, respectively). Conversely, Bridge Creek and Little Blitzen trout had similar values for $Mo_{2\ max}$ and metabolic power at 24°C. U_{crit} of Little Blitzen trout was similar at the two temperatures (61 ± 3 vs. 57 ± 4 cm s^{-1}). However, the U_{crit} for Bridge Creek trout increased from 62 ± 3 cm s^{-1} to 75 ± 3 cm s^{-1} when water temperature was raised from 12° to 24°C, and the U_{crit} value at 24°C was significantly greater than for Little Blitzen fish. Cost of transport was lower for Bridge Creek trout at both 12° and 24°C, indicating that these trout swim more efficiently than those from the Little Blitzen. Possible explanations for the greater metabolic power of Little Blitzen redband trout at 12°C include increased relative ventricular mass (27%) and an elevation in epaxial white muscle citrate synthase activity (by 72%). Bridge Creek trout had 50% higher lactate dehydrogenase activity in white muscle and presumably a greater potential for anaerobic metabolism. Both populations exhibited a preferred temperature of approximately 13°C and identical mitochondrial haplotypes and p53 gene allele frequencies. However, Bridge Creek trout had a more robust body form, with a relatively larger head and a deeper body and caudal peduncle. In summary, despite the short distance (~10 km) and genotypic similarity between study streams, our results indicate that phenotypic reorganization of anatomical characteristics, swimming ability at environmentally pertinent temperatures and white axial muscle ATP-producing pathways occurs in redband trout.

Introduction

Redband trout (*Oncorhynchus mykiss* ssp.) are found in the desert basins of western North America and inhabit high-elevation streams that are characterized by extreme variation in seasonal flow and water temperature (Behnke 1992; Vinson and Levesque 1994; Zoellick 1999). For example, summer stream flows can become intermittent, daily maximum water temperatures can exceed 29°C, and diel temperature fluctuations of 8°–12°C are common (Zoellick 1999; K. J. Rodnick et al., unpublished data). Based on the "harsh" environmental conditions in which these trout reside, and observations of feeding activity at water temperatures approaching 28°C, it has been assumed that the redband trout has evolved adaptations for

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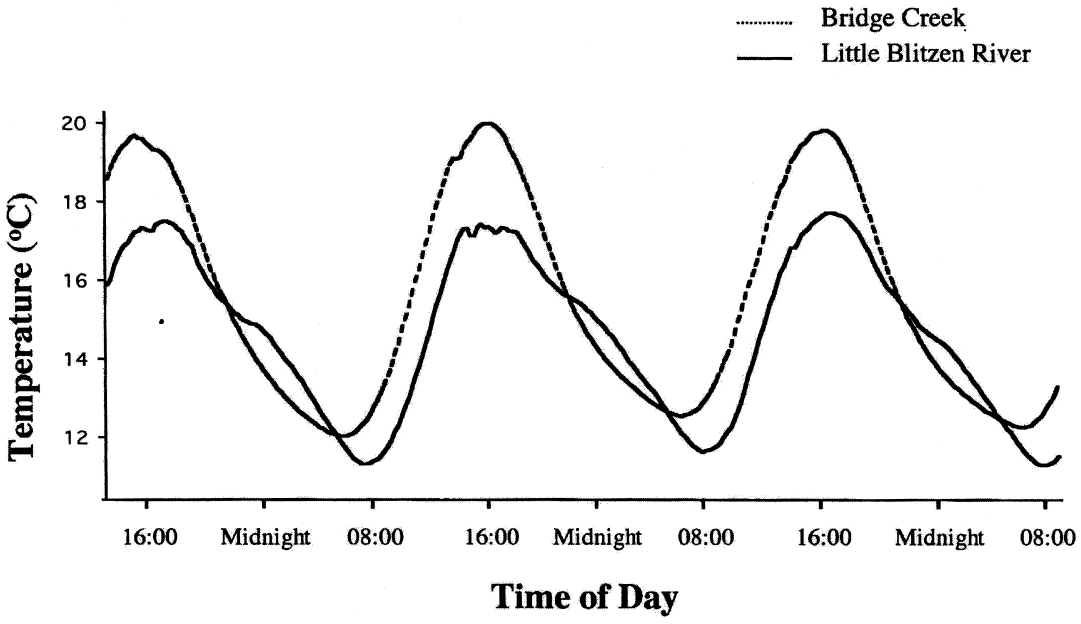

Figure 1. Diurnal fluctuations in water temperature in Bridge Creek and the Little Blitzen River during July 1999

warm-water tolerance (Behnke 1992). However, 11 redband trout populations have become extinct and 10 others are at risk (Nehlsen et al. 1993), and the U.S. Fish and Wildlife Service is monitoring the redband trout as a sensitive species. This suggests that "natural" thermal conditions in streams already force redband trout to operate near their physiological limits and that their ability to perform functions such as swimming or to withstand further environmental perturbations is severely restricted.

There is limited information on the physiological ecology of redband trout (Behnke 1992; Vinson and Levesque 1994; Zoellick et al. 1999) and on the ability of salmonid species to acclimatize to both high and variable stream temperatures (Hokanson et al. 1977; Thomas et al. 1986; Houston and Schrapp 1994; Dickerson and Vinyard 1999). To promote a better understanding of the effects of high stream temperatures on this species, and to define the temperature optima for physiological function, we measured (1) critical swimming speed (U_{crit}) and oxygen consumption in the field at 12° and 24°C; (2) biochemical indices of energy metabolism in the heart (ventricle), axial white skeletal muscle, and blood; and (3) preferred temperature in a laboratory thermal gradient. Studies were conducted on redband trout from two streams in the High Desert Ecoregion of Oregon with different thermal characteristics. During the late summer (July–August), both streams have minimum daily temperatures of approximately 12°C. However, maximum temperatures in the cooler stream (Little Blitzen River) rarely exceed 18°C, whereas those in the warmer stream (Bridge Creek) can reach or exceed 24°C.

Material and Methods

Study Sites

The two streams selected for study in the summer of 1999, Bridge Creek and the Little Blitzen River, originate on Steens Mountain in southeastern Oregon and eventually drain into the Malheur National Wildife Refuge. The water supplying the Little Blitzen River is predominantly snowmelt, and the riparian vegetation along this stream is extensive. These features, along with topographical shading, keep this stream relatively cool (<18°C) during the summer. In the spring and early summer, snowmelt also contributes significantly to flow in Bridge Creek; however, during most of the summer, flow in this stream depends on groundwater from springs. The largest of these springs provides water with a constant temperature of 15°C and accounts for approximately 50% of stream flow. Unlike the Little Blitzen River, Bridge Creek has very limited riparian vegetation, and water temperatures can reach 24°C on very hot summer days (air temperature = 34°–38°C).

In 1999, snowpack on the Steens Mountain was 180% of normal, and the summer was unusually cool. As a result, stream temperatures were cooler than average, although maximum temperatures in Bridge Creek (20°C) exceeded those in the Little Blitzen River (17°C; Fig. 1).

Swimming Performance and Metabolism

Experiments were conducted streamside at the Little Blitzen River from July 20 to 30, and at Bridge Creek from August 6

to 16. To minimize capture stress and injury, redband trout of similar size (fork length ≈ 15–20 cm, body weight range = 40–105 g) were collected by anglers using dry flies and barbless hooks, and kept in stream cages for 2–5 d before experiments. These stream cages were large (200 L) plastic containers that had numerous large (1.6 cm in diameter) holes to allow water and suspended material (including invertebrate drift) to pass through, and they contained several large rocks for cover. Twenty-four to 48 h before putting fish into the Blazka-type swim-tunnel respirometers (see Cech 1990; volume = 6.8 L; Waterloo Biotelemetry Institute, University of Waterloo, Canada), fish were netted, placed into clear Perspex tubes containing mesh (1 cm^2) caps, and held within their native stream. This procedure allowed each fish to acclimate to confinement before being placed into one of the two respirometers. Each Perspex tube was 42.75 cm long and 8.75 cm in internal diameter, dimensions equal to that of the swimming section of the respirometers. The night before (i.e., between 1800 and 2000 hours) swimming and metabolic studies, fish were put into the respirometers and given a brief (10–15 min) training session to orient them to the current and to allow them to experience fluctuations in water velocity. All trout were then left at a current velocity of approximately 0.5 BL s^{-1} (BL = body lengths) until experiments began at 0700–0800 hours the next morning. During the overnight acclimation period, stream water was continuously pumped through the swim tunnels at 2 L min^{-1} using submersible pumps (Little Giant Pump Co., Oklahoma City, Okla.). To limit disturbance to the fish during habituation and U_{crit} tests, the swimming section of each respirometer was covered with a sheet of black plastic. Portable gasoline generators (models XL 5000 and EXL 6500, Generac Power Systems, Waukesha, Wis.) provided the electrical power required to run the respirometers and all associated equipment.

Experiment 1: Swimming Performance at 12°–14°C. A modified U_{crit} test (Brett 1964) was used to determine the swimming and metabolic capacity of individual fish. In this protocol, current velocity was increased by 10 cm s^{-1} every 20 min until a swimming speed of 40 cm s^{-1} (ca. 2 BL s^{-1}) was achieved, and by 5 cm s^{-1} thereafter. At each swimming speed, oxygen consumption was measured for 6–10 min, the period of oxygen measurement beginning 3 min after swimming speed was increased. Exhaustion was determined as the inability of the fish to separate itself from the rear grid of the respirometer after three successive, mild (<5 V) electrical shocks.

Experiment 2: Swimming Performance at 24°C. We assessed the influence of temperature on routine metabolic rate by measuring oxygen consumption as water temperature was increased from 12° to 24°C. Water temperature was increased by 2°C per hour, and swimming velocity was maintained at approximately 0.5 BL s^{-1}. This rate of temperature increase approximated the maximum rate of heating that redband trout experience during a summer diurnal cycle of stream temperature (Fig. 1). After routine levels of oxygen consumption were measured at 24°C, each fish was given a modified U_{crit} test, as described in experiment 1.

At the end of U_{crit} measurements, fish were anesthetized (MS-222, 0.1 g L^{-1}; $NaHCO_3$, 0.1 g L^{-1}), and fork length and body mass were recorded. Body width and depth were also measured just anterior to the dorsal fin using calipers and were used to correct measurements of swimming speed for solid-blocking effects according to Jones et al. (1974).

Measurements and Calculations. Water temperature and oxygen content within each swim tunnel were continuously measured by pumping water through an external circuit using a peristaltic pump (Masterflex Model 7523-20, Cole-Parmer). This circuit was constructed of tubing with extremely low gas permeability (Tygon Food, ser. 6419, Cole Palmer Instrument) and contained a customized flow chamber that housed a galvanic oxygen electrode equipped with thermal sensor (Model CellOx 325, WTW, Germany). This oxygen electrode was connected to an oxygen meter (Model Oxi 340, WTW) equipped with automatic temperature and altitude compensation. When stream temperatures were not at the desired experimental temperature, the submersible pumps supplying water to each swim tunnel were placed into an insulated reservoir (120 L). Water temperature in this reservoir was controlled using a recirculating water bath (Neslab Model RTE-100, Portsmouth, N.H.), and water oxygen levels were maintained at saturation levels by bubbling air and/or pulsing pure oxygen into the water. Oxygen consumption (Mo_2) was measured at the beginning of each experiment and at all swimming speeds by stopping the flow of fresh water into the swim tunnel for 6–10 min and recording the drop in water oxygen content (Cech 1990). Oxygen consumption (in mg O_2 h^{-1}) was calculated as

$$\mathrm{Mo}_2 = \left[\frac{(C_i - C_f)}{T} \times V_c\right] \times 60,$$

where C_i = water oxygen content (mg L^{-1}) at the start of Mo_2 measurement, C_f = water oxygen content at the end of Mo_2 measurement, V_c = volume of the respirometer and external circuit (6.81 L), and T = time (min) required to make MO_2 measurement.

Metabolic power (mg O_2 h^{-1}) was calculated as maximum MO_2 ($Mo_{2\,max}C$; measured at maximum swimming speed) minus routine Mo_2 (RMo_2; at 0.5 BL s^{-1}). U_{crit} was calculated as

$$U_{crit} = V + \frac{(T_f \times V_i)}{T_i},$$

where V = the velocity at which the fish swam for the entire

time increment, V_i = the velocity increment (5 or 10 cm s^{-1}), T_f = time elapsed from the last change in current velocity to fatigue, and T_i = time increment, the time between step increases in velocity (20 min), and swimming speed was corrected for the effect of solid blocking (Jones et al. 1974).

Swimming efficiency was measured as cost of transport (COT) using an oxycalorific coefficient of 3.25 cal mg O_2^{-1} (Parsons and Sylvester 1992). For each fish, a second-order regression was fitted to the relationship between swimming speed (cm s^{-1}) and COT, and the minimum COT and swimming speed at minimum COT were calculated from the derived relationship. Condition factor was calculated as [(mass in g/fork length in cm)^3 × 100].

Temperature Preference

After capture, fish (n = 24, size range = 10–20 cm) from both Bridge Creek and the Little Blitzen River were transported to Portland State University in an insulated 50-L tank. During the 7–8-h trip, water temperature was maintained between 9° and 17°C using ice, and oxygen saturation was maintained by periodic bubbling with air. At Portland State University, fish were housed in 1,000-L insulated tanks at 15° ± 1°C for 36–48 h before experiments were conducted. This temperature approximated the mean daily temperature for the two streams. Photoperiod was 12L : 12D. To determine thermal preference, trout were placed in a thermal gradient (Wollmuth et al. 1987) composed of nine separate lanes (2.5 m long × 28 cm wide; 10 cm deep), each equipped with thermocouples every 12.5 cm. Temperature within each lane ranged from approximately 8° to 30°C. The gradient was filled to a depth of 7.5 cm with water from the holding tanks, and trout were allowed 3 h within the gradient to select their preferred temperature before data collection began. Thermal preference was determined as the average temperature selected during the fourth experimental hour. To accomplish this, the position of each fish was recorded at 5-s intervals using a wide-angle camera located above the gradient. The recorded image was then digitized using a frame grabber (Data Translation, Marlboro, Mass.). Finally, the position of the fish was converted to a temperature using the data retrieved from the thermocouples and customized software. Preliminary experiments revealed that selected temperature was not affected by longer exposures to the gradient (up to 8 h). After determination of preferred temperature, the trout were anesthetized, the mass and length of each fish was recorded, fin clips were taken for genetic analysis, and the fish were frozen for subsequent morphometric analysis.

Tissue Collection for Biochemical Analyses

Additional redband trout were held in stream cages for approximately 2 d before sampling. Animals were anesthetized with buffered MS-222, weighed, and measured (fork length), and blood samples (1 mL) were drawn from the caudal vessels. The blood was allowed to clot on ice, and then the serum was separated by centrifugation, placed in cryovials, and flash frozen in liquid nitrogen.

The ventricle was rapidly excised, rinsed in ice-cold 1.0% NaCl, blotted dry, weighed, and frozen rapidly with aluminum clamps cooled to the temperature of liquid nitrogen. Epaxial white muscle on the right side of each fish was excised from beneath the dorsal fin and dissected free of bone, fat, and connective tissue. All samples were transported to Idaho State University under liquid nitrogen and stored at −80°C until assays were conducted.

Serum/Plasma Osmolality Electrolytes, Energy Substrates, and Proteins

We used vapor osmometry (Wescor model 5520, Logan, Utah) to determine serum osmolality, flame photometry (Instrumentation Laboratory model 943, Lexington, Mass.) to measure serum sodium and potassium concentrations, and a calcium-binding reagent (Arsenazo III, Sigma Procedure 588) for calcium levels. Free (nonesterified) fatty acids (FFAs) were measured by an enzymatic method (NEFAC kit, Wako Chemicals, Richmond, Va.), and triglycerides were determined using the INT reagent (Sigma Procedure 336). Albumin concentration q5
was measured with the bromocresol green binding technique (Sigma Procedure 631) with a standard curve generated using bovine serum albumin (BSA). Total protein was determined by the Bradford dye-binding method (Bio-Rad Laboratories, Hercules, Calif.) with BSA as the standard.

It was apparent that allowing blood to clot on ice for even brief periods (<20 min) promoted erythrocyte uptake of potassium and precluded accurate estimates of extracellular concentrations in field-caught redband trout. Thus, we subsequently collected plasma from thermally acclimated rainbow trout (see below) using lithium heparin (20 U mL^{-1}) as the anticoagulant.

Assay of Maximal Enzyme Activity and Thermal Sensitivity

We measured maximal activities of enzymes that provide indices of anaerobic (lactate dehydrogenase [LDH]) and aerobic (citrate synthase [CS]) energy metabolism. Kinetic assays were conducted at 15° and 25°C (redband trout samples) or only 15°C (hatchery rainbow trout samples) with saturating concentrations of substrates and cofactors using a thermostatically controlled Perkin-Elmer Lambda 6 UV/VIS spectrophotometer (Norwalk, Conn.). The thermal sensitivity of redband trout enzymes was calculated using the formula $Q_{10} = (R_2/R_1)^{10}/T_2 - T_1$, where T_2 and T_1 were 25° and 15°C, respectively. We determined the thermal stability of LDH in redband trout white muscle by raising the assay temperature to 30°C and then increasing temperature by 2° increments. We iden-

tified thermal transitions or breakpoints using Arrhenius plots (natural log) of temperature (°K) versus maximal enzyme activity.

For all enzyme assays, frozen samples (ca. 25 mg) of ventricular and epaxial white skeletal muscle were homogenized in 19 vol of ice-cold extraction medium using motor-driven Duall-21 ground-glass homogenizers (Kontes Glass, Vineland, N.J.). Enzyme activities are expressed as units (U) per gram wet tissue mass, where 1 U denotes the conversion of 1 μmol of substrate to product per minute. The final volume for each assay was 1.0 mL, and all activities were linear over the reaction period (LDH, 5 min at 340 nm; CS, 7 min at 412 nm).

Lactate Dehydrogenase (*EC 1.1.1.27*). The extraction medium consisted of (in mmol L^{-1}): 50 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), 1 ethylenediaminetetraacetic acid (EDTA), and 2 dithiothreitol (DTT), pH 7.5 at 15°C. Whole homogenates were used at final dilutions ranging from 1 : 1,000 to 1 : 2,000. The reaction mixture contained (in mmol L^{-1}): 50 Hepes, 1 KCN, 0.17 nicotinamide adenine dinucleotide, reduced form (NADH), and either 1.0 mmol L^{-1} pyruvate (white skeletal muscle) or 0.25 mmol L^{-1} pyruvate (cardiac muscle), adjusted to pH 7.5 at 15°C. Pyruvate was omitted from controls.

Citrate Synthase (*EC 4.1.3.7*). Extraction medium consisted of (in mmol L^{-1}): 20 Hepes, 1 ethylene glycol-bis (β-aminoethyl ether)-*N*, *N*, *N*′, *N*′-tetraacetic acid (EGTA), pH 7.5 at 15°C. Homogenates of frozen tissue were taken through a freeze-thaw cycle to liberate this mitochondrial matrix protein and maximize enzyme activity. The final dilution of homogenates ranged from 1 : 500 (skeletal muscle) to 1 : 8,000 (cardiac muscle). The assay reaction mixture consisted of (in mmol L^{-1}): 20 Hepes, 1 EGTA, 220 sucrose, 40 KCl, 0.1 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB), 0.10 acetyl CoA, and 0.10 oxaloacetate (omitted in controls).

Triglyceride and Water Content of Axial White Skeletal Muscle

Muscle triglycerides were isolated using a modification of the methods described by Folch et al. (1957) and Carr et al. (1993). Triglyceride concentration was measured enzymatically by adding the prepared tissue sample or standards to a triglyceride INT reagent (Sigma Procedure 336). We also determined water content in the skeletal muscle by lyophilizing frozen tissue (20–30 mg) to a constant mass

Thermal Acclimation of Hatchery-Reared Rainbow Trout

We extended our study to include tissues from hatchery-reared rainbow trout (10-mo-old females weighing 300–400 g; Clear Springs Foods, Buhl, Idaho) that were acclimated to 5° or 15°C to examine the importance of thermal history in mediating the observed differences in tissue adaptability and biochemical characteristics between redband trout populations. Hatchery fish, which were reared and maintained in flow-through outdoor concrete raceways receiving spring water at 15°C, were randomly assigned to one of two temperature-controlled, 1,000-L circular tanks After a 2-wk initiation period at 15°C, the temperature of the cold acclimation tank was lowered by 1°C d^{-1} until 5°C was reached. All fish remained at their respective temperatures for an additional 8 wk. To promote similar rates of body weight gain, cold- (5°C) and warm- (15°C) acclimated trout received 1.0% and 1.25% of their mean body weight, respectively, of a commercial trout chow every other day. To examine temperature effects independent of photoperiod cues, fish were kept under controlled photoperiod (12L : 12D). Fish were fasted for 48 h before tissue sampling, anesthetized, measured, and processed for collection of blood and tissues.

Morphometrics

To assess potential morphological differences between redband trout populations, we measured a series of external features from preserved (10% formalin, followed by 37% isopropanol) specimens collected from the Little Blitzen River and Bridge Creek. We measured nine external body features as an estimate of external morphology. These variables included pectoral fin length, pelvic fin length, premaxilla length, mouth width, head length and width, eye diameter, and body and caudal peduncle depth. Measurements were collected using digital calipers connected to a personal computer that compiled the measurements using a software package (WinWedge, version 1.2, Tal Technologies, Philadelphia).

Genetic Analyses

Fin samples of redband trout from the Little Blitzen River and Bridge Creek were taken and stored in 70% ethanol or frozen at −80°C until DNA was extracted using methods modified from Sambrook et al. (1989) and Hillis et al. (1996). Total genomic DNA was isolated and amplified using the polymerase chain reaction (PCR) and nucleotide primers specific for loci of interest. Amplification products for nuclear and mitochondrial restriction fragment length polymorphisms (RFLPs) were digested separately with 15 restriction enzymes (*Ava* I, *Bcl* I, *Bgl* II, *Dde* II, *Dpn* II, *Hae* III, *Hha* I, *Hinc* II, *Hind* III, *Hinf* I, *Mse* I, *Msp* I, *Nhe* I, *Pvu* I, and *Rsa* I). The resulting fragments were separated by electrophoresis, visualized, and scored using the methods of Paragamian et al. (1999). Resulting haplotypes and genotypes were compared between study streams and against previously analyzed populations of redband trout from several locations (M. S. Powell, unpublished data).

Table 1: Temperature-dependent differences in routine and active oxygen consumption (Mo_2) between juvenile trout from Bridge Creek and the Little Blitzen River

	N	Mass (g)	Routine Mo_2	Maximum Mo_2[a]	Metabolic Power[a]
Bridge Creek:					
12°–14°C	8	92 ± 11[b]	121 ± 8[c]	572 ± 45[b,c]	451 ± 44[b,c]
			(88 ± 4)	(431 ± 31)	(350 ± 32)
24°C	7	108 ± 12[b]	304 ± 28	937 ± 62	633 ± 69
			(227 ± 19)	(723 ± 41)	(503 ± 53)
Little Blitzen:					
12°–14°C	9	58 ± 6	165 ± 12[c]	827 ± 52	662 ± 47
			(114 ± 8)	(592 ± 36)	(492 ± 33)
24°C	9	71 ± 5	383 ± 38	960 ± 42	576 ± 47
			(272 ± 25)	(705 ± 27)	(438 ± 34)

Note. For each temperature-stream combination, units are mg O_2 kg^{-1} h^{-1}; mass-adjusted values are in parentheses (resting Mo_2, mass $^{-0.830}$; maximum Mo_2, mass $^{-0.870}$; metabolic power, mass$^{-0.882}$). Differences in mass between groups were initially examined using a 2 × 2 ANOVA. Values are mean ± SEM.

[a] When stream × temperature interactions were significant ($P < 0.05$), a one-way ANOVA followed by Fisher's LSD test was used to identify differences between groups.

[b] Indicates a significant difference between streams within each temperature.

[c] Indicates a significant difference between temperatures within each stream.

Statistical Analyses

Swimming Speed and Metabolism. A one-way ANCOVA was used to examine whether the slopes of the regression lines between log oxygen consumption (i.e., RMo_2, $Mo_{2\,max}$, and metabolic power; in mg O_2 h^{-1}) and log weight (kg) were different between groups. Because the slopes of the regression lines were not significantly different between groups ($P > 0.05$), these parameters were converted into mass-independent values (Cech 1990; Myrick and Cech 2000) using mg O_2 $kg^{-0.830}$ h^{-1}, mg O_2 $kg^{-0.870}$ h^{-1}, and mg O_2 $kg^{-0.882}$ h^{-1}, respectively. These $\log_{10}$ transformations were based on data for Bridge Creek redband trout that ranged in size from 45 to 1,400 g (K. J. Rodnick et al., unpublished data). After adjusting for body mass, a 2 × 2 ANOVA was used to examine whether stream or test temperature significantly affected metabolic parameters. When the stream × temperature interaction was significant, one-way ANOVAs followed by Tukey's post hoc test were used to compare groups.

A 2 × 2 ANCOVA was performed initially to examine whether swimming speed (cm s^{-1}) differed between treatment groups. However, because the stream × temperature interaction was significant, a one-way ANCOVA (with fish length as the covariate) followed by Tukey's post hoc procedure was used to compare groups.

Fish length, fish mass, condition factor, and minimum COT (cal $kg^{-0.870}$ km^{-1}) were compared between groups using a two-way ANOVA. Swimming speed at minimum COT (cm s^{-1}) was compared between groups using a two-way ANCOVA, with length as the covariate. Q_{10} values for RMo_2 were compared between Bridge Creek and Little Blitzen River trout using a one-way ANOVA. Measurements for U_{crit} (BL s^{-1}) and metabolism (mg O_2 kg^{-1} h^{-1}) are also presented (Tables 1, 2) so that our data can be compared directly with literature values.

Biochemistry. We tested for physical and biochemical differences between redband trout from Bridge Creek and the Little Blitzen River, and between cold- and warm-acclimated rainbow trout, using either ANOVA or ANCOVA, and Tukey's post hoc test when the initial analysis was significant. Because our data set included a wide range of body masses for redband trout, and previous studies have noted scaling of enzyme activities of anaerobic and aerobic pathways in fish striated muscle (Kieffer et al. 1996; Norton et al. 2000), we used body mass as the covariate for our ANCOVA. We kept redband trout separate from thermally acclimated rainbow trout during data analysis because of anticipated differences in factors such as diet, growth rate, condition factor, genetics, life history, activity, thermal history, and water characteristics.

Temperature Preference and Morphometrics

Preferred temperature was compared between the Bridge Creek and Little Blitzen trout using an ANCOVA, with weight and length as covariates. To determine whether there were morphological differences between the two populations, after controlling for potential body size differences, we transformed morphological measurements into size-adjusted measurements. To accomplish this, we $\log_{10}$-transformed all morphological features, regressed each trait against $\log_{10}$ fork length using an ordinary least squares regression, and then used the residual variation from each of the nine regressions as our estimate of morphology (Reist 1985). We then performed a multivariate

Table 2: Swimming performance of redband trout from Bridge Creek and the Little Blitzen River at 12° and 24°C

	N	Fork Length (cm)	Condition Factor	U_{crit} (cm s^{-1})	U_{crit} (BL s^{-1})
Bridge Creek:					
12°–14°C	8	20.3 ± .9[a]	1.07 ± .02	62 ± 3	3.1 ± .2
24°C	7	21.4 ± .9[a]	1.07 ± .02	75 ± 3[a,b]	3.5 ± .2
Little Blitzen:					
12°–14°C	9	17.6 ± .5	1.03 ± .02	61 ± 3	3.5 ± .1
24°C	9	18.8 ± .4	1.05 ± .02	57 ± 4	3.2 ± .2

Note. Length and condition factor were compared between groups using a 2 × 2 ANOVA. Swimming speed in cm s^{-1} was initially compared between groups using a 2 × 2 ANCOVA with length as the covariate. However, because the stream × temperature interaction was significant ($P < 0.05$), a one-way ANCOVA followed by Fisher's LSD test was used to identify differences between groups. Values are mean ± SEM.

[a] Indicates a significant difference between streams within each temperature.

[b] Indicates a significant difference between temperatures within each stream.

analysis of variance (MANOVA) on the nine size-adjusted morphological variables to determine whether there was an overall statistical difference in morphology between the two populations of trout. Finally, in order to describe how morphology might differ between populations, we performed a discriminant analysis using all nine size-adjusted measurements to determine which traits contributed to population separation. Univariate ANOVA was used as a test of significance for each morphological feature in the discriminant analysis.

All statistical analyses were performed using Statview or SAS statistical software (SAS Institute), and differences were considered significant when $P < 0.05$. Values presented in figures, tables, and throughout the text are means ± SEM.

Results

For both physiological and biochemical studies, the length and mass of Bridge Creek redband trout were significantly greater ($P < 0.05$; 2 × 2 ANOVA) as compared with trout from the Little Blitzen River. In contrast, there was no difference in condition factor between the two streams (Tables 1–3). By design, length, weight, and condition factor did not differ between the two groups of thermally acclimated rainbow trout (Table 3). q9

Metabolism

RMo_2 at 12°C was not significantly different between trout from Bridge Creek and the Little Blitzen River (85 ± 4 and 110 ± 8 mg O_2 $kg^{-0.830}$ h^{-1}, respectively; Table 1). Increasing water temperature acutely from 12° to 24°C elevated RMo_2 in trout from both Bridge Creek (Q_{10} = 2.0 ± 0.4) and the Little Blitzen River (2.3 ± 0.2). However, RMo_2 was also not significantly different between groups at 24°C.

Metabolic rate increased in a curvilinear fashion as swimming speed increased (Fig. 2). At 12°C, $Mo_{2\,max}$ was significantly (37%) greater in Little Blitzen River trout as compared with those from Bridge Creek. This difference in $Mo_{2\,max}$ was reflected in metabolic power, which was 142 mg O_2 $kg^{-0.882}$ h^{-1} greater ($P < 0.05$) for Little Blitzen River trout (Table 1). Bridge

Table 3: Physical characteristics of native redband trout and hatchery rainbow trout used for serum and tissue biochemical analyses

	Redband Trout		Rainbow Trout	
Variable	Little Blitzen (N = 14)	Bridge Creek (N = 14)	Clear Springs, 5°C (N = 12)	Clear Springs, 15°C (N = 12)
Fork length (cm)	19.0 ± .6	22.7 ± 1.2[a]	33.9 ± .6	34.2 ± .6
Body mass (g)	74 ± 8	144 ± 23[a]	495 ± 20	524 ± 30
Condition factor	1.04 ± .02	1.10 ± .03	1.27 ± .05	1.30 ± .05
Ventricle mass (mg)	86 ± 13	125 ± 20[a]	515 ± 24	421 ± 25[a]
RVM (%)	.112 ± .005	.088 ± .003[a]	.106 ± .004	.081 ± .003[a]

Note. Eight males and six females were used from the Little Blitzen River, and five males and nine females from Bridge Creek. All of the Clear Springs trout were females. Relative ventricle mass (RVM) was calculated as (100 × ventricle mass [g] × body $mass^{-1}$). All values are mean ± SEM.

[a] Indicates a significant difference ($P < 0.05$) between streams, or between acclimation temperatures for rainbow trout, as determined by one-way ANOVAs.

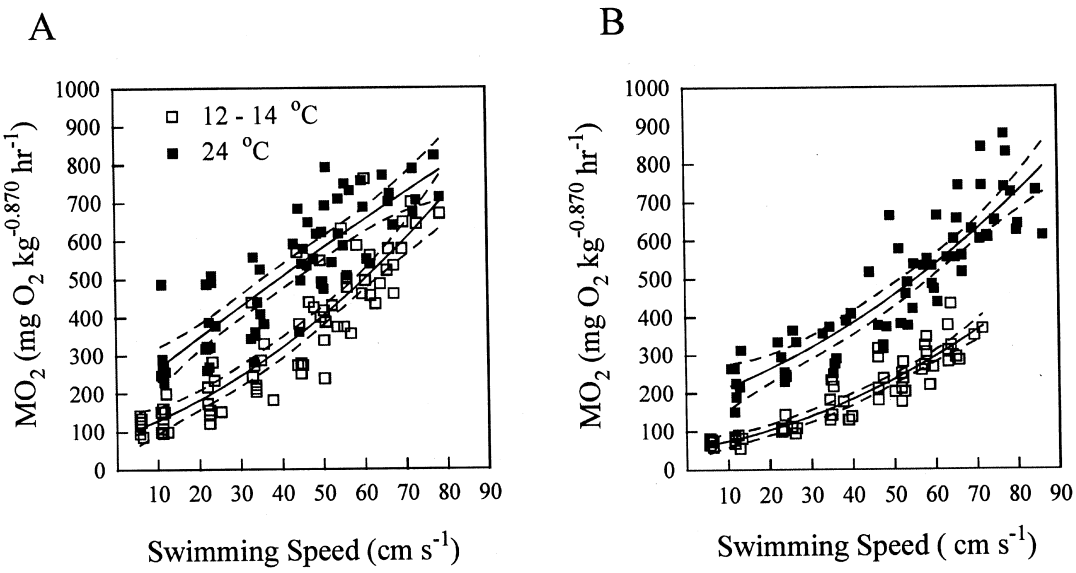

Figure 2. Relationship between mass-adjusted oxygen consumption (Mo_2) and swimming speed for redband trout from (*A*) the Little Blitzen River and (*B*) Bridge Creek. The fitted lines represent second-order regressions of Mo_2 versus swimming speed for each stream/temperature combination. The broken lines represent the 95% confidence intervals about each fitted line. $N = 7–9$ per group.

Creek fish had significantly higher values for $Mo_{2\,max}$ and metabolic power at 24°C (by 67% and 41%, respectively) as compared with 12°C. In contrast, $Mo_{2\,max}$ and metabolic power in Little Blitzen River trout were unaffected by the 12°C increase in temperature. Because of the differential effects of temperature on $Mo_{2\,max}$ and metabolic power, these parameters were not significantly different between the two streams at 24°C.

Swimming Performance and Cost of Transport

Critical swimming speed (U_{crit}) for Bridge Creek redband trout increased from 62 ± 3 cm s^{-1} (3.1 BL s^{-1}) at 12°C to 75 ± 3 cm s^{-1} (3.5 BL s^{-1}) at 24°C. In contrast, the U_{crit} for Little Blitzen River trout was similar at the two temperatures (61 ± 3 vs. 57 ± 4 cm s^{-1}). Based on measurements of metabolic power, it was expected that the U_{crit} for Bridge Creek trout would be lower at 12°C, and similar at 24°C, as compared with trout from the Little Blitzen River. However, this was not the case. U_{crit} values for trout from the two streams were similar at 12°C, and Bridge Creek trout had a significantly greater U_{crit} (by 18 cm s^{-1}) at 24°C.

For trout from both streams, the minimum COT was less for fish swimming at 12°C as compared with 24°C. However, because the minimum COT occurred at a significantly lower swimming speed at 12°C (Fig. 4*A*), COT was only lower at 12°C below swimming speeds of approximately 60 cm s^{-1} (Fig. 3*C*, 3*D*). When the relationship between COT and swimming speed is examined at 12°C (Fig. 3*A*) or 24°C (Fig. 3*B*), it is clear that trout from Bridge Creek swam more efficiently than those from the Little Blitzen River. Minimum COT was significantly lower for Bridge Creek trout at both 12° (by 168 cal $kg^{-0.870}$ km^{-1}) and 24°C (by 232 cal $kg^{-0.870}$ km^{-1}). However, there was no significance difference in the swimming speed at minimum COT between streams at either temperature (Fig. 4*A*).

Temperature Preference

Despite different thermal histories, redband trout from the Little Blitzen River and Bridge Creek trout selected identical temperatures in the thermal gradient (just below 13°C; Table 4). In addition, temperature preference was not significantly affected by fish mass or length.

Tissue Characteristics and Biochemistry

Relative ventricle mass (RVM) was 27% greater in fish from the Little Blitzen River. This difference was almost identical to that measured between rainbow trout acclimated to 5° or 15°C under controlled conditions. Absolute and relative ventricular mass were 22% and 31% higher in 5°C trout, respectively, as compared with those held at 15°C. These data provide compelling evidence that thermal history and water temperature modulate ventricle size in both wild and laboratory-reared trout.

The ANCOVA for maximal LDH activity in redband trout white muscle indicated that the interaction and covariate (mass) terms were significant. After accounting for this relationship,

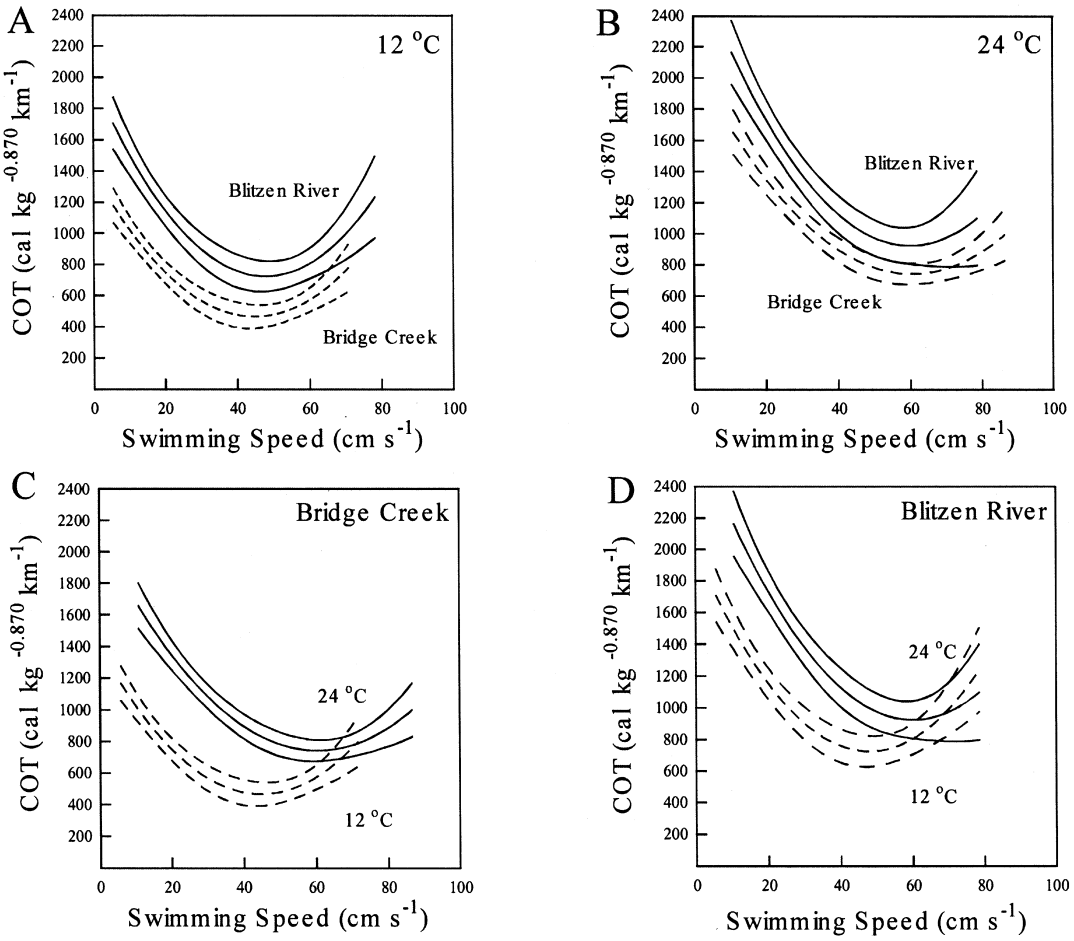

Figure 3. Influence of stream (Blitzen River [*A*] vs. Bridge Creek [*B*]) and water temperature (12°C [*C*] vs. 24°C [*D*]) on the relationship between mass-adjusted cost of transport (COT) and swimming speed in redband trout. In each figure, the fitted lines represent second-order regressions (with 95% condfidence intervals) of COT versus swimming speed. $N = 7$–9 per stream/temperature combination.

Bridge Creek redbands had 35% higher LDH activities than fish from the Little Blitzen River (Table 5). In contrast, the thermal characteristics (Q_{10} and inactivation break point) of LDH proteins in redband trout muscle did not differ between streams (1.7 and 37°C, respectively). There was no relationship between body size and CS activity, and the thermal sensitivity (Q_{10}) of CS did not differ between the groups of redband trout. However, Bridge Creek trout had significantly lower values of white muscle CS (by 40%) than trout from the Little Blitzen River. Despite differences in RVM and in white muscle CS activity between the two streams, similar levels of CS activity were measured in the cardiac muscle of redband trout from the Little Blitzen River (19.6 ± 0.7 U g wet wt^{-1}) and Bridge Creek (18.6 ± 0.5 U g wet wt^{-1}).

A decrease in acclimation temperature of 10°C (from 15° to 5°C) caused changes in white muscle enzyme activities that were of a similar magnitude to the differences observed between Bridge Creek and Little Blitzen redband trout (Table 5). Acclimation of hatchery-reared trout to 5°C increased white muscle aerobic capacity (increased CS activity) but decreased anaerobic capacity (lower LDH activity; Table 5). Further, as with redband trout, exposure to cold temperatures had no effect on myocardial CS activity (5°C, 21.2 ± 1.5 U g wet wt^{-1}; 15°C, 22.2 ± 3.0 U g wet wt^{-1}).

White muscle water content and stored triglyceride concentrations were comparable between redband trout from the Little Blitzen River and Bridge Creek and between hatchery rainbow trout acclimated to 5° and 15°C (Table 5).

Serum/Plasma Composition

Data for serum (wild redband trout) and plasma (hatchery rainbow trout) ions, lipids, osmolality, and proteins are summarized in Table 6. Bridge Creek trout had slightly lower (by

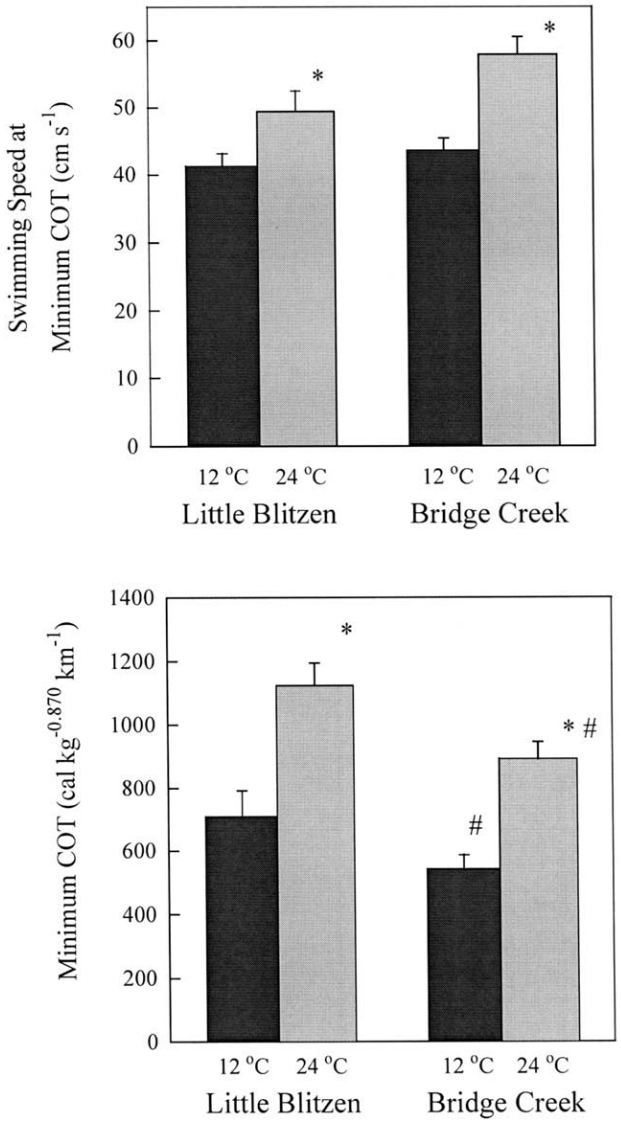

Figure 4. Minimum cost of transport (COT) and swimming speed at minimum COT for redband trout from Bridge Creek and the Little Blitzen River. Asterisks indicate a significant difference ($P < 0.05$) between temperatures within a stream. Pound signs indicate a significant difference between streams within each temperature. $N = 7$–9 per stream/temperature combination. All values are mean ± SEM.

9%) osmolality compared with those from the Little Blitzen. However, this was the only difference detected in serum biochemistry. No differences were observed in plasma from hatchery rainbow trout acclimated to 5° versus 15°C.

Morphometrics

Our analysis of the nine size-adjusted characteristics revealed significant differences in morphology between the two populations of rainbow trout (MANOVA, Wilk's $\lambda = 0.58$, $F_{9,34} = 2.69$, $P = 0.018$). The discriminant function correctly classified 39 of the 44 specimens used in the analysis. The first canonical axis accounted for 71% of the variability in morphological features and was most significantly correlated with four characteristics: caudal peduncle depth, body depth, head width, and mouth width (Table 7). Fish from the Bridge Creek population had higher discriminant scores based on the first canonical axis than those from the Little Blitzen River ($t = 5.47$, $P < 0.0001$), indicating a more robust form with a relatively larger head, and a deeper body and caudal peduncle (Fig. 5).

Genetics

Table 8 shows that there were no significant differences in mitochondrial haplotype or p53 gene allele frequency between fish from the Little Blitzen River and Bridge Creek. However, when fish from these streams are compared to other redband populations within the same basin (e.g., Mud Creek) and adjacent basins (Catlow and Warner Lakes), it is clear that significant differences ($P \geq 0.05$) in haplotype and allele frequencies do occur among wild redband trout populations in southeastern Oregon.

Discussion

The three main objectives of our multifaceted study were (1) to determine whether physiological and biochemical measurements support anecdotal evidence of exceptional warm-water tolerance in redband trout; (2) to define the temperature optima for physiological function in this species; and (3) to examine whether biochemical, genetic, morphological, and/or physiological traits vary between redband trout living in streams with different thermal characteristics. Our results provide direct evidence that redband trout can tolerate acute exposure to at least 24°C and that thermal history does not affect the redband trout's preferred temperature. Further, we show that despite a similar genotype, thermal preference, and geographic proximity, redband trout display differences in physiology, biochemistry, and morphology. Below we discuss how the unique environment inhabited by these trout may have influenced their physiology and compare the current data for redband trout with previously published results for other salmonids.

Metabolism/Swimming Performance: Comparison with Data for other Oncorhynchus mykiss

In this study, we measured oxidative metabolism at a current velocity of 10 cm s^{-1} (ca. 0.5 BL s^{-1}) and refer to these values as "routine Mo_2" or RMo_2. We justified this because wild redband trout (unlike hatchery-reared rainbow trout) become highly agitated in respirometers when no current is present, and wild fish are always exposed to some current in their native

Table 4: Preferred temperatures for redband trout from Bridge Creek and the Little Blitzen River

	N	Fork Length (cm)	Mass (g)	Selected Temperature (°C)
Bridge Creek	13	12.3 ± .4[a]	19.8 ± 2.2[a]	12.9 ± .5
Little Blitzen	11	17.7 ± 2.0	35.9 ± 3.7	12.7 ± .6

Note. Selected temperatures were compared between groups using a one-way ANCOVA with length as the covariate. Mass and length were compared using a one-way ANOVA. Values are mean ± SEM.

[a] Indicates a significant difference ($P < 0.05$) between groups.

streams (i.e., extrapolating O_2 consumption to 0 cm s^{-1} is not ecologically relevant). When redband trout values for RMo_2 at 12°–14°C (88–114 mg $kg^{-0.83}$ h^{-1}) are compared with those for hatchery-reared rainbow trout at 15°C and 15% of U_{crit} (98 mg $kg^{-0.83}$ h^{-1}; Burgetz et al. 1998) or wild rainbow trout at 0.5 BL s^{-1} (105.9 mg $kg^{-0.83}$ h^{-1}; Facey and Grossman 1990), it appears that RMo_2 among all three groups is similar.

The mass-adjusted $Mo_{2\,max}$ of redband trout from the Little Blitzen River and Bridge Creek ranged from 431 to 723 mg $kg^{-0.87}$ h^{-1}, depending on temperature. This range is comparable to measurements taken on rainbow trout collected from the wild (498 mg $kg^{-0.87}$ h^{-1}; Facey and Grossman 1990) and tested at 15°C. However, these values are significantly greater than those reported for hatchery-reared trout (ca. 350–375 $kg^{-0.87}$ h^{-1}; Kiceniuk and Jones 1977; Alsop and Wood 1997; Burgetz et al. 1998, Fig. 9). These results strongly suggest that wild *Oncorhynchus mykiss* exhibit a level of "aerobic fitness" that is at least one-third greater than that of hatchery-reared individuals. However, the degree to which this difference in $Mo_{2\,max}$ was influenced by genotypic or phenotypic characteristics is unclear at this time. Dickson and Kramer (1971) reported that Mo_2 and metabolic power are similar in wild and domesticated rainbow trout reared under hatchery conditions, suggesting that performance differences between wild and domesticated *O. mykiss* are primarily environmental in nature. In contrast, the high metabolic rates reported for wild hatchery-reared sockeye salmon at U_{crit} (ca. 600 mg $kg^{-0.87}$ h^{-1} at 15°C; Brett and Glass 1973) suggest that genotypic differences can also influence $Mo_{2\,max}$ in salmonids. Clearly, more work must be conducted in this area before accurate bioenergetic models can be constructed for wild salmonids.

Although metabolic power in our wild redband trout (300–500 mg $kg^{-0.882}$ h^{-1}) was also approximately 50% greater than values for hatchery rainbow trout (e.g., 264 mg $kg^{-0.882}$ h^{-1} at 15% of U_{crit}; Burgetz et al. 1998), absolute U_{crit} values (57–75 cm s^{-1}; Table 2) were less than or equal to those achieved by domesticated fish tested using similar U_{crit} protocols and temperatures (mean = 70.4 cm s^{-1}; Table 9). Wild redband trout could be poor swimmers as compared with hatchery-reared salmonids. However, this conclusion would not fit with

Table 5: Biochemical characteristics of white axial muscle from native redband trout and thermally acclimated hatchery rainbow trout

			Clear Springs	
Variable	Little Blitzen	Bridge Creek	5°C	15°C
LDH:				
15°C	411 ± 30	619 ± 26[a,b]	519 ± 42	779 ± 45[b]
25°C	670 ± 32	1,058 ± 77[a,b]	ND	ND
Q_{10} (15°–25°C)	1.63 ± .08	1.71 ± .11	ND	ND
LDH break point (°C)	37.0 ± .5	36.9 ± .4	ND	ND
CS:				
15°C	2.97 ± .18	1.72 ± .14[b]	2.91 ± .14	2.27 ± .15[b]
25°C	5.08 ± .30	3.06 ± .25[b]	ND	ND
Q_{10} (15°–25°C)	1.72 ± .06	1.79 ± .08	ND	ND
Water content (%)	78.6 ± 1.4	78.6 ± 2.4	77.7 ± 2.0	77.2 ± 2.8
Triglyceride (mg g^{-1} wet wt)	8.5 ± 1.6	9.4 ± 1.3	7.0 ± 1.7	3.9 ± .5

Note. $N = 8$ for all groups. LDH = lactate dehydrogenase; CS = citrate synthase. ND = not determined. Values are mean ± SEM.

[a] Indicates that ANCOVA revealed a significant interaction ($P < 0.05$) between the covariate mass and stream.

[b] Significantly different by ANOVA ($P < 0.05$).

Table 6: Serum components of redband trout from Bridge Creek and the Little Blitzen River (Oregon) and plasma characteristics from thermally acclimated rainbow trout (Clear Springs, Idaho)

Component	Little Blitzen ($N = 14$)	Bridge Creek ($N = 14$)	ANOVA	Clear Springs ($N = 12$) 5°C	15°C	ANOVA
Albumin (g L^{-1})	28.0 ± 2.4	31.3 ± 1.9	NS	25.2 ± 1.2	22.8 ± 1.2	NS
Calcium (mg dL^{-1})	2.5 ± .2	2.6 ± .2	NS	2.2 ± .1	2.1 ± .1	NS
FFA (mM)	1.07 ± .11	.88 ± .10	NS	.25 ± .04	.27 ± .02	NS
Osmolality (mOsm kg^{-1})	293 ± 4	267 ± 8	Y	311 ± 3	306 ± 2	NS
Potassium (meq L^{-1})	ND	ND		2.6 ± .1	2.5 ± .1	NS
Sodium (meq L^{-1})	134 ± 5	124 ± 6	NS	157 ± 1	158 ± 1	NS
Total protein (g L^{-1})	44.2 ± 2.0	44.3 ± 1.8	NS	31.9 ± 1.0	31.9 ± 2.1	NS
Triglycerides (mg dL^{-1})	333 ± 19	301 ± 27	NS	154 ± 16	174 ± 16	NS

Note. FFA = free fatty acids; NS = not significantly different; Y = significantly different, $P < 0.05$; ND = not determined. Values are mean ± SEM.

existing data. First, McDonald et al. (1998) and Rimmer et al. (1985) demonstrate that wild-caught 1^+ Atlantic salmon are superior swimmers as compared with hatchery-reared conspecifics. Second, the summer (15°C) and fall (10°C) acclimatized wild rainbow trout studied by Facey and Grossman (1990) had size-adjusted U_{crit}'s of 85.3 cm s^{-1} and 104 cm s^{-1}, respectively (Table 9). It is possible that the relatively short period between capture and testing (2–5 d), high levels of stress during swim tunnel confinement, and/or some unknown aspect of our experimental procedures led to reduced swimming performance in redband trout from southeastern Oregon. However, we believe it is more likely that selective pressures in small, shallow streams like the Little Blitzen River and Bridge Creek promote burst swimming performance over sustained, aerobic swimming capability. Although we measured only the latter in the current study, trade-offs between U_{crit} and burst swimming performance have been reported for other fish species (Reidy et al. 2000).

Thermal/Population Effects on Redband Physiology

The definition of optimum temperature in fishes poses a significant challenge because of the wide variety of physiological processes affected by temperature, the potential importance of environmental history, and other factors like life stage and reproductive status. In this study, we did not determine the optimum temperatures for U_{crit} and metabolic power for redband trout. However, the fact that we noted similar or 20% higher values for these parameters at 24° versus 12°–14°C strongly suggests that the swimming performance of these fish is not negatively affected by acute exposure to stream temperatures of 24°C, and that the thermal optimum for some populations (Tables 1, 2) may be shifted toward the upper end of their thermal tolerance zone (critical thermal maximum = 29.0°–29.5°C; Rodnick et al., unpublished data). Previous data on the relationship between temperature and swimming performance for other salmonids indicates that U_{crit} is maximized at temperatures below 18°C (Brett 1964; Taylor et al. 1996) or declines significantly at temperatures above 23°C (Griffiths and Alderdice 1972).

Although our analysis of mitochondrial haplotype and nuclear allele frequencies indicate that the genotype of redband trout from southeast Oregon varies between basins, little or no genetic variation was found between redband trout from Bridge Creek and the Little Blitzen River. This implies that environmental influences were primarily responsible for the temperature-dependent differences in U_{crit}, $Mo_{2\,max}$/metabolic scope, and COT between Bridge Creek and Little Blitzen trout. The importance of rearing conditions in mediating differences in the thermal sensitivity of physiological performance in wild *O. mykiss* is also evident when our data are compared to those of Myrick and Cech (2000). These authors measured RMo_2 and U_{crit} in rainbow trout from Eagle Lake (a high-elevation, alkaline

Table 7: Correlations between nine rainbow trout morphological features and the first canonical factor from a discriminant analysis for fish from Bridge Creek and Little Blitzen River, Oregon

Variable	Canonical Structure	*F* Value[a]	*P* Value[a]
Caudal peduncle depth	.73	11.79	.0014
Body depth	.93	23.35	.0001
Pectoral fin length	.34	2.11	.15
Pelvic fin length	.32	1.83	.18
Head length	.35	2.29	.14
Head width	.53	5.52	.024
Premaxilla length	−.24	1.06	.31
Mouth width	.48	4.48	.04
Eye diameter	.15	.40	.53

[a] Based on univariate ANOVA for each size-adjusted morphological feature.

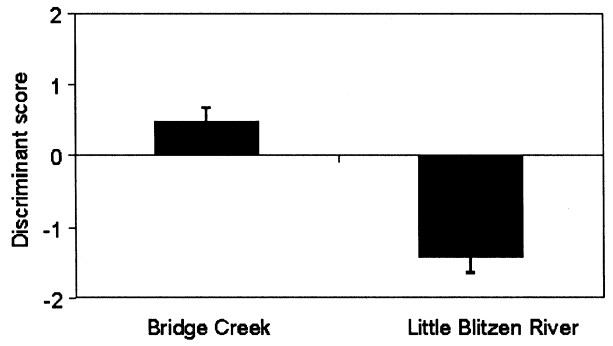

Figure 5. Population scores (mean ± SEM) from the first canonical axis of a discriminant analysis of nine morphological features of Oregon redband trout.

q16 lake) and the Mount Shasta State Fish Hatchery ("a highly inbred stock of generic rainbow trout" [Myrick and Cech 2000, p. 000]) and found no difference in either parameter at temperatures ranging from 10° to 25°C, although the Mount Shasta strain grew faster than the Eagle Lake strain at 22°C. However, in contrast to the redband trout in the current study, both strains of rainbow trout were hatchery reared at the Mount Shasta facility at a constant temperature of 14°C before testing.

Although many environmental parameters can influence the
q17 metabolic and exercise physiology of fishes (Randall and Brauner 1991; Hammer et al. 1995), we believe that temperature was the primary determinant of the physiological differences observed between these two populations of redband trout. Support for this conclusion is twofold: (1) current velocity and water quality in the two streams were similar (pH 8.0–8.7, dissolved oxygen > 8.5 mg L^{-1}, current velocity = 25–66 cm s^{-1}), and (2) acclimation of genetically identical groups of hatchery rainbow trout to cold (5°C) versus warm (15°C) temperatures resulted in differences in white muscle biochemistry and ventricle mass that were representative of those for Little Blitzen and Bridge Creek trout, respectively (Table 5). Whether the disparity in physiological performance/characteristics between the two streams is due to seasonal (summer) differences in their thermal environment or annual variations in stream temperatures (Bridge Creek, 6°–24°C; Little Blitzen River, 0°–18°C) is unclear because no studies have directly examined the influence of acclimatization to diurnally fluctuating temperatures on fish swimming capacity. However, it is noteworthy that the marked difference in temperature-dependent swimming performance between these two populations of redband trout is exactly what would be predicted by Guderley and Blier (1988). These authors suggest that fish that experience/tolerate a broad range of temperatures (i.e., Bridge Creek) have an optimum for locomotion that is shifted toward high temperatures and have markedly restricted locomotor capacity at decreased temperatures. Conversely, fish exposed to a more stable thermal environment (i.e., the Little Blitzen River) have an optimum for swimming performance that is centrally located within their range of tolerated temperatures and do not suffer decreased locomotor function at low temperatures.

Mechanistic Explanations for Differences in Performance

From the comparative analysis of metabolism and U_{crit}, it is clear that (1) trout from the Little Blitzen River are better able to maintain metabolic scope in the "cold" (12°C; Table 2) and

Table 8: Mitochondrial haplotype and nuclear allele frequencies among Little Blitzen River and Bridge Creek redband trout as compared with those from other Oregon Basins

		mtDNA		p53 Locus	
Basin	*N*	RBT-1	RBT-2	RBT-A	RBT-B
Harnev/Malheur:					
Bridge Creek	22	.875	.125*	.636	.364*
Little Blitzen	14	.857	.143*	.643	.357*
Mud Creek	6	.167	.833	.333	.667
Catlow (12 Mile Creek)	12	.083	.917	.667	.333*
Warner Lakes (Rock Creek)	8	.375	.625	.437	.563
Total	62				

Note. Monte Carlo χ^2 analysis and Fisher's exact tests show significant geographic differences ($P \leq 0.05$) in the distributions of haplotypes (Rolf and Bentzen 1989; Motulsky 1995). Allele frequences also show significant differences ($P \leq 0.05$) between Bridge Creek, Little Blitzen River, Catlow Basin as compared with other locations. (Z power = 2.33, power = 98.93%).

* $P \leq 0.05$.

Table 9: Critical swimming speeds (cm s^{-1}) for hatchery-reared and wild *Oncorhynchus mykiss* sp. at temperatures ranging from 10° to 18°C

Study	Temperature (°C)	Fish Length (cm)	Condition Factor	U_{crit}
Jones 1971	12	10.9	1.3	97.5
Jones et al. 1974	12–13	32.8	NA	71.1
Keen et al. 1994	18	34.9	1.1	72.8
Alsop and Wood 1997	15	11	NA	68.2
Waiwood and Beamish 1978	12	9	NA	76.1
Webb 1971	15	12	?	74.8
Kiceniuk and Jones 1977	10	30	NA	69.4
Burgetz et al. 1998	17.5	35.8	1.07	57.3
Keiffer et al. 1998	15	11.5	~1.1	46.5
Mean				70.4
Present study	12–24	~20	~1.05	57–75
Facey and Grossman 1990	10 (fall)	7.8	2.08	104
Facey and Grossman 1990	15 (summer)	7.8	1.75	84

Note. All U_{crit} values were adjusted to a length of 20 cm using the formula $U_{crit} = U_{crit}$ reported/[(fish length/20 cm)$^{0.5}$]. The exponent 0.5 was derived by Brett (1964) for sockeye salmon. Only data for U_{crit} protocols where the time increment was 20 min or greater were utilized because periods shorter than this can artificially inflate U_{crit} values (Beamish 1978; Hammer 1995). All values are mean ± SEM. NA = data not available.

(2) COT is significantly lower for Bridge Creek fish at both temperatures (Fig. 3).

Mechanisms of "Cold Adaptation" in Little Blitzen Trout. Trout from the Little Blitzen River experience cooler water in the summer and a significantly colder winter environment than Bridge Creek trout (see above). We believe that these conditions promoted adaptations that enhanced the ability of Little Blitzen trout to preserve kinetic potential and aerobic capacity when they swam at 12°C. The energy consumed by contracting axial skeletal muscles represents the main cost of aquatic locomotion. Higher rates of oxygen consumption by Little Blitzen trout at 12°C could be achieved by elevating several related mechanisms that promote transfer of oxygen from the water to mitochondria in skeletal muscle. First, cold-induced increases in the heart size of hatchery-reared rainbow trout have been reported previously (Keen and Farrell 1994; Aho and Vornanen 2001) and correlate directly with changes in luminal volume of the heart. Thus, we would predict that the 27% larger ventricle found in Little Blitzen fish would promote a proportionately greater stroke volume, cardiac output, and therefore oxygen delivery to the skeletal muscle. Second, we document a higher aerobic capacity (CS activity) in the axial white muscle of Little Blitzen trout. This finding is consistent with experiments on cold-acclimated or acclimatized hatchery rainbow trout (this study; q18 Guderley and Gowlicka 1992; Cordiner and Egginton 1997) and winter-acclimatized pickerel (Kleckner and Sidell 1985) and strongly suggests that parallel changes occurred in the red axial muscle (Guderley and Gowlicka 1992; Cordiner and Egginton 1997). Red muscle is responsible for the majority of propulsive force at swimming speeds up to 75%–85% of U_{crit}, and its function is highly correlated with aerobic capacity (Webb 1971; Taylor et al. 1996). Given the genetic similarity between fish from the two study streams, and the fact that thermal acclimation rarely leads to qualitative changes in the enzyme isoform expressed in skeletal muscle (Guderley and Blier 1988), it is unlikely that the higher CS activity in axial muscle of Little Blitzen redband trout was mediated by a change in CS isoform. The higher CS activity in the axial muscle of Little Blitzen River trout was probably associated with a cold-induced increase in the amount of enzyme per mitochondrion and/or an increase in mitochondrial density (Dean 1969; Sidell 1983; Egginton q19 and Sidell 1989; Rodnick and Sidell 1994). Although both of these adaptations would have led to increased oxidative/catalytic capacity, an increased mitochondrial density would also have ameliorated the negative effects of cold temperatures on the diffusive exchange of oxygen and metabolites between cellular compartments (Guderley and Blier 1988; Egginton and Sidell 1989).

Lipid, either circulating as nonesterified fatty acids or stored as intracellular triglyceride, is a major fuel of aerobic exercise in rainbow trout (Kieffer et al. 1998). Previous studies have shown that white muscle from rainbow trout will metabolize fatty acids and that cold acclimation increases the catalytic potential for fatty acid oxidation in this tissue (Dean 1969; Guderley and Gowlicka 1992). In the current study, we found no differences in white muscle triglyceride content or plasma FFA levels between the two groups of wild redband trout or between

thermally acclimated hatchery rainbow trout. The former finding agrees with studies by Dean (1969) showing similar total lipids in white muscle of cold- (5°C) versus warm- (18°C) acclimated rainbow trout. Unfortunately, the concentration of fatty acids, alone, without accompanied measurements of metabolic flux, cannot define the rates of lipid storage and utilization in a tissue like white muscle or in vivo. Given the enhanced aerobic scope of Little Blitzen trout at 12°C and higher activities of CS in white muscle, we might predict increased lipid utilization for energy production in these animals. However, Keiffer et al. (1998) found that overall energy metabolism of cold-acclimated (5°C) rainbow trout had a decreased reliance on lipids (and increased dependence on carbohydrates) when swimming as compared with fish acclimated to 15°C. Unlike white muscle, we report similar weight-specific cardiac CS activities in Little Blitzen and Bridge Creek trout, and in hatchery rainbow trout acclimated to 5° or 15°C. However, given the 27% and 23% larger ventricles in the Little Blitzen trout and 5°C-acclimated rainbow trout, respectively, we would argue that the total mass of ventricular mitochondria increased to maintain metabolic capacity and function of this organ at colder temperatures. This conclusion agrees with a previous study on cold- (5°C) versus warm- (25°C) acclimated striped bass (*Morone saxatilis*; Rodnick and Sidell 1994). However, our results are in contrast to those of Cordiner and Egginton (1997), who found that rainbow trout acclimatized to 18°C had lower cardiac CS activity than fish acclimatized to 4° or 11°C. Unfortunately, these latter authors did not report ventricle mass at the different acclimatization temperatures.

Although not examined in the current study, there are a number of other morphological and biochemical alterations that may have enhanced the aerobic capacity of Little Blitzen trout in the cold (12°C). These include increases in the mass (Jones and Sidell 1982; Egginton and Taylor 1996), myoglobin concentration (Cordiner and Egginton 1997), and capillary : fiber ratio (Egginton and Cordiner 1997) of locomotory red muscle and an elevated heart rate at cold temperatures (Aho and Vornanen 2001). However, it is unlikely that alterations in blood oxygen carrying capacity were involved because changes in hematocrit in response to temperature acclimation are neither substantial nor consistent (Farrell 1997), and hemoglobin-oxygen affinity in rainbow trout changes little with acclimation to different temperatures (Eddy 1971).

Swimming Efficiency of Bridge Creek Trout. COT values were generally lower for Bridge Creek trout than for Little Blitzen Trout (Fig. 3). Further, minimum COT values were 24% and 21% lower for Bridge Creek fish at 12° and 24°C, respectively. We believe that several factors may have contributed to the increased swimming efficiency demonstrated by Bridge Creek redband trout. In our studies, we did not measure or take into account the contribution of anaerobic metabolism to swimming performance. Bridge Creek trout had higher (40%–60%) white muscle LDH activities than Little Blitzen trout (Table 5). This increased capacity to generate ATP via anaerobic pathways may have allowed Bridge Creek trout to swim faster at 24°C despite similar levels of $Mo_{2\ max}$/metabolic power, and to swim at comparable swimming speeds at 12°C even though their Mo_2/metabolic power was approximately 28% lower than for Little Blitzen redband trout. This explanation is consistent with the findings of Nelson et al. (1994) and Kolok (1992). Nelson et al. (1994) reported that Bras d'or Lake cod, which inhabit an environment characterized by seasonal fluctuations in temperature and salinity, had an increased reliance on anaerobic metabolism when swimming maximally as compared with cod from the Scotian Shelf (a more stable environment). Kolok et al. (1992) found that white muscle LDH activity was the only significant correlate with U_{crit} in summer-acclimatized largemouth bass (*Micropterus salmoides*). However, increased anaerobic potential cannot explain the lower COT values of Bridge Creek trout when swimming at speeds below 40 cm s^{-1}. White muscle is not recruited until salmonids reach swimming speeds of 70%–80% of U_{crit} (Webb 1971; Burgetz et al. 1998), and thus anaerobic metabolism should have minimal contribution to overall metabolism at slower swimming speeds. Clearly, other physiological or morphological features must have mediated the reported difference in COT between populations at slow swimming speeds. It is also possible that increased muscle efficiency (η_m) and or caudal propeller efficiency (η_p) also contributed to the improved COT of Bridge Creek redband trout. A change in either of these parameters would result in a higher thrust power (P_T) for a given metabolic power (P_{aerob}; $P_T = P_{aerob} \times \eta_m \times \eta_p$; Webb 1977). Although we have no evidence for increased muscle efficiency in Bridge Creek trout, it is likely that η_p was substantially greater in this population. Bridge Creek trout had deeper caudal peduncles (Table 8), and several authors (Taylor and McPhail 1985; Taylor and Foote 1991; Hawkins and Quinn 1996) indicate that salmonids with longer/deeper caudal regions are well suited to sustained swimming at high velocities (i.e., have higher U_{crit}'s). However, the interpretation of how differences in morphology might have affected swimming performance and efficiency is not straightforward. Bridge Creek trout also had deeper bodies and larger heads than individuals from the Little Blitzen River. A more robust body form would have a greater wetted surface area (and thus drag; Webb 1977), and Hawkins and Quinn (1996) suggest that this body shape is least suited to sustained swimming. Clearly, additional studies that relate metabolism, morphology, and swimming kinematics (stride length, tail beat frequency, tail beat amplitude) are required before the differences in swimming efficiency between these two populations can be completely understood.

Preferred Temperature

Despite the difference in maximum summer temperatures and diurnal temperature fluctuations between streams, the preferred

temperature of both groups of redband trout was approximately 13°C (Table 3). This value falls within the narrow range of preferred temperatures reported for 1^+ rainbow trout (10°–15°C; McCauley and Huggins 1979), and the insensitivity of preferred temperature to differences in thermal environment is consistent with data presented by McCauley and Huggins (1979) for 1^+ rainbow trout and by Brett (1952) for chum (*Oncorhynchus keta*) and sockeye salmon (*Oncorhynchus nerka*). Taken together, these data suggest that the thermal preference of juvenile and adult salmonids is genetically determined and relatively independent of immediate thermal history.

There was a fundamental difference in the relationship between optimum temperature for U_{crit}/metabolic power (ca. 24°C) and preferred temperature (ca. 13°C) in Bridge Creek trout, as compared with previous studies on fishes acclimated to different temperatures. Brett (1971) and Kelsh (1996) show that the U_{crit}/metabolic power of sockeye salmon and bluegill (*Lepomis macrochinus*), respectively, are maximized at their preferred temperature. Further, a model constructed by Kelsh and Neill (1990) predicts that even fish with preferred temperatures that are independent of acclimation temperature (salmonids; blue tilapia, *Tilapia aurea*, etc.) will perform best at their preferred temperature. Thus, our results for wild redband trout challenge the generally held opinion that Fry's scope of activity is always an accurate predictor of a fish's preferred temperature. As pointed out by Kelch and Neill (1990), "fishes prefer temperatures that are an integrated optimum for metabolic processes." Given the fluctuating diurnal temperatures (Fig. 1) and temporal variations in food availability to which redband trout are exposed (personal observations), it is likely that the preferred temperature of this subspecies of *O. mykiss* is closely linked to optimal growth (Jobling 1981) or is a compromise between that for optimal growth and that for maximum metabolic power/U_{crit}.

Conclusions

We performed a number of field and laboratory studies on wild redband trout from the High Desert in southeastern Oregon to better understand their physiology and biology. This research provides the first direct evidence that these trout are able to tolerate at least short exposure to temperatures approaching 24°C. Further, our results suggest that differences in winter and/or summer thermal conditions result in phenotypic differences in temperature-dependent swimming performance and metabolism that are the result of alterations in cardiac mass, axial muscle biochemistry, and/or body morphometrics. In contrast to our results for metabolism and swimming performance, we found no evidence for a stream-specific difference in preferred temperature. This result confirms previous work on salmonids acclimated to various temperatures and supports the hypothesis that preferred temperature in adult salmonids is largely genetically determined. Given the discrepancy between this study and that of Myrick and Cech (2000), the marked influence of seasonal acclimatization on fish physiology (Facey and Grossman 1990; Adams and Parsons 1998), and the inability of alterations in hatchery practices to narrow the performance difference between wild and hatchery fish (McDonald et al. 1998), it is clear that accurate data on environmental adaptation in wild fishes will be obtained only if fish are reared and tested under natural conditions.

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