Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon

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Streamside measurements of critical thermal maxima (T_{crit}), swimming performance (U_{crit}), and routine (R_r) and maximum (R_{max}) metabolic rates were performed on three populations of genetically distinct redband trout *Oncorhynchus mykiss* in the high-desert region of south-eastern Oregon. The T_{crit} values ($29 \cdot 4 \pm 0 \cdot 1°$ C) for small (40–140 g) redband trout from the three streams, and large (400–1400 g) redband trout at Bridge Creek were not different, and were comparable to published values for other salmonids. At high water temperatures (24–28° C), large fish incurred higher metabolic costs and were more thermally sensitive than small fish. U_{crit} ($3 \cdot 6 \pm 0 \cdot 1\, L_F\, s^{-1}$), R_r ($200 \pm 13\, mg\, O_2\, kg^{-0 \cdot 830}\, h^{-1}$) and metabolic power ($533 \pm 22\, mg\, O_2\, kg^{-0 \cdot 882}\, h^{-1}$) were not significantly different between populations of small redband trout at 24° C. R_{max} and metabolic power, however, were higher than previous measurements for rainbow trout at these temperatures. Fish from Bridge Creek had a 30% lower minimum total cost of transport (C_{min}), exhibited a lower refusal rate, and had smaller hearts than fish at 12-mile or Rock Creeks. In contrast, no differences in U_{crit} or metabolism were observed between the two size classes of redband trout, although C_{min} was significantly lower for large fish at all swimming speeds. Biochemical analyses revealed that fish from 12-mile Creek, which had the highest refusal rate (36%), were moderately hyperkalemic and had substantially lower circulating levels of free fatty acids, triglycerides and albumin. Aerobic and anaerobic enzyme activities in axial white muscle, however, were not different between populations, and morphological features were similar. Results of this study: 1) suggest that the physiological mechanisms that determine T_{crit} in salmonids are highly conserved; 2) show that adult (large) redband trout are more susceptible to the negative affects of elevated temperatures than small redband trout; 3) demonstrate that swimming efficiency can vary considerably between redband trout populations; 4) suggest that metabolic energy stores correlate positively with swimming behaviour of redband trout at high water temperatures; 5) question the use of T_{crit} for assessing physiological function and defining thermal habitat requirements of stream-dwelling salmonids like the redband trout.   © 2004 The Fisheries Society of the British Isles

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INTRODUCTION

Redband trout *Oncorhynchus mykiss* (Walbaum) are a loosely classified group of rainbow trout subspecies known in Oregon as *O. mykiss newberrii* and *O. mykiss gairdneri*, and in the Sacramento River Valley (California) as *O. mykiss stonei* (Behnke, 1992). These fish commonly inhabit high elevation streams in arid regions that are characterized by extreme variation in seasonal water flow, temperature and dissolved oxygen levels (Behnke, 1992; Vinson & Levesque, 1994; Zoellick, 1999). Despite their wide distribution and apparent adaptability to harsh environmental conditions, however, the redband trout is considered a species at risk (Marshall *et al.*, 1996) due to the recent decline and disappearance of several populations (Nehlsen *et al.*, 1991). At present, the management of redband trout populations is problematic because the physical and biological factors that limit the distribution and physiological tolerance of these fish are not well understood, and very little is known about the effects of environmental factors on the metabolic performance of wild populations of native salmonids.

Given that the physiological performance and bioenergetics of ectotherms are highly dependent on ambient temperature, one possible explanation for the decline in redband trout populations is habitat modification that resulted in elevated stream temperatures. Recent data (Gamperl *et al.*, 2002), however, shows that small (35–110 g) redband trout from two streams (Bridge Creek and the Little Blitzen River, Malheur County, Oregon) can tolerate short-term (<2 h) exposure to warm water temperatures (>24° C), and suggests that thermal history influences the temperature-dependent swimming performance and aerobic metabolism of these fish. Specifically, these data showed that: 1) redband trout from the Little Blitzen River, with a colder thermal history, have similar values for metabolic power and swimming performance at 12 and 24° C; 2) redband trout from Bridge Creek with a warmer thermal history, exhibited greater metabolic power and swimming performance at 24 *v.* 12° C; 3) that thermal history influences temperature-dependent swimming performance, aerobic metabolism, muscle biochemistry and cardiac mass. These data support anecdotal observations that some populations of redband trout may tolerate high water temperatures (Behnke, 1992, Zoellick, 1999), and suggest that biochemical indices may prove valuable for identifying redband populations that are warmwater tolerant. Moreover, despite similar genotypes between Bridge Creek and Little Blitzen redband trout, significant inter-stream differences in body morphology were noted and these variations could have contributed to differences in swimming performance.

Although the results of initial studies (Gamperl *et al.*, 2002) provided new insights into the physiological, biochemical, genetic and morphological characteristics of Oregon redband trout, the research focused on small (1 + year-old), genetically indistinguishable, redband trout from just two streams in close proximity. Consequently, to promote a more extensive understanding of redband trout physiology, the current studies were designed to include different life stages of redband trout and genetically distinct populations. Whether or not the redband trout can be designated as warmwater tolerant relative to other salmonids remains unclear, and such data are essential to the establishment of appropriate stream temperature criteria for these fish. Further, these data are

an important test of hypotheses that salmonids (with the exception of those restricted to high latitudes) have similar thermal tolerances irrespective of origin (Myrick & Cech, 2000) and that the upper thermal tolerance (T_{crit}) of salmonids is phylogenetically conservative (Beitinger *et al.*, 2000).

The specific objectives of this study were: 1) to compare the thermal tolerance and metabolic and performance physiology of adult and juvenile redband trout; 2) to determine the influence of water temperature and morphology on the bioenergetics, thermal tolerance, swimming performance and metabolic biochemistry of genetically distinct redband trout from other tributaries in south-eastern Oregon. To accomplish this, Bridge Creek was revisited to compare the physiology of small and large redband trout, and small redband trout in Bridge Creek were compared with populations from two other streams.

MATERIALS AND METHODS

STUDY SITES AND FISH SAMPLING

The three streams were studied in July and August 2000 (Fig. 1). They were selected for high ($\geq 20°$ C) summer water temperatures and the presence of genetically distinct populations of redband trout (Gamperl *et al.*, 2002). Bridge Creek originates in the Steens Mountain and drains into the Malheur National Wildlife Refuge. During spring and early summer, snowmelt contributes significantly to flow in Bridge Creek, however, mid
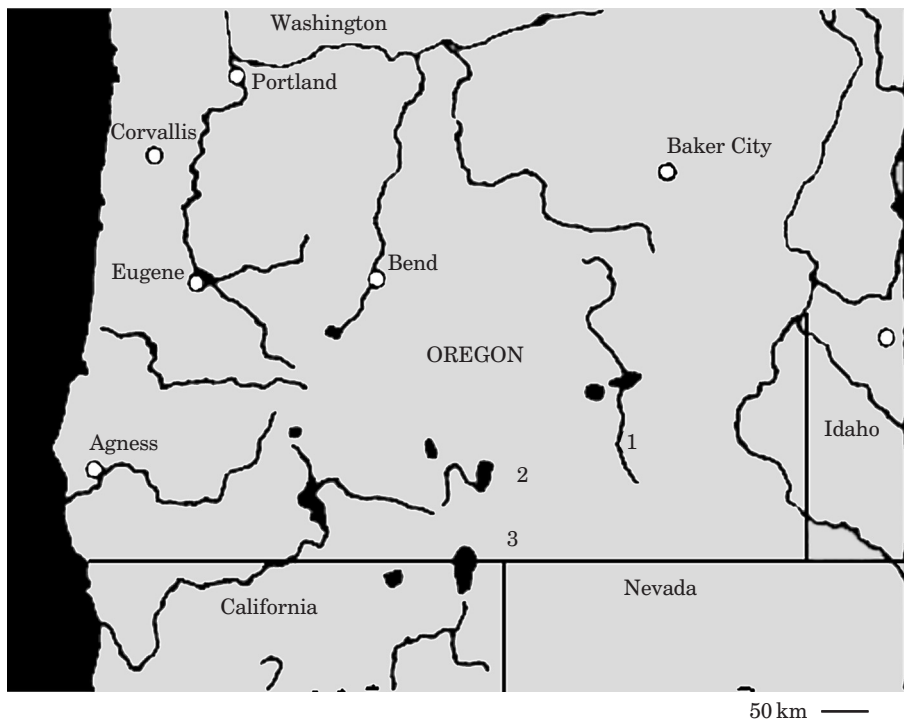

FIG. 1. Study sites in south-eastern Oregon: Bridge Creek (1), Rock Creek (2) and 12-mile Creek (3).

to late summer flow depends primarily upon groundwater from springs providing constant 15° C water. The influence of these springs on stream temperatures is evident when the thermal profile in the upper section of the river is compared with East Canal, which is downstream (Fig. 2). Rock Creek is located in the Guano, Nevada sub-basin and flows through Hart Mountain National Antelope Refuge. Finally, 12-mile Creek is located 65 km south-west of Rock Creek in the Warner Lakes sub-basin, just north of the Nevada border. All three of these streams have very limited riparian vegetation. Rock and 12-mile Creeks, however, were much warmer in the summer months than Bridge Creek (Fig. 2). The average annual precipitation is similar for all three streams [23·8 cm for 12-mile Creek, 25·5 cm for Bridge Creek and 28·8 cm for Rock Creek (Western Regional Climate Center, unpubl. data)].

Redband trout were collected by anglers using dry flies and barbless hooks. In addition, some fish in 12-mile Creek were scooped by hand from undercut banks. Following capture, fish were held in stream cages constructed of PVC pipe (1·9 cm diameter) and 0·6 cm rigid plastic mesh. These cages (0·6 m wide × 1·2 m long or 0·9 m wide × 1·5 m long) were anchored in the stream at depths ranging from 0·5 to 1·0 m, and provided the fish with adequate water flow and protection from sun exposure. Redband trout were held in stream cages for a minimum of 36 h prior to experiments. This study was approved by Animal Welfare Committees at Idaho State and Portland State Universities.

THERMAL TOLERANCE AND SWIMMING PERFORMANCE

Fish were categorized into two size classes according to the dimensions of the respirometers. Small (40–140 g) redband trout 1–2 year-old (based on scale determinations) were studied from all three streams whereas large (400–1400 g) fish 3–5 year-old were only tested at Bridge Creek. All metabolic experiments were conducted using modified Blažka
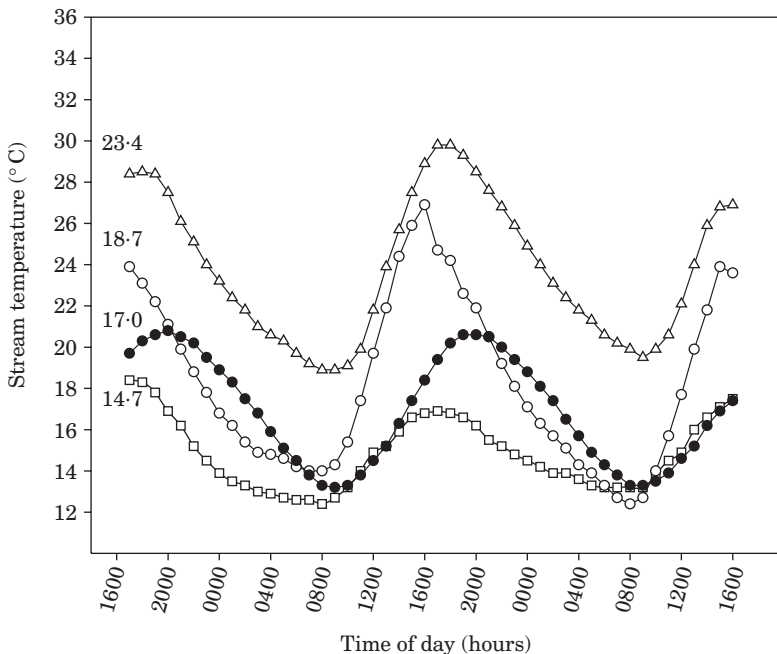

FIG. 2. Diurnal fluctuations in water temperature one day before and after maximum water temperatures were recorded in each stream [△, 12-mile Creek; ○, Rock Creek; ●, Bridge Creek (lower East Canal); □, Bridge Creek (upper)] during July to August 2000. Numbers at the start of each profile represent the mean water temperature for each stream during a 24 h period.

field respirometers with honeycombed plastic grids to promote uniform water flow (Waterloo Biotelemetry Institute, University of Waterloo, Canada). Respirometers were set-up streamside at locations with good vehicle accessibility and where conditions were optimum for placing the stream cages. The front portion of each respirometer was covered with black plastic to provide a darkened refuge and minimize disturbance of the fish due to external stimuli. The rear of the swim section of each respirometer contained a stainless steel grid. To prevent fish from resting on the grid during swimming experiments, electrodes were connected to the grid for application of small ($<5\,\mathrm{V}$) electrical stimuli. Electrical power to run the respirometers and associated equipment was supplied by portable gasoline generators (Models XL 5000 and EXL 6500, Generac Power Systems, Inc., Waukesha, WI, U.S.A.). Water was supplied to the respirometers at $2\,\mathrm{l\,min^{-1}}$ by submersible pumps (Little Giant Pump Co., Oklahoma City, OK, U.S.A.) placed either in the stream (overnight), or in large ($120\,\mathrm{l}$) temperature-controlled reservoirs adjacent to the respirometers (during actual experiments). The water temperature in each reservoir was controlled using thermostatically controlled circulators (Model 1013S, Fisher Scientific, Pittsburgh, PA, U.S.A.), which pumped water through stainless steel coils immersed in the reservoir. Oxygen tensions were maintaied at saturation levels by bubbling air and pulsing pure oxygen into the water.

CRITICAL SWIMMING VELOCITY

A modified critical swimming velocity (U_{crit}) test (Brett, 1964), was used to determine the swimming and metabolic capacity of individual fish. Fish were transferred into the respirometers the evening prior to experiments and exposed to a slow ($0{\cdot}5\,L_{\mathrm{F}}\,\mathrm{s}^{-1}$) current. Routine oxygen consumption (R_{r}) was recorded at $14^\circ\,\mathrm{C}$, thereafter water temperature was increased by $2^\circ\,\mathrm{C\,h^{-1}}$ to $24^\circ\,\mathrm{C}$, and oxygen consumption (M_{O_2}) was measured again. Current velocity was then increased by $10\,\mathrm{cm\,s^{-1}}$ every $20\,\mathrm{min}$ until a swimming speed of $40\,\mathrm{cm\,s^{-1}}$ ($c.\ 2\,L_{\mathrm{F}}\,\mathrm{s}^{-1}$) was achieved, and by $5\,\mathrm{cm\,s^{-1}}$ (small fish) or $10\,\mathrm{cm\,s^{-1}}$ (large fish) thereafter until fish fatigued. At each swimming speed, M_{O_2} was measured for 6–$10\,\mathrm{min}$, beginning $3\,\mathrm{min}$ after the speed was increased. Exhaustion was determined by the inability of the fish to separate itself from the rear grid of the respirometer after successive, mild ($<5\,\mathrm{V}$), electrical shocks. At the end of U_{crit} measurements, fish were anaesthetized (MS 222 $0{\cdot}1\,\mathrm{g\,l^{-1}}$; NaHCO$_3$ $0{\cdot}1\,\mathrm{g\,l^{-1}}$), and L_{F} (cm) and body mass (M, g) were recorded. Body width and depth were also recorded to correct measurements of swimming speed for solid-blocking effects according to Jones *et al.* (1974). The condition factor (K) was calculated from $K = 100\,M\,L_{\mathrm{F}}^{-3}$.

Water temperature and oxygen content within each respirometer were measured continuously by pumping water through an external circuit containing a custom flow chamber and a galvanic oxygen electrode equipped with thermal sensor (Model CellOx 325 WTW Inc., Weilheim, Germany). This oxygen electrode was connected to an oxygen meter (Model Oxi 340, WTW Inc.) equipped with automatic temperature and altitude compensation. When stream temperatures did not match the desired experimental temperatures, the respirometer received water from thermostatically controlled reservoirs, and oxygen tensions were maintained at saturation levels. Oxygen consumption was measured at the beginning of each experiment, and at all swimming speeds by stopping the flow of fresh water into the respirometer and recording the drop in water oxygen content. M_{O_2} ($\mathrm{mg\,O_2\,h^{-1}}$) was calculated as $M_{\mathrm{O}_2} = 60 V_{\mathrm{c}}\ (C_{\mathrm{i}} - C_{\mathrm{f}})\ t^{-1}$ where $C_{\mathrm{i}} =$ water oxygen content ($\mathrm{mg\,l^{-1}}$) at the start and $C_{\mathrm{f}} =$ water oxygen content at the end of the measurement, $V_{\mathrm{c}} =$ volume of the respirometer and external circuit ($6{\cdot}8\,\mathrm{l}$ and $8\,\mathrm{l}$ for the small and large respirometers, respectively), and $t =$ time (min) required to make the M_{O_2} measurements. Metabolic power ($\mathrm{mg\,O_2\,h^{-1}}$) was calculated as maximum M_{O_2} (R_{max}; measured at maximum swimming speed) minus routine M_{O_2} (R_{r} at $0{\cdot}5\,L_{\mathrm{F}}\,\mathrm{s}^{-1}$). Metabolic variables ($R_{\mathrm{max}}$, metabolic power, and R_{r}) for Bridge Creek fish were plotted with body M on log scales, and slopes from these equations were used to control for the effects of body mass for all redband trout.

Critical Swimming Speed (U_{crit}) was calculated as: $U_{\mathrm{crit}} = V + t_{\mathrm{f}}\ V_{\mathrm{i}}\ t_{\mathrm{i}}^{-1}$, where $V =$ velocity at which the fish swam for the entire time increment, $V_{\mathrm{i}} =$ velocity increment (5 or $10\,\mathrm{cm\,s^{-1}}$); $t_{\mathrm{f}} =$ 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). Swimming efficiency was measured as cost of transport (C) (Schmidt-Nielsen, 1972), using an oxycalorific coefficient of 13·61 J from (3·25 cal) mg O_2^{-1} (Bradfield & Solomon, 1972). For each fish, a second-order regression was fitted to the relationship between swimming speed (cm s^{-1}) and C, and the minimum C (C_{min}) and swimming speed (U_{crit}) at C_{min} were estimated from the derived relationship.

CRITICAL THERMAL MAXIMUM

The evening before each experiment, individual fish were transferred into a respirometer. Water was supplied directly from the stream overnight *via* a submersible pump, and current velocity was maintained at *c.* $0.5 L_F$ s^{-1}. The following morning, the submersible pump was transferred to a reservoir containing stream water at 14° C. Testing began at 0700–0900 hours and lasted *c.* 8 h. In these experiments, water temperature was increased by 2° C h^{-1} until the fish lost equilibrium. This rate of temperature increase approximated the maximum rate of heating that redband trout experience during a summer diurnal cycle of stream temperature (Zoellick, 1999; Gamperl *et al.*, 2002; Fig. 2). The R_r was normally measured over 8–12 min intervals after every 2° C increase in temperature. At the highest temperatures (>28° C), however, measurements were taken every 1° C to obtain data just prior to the loss of equilibrium. Given that spontaneous activity varied considerably between temperatures for individual fish and between fish, activity was scored every 2 min during each MO_2 measurement according to a 0–10 scale. When the average activity score was high (>4), measurements were excluded from the analysis. When high activity occurred at ≤24° C, temperature increases were delayed for a short time (<30 min), and measurements of MO_2 were repeated if possible. Measurements were not repeated at higher temperatures to avoid varying the duration of exposure to sublethal extremes.

To assess the effects of extreme temperature on metabolic rate, thermal sensitivity (Q_{10}) was calculated over two temperature ranges: 14–24 and 24–28° C for small redband trout and one range (14–26° C) for large fish since data were only collected on a limited number of individuals ($n = 3$) at 28° C. Immediately after the T_{crit} was recorded, fish received a cooler (4–6° C below T_{crit}) source of water, and temperature was reduced further (by 5–10° C h^{-1}) until temperature in the respirometers reached stream values. Fish were anaesthetized with clove oil (0·1 ml l^{-1}), weighed (nearest g) and measured (L_F and body width and depth at the anterior margin of the dorsal fin) using calipers. Fish had their adipose fins clipped, were allowed to recover from anaesthesia, and were released back into their native stream.

TISSUE COLLECTION

Additional redband trout were anaesthetized with buffered MS-222, weighed, measured (L_F), and blood was drawn from the caudal vessels using sterile syringes and lithium heparin as the anticoagulant. Plasma was separated by a centrifuge, and samples of both plasma and whole blood were frozen in liquid nitrogen. The liver and cardiac ventricle were excised, rinsed in ice-cold 1·0% (w/v) NaCl, blotted dry, and weighed to the nearest 0·01 g. Epaxial white muscle on the right side of each fish was excised from beneath the dorsal fin and dissected free of obvious bone, fat and connective tissue, and freeze clamped at the temperature of liquid nitrogen. All samples were transported to Idaho State University under liquid nitrogen and stored at −80° C until assays were conducted.

PLASMA AND WHOLE BLOOD ANALYSES

Plasma osmolality was determined by vapour osmometry (Wescor Model 5520, Logan UT, U.S.A.). Sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratory Model 943, Lexington, MA, U.S.A.). Total plasma calcium was estimated by a binding reagent (Arsenazo III, Sigma Procedure No. 588). Free (non-esterified) fatty acids (FFA) were measured by an enzymatic method (NEFA C kit, Wako

Chemicals, Richmond, VA, U.S.A.) and triglycerides were determined using the INT reagent (Sigma Procedure No. 336) according to the manufacturer's instructions. Plasma albumin concentration was measured with the bromocresol green binding technique (Sigma Procedure No. 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, CA, U.S.A.) with BSA as the standard. Haemoglobin concentration in whole blood was quantified using the Sigma Diagnostic Procedure (Number 525). The mixture (20 µl of whole blood and 5 ml of Drabkin's reagent) was centrifuged for 3 min at $10\,000\,g$ and $4°$ C, and absorbance of the resulting supernatant at 540 nm was recorded and compared to haemoglobin standards.

TISSUE BIOCHEMISTRY

Assays of maximal lactate dehydrogenase (LDH) and citrate synthase (CS) activities were conducted on whole homogenates of frozen white muscle at $15°$ C according to the methods provided in Gamperl *et al.* (2002). These two enzymes provide indices of anaerobic and aerobic energy metabolism, respectively. For both assays, *c.* 25 mg of tissue was homogenized in 19 volumes of ice-cold extraction medium using motor-driven Duall-21 ground-glass homogenizers (Kontes Glass Co., Vineland, NJ, U.S.A.). Enzyme activities are expressed as units (U) g^{-1} wet tissue mass, where 1 U denotes the conversion of 1 µmol of substrate to product per min. The final volume for each assay was $1·0$ ml, and all activities were linear over the reaction period. Chemicals were of analytical grade and purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.).

Muscle triglycerides were isolated using chloroform/methanol methods as described by Kinney LaPier & Rodnick (2000). Triglyceride concentration was measured enzymatically by adding the prepared tissue sample or standards to a triglyceride INT reagent (Sigma Procedure No. 336). Per cent water content in the skeletal muscle was determined by lyophilizing frozen tissue to a constant mass and expressing water as a percentage of tissue mass per cent water $= 100 - 100$ (dry mass) (wet mass)$^{-1}$.

MORPHOMETRICS

To assess potential morphological differences between redband trout populations, external and internal features were measured from specimens collected from the three study streams. Specimens were preserved in 10% formalin for 2 weeks, and then placed in 37% isopropanol before measurements were made. Nine body features were assessed 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 data using a software package (WinWedge, version 1.2, Tal Technologies, Philadelphia, PA, U.S.A.). Redband trout of different sizes were also examined for absolute and relative mass of red and white axial muscle compartments. Preserved specimens were cross-sectioned at the anterior margin of the anal fin and photographed with a digital camera. Images were imported into Scion Image Software (Scion Corp., Frederick, MD, U.S.A.), red and white muscle cross-sectional areas were traced with a cursor, and calculated automatically.

STATISTICS

Statistical analyses were performed using StatView and SAS software (SAS, 1999). All data presented in the text, figures and tables are means $\pm$ S.E. and statistical significance for all statistical tests was set at $P < 0·05$. Given the size ranges of animals studied, all metabolic data were mass-adjusted. A one-way ANCOVA was used to examine whether the slopes of the regression lines between $\log_{10}$ oxygen consumption (*i.e.* R_r, R_{max} and metabolic power; mg $O_2\,h^{-1}$) and $\log_{10} M$ (kg) were different between the groups of small redband trout. Because the slopes of the regression lines were not significantly different between groups ($P > 0·05$), these variables were converted into mass-independent values

(Cech, 1990; Myrick & 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 1400 g (Fig. 3).

ANCOVA, with M as the covariate, was used to examine whether biochemical characteristics were different between streams or size classes. One-way ANOVAs were performed, followed by Tukey-Kramer *post hoc* tests, to assess differences between streams and size classes in U_{crit}, T_{crit}, C_{min}, R_r, R_{max}, metabolic power and Q_{10} values. Repeated measures ANOVA was used to test for between stream differences in R_r as the temperature was increased from 14° C until loss of equilibrium, and for differences in Q_{10} values between temperature ranges (14–24 *v*. 24–28° C) within each stream.

To determine if there were morphological differences between the populations of redband trout, morphological measurements were $\log_{10}$-transformed, and each trait was regressed against its corresponding $\log_{10}$-L_F using least-squares regression. The common within group slope method was then used to adjust for body size differences among specimens (Reist, 1985). As an overall assessment of morphological differences across populations, a multivariate analysis of variance (MANOVA) was then performed on the nine size-adjusted morphological variables. To identify which traits varied significantly, a univariate ANOVA was employed for each morphologic trait using the Tukey-Kramer multiple comparison method to identify which population means differed from each other. Statistical significance was set at $P < 0.05$ using type III sum-of-squares (SAS, 1999).

RESULTS

CRITICAL THERMAL MAXIMUM

The T_{crit} for redband trout was highly conserved between size classes (Bridge Creek) and between streams (Table I). Values for individual fish ranged from
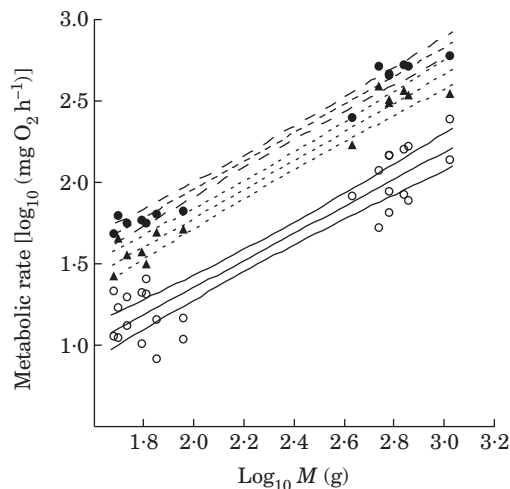

FIG. 3. $\log_{10}$ mass of redband trout and $\log_{10}$ routine metabolic rate (○—R_r, 14° C, $n = 13$; 24° C, $n = 14$; $y = 0.83x - 0.303$, $r^2 = 0.99$), metabolic power (▲–, 24° C, $n = 14$; $y = 0.882x + 0.018$; $r^2 = 0.98$) and maximum oxygen consumption (●– R_{max}, 24° C, $n = 14$; $y = 0.87x + 0.219$; $r^2 = 0.94$), as measured during critical swim speed (U_{crit}) tests in combined size classes (40–140 and 400–1400 g) of fish from Bridge Creek. Lines represent linear regression equations with 95% CI. Slopes from these equations were used for mass correction of all other data (Cech, 1990).

TABLE I. Critical thermal maximum values for small (40–140 g) redband trout from three streams and large (400–1400 g) fish at Bridge Creek. One-way ANOVA showed that there were no significant ($P < 0.05$) differences between groups

Stream	Mean ± S.E. T_{crit} (°C)
12-mile $n = 8$	29·7 ± 0·3
Rock $n = 8$	29·6 ± 0·1
Bridge (small) $n = 7$	29·0 ± 0·2
Bridge (large) $n = 5$	29·0 ± 0·4

28·0–30·8° C, and the overall mean for all streams and size classes ($n = 27$) was 29·4 ± 0·1° C.

ROUTINE OXYGEN CONSUMPTION AND Q_{10}

Initially, R_r increased at a relatively constant rate as water temperature was increased from 14° C. The values of R_r, however, began to increase at a substantially faster rate when large redband trout reached 22° C and small fish reached 24° C (Fig. 4). For small redband trout, mass-adjusted R_r was not significantly different between streams at 14, 24 or 28° C, and no differences were detected in Q_{10} values between streams from 14 to 24° C (2·2 ± 0·3, $n = 21$) or from 24 to 28° C (4·9 ± 0·6, $n = 18$) (Table II). Mass–adjusted R_r was similar between size classes at 14–22° C, but was 40–60% higher for larger redband
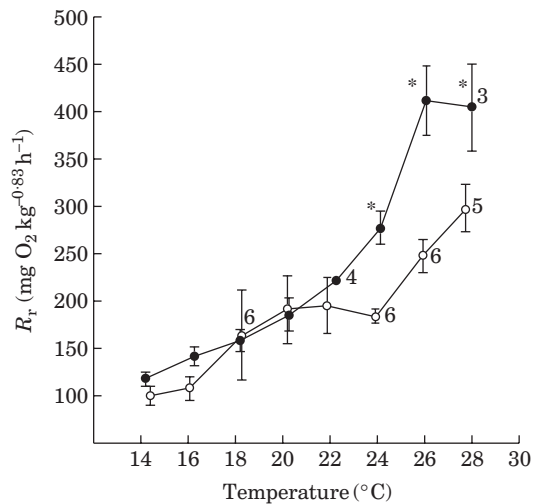

FIG. 4. Water temperature and mean ± S.E. routine metabolic rate for large (●, 400–1400 g; $n = 5$) and small (○, 40–140 g; $n = 7$) redband trout as measured during critical thermal maximum (T_{max}) tests at Bridge Creek. Numbers next to symbols indicate reduced sample size due to variability in the T_{max} measurement or exclusion of redband trout exhibiting high (≥4) activity levels. The mass exponent (−0·83) was derived from regression equations shown in Fig. 3. *, Significant difference between large and small redband trout within each temperature ($P < 0.05$).

TABLE II. Routine metabolic rate (mg O_2 kg$^{-0.83}$ h^{-1}) and Q_{10} values for small (40–140 g) and large (400–1400 g) redband trout as measured during critical thermal maximum (T_{crit}) tests. Values are means ± S.E. The mass exponent (−0.83) was derived using regression equations shown in Fig. 3. Comparisons between streams (or size classes at Bridge Creek) were made using one-way ANOVA followed by Tukey-Kramer *post hoc* tests. Dissimilar superscript letters indicate a significant difference ($P < 0.05$) between small redband trout from 12-mile, Rock and Bridge Creeks. *, A significant difference between the two size classes of fish from Bridge Creek. Q_{10} values from 24 to 28° C were compared with those from 14 to 24° C using repeated measures ANOVA, [+]a significant ($P < 0.05$) difference within each stream. Superscript numbers indicate reduced sample size due to variability in the T_{crit}, exclusion of redband trout exhibiting high (≥4·0) activity levels, or loss of data due to equipment problems. The Q_{10} from 24 to 28° C was not calculated for large Bridge Creek redband trout since data at 28° C were only collected for three individuals

Stream	K	M (g)	R_r (14° C)	R_r (24° C)	R_r (26° C)	R_r (28° C)	Q_{10} 14–24° C	Q_{10} 14–26° C	Q_{10} 24–28° C
12-mile n = 8	0·88 ± 0·07	94 ± 5[a]	74·5 ± 6·6	150·9 ± 10·7	214·1 ± 29·9	254·3 ± 27·9[6]	2·1 ± 0·1	2·4 ± 0·4	4·7 ± 1·3[+6]
Rock n = 8	0·92 ± 0·02	54 ± 3[b]	74·3 ± 13·1	154·9 ± 22·1[7]	239·2 ± 37·8	291·2 ± 21·8	2·5 ± 0·6[7]	2·4 ± 0·6	5·4 ± 0·8[+7]
Bridge (small) n = 7	1·04 ± 0·05	79 ± 8[a]	100·2 ± 10·3	184·2 ± 8·0[6]	248·3 ± 17·6	296·8 ± 25·0[5]	2·1 ± 0·2	2·1 ± 0·1	4·2 ± 1·3[+5]
Bridge (large) n = 5	1·05 ± 0·06	1044 ± 17*	117·8 ± 8·1	277·5 ± 17·3*	411·5 ± 36·4*	405·9 ± 47·5*[3]	2·4 ± 0·2	2·9 ± 0·3*	—

trout at 24, 26 and 28° C (Fig. 4 and Table II). These data suggest that larger redband trout are more thermally sensitive and incur higher metabolic costs than small redband trout during acute exposure to high water temperatures.

SWIMMING PERFORMANCE AND METABOLISM

The R_r, R_{max} and metabolic power at 24° C were not significantly different between streams, averaging 200 ± 13 mg O_2 kg$^{-0.83}$ h^{-1}, 739 ± 20 mg O_2 kg$^{-0.87}$ h^{-1} and 533 ± 22 mg O_2 kg$^{-0.882}$ h^{-1}, respectively (Table III). Absolute U_{crit} was 20% higher in small redband trout from Bridge Creek as compared with Rock and 12-mile Creek, although relative U_{crit} (L_F s^{-1}) was not different between streams (overall mean $= 3.59 \pm 0.11$ L_F s^{-1}; $n = 24$; Table IV).

Both absolute and relative U_{crit} for small redband trout were positively correlated with metabolic power (Fig. 5). After controlling for differences in metabolic power with ANCOVA, relative U_{crit} was higher for Bridge Creek redband trout than fish from 12-mile Creek, and absolute U_{crit} was higher at Bridge Creek than at Rock and 12-mile Creeks (Table IV). These data reflect greater metabolic efficiency during maximal swimming by Bridge Creek redband trout.

It is noteworthy that a dramatic difference was observed in the willingness of small redband trout to swim in the respirometers. Absolute refusals to swim occurred only once in eight trials (12%) involving Bridge Creek redband trout, whereas Rock Creek fish refused four out of 12 tests (33%) and 12-mile Creek fish failed to perform in five out of 14 tests (36%). Further, among the fish that did swim to U_{crit}, the average number of temporary refusals observed prior to the highest velocity increment (where a 'refusal' is defined by cessation of voluntary swimming requiring electrical stimulation and momentarily reduced current velocity to induce the fish to resume swimming) was also substantially lower at Bridge Creek (0.3 refusals per trial) compared with Rock (1.5 refusals per trial) and 12-mile (2.2 refusals per trial) Creeks.

Relative U_{crit} did not differ significantly between size classes at Bridge Creek (mean $\pm$ S.E. $= 3.55 \pm 1.84$ L_F s^{-1}; $n = 14$), even though absolute U_{crit} was *c.* 76% higher for the large trout (Table IV). In contrast to small redband trout, metabolic power did not explain a significant amount of variance in relative or absolute U_{crit} for large fish (Fig. 5).

COST OF TRANSPORT

The relationship between total cost of transport and swimming velocity, described by second-order regressions, was similar between streams at low velocities (<40 cm s^{-1}). At swimming velocities >40 cm s^{-1}, however, small Bridge Creek redband trout were more efficient [Fig. 6(a)]. Although the minimum total cost of transport was 30% lower at Bridge Creek compared with Rock and 12-mile Creeks (Table IV), the optimum swimming speed was not different between streams (overall mean $\pm$ S.E. 2.67 ± 0.11 L_F s^{-1}, $n = 23$; Table IV) and corresponded to 70–75% of U_{crit}. Large redband trout from Bridge Creek were more efficient than small fish at all swimming velocities [Fig. 6(b)], although relative U_{opt} (optimum swimming velocity) was similar for different size fish (Table IV).

TABLE III. Mean ±s.e. metabolic variables for small (40–140 g) and large (400–1400 g) redband trout as measured during critical swimming velocity tests at 24° C. The mass exponents (b) were derived using regression equations shown in Fig. 3. One-way ANOVA showed that there were no significant ($P < 0.05$) differences in any metabolic variables between streams

Stream	M (g)	R_r $b = 0.830$ (mg O_2 kg^{-b} h^{-1})	Routine activity score	R_{max} $b = 0.870$ (mg O_2 kg^{-b} h^{-1})	Metabolic power $b = 0.882$ (mg O_2 kg^{-b} h^{-1})
12-mile $n = 9$	56·5 ± 3·8	201·2 ± 30·2	2·3 ± 0·2	775·6 ± 21·6	568·9 ± 35·1
Rock $n = 8$	50·4 ± 2·9	197·6 ± 8·5	1·8 ± 0·4	756·8 ± 41·0	553·5 ± 35·4
Bridge (small) $n = 7$	62·9 ± 5·6	200·5 ± 22·9	2·2 ± 0·3	672·0 ± 35·9	462·6 ± 35·7
Bridge (large) $n = 7$	662·6 ± 74·7	212·2 ± 8·7	1·0	689·4 ± 42·4	476·2 ± 42·1

TABLE IV. Swimming performance and efficiency variables (means ± S.E.) for small (40–140 g) and large (400–1400 g) redband trout as measured during critical swimming velocity tests at 24° C. Dissimilar small letters indicate significant ($P < 0.05$) differences in a variable between streams, detected using one-way ANOVA followed by Tukey-Kramer *post hoc* tests. Dissimilar capital letters indicate significant ($P < 0.05$) differences in small redband trout U_{crit} between streams, after controlling for differences in metabolic power with ANCOVA. *, A significant difference between large and small redband trout from Bridge Creek. Superscript numbers indicate reduced sample size due to exclusion of fish from C_{min} and U_{opt} analyses because values could not be accurately calculated from fitted curves

| Stream	K	L_F (cm)	U_{crit} ($L_F\,s^{-1}$)	U_{crit} ($cm\cdot s^{-1}$)	U_{opt} ($cm\,s^{-1}$)	U_{opt} ($L_F\,s^{-1}$)	C_{min} $J\,g^{-0.87}\cdot h^{-1}$ ($cal\,g^{-0.87}\,h^{-1}$)
12-mile $n = 9$	0·97 ± 0·02	17·9 ± 0·4[ab]	3·41 ± 0·17[A]	60·7 ± 2·4[aA]	43·1 ± 2·8[a7]	2·43 ± 0·19[7]	9·72 ± 0·84[a7] (2·32 ± 0·20)
Rock $n = 8$	1·09 ± 0·07	16·7 ± 0·4[b]	3·55 ± 0·19[AB]	59·1 ± 3·0[aA]	44·7 ± 2·4[b]	2·68 ± 0·17	9·93 ± 0·63[a] (2·37 ± 0·15)
Bridge (small) $n = 7$	0·99 ± 0·01	18·5 ± 0·5[a]	3·88 ± 0·23[B]	71·2 ± 3·1[bB]	52·8 ± 2·4[b]	2·89 ± 0·20	6·96 ± 0·46[b] (1·66 ± 0·11)
Bridge (large) $n = 7$	1·05 ± 0·04	39·6 ± 1·6*	3·21 ± 0·23	125·5 ± 5·7*	101·7 ± 8·1*[5]	2·63 ± 0·35[5]	3·98 ± 0·46*[5] (0·95 ± 0·11)
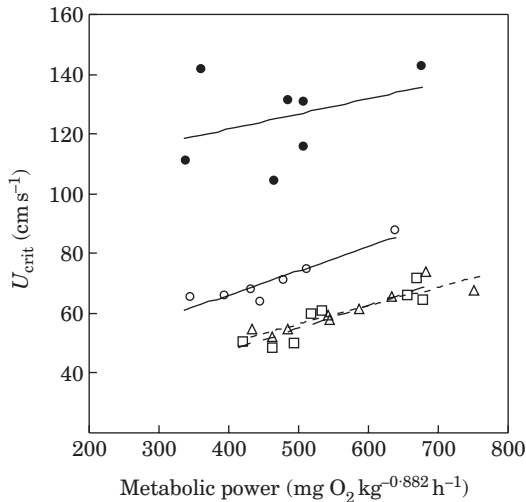
FIG. 5. Metabolic power and critical swimming velocity for redband trout at 24° C. The curves were fitted by: $y = 0.081x + 33.9$ ($n = 7$; $r^2 = 0.87$) for small (40–140 g) redband trout at Bridge Creek (○), $y = 0.077x + 16.7$ ($n = 8$; $r^2 = 0.82$) at Rock Creek (□) and $y = 0.061x + 25.8$ ($n = 9$; $r^2 = 0.82$) at 12-mile Creek (□). Metabolic power did not explain a significant amount of variance in U_{crit} for large (400–1400 g) redband trout at Bridge Creek (●; $P > 0.05$). ANCOVA showed that the slopes for small fish were not significantly different between streams. The mass exponent (0.882) was derived using regression equations shown in Fig. 3.

PLASMA AND TISSUE BIOCHEMISTRY

The L_F, M and K of redband trout used for biochemical analyses were not equal (Table V). Fish from Rock Creek were significantly smaller than fish from Bridge and 12-mile Creeks, and had lower K than fish from Bridge Creek. In addition, the relative ventricle mass of Bridge Creek redband trout was lower (21–33%) as compared with the other populations. Most of the plasma and blood variables were similar between populations, however, potassium levels showed distinct differences between streams (Table V). Fish from 12-mile Creek appeared hyperkalemic. Blood lipids (free fatty acids and triglycerides) were substantially lower in Rock and 12-mile Creeks as compared with Bridge Creek, however these variables were only significantly reduced in redband trout from 12-mile Creek. Albumin concentrations were lower in Rock and 12-mile Creek fish. Evidence for a specific reduction in albumin levels is provided by a lower albumin: total protein ratio in fish from 12-mile Creek.

Maximal activities of LDH in axial white muscle increased significantly with increasing body mass [Fig. 7(a)]. No additional variance in LDH activity could be explained when accounting for differences among populations in either the elevation (ANCOVA, d.f. = 2 and 45, $P = 0.17$) or slope (ANCOVA, d.f. = 3 and 20, $P = 0.31$) of the relationship [Fig. 7(a)]. This analysis provides evidence for positive allometry of anaerobic metabolism over the size range of redband trout examined. As expected, white muscle exhibited much lower CS activity and this enzyme did not scale with body mass [Fig. 7(b)]. The water content of white muscle was similar between populations, averaging 79·4–80·0%, as were
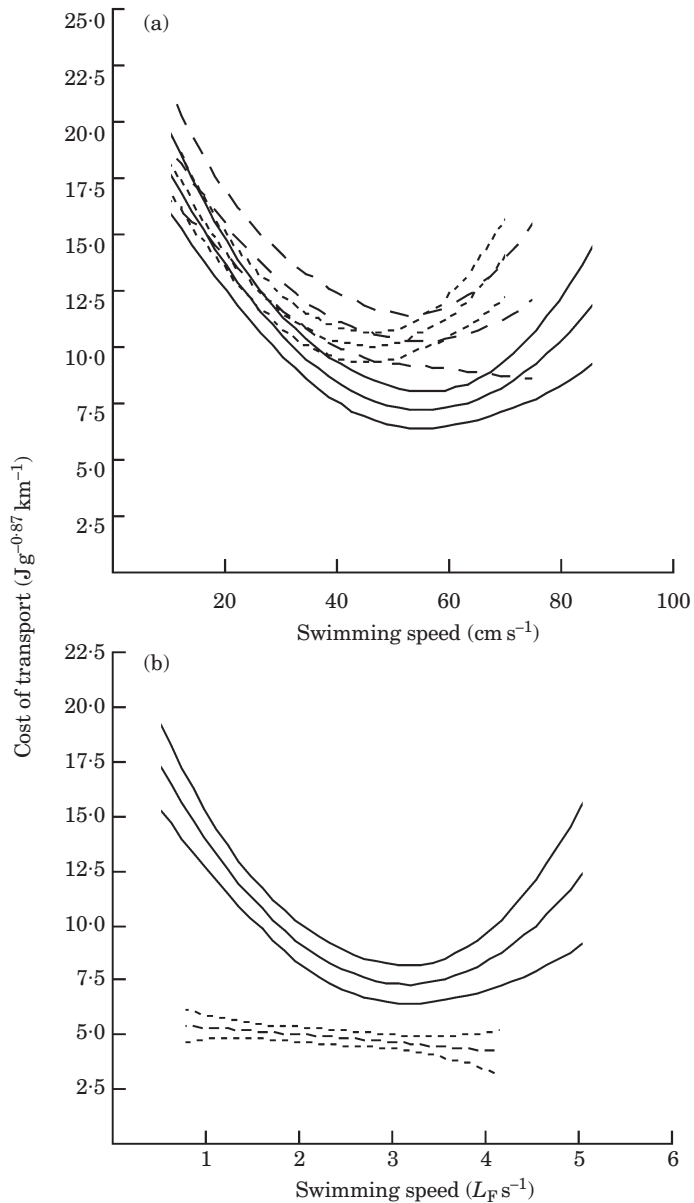
FIG. 6. Swimming velocity and total cost of transport for (a) small (40–140 g) redband trout from Bridge (——) ($y = 0{\cdot}0051x^2 - 0{\cdot}571x + 23{\cdot}1$; $r^2 = 0{\cdot}75$; $P < 0{\cdot}0001$), Rock (- - -) ($y = 0{\cdot}0066x^2 - 0{\cdot}602x + 23{\cdot}71$; $r^2 = 0{\cdot}77$; $P < 0{\cdot}0001$) and 12-mile (— —) ($y = 0{\cdot}0044x^2 - 0{\cdot}48x + 23{\cdot}37$; $r^2 = 0{\cdot}57$; $P < 0{\cdot}0001$) Creeks, and for (b) large (- - -) (400–1400 g; $y = -0{\cdot}0146x^2 - 0{\cdot}283x + 5{\cdot}631$; $r^2 = 0{\cdot}268$; $P > 0{\cdot}05$) and small (——) (40–140 g; $y = 0{\cdot}0051x^2 - 0{\cdot}571x + 23{\cdot}1$; $r^2 = 0{\cdot}75$; $P < 0{\cdot}0001$) redband trout from Bridge creek at 24° C. Lines show fitted least-squares regression curves with 95% CI. The mass exponent (0·87) was derived using regression equations shown in Fig. 3.

TABLE V. Physical and plasma characteristics (means ± S.E.) for redband trout from three streams in south-eastern Oregon. Comparisons between populations were made using one-way ANOVA followed by Tukey-Kramer *post hoc* tests. *, Significant differences between means ($P < 0.05$). Dissimilar superscript letters indicate differences between groups

Physical Variables	Bridge Creek	Rock Creek	12-mile Creek
n	8	9	8
L_F (cm)	28.8 ± 3.6^A	$17.4 \pm 1.0^{*B}$	27.2 ± 1.4^A
M (g)	334 ± 96^A	$51 \pm 9^{*B}$	207 ± 32^A
K	1.07 ± 0.02^A	$0.90 \pm 0.02^{*B}$	$0.97 \pm 0.03^{*B}$
Relative ventricle mass (%)	$0.091 \pm 0.004^{*A}$	0.110 ± 0.006^B	0.121 ± 0.006^B
Plasma variables			
Osmolality (mmol kg H_2O^{-1})	292 ± 5	300 ± 3	307 ± 3
Sodium (mmol kg H_2O^{-1})	149 ± 4	158 ± 1	154 ± 1
Potassium (mmol kg H_2O^{-1})	2.5 ± 0.2^A	2.0 ± 0.1^A	$3.6 \pm 0.1^{*B}$
Calcium (mmol l^{-1})	2.1 ± 0.1	1.8 ± 0.1	2.1 ± 0.1
Free fatty acids (mmol l^{-1})	0.53 ± 0.05	0.47 ± 0.05	$0.41 \pm 0.05^*$
Triglycerides (mg dl^{-1})	217 ± 35	118 ± 22	$88 \pm 10^*$
Albumin (mg ml^{-1})	26.5 ± 1.8^A	$18.0 \pm 1.7^{*B}$	$21.0 \pm 0.8^{*C}$
Total protein (mg ml^{-1})	36.2 ± 1.9^A	$28.1 \pm 1.3^{*B}$	37.9 ± 1.0^A
Albumin : total protein	0.72 ± 0.03^A	0.63 ± 0.03^A	$0.56 \pm 0.01^{*B}$
Blood variable			
Haemoglobin (g dl^{-1})	10.8 ± 0.5	11.0 ± 0.6	9.8 ± 0.5

the low concentrations of triglyceride (Bridge Creek: 4.0 ± 2.0 mg g^{-1}, Rock Creek: 2.1 ± 0.3 mg g^{-1} and 12-mile Creek: 2.4 ± 0.8 mg g^{-1}). Even after controlling for any body size effects, muscle triglyceride concentrations did not differ among populations (ANCOVA, d.f. = 3 and 20, $P = 0.82$).

MORPHOLOGY

Overall, there were significant differences in morphology among the three populations of redband trout (MANOVA, d.f. = 18 and 72, $P < 0.0001$). Only five of the nine traits, however, showed a significant level of divergence (Fig. 8). Pectoral fin length was larger in fish from Rock Creek, than in either the Bridge or 12-mile populations [Fig. 8(c); ANOVA, d.f. = 2 and 44, $P = 0.011$], but pelvic fin length in Rock Creek fish was only different from Bridge Creek fish [Fig. 8(d); ANOVA, d.f. = 2 and 44, $P = 0.025$]. In addition to fish size, Rock Creek fish also had a larger eye diameter than each of the other two populations [Fig. 8(i); ANOVA, d.f. = 2 and 44, $P = 0.0013$]. Conversely, fish from Bridge Creek had significantly shorter premaxilla length [Fig. 8(f); ANOVA, d.f. = 2 and 44, $P = 0.016$], and narrower mouths [Fig. 8(g); ANOVA, d.f. = 2 and 44, $P = 0.022$] than fish from 12-mile Creek.

There was significant allometry between muscle cross-sectional area at the posterior edge of the anal fin and M for all three populations of redband trout. Although slight, Bridge Creek trout had significantly higher red muscle area than the other groups. In contrast, it was not possible to detect any differences in white muscle area between streams (Fig. 9).
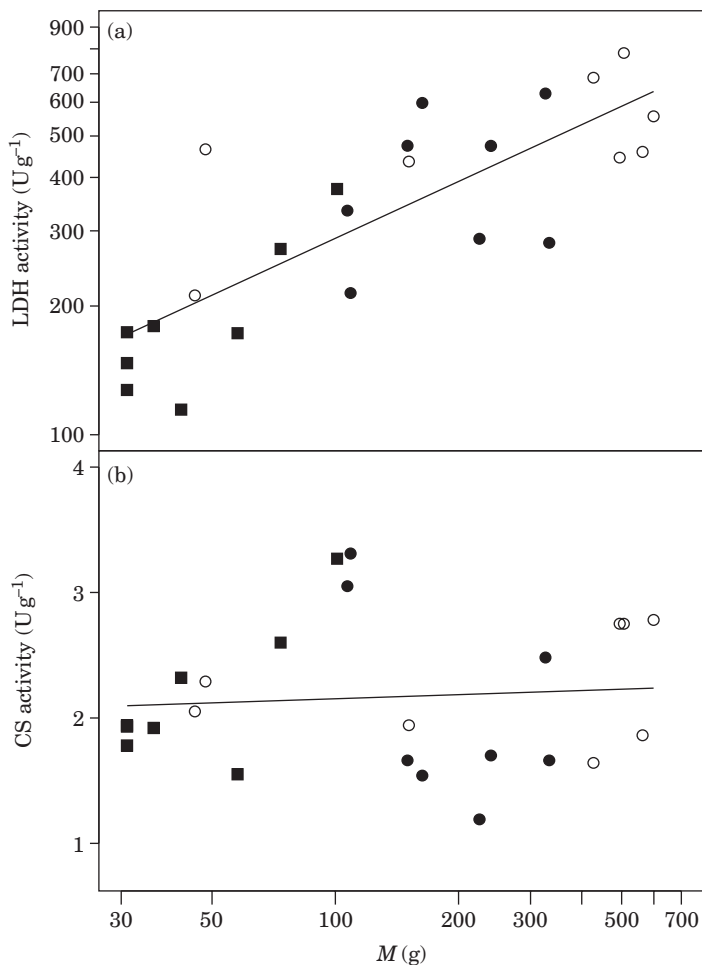
FIG. 7. (a) The relationship between redband trout body mass and lactate dehydrogenase activity in white muscle. The curve was fitted by: $\log_{10}y = 0.44 \log_{10}x - 1.58$, $n = 24$, $r^2 = 0.65$, $P < 0.0001$. Fish from Bridge Creek (○) 12-mile Creek (●) and Rock Creek (■). (b) The relationship between redband trout body mass and citrate synthase activity in white muscle. The curve was fitted by: $\log_{10}y = 0.11 \log_{10}x - 1.94$, $n = 24$, $r^2 = 0.01$, $P = 0.70$. Fish as in (a).

DISCUSSION

The main purpose of this multi-disciplinary study was to assess the thermal tolerance, swimming ability and metabolism of wild redband trout in south-eastern Oregon. A previous study on populations of small fish from Bridge Creek and the Little Blitzen River suggested that environmental variability could modify swimming performance, metabolism, biochemical characteristics and related morphology of genetically similar animals (Gamperl *et al.*, 2002). The current study extends these results to include different size redband trout and three populations of genetically-distinct fish from a wider geographic range.
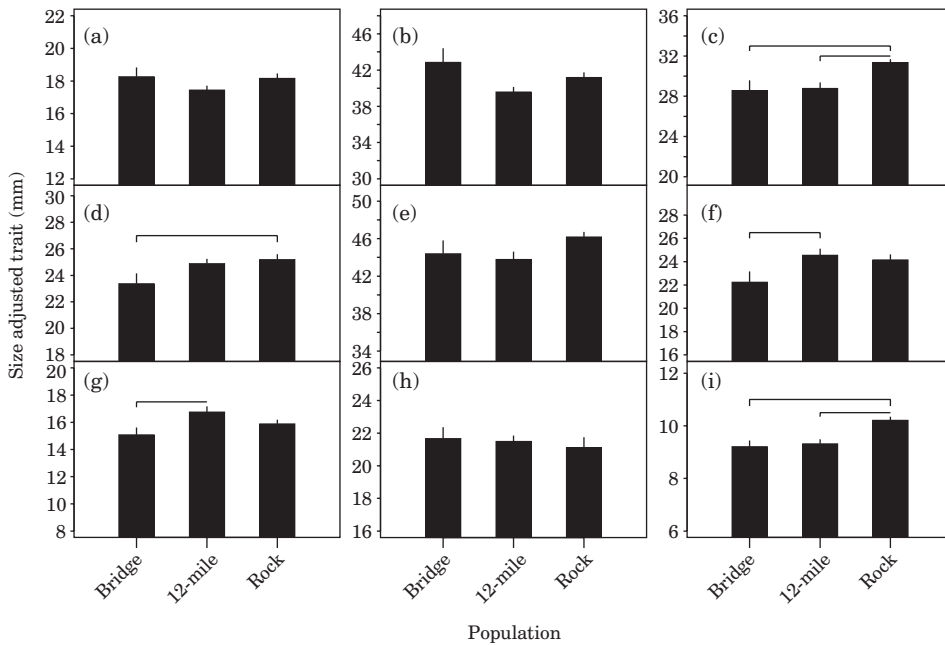
FIG. 8. Size-adjusted measurements (means ± S.E.) of (a) caudal peduncle depth, (b) body depth, (c) pectoral fin length, (d) pelvic fin length, (e) head length, (f) premaxilla length, (g) mouth width, (h) head width and (i) eye diameter of three redband trout populations. Horizontal lines indicate means that are significantly ($P < 0.05$) different from each other by Tukey's *post hoc* test.

CRITICAL THERMAL MAXIMUM

The T_{crit} of a fish is the temperature at which it becomes incapacitated after being acutely exposed to a constant rate of heating. Using an ecologically relevant heating rate of $2° C h^{-1}$ (Fig. 2) and native water during the hottest period of the summer, it was determined that the T_{crit} for three distinct populations of wild redband trout in south-eastern Oregon was not different, and averaged $29.4 ± 0.1° C$. This value is very similar to reported literature values for rainbow trout and other salmonids acclimated to $\geq 15° C$ ($29.1–30° C$), with the exception of Atlantic Salmon *Salmo salar* L. ($32.7–32.9° C$; Beitinger *et al.*, 2000). Thus, the present results support Myrick & Cech's (2000) hypothesis that salmonids (with the exception of those restricted to high latitudes) have similar thermal tolerances irrespective of origin, and that the upper thermal tolerance of wild salmonids is phylogenetically conservative and resistant to evolutionary change (Beitinger *et al.*, 2000). This finding may seem surprising given the high maximum summer temperatures ($24–30° C$) and large diel temperature fluctuations ($6–10° C$) to which these redband trout were exposed (Fig. 2). Many studies, however, have also demonstrated that thermal history has minimal effects on the T_{crit} or upper thermal tolerance of salmonids (Heath, 1963; Kaya, 1978; Lee & Rinne, 1980; Konecki *et al.*, 1995).

The present data also demonstrated that the T_{crit} of redband trout remained constant over a 35-fold size range (40–1400 g). As far as is known, this is the first experimental study that has investigated whether the T_{crit} of juvenile and
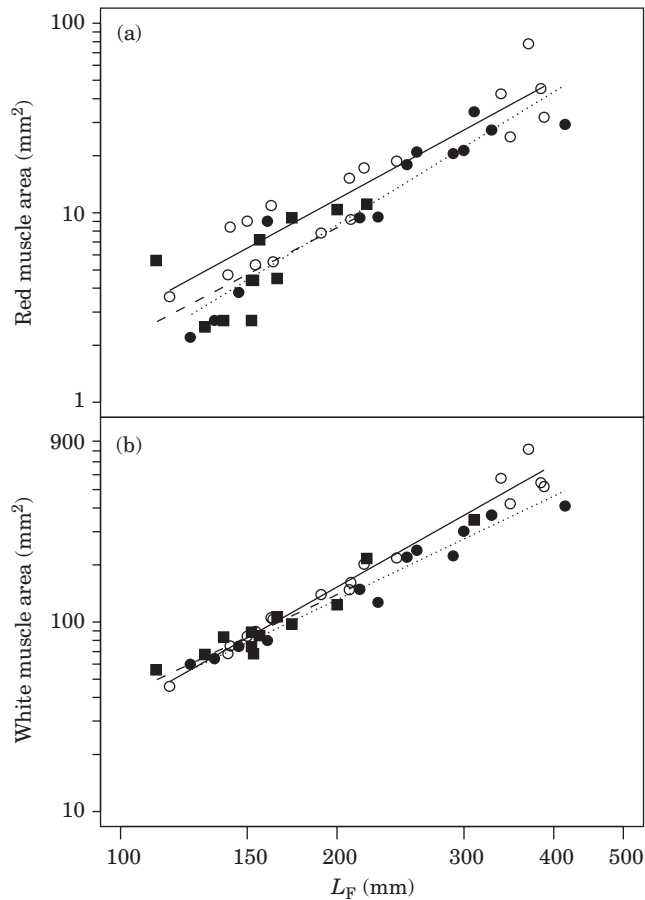
FIG. 9. (a) The relationship between body length and red muscle cross-sectional area in redband trout. The curves were fitted by: Bridge Creek (—○—) $-\log_{10}y = 2\cdot10\ \log_{10}x - 3\cdot76$, $n = 22$, $r^2 = 0\cdot87$, $P < 0\cdot0001$, 12-mile Creek (…●…) $-\log_{10}y = 2\cdot33\ \log_{10}x - 4\cdot43$, $n = 13$, $r^2 = 0\cdot90$, $P < 0\cdot0001$ and Rock Creek (–■–) $-\log_{10}y = 2\cdot06\ \log_{10}x - 3\cdot82$, $n = 14$, $r^2 = 0\cdot48$, $P = 0\cdot0092$. (b) The relationship between body length and white muscle cross-sectional area. The curves were fitted by: Bridge Creek $\log_{10}y = 2\cdot10\ \log_{10}x - 2\cdot64$, $n = 22$, $r^2 = 0\cdot98$, $P < 0\cdot0001$, 12-mile Creek $\log_{10}y = 1\cdot82\ \log_{10}x - 2\cdot06$, $n = 13$, $r^2 = 0\cdot96$, $P < 0\cdot0001$ and Rock Creek $\log_{10}y = 1\cdot82\ \log_{10}x - 2\cdot05$, $n = 14$, $r^2 = 0\cdot84$, $P < 0\cdot0001$. Fish as in (a).

adult salmonids differs. The lack of a size or age effect on the T_{crit} of redband trout agrees with the majority of published data. For example, a recent study by Carline & Machung (2001) showed that neither length nor condition influenced the T_{crit} of wild and domestic strains of brown *Salmo trutta* L. and rainbow trout. In addition, the T_{crit} values reported by Currie *et al.* (1998) for 4 cm (standard length) rainbow trout acclimated to temperatures between 10 and 20° C are within 0·4° C of those reported by Lee & Rinne (1980) for 20 cm (total length) conspecifics at similar temperatures. There is one study, however, that showed that the upper thermal tolerance of brown trout and Atlantic salmon parr is 2–4° C greater than for alevins (Spaas, 1960).

ROUTINE OXYGEN CONSUMPTION AND Q_{10}

The mass exponent calculated for redband trout R_r (0·83) is extremely similar to other data on salmonids (Fry, 1971; Brett & Glass, 1973; Cho, 1992) and to that measured for Atlantic cod *Gadus morhua* L. (Saunders, 1963; Reidy *et al.*, 1995). Thus, it appears that the mass exponent for R_r varies little between strains of rainbow trout, or even between temperate fish species.

The mass-adjusted R_r of redband trout at 14° C ranged from *c.* 60 to 120 mg $O_2 \, kg^{-0.83} \, h^{-1}$ and is within the range of values published for salmonids at similar temperatures (Brett & Groves, 1979; Facey & Grossman, 1990; Burgetz *et al.*, 1998; Gamperl *et al.*, 2002). The R_r for redband trout (200–300 mg $O_2 \, kg^{-0.83} \, h^{-1}$) at 24° C was also comparable to most salmonid studies at elevated temperatures (19–25° C; Brett, 1971; Heath & Hughes, 1973; Woodward & Smith, 1985), with the exception of Henry & Houston (1984) (145 mg $O_2 \, kg^{-0.83} \, h^{-1}$). While these data indicate that the redband trout does not have reduced routine metabolic costs within this temperature range, as compared with other salmonids, it should not be concluded that the redband trout and rainbow trout have similar thermal sensitivities. For example, when rainbow trout (430–600 g) were acclimated to 16° C and subjected to a 1·5° C h^{-1} increase in temperature until loss of equilibrium (Heath & Hughes, 1973), the Q_{10} for R_r increased dramatically after 20° C (Q_{10} between 20 and 24° C = 4·96) and R_r plateaued at temperatures >24° C. In contrast, the breakpoint in the temperature–R_r relationship for large redband trout (400–1400 g) did not occur until 22° C and R_r did not plateau until 26° C (Fig. 4). Thus, it appears that adult redband trout can tolerate a 2° C higher acute increase in temperature before experiencing thermal stress than hatchery rainbow trout.

From the present results, it is also apparent that size and age can affect the temperature at which redband trout experience thermal stress. In juvenile redband trout the breakpoint in the temperature–R_r relationship did not occur until 24° C, and R_r never plateaued. Further, R_r was significantly lower (*c.* 40–60%) for juvenile fish at 24, 26 and 28° C. The finding that larger redband trout have an increased thermal sensitivity is consistent with a study by Linton *et al.* (1998) that investigated the effect of simulated climate warming on the growth, appetite and metabolism of rainbow trout over the annual temperature cycle. It was reported by Linton *et al.* (1998) that a 2° C increase in summer water temperatures had a significant negative effect on conversion efficiency during the second year of the study (fish mass *c.* 360 g) but not during the first year (fish mass *c.* 20 g). Clearly, the added energetic cost of R_r at temperatures >24° C could reflect a loss of homeostatic capability and be of ecological significance for adult redband trout.

SWIMMING PERFORMANCE AND METABOLISM

In this study, maximum metabolic rate scaled with body mass[0·87]. As far as is known, there is only one other study that reports mass exponents for maximum metabolic rate (R_{max}) in salmonids (Brett & Glass, 1973). These authors reported that the mass exponent for R_{max} in sockeye salmon *Oncorhynchus nerka* (Walbaum) ranged from 0·963 to 0·999, depending on temperature. While this comparison suggests that interspecific differences in the scaling of R_{max} with

body size exist, this interpretation is confounded because metabolic and swimming performance measurements were made on hatchery-reared fry and fingerlings, but wild-caught 'jacks' and adults (Brett & Glass, 1973). The use of wild-caught fish of large size may have artificially inflated their mass exponent since wild-caught individuals are thought to be more fit than hatchery-reared conspecifics.

Maximum metabolic rate (R_{max}) and metabolic power for redband trout in this study averaged 723 mg O_2 kg$^{-0.87}$ h^{-1} (986 mg O_2 kg^{-1} h^{-1}) and 515 mg O_2 kg$^{-0.882}$ h^{-1} (683 mg O_2 kg^{-1} h^{-1}), respectively at 24° C, and these are the highest published values for any salmonid with the exception of the sockeye salmon tested by Brett (1964, 1971). The current values are well above those measured for hatchery-reared rainbow trout at 10–15° C (*c.* 262–554 kg$^{-0.87}$ h^{-1} and 174–438 mg O_2 kg$^{-0.882}$ h^{-1}; Dickson & Kramer, 1971; Webb, 1971; Kiceniuk & Jones, 1977; Waiwood & Beamish, 1978; Alsop & Wood, 1997; Burgetz *et al.*, 1998), wild rainbow trout swum at normal summer temperatures (15° C; 498 mg kg$^{-0.87}$ h^{-1}, 345 mg O_2 kg$^{-0.882}$ h^{-1}; Facey & Grossman, 1990), and hatchery-reared rainbow trout from wild and domestic parents when tested at 24° C (388–488 mg kg$^{-0.87}$ h^{-1}; 273–395 mg O_2 kg$^{-0.882}$ h^{-1}; Dickson & Kramer, 1971). These results show that redband trout have more available energy than rainbow trout at 24° C, and strongly suggest that an enhanced aerobic fitness may be an important factor which allows them to populate streams where summer stream temperatures are near or above 24° C for several hours during the day (Fig. 2).

The swimming and metabolic performance of salmonids has been studied extensively. There are no published investigations, however, with the exception of Gamperl *et al.* (2002), that conducted stream-side measurements using native water at environmentally relevant temperatures. The present study shows that although the relative critical swimming speed (U_{crit} in L_F s^{-1}) of the three populations of redband trout were similar, the oxygen consumption required to fuel this swimming effort was *c.* 30% lower at Bridge Creek [Figs 5, 6(a) and Table IV]. It is probable that the difficulty experienced in getting fish from 12-mile and Rock Creeks to complete their U_{crit} tests contributed significantly to this difference. Variance in compliance during U_{crit} tests is not uncommon (Beamish, 1978), and may be indicative of excess stress and result in elevated metabolic rates that are independent of physical activity. It is unlikely, however, that differences in stress were solely responsible for the difference in swimming efficiency between redband trout from Bridge Creek and those from the other two streams: 1) R_r was not different between the three populations and 2) previous data showed that cost of transport was significantly lower for Bridge Creek fish at both 12 and 24° C as compared with fish from the Little Blitzen River, even though few electric shocks were needed to induce either population to swim (Gamperl *et al.*, 2002).

It is possible that nutritional state and imbalances in potassium homeostasis explain the differences in swimming efficiency and the reluctance of fish at 12-mile and Rock Creeks to swim (refusal rate 33–36%). Fish sampled from Rock and 12-mile Creeks had a significantly lower K, and fish from 12-mile Creek had reduced levels of plasma lipids and elevated plasma potassium levels, as compared with those at Bridge Creek (Table V). Lipids are the principal energy fuel

source during sub-maximal sustained swimming (Kieffer *et al.*, 1998), and reductions in plasma lipids could reflect an imbalance between lipid utilization and availability, and force the use of other metabolic fuels for energy production. The hyperkalemia in fish from 12-mile Creek could indicate ion loss from the swimming musculature due to rhabdomyolysis (myocyte necrosis) or an inability of the Na^+/K^+ pump to keep pace with the K^+ efflux from fast-twitch skeletal muscle cells (Everts *et al.*, 1993). Ultimately, elevations in plasma potassium could lead to reduced excitability of cardiac and skeletal muscle cells, and thus diminish the strength of muscular contraction.

What other mechanisms could be responsible for the lower oxygen consumption and enhanced swimming efficiency exhibited by Bridge Creek redband trout? Given the smaller size of the cardiac pump in redband trout from Bridge Creek, a lower stroke volume, reduced cardiac output and lower convective transport of oxygen to the tissues would be anticipated. This is especially true since haemoglobin levels (a measure of blood O_2 carrying capacity) were similar between the three populations of redband trout (Table V). Overall, a reduced oxygen requirement during activity may compensate for a lower blood–oxygen transport capacity and help maintain equivalent aerobic swimming performance (Stillwell & Benfey, 1996). Although beyond the scope of the current study, it is also possible that there were: 1) phenotypic differences in the myofibrillar contractile elements which promoted more efficient skeletal muscle contraction in Bridge Creek redband trout (Wakeling *et al.*, 2000) and 2) subtle differences in the mechanical properties of the propulsive systems (*i.e.* increased propeller efficiency, Webb, 1971) or swimming kinematics.

MUSCLE ENZYMES AND MORPHOLOGY

Similar to studies by Somero & Childress (1980) and Norton *et al.* (2000) on active teleosts, positive scaling of anaerobic (LDH) enzyme activity was observed in redband trout axial white muscle over a 35-fold size range (40–1400 g). This finding is probably related to the high cost of burst swimming in larger fishes (Webb, 1975). In contrast, the marker enzyme of aerobic metabolism was mass-independent in redband trout white muscle. The observation that mass-corrected enzyme activities in white muscle were not different between these three populations of redband trout contrasts with the earlier study on redband trout from Bridge Creek and the Little Blitzen River (Gamperl *et al.*, 2002). Small redband trout from the Little Blitzen River (a cooler stream than Bridge Creek) had higher aerobic potential and lower anaerobic potential in their white muscle than similar-sized fish from Bridge Creek. Given the lack of genetic variability between these populations, and results from laboratory thermal acclimation experiments, intraspecific differences in enzyme activities were attributed to dissimilar thermal histories (Gamperl *et al.*, 2002). Because fish from the Little Blitzen River, Rock Creek and 12-mile Creek all experience cold winter temperatures (0–2° C) as compared with Bridge Creek (6–10° C), and no variations in maximal enzyme activities were detected between populations in the current study, it appears that differences in stream thermal characteristics during the summer were primarily responsible for the differences in LDH and CS activities observed between Bridge Creek and the Little Blitzen River

(Gamperl *et al.*, 2002). This hypothesis, however, requires further experimental verification. For example, it is also possible that differences in fish condition in the current study may have impacted enzyme activities independent of thermal histories.

EXTERNAL MORPHOLOGY

Swimming ability, including U_{crit}, in salmonids has been correlated with body shape (Taylor & McPhail, 1985). Despite a previous determination that mitochondrial haplotype and nuclear allele frequencies were identical between redband trout from Bridge Creek and the Little Blitzen River, fish from the Bridge Creek population had a more robust body form with a larger head, and a deeper body and caudal peduncle (Gamperl *et al.*, 2002). These morphological differences were presumably driven by environmental factors and could have contributed to the improved swimming performance of the Bridge Creek fish, although deep robust body forms are considered better for burst swimming performance (Webb, 1978). In the current study, there was little evidence for genetically or environmentally induced morphological differences between populations of redband trout, and thus no relationship between body form and swimming performance can be established. The morphological analysis did show that Rock Creek fish had longer pectoral fins and larger eye diameters than fish from Bridge and 12-mile Creeks, and that fish from Bridge Creek had significantly shorter premaxilla length and narrower mouths than fish from 12-mile Creek. Although longer pectoral fins may impart some hydrodynamic advantage for Rock Creek redband trout, the greater swimming efficiency of Bridge Creek redband trout does not appear to be correlated with any obvious differences in body morphology.

ARE REDBAND TROUT WARMWATER TOLERANT?

Given that stream-dwelling fishes are in thermal equilibrium with their environment, and temperature affects virtually all physiological, biochemical and life-history activities of fishes, it is not surprising that thermal tolerance is an important physiological trait that defines suitable habitat. It is clear, however, from this study that the indices used to define 'thermal tolerance' must be chosen carefully for fishes living in habitats with high summer temperatures. Based on field observations of redband trout in desert basins of western North America (Behnke, 1992; Zoellick, 1999), it was proposed that these fish can tolerate higher maximum temperatures than other salmonids. Given that the T_{crit} for redband trout in south-eastern Oregon differs very little from rainbow trout and most other salmonids, it could be concluded that the redband trout is not 'uniquely' tolerant to warm water temperatures. While measures of thermal tolerance such as T_{crit} provide an ecologically relevant lethal index, it should be emphasized that T_{crit} values only define the acute temperature tolerance of animals and relate to short-term survival. Thus, T_{crit} data are not very useful when assessing the thermal and habitat requirements of a species like the redband trout which lives in a temporally and spatially heterogeneous thermal environment, or the physiological status of fishes in general. In a recent review paper by Pörtner (2002), it was elegantly pointed out that thermal tolerance limits are

linked with aerobic scope (metabolic power) and the capacity of the whole animal, and that adjustments in these variables can be crucial in determining the geographical distribution of ectothemic vertebrates (including fishes). Clearly, the elevated R_{max} and metabolic power of redband trout at 24° C (as compared with data for rainbow trout) strongly supports the idea that the redband trout does have an enhanced capacity to function, and probably flourish, at warmer temperatures than most salmonids. This conclusion is supported by the work of Behnke (1992) and Zoellick (1999), and by the failure of rainbow trout stocking efforts in the Malheur National Wildlife Refuge (R. Roy, pers. comm.).

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