Use of an Annular Chamber for Testing Thermal Preference of Westslope Cutthroat Trout and Rainbow Trout

Thomas E. McMahon[a] and Elizabeth A. Bear[b]
Ecology Department, Fish and Wildlife Program
and
Alexander V. Zale
U.S. Geological Survey, Montana Cooperative Fishery Research Unit

Montana State University
Bozeman, Montana 59717 USA

ABSTRACT

Remaining populations of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) in western North America are primarily confined to cold headwaters whereas nonnative rainbow trout (*Oncorhynchus mykiss*) predominate in warmer, lower elevation stream sections historically occupied by westslope cutthroat trout. We tested whether differing thermal preferences could account for the spatial segregation observed in the field. Thermal preferences of age-1 westslope cutthroat trout and rainbow trout (125 to 150 mm total length) were assessed in the laboratory using a modified annular preference chamber at acclimation temperatures of 10, 12, 14, and 16°C. Final preferred temperature of westslope cutthroat trout (14.9°C) was similar to that of rainbow trout (14.8°C) when tested in a thermal gradient of 11-17°C. The high degree of overlap in thermal preference indicates the two species have similar thermal niches and a high potential for competition. We suggest several modifications to the annular preference chamber to improve performance in future studies.

INTRODUCTION

Three main metrics are used to define thermal requirements for fishes – upper and lower survival (tolerance) limits, growth range and optima, and thermal preference and avoidance (Fry 1947). The first two criteria are physiological measures of performance, whereas thermal preference and avoidance represent temperature ranges that fish select or avoid through thermoregulatory movement. The preferred temperature of a species often corresponds closely to the optimal temperature range for growth and metabolism, thereby providing a behavioral mechanism to maximize survival and reproduction (Jobling 1981). Thermal preference information can help define optimal thermal habitat for a species as well as identify the potential for thermal niche overlap among competitors or predators (Edsall and Cleland 2000; Larsson 2005).

The acute preference method has been commonly used to determine thermal preference by acclimating fish to a certain temperature and then placing them into a thermal gradient for a short (typically 2 h or less) time period (Fry 1947). Horizontal and vertical gradient chambers (Javaid and Anderson 1967, Kwain and McCauley 1978, Peterson et al. 1979, Edsall and Clelland 2000) and shuttle boxes that produce a varying thermal gradient controlled by the organisms' movement (McCauley 1977, Konecki et al. 1995) have traditionally been employed to measure thermal preference. However, these test systems may be limited by the presence of confounding variables inherent to their design such as varying water depth, cover, and light intensity that may mask or bias temperature preferences (Richards et al. 1977, Myrick et al. 2004). To minimize the

---

[a]Corresponding author; E-mail: tmcmahon@montana.edu
[b]Present address: Wyoming Game and Fish Department, 528 South Adams Street, Laramie, Wyoming 82070 USA

potential for such biases, an annular chamber with no corners or other cover and uniform illumination and water depth was recently developed for thermal preference studies (Myrick et al. 2004).

The objective of this study was to compare thermal preference of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*O. mykiss*) using a modified annular thermal preference chamber. Westslope cutthroat trout, once widespread throughout the northwestern United States and Canada are now primarily confined to cold headwater streams (Shepard et al. 2005), and rainbow trout and other nonnative salmonids dominate warmer, lower elevation sites historically occupied by westslope cutthroat trout (Paul and Post 2001). The distinctive elevational gradients in distribution between westslope cutthroat trout and rainbow trout and other nonnative salmonids, with relatively little spatial overlap, suggest that differential responses to temperature are likely important in mediating their spatial distributions within and among watersheds (Paul and Post 2001, Shepard 2004, Sloat et al. 2005). We assessed to what degree differences in thermal preference could explain the observed spatial segregation of westslope cutthroat trout and rainbow trout and evaluated the performance of an annular chamber for determining thermal preference.

METHODS AND MATERIALS

Test fish

Westslope cutthroat trout were obtained as eyed eggs from a wild broodstock maintained at the Westslope Trout Company (Ronan, Montana), and Fish Lake strain rainbow trout eggs were obtained from the Ennis National Fish Hatchery (Ennis, Montana). Fish used in trials were age-1 juveniles ranging in total length from 125 to 150 mm. Individuals of each species were separated into each of four separate 75-L flow-through acclimation tanks (3 L/min flow rate). Tank temperatures were originally 14°C but were adjusted at 1°C/d until acclimation temperatures of 10, 12, 14, and 16°C were reached. Test species were then reared in separate tanks at the four respective acclimation temperatures for two weeks prior to testing. Fish were fed to excess daily using pellcted trout food delivered over an 8-h period with a belt feeder. Tanks were cleaned daily and test fish were not fed for 24 h prior to testing.

Test apparatus

Preference testing was performed in an annular preference apparatus based on the design described by Myrick et al. (2004). We modified our apparatus from the original design in order to accommodate larger fish and higher flow rates. The diameter of the apparatus was 118 cm. Cross-sectional widths of the circular outer mixing and middle preference channels were 10 cm and 20 cm, respectively. The radius of the inner effluent channel with center drain was 29 cm. Water supplied from cold (8.5°C) and warm (22.4°C) constant temperature springs was mixed in four head tanks to achieve test temperatures of 8.4, 12.3, 17.0, and 22.3 °C. Head tank temperatures were monitored with electronic thermographs and adjusted accordingly at the start of each trial. Aerated head tank water was supplied by 2-m-long, 1.25-cm-diameter hoses to eight separate mixing sections positioned around the outer edge of the test apparatus at a flow rate of 3 L/min, yielding a total flow-through rate of the apparatus of 24 L/min. A standpipe on the center drain maintained water depth at 10 cm. Dissolved oxygen concentrations measured in head tanks were greater than 7 mg/L (70% saturation) during the study.

Unequal availability of temperatures and sharp temperature gradients within the preference channel were noted drawbacks of the original chamber design (Myrick et al. 2004). Therefore, to provide an equal area of available temperatures, water from each of the four head tanks was delivered to two separate mixing sections creating two equal areas of the four test temperatures. To minimize sharp temperature gradients and ensure laminar radial flow across the preference channel, 16 tubes (0.6-cm-diameter) were placed in a zig-zag pattern along the inner wall of each mixing chamber to achieve adequate mixing and uniform delivery to the preference channel. In addition, mixing sections were sequentially arranged as A, B. C, D, D, C, B, A, where A received head tank temperature of 8.4°C, B received 12.3°C, C received 17.0°C, and D received 22.3°C. Dye testing showed that flow from each mixing section across the preference chamber was relatively uniform, with some eddy currents developing near transition areas. Temperatures in the annular preference chamber consistently produced a thermal gradient of about 6°C, with average temperatures ranging from about 11 to 17°C and with temperature extremes of 10.5 to 18°C (Fig. 1). Temperatures varied from about 0.5 to 1.0°C between adjacent temperature measurement points positioned about 15 cm apart along the midline of the preference channel.

The test apparatus was covered with a shroud to minimize disturbance. Fish movement in the preference channel was recorded remotely with a Loligo Video Tracking System (Hobro, Denmark). The camera was mounted 2 m from the water surface to capture the complete field of view of the entire annular preference apparatus. White acetate sheets were mounted on the top of the shroud to provide diffuse, uniform lighting from overhead lights.

Test protocol

Twenty-four trials were run, 12 with each species, with three replicates at each of the four acclimation temperatures. The sequence of the trials was chosen randomly prior to testing. To minimize the chance of preference of certain areas of the test apparatus independent of temperature, the positioning of the head tank delivery hoses was also randomly assigned prior to testing. Each head tank hose was labeled 1 through 8 and the position of hose 1 was randomly assigned to a mixing section and the remaining hoses were sequentially positioned as described above.

Following hose adjustment, water temperatures were allowed to equilibrate for 30 min. Temperature was then measured with a YSI meter (Yellow Springs, Ohio) 1 cm off

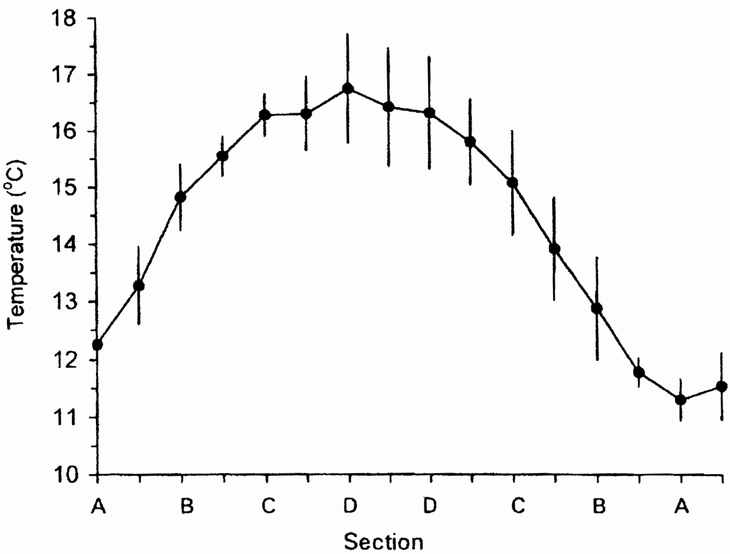

Figure 1. Water temperature (mean ± standard deviation) from 15 randomly selected trials measured at 16 equidistant points along the centerline of the annular preference channel. Letters in the x-axis refer to the eight different mixing sections arranged sequentially around the perimeter of the preference chamber.

the bottom in the middle of the preference channel corresponding to the middle and edges of each mixing section for a total of 16 measurements. Test fish were selected from the appropriate acclimation tank, measured for length (mm), and introduced into the preference channel in a section most closely corresponding to their acclimation temperature. The video system recorded fish position continuously for the next 3 h. At the end of a trial, the test fish was removed, and temperature were re-measured. The test apparatus was then drained and hoses were repositioned as described above in preparation for the next trial.

Data analysis

Temperatures in the preference channel were calculated as the averages of pre- and post-trial temperatures. Temperatures between point measurements were calculated by interpolation of the averages of the two adjoining measurements. Fish locations (nose position) in relation to available temperatures were recorded from the video file every 1 min over the final 2 h of the 3 h test period for a total of 120 fish locations per trial; the first hour was deemed an acclimation period and fish locations were not recorded. Fish that failed to acclimate to the test apparatus, as evidenced by continuous swimming and lack of selection for any channel sections during the 2-h test period, were omitted from the data set.

Acute preference temperature for each trial was calculated as the mean temperature of the individual fish locations, and a frequency distribution of selected temperatures for each acclimation temperature was constructed for species comparisons (Richards et al. 1977, Edsall and Clelland 2000). Differences in acute preference temperature were compared among acclimation temperatures and between species using two-way analysis of variance. Potential linear and nonlinear relationships between preferred temperatures and acclimation temperatures were analyzed with a curve-fitting program (Sigma Plot 2002). The final preferendum for each species was determined as the point where the preferred temperature was equal to the acclimation temperature on the preference-acclimation curve (Richards et al. 1977, Hofmann and Fischer 2002).

RESULTS

Temperature selection by westslope cutthroat trout and rainbow trout was similar at each acclimation temperature (Fig. 2). At the lower acclimation temperatures of 10 and 12°C, both species selected temperatures 2 to 4°C warmer than acclimation temperatures. At 14°C acclimation, temperature selection for both species was bimodal, with peaks in selected temperatures about 2°C lower and higher than acclimation temperature. At the highest acclimation temperature (16°C), both species showed strong selection for temperatures below the acclimation temperature with westslope cutthroat trout selecting lower temperatures more frequently than rainbow trout.

Acute preference temperatures for westslope cutthroat trout and rainbow trout ranged from 14.5 to 15.8°C and varied only slightly (difference 0.17 to 0.29°C) between the two species at any acclimation temperature (Fig. 3). Acute preference temperature was not significantly different among acclimation temperatures ($F = 2.61$, $P = 0.10$) or between species ($F = 0.27$, $P = 0.61$). The overall mean acute preference temperature for westslope cutthroat trout was 15.4 ± 0.2°C SE (n = 6) and 15.3 ± 0.2°C SE (n = 11) for rainbow trout.

There was no significant relationship between acclimation temperature and acute preference temperature for westslope cutthroat trout (linear, $r^2 = 0.01$, $P = 0.89$; curvilinear, $r^2 = 0.12$, P = 0.94) or rainbow trout (linear, $r^2 = 0.10$, $P = 0.68$; curvilinear, $r^2 = 0.35$, $P = 0.81$). Therefore, the final preferenda of 14.9°C for westslope cutthroat trout and 14.8°C for rainbow trout were determined by plotting the relationship between acclimation and acute preference temperatures using a graphical smoothing function and interpolating where the two temperatures were equal on the curves (Fig. 3).

DISCUSSION

Juvenile westslope cutthroat trout and rainbow trout exhibited equivalent thermal preferences, with final preferred temperatures of 14.8-14.9°C when tested in a thermal gradient of 11-17°C. The similarity of thermal preferences parallels previous results showing the two species also have very similar optimum growth temperatures (13.6°C for westslope cutthroat trout and 13.1°C for rainbow trout; Bear et al. 2007). Therefore, both species have nearly identical physiological optimum temperatures, defined as the average of the optimum growth temperature and final temperature preferendum (McCullough et al. 2001) – 14.2°C for westslope cutthroat trout and 14.0°C for rainbow trout. Our results support laboratory (Bear et al. 2007) and field distribution data (Shepard 2004, Sloat et al. 2005) indicating that temperatures less than 15°C are optimal for long term growth and survival of westslope cutthroat trout.

Greater variability in occupied temperatures among fish acclimated to 14 and 16 °C than to 10 and 12°C may reflect the proximity of the acclimation temperatures to the final preferenda. Fish acclimated to the lower temperatures may have more uniformly selected high temperatures in an effort to rapidly achieve high core body temperatures and compensate for time spent at suboptimal temperatures. Cherry et al. (1975) noted that fish were more mobile and more variability in temperature selection occurred between replicates at temperatures that were near or above final preferenda than at lower acclimation temperatures.

The high degree of overlap in physiological and behavioral responses to

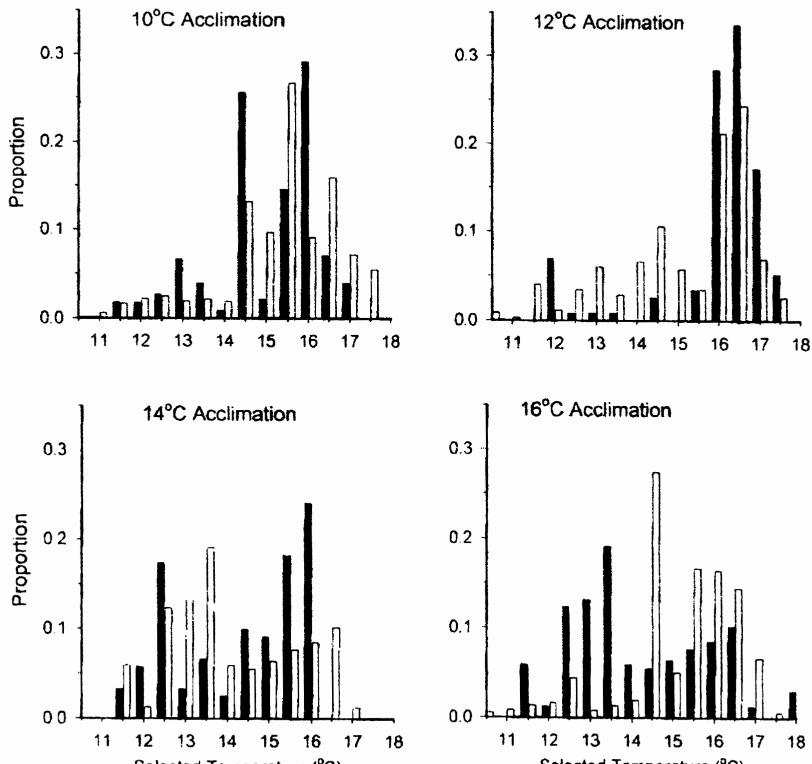

Figure 2. Frequency (shown as proportion of total observations) of temperatures selected by juvenile westslope cutthroat trout (black bars) and rainbow trout (open bars) acclimated to 10, 12, 14, and 16°C.

temperature indicates westslope cutthroat trout and rainbow trout have similar thermal niches and a high potential for competition. The ability of rainbow trout to survive prolonged exposure to temperatures greater than 20°C and to grow over a wider range of temperatures than westslope cutthroat trout (Bear et al. 2007) likely accounts for the increased occurrence of rainbow trout at lower elevations and warmer temperatures. Although we found low selection by both species of temperatures at the lower (10.5-12°C) and upper (17.0-18.0°C) range of test temperatures, assessing temperature preferences over a wider range than we examined would identify avoidance temperatures of each species and help elucidate if rainbow trout more strongly avoid colder temperatures and if westslope cutthroat trout avoid warmer temperatures.

Preferred temperature of the Fish Lake strain rainbow trout in our study was about 2°C lower than that reported for other strains in previous studies. Juvenile rainbow trout acclimated to 15°C selected acute preference temperatures of 16.9°C (Kwain and McCauley 1978), 17.5°C (Javaid and Anderson 1967), and 18.4°C (McCauley and Pond 1971) compared to our estimate of 15.0°C. Although differences in test equipment may account for some of the differences in preferred temperature of rainbow trout among studies (Myrick et al. 2004), rainbow trout in our study also exhibited about 4°C lower optimum growth temperature than other rainbow trout (Bear et al. 2007), suggesting the possibility for stock differences in thermal requirements for this widely distributed species and the need for more extensive thermal testing among different stocks and strains using standardized methods (see also Myrick and Cech 2004, Konecki et al. 1995). We found no reports in the literature on thermal preference testing of westslope cutthroat trout.

Overall, we found the annular chamber (Myrick et al. 2004) performed well for measuring temperature preference. Our modifications from the original prototype resulted in equal availability of temperatures and relatively gradual temperature gradients within the preference channel. Random assignment of temperature placement also precluded the need for testing whether fish preferred certain areas of the channel independent of temperature (Despatie et al. 2001, Hofmann and Fischer 2002). However, we did encounter several limitations in the study design. Although we were able to establish a consistent thermal gradient, the gradient of 11-17°C was narrower than we had planned, given head tank temperatures of 8-22°C. We originally tested the apparatus with higher flow rates, but the larger volumes of water created unstable and unpredictable

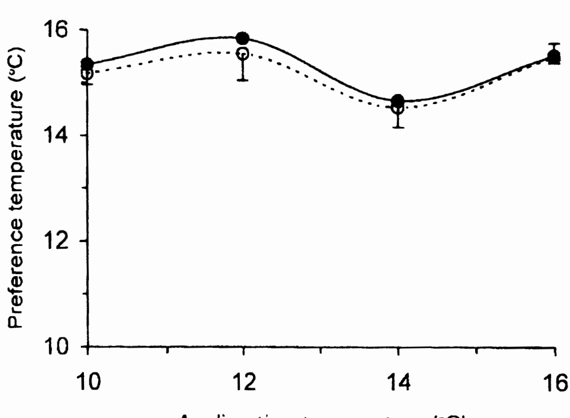

Figure 3. Acute temperature preferenda of juvenile westslope cutthroat trout (solid line; mean + standard deviation, SD) and rainbow trout (dotted line; mean - SD) in relation to acclimation temperature.

thermal gradients in the preference channel. We were able to achieve a more stable, albeit narrower, thermal gradient by reducing inflow rates and allowing for more complete mixing in each mixing section by blocking off the inner wall of the mixing sections and inserting outflow tubes along the wall. Another drawback to our apparatus design was uncertainty associated with the actual temperatures selected by individual fish, as repeat temperature measurements at the same channel locations at the start and end of a trial sometimes varied by as much as 1°C near transition areas. Placement of temperature sensors along the channel coupled with the video recording of position (e.g., Edsall and Clelland 2000, Hofmann and Fischer 2002, Myrick et al. 2004) would provide simultaneous measurement of water temperature and fish location and reduce ambiguity in the actual temperature fish were occupying. Finally, we had difficulty achieving consistent acclimation to the test apparatus with westslope cutthroat trout. We do not believe this was related to the test apparatus, as only one rainbow trout failed to show normal preference behavior. For some species, incorporating a pre-testing period longer than one hour may be required to enhance acclimation to the annular chamber (Morgan and Metcalfe 2001).

ACKNOWLEDGMENTS

We thank Cal Fraser, Montana State University Wild Trout Laboratory, for constructing the annular preference chamber. Chris Myrick, Colorado State University, graciously provided photos of his original annular chamber design, which aided us during construction of our own chamber. We thank the U.S. Fish and Wildlife Service, Bozeman Fish Technology Center, for allowing us to conduct the study at its facility, and Matt Toner, Bill Krise, Rick Barrows, Jim Bowker, and Yvette Converse of the Center staff for technical assistance. The Wild Fish Habitat Initiative, Montana University Water Center, and U.S. Fish and Wildlife Service, Partners in Fish and Wildlife Program, funded the study. We thank Liz Galli-Noble for helpful administrative support of the project and Mike Meeuwig for manuscript review.

LITERATURE CITED

Bear, B.A., T.E. McMahon, and A.V. Zale. 2007. Comparative thermal requirements of westslope cutthroat trout and rainbow trout: implications to species interactions and development of thermal protection standards. Transactions of the American Fisheries Society 136:1113-1121.

Cherry, D.S., K.L. Dickson, J. Cairns Jr., and J.R. Stauffer. 1977. Preferred, avoided, and lethal temperatures of fish during rising temperature conditions. Journal of the Fisheries Research Board of Canada 34: 239-246.

Despatie, S.-P., M. Castonguay, D. Chabot, and C. Audet. 2001. Final thermal preferendum of Atlantic cod: effect of food ration. Transactions of the American Fisheries Society 130: 263-275.

Edsall, T.A. and J. Cleland. 2000. Optimum temperature for growth and preferred temperatures of age-0 lake trout. North American Journal of Fisheries Management 20: 804-809.

Fry, F.E.J. 1947. Effects of the environment on animal activity. University of Toronto Studies, Biological Series 55. Publication of the Ontario Fisheries Research Laboratory 68: 1-62.

Hofmann, N. and P. Fischer. 2002. Temperature preferences and critical thermal limits of burbot: implications for habitat selection and ontogenetic habitat shift. Transactions of the American Fisheries Society 131: 1164-1172.

Javaid, M.Y. and J.M. Anderson. 1967. Thermal acclimation and temperature selection in Atlantic salmon, *Salmo salar*, and rainbow trout, *S. gairdneri*. Journal of the

Fisheries Research Board of Canada 24: 1507-1513.
Jobling, M 1981. Temperature tolerance and the final preferendum-rapid methods for the assessment of optimum growth temperatures. Journal of Fish Biology 9: 439-455.
Konecki, J.T., C. A. Woody, and T.P. Quinn. 1995. Temperature preference in two populations of juvenile coho salmon, *Oncorhynchus kisutch*. Environmental Biology of Fishes 44: 417-421.
Kwain, W. and R.W. McCauley. 1978. Effects of age and overhead illumination on temperatures preferred by underyearling rainbow trout, *Salmo gairdneri*, in a vertical temperature gradient. Journal of the Fisheries Research Board of Canada 35: 1430-1433.
Larsson, S. 2005. Thermal preference of Arctic charr, *Salvelinus alpinus*, and brown trout, *Salmo trutta* - implications for their niche segregation. Environmental Biology of Fishes 73: 89-96.
McCauley, R.W. 1977. Laboratory methods for determining temperature preference. Journal of the Fisheries Research Board of Canada 34: 749-752.
McCauley, R.W. and W.L. Pond. 1971. Temperature selection of rainbow trout (*Salmo gairdneri*) fingerlings in vertical and horizontal gradients. Journal of the Fisheries Research Board of Canada 28: 1801-1804.
McCullough, D.A., S. Spalding, D. Sturdevant, and M. Hicks. 2001. Issue Paper 5: Summary of technical literature examining the physiological effects of temperature on salmonids. U.S. Environmental Protection Agency EPA 910-D-01-005.
Morgan, I.J. and N.B. Metcalfe. 2001. The influence of energetic requirements on the preferred temperature of overwintering juvenile Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 58: 762-768.
Myrick, C.A. and J.J. Cech, Jr. 2004. Temperature effects on juvenile salmonids in California's central valley: what don't we know? Reviews in Fish Biology and Fisheries 14: 113-123.
Myrick, C.A., D.K. Folgner, and J.J. Cech, Jr. 2004. An annular chamber for aquatic animal preference studies. Transactions of the American Fisheries Society 133: 427-433.
Paul, A.J. and J.R. Post. 2001. Spatial distribution of native and nonnative salmonids in streams of the eastern slopes of the Canadian Rocky Mountains. Transactions of the American Fisheries Society 130: 417-430.
Peterson, R.H., A.M. Sutterlin, and J.L. Metcalfe. 1979. Temperature preference of several species of *Salmo* and their hybrids. Journal of the Fisheries Research Board of Canada 36: 1137-1140.
Richards, F. P., W.W. Reynolds, and R.W. McCauley. 1977. Temperature preference studies in environmental impact assessments: an overview with procedural recommendations. Journal of the Fisheries Research Board of Canada 34: 729-761.
Shepard, B.B. 2004. Factors that may be influencing nonnative brook trout invasion and their displacement of native westslope cutthroat trout in three adjacent southwestern Montana streams. North American Journal of Fisheries Management 24: 1088-1100.
Shepard, B.B., B.E. May, and W. Urie. 2005. Status and conservation of westslope cutthroat trout within the western United States. North American Journal of Fisheries Management 25: 1426-1440.
SigmaPlot. 2002. SigmaPlot® version 8.0. SPSS Publishing, Chicago, Illinois.
Sloat, M.R., B.B. Shepard, and R.G. White. 2005. Influence of stream temperature on

the spatial distribution of westslope cutthroat trout growth potential within the Madison River basin, Montana. North American Journal of Fisheries Management 25: 225-237.

Received: 4 June 2007 Accepted: 30 August 2007