Thermal Requirements of Westslope Cutthroat Trout

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Abstract

Westslope cutthroat trout *Oncorhynchus clarki lewisi*, have declined throughout their native range in the Northern Rockies and were considered for listing under the federal Endangered Species Act. Water temperature is widely regarded as playing a key role in determining their persistence, but specific thermal optima and lethal levels for this cutthroat trout subspecies have not been precisely defined. This laboratory study used the acclimated chronic exposure method to determine thermal optima and tolerances for westslope cutthroat trout and for rainbow trout *Oncorhynchus mykiss*, a potential nonnative competitor now occupying much of the former range of westslope cutthroat trout. Optimum growth temperature for westslope cutthroat trout (13.6°C; 95% CI, 10.3 - 17.0°C) over the 60-d test period was, unexpectedly, similar to that of rainbow trout (13.1°C; 95% CI, 6.8 - 18.2°C). However, rainbow trout were predicted to grow better over a wider range of temperatures than cutthroat trout, with growth significantly better at temperatures below 6.8°C and above 20.8°C. In addition, the ultimate upper incipient lethal temperature (temperature at which 50% of the population survives for 60-d) of rainbow trout (24.2°C; 95% CI, 20.3 - 24.2°C) was 4°C higher than that of westslope cutthroat trout (19.7°C; 95% CI, 19.1 - 20.3°C). The higher upper temperature tolerance and wider growth range of rainbow trout may account for its increased occurrence at lower elevations than cutthroat trout. We hypothesized that cutthroat-rainbow trout hybrids would have intermediate temperature requirements, but poor survival of hybrid eggs precluded testing this hypothesis. Disease outbreaks also precluded planned experiments of temperature effects on competitive interactions between rainbow trout, brook trout and westslope cutthroat trout. However, the thermal requirement information we did establish in this study can help guide protection and restoration efforts for this unique cutthroat trout subspecies.

Introduction

Historically, westslope cutthroat trout *Oncorhynchus clarki lewisi* ranged widely over western Montana, Idaho, and portions of eastern Washington and Oregon (Behnke 1992; Shepard et al. 2003). Along with many other cutthroat and native trout species, westslope cutthroat trout now persist in only a small portion of their native range, are listed as a "species of special concern" in Montana (MTNHP 2004), and were recently evaluated for listing as a federally threatened species under the Endangered Species Act (USFWS 2003). Leading causes for the decline of westslope cutthroat trout were habitat degradation, competition with nonnative rainbow *Oncorhynchus mykiss*, brook *Salvelinus fontinalis*, and brown trout *Salmo trutta*, and hybridization with rainbow trout (Hanzel 1959; Allendorf and Leary 1988; Liknes and Graham 1988; Allendorf et al. 2004).

Competition with nonnative species has led to a reduction in westslope cutthroat trout populations, but the specific mechanisms involved have not been clearly demonstrated (Griffith 1988). Water temperature is thought to be one of the primary mechanisms influencing competitive interactions (DeStaso and Rahel 1994; Taniguchi et al. 1998; Reese and Harvey 2002). The interacting effects of increased water temperature and competitive behavior may lead to decreases in growth and survival of some species (Magoulick and Wilzbach 1998; T.E. McMahon, Montana State University, unpublished data). For example, brook trout exhibited competitive dominance over Colorado cutthroat trout *Oncorhynchus clarki pleuriticus* when water temperature was at 20ºC, whereas at 10ºC neither species showed clear dominance (De Staso and Rahel 1994). This competitive dominance suggests that brook trout may have an increased thermal tolerance compared to Colorado cutthroat trout, shifting the competitive advantage to brook trout at warmer water temperatures. Therefore, testing the effect that rainbow trout and brook trout may have on growth, feeding, and survival of westslope cutthroat trout at various water temperatures will help managers conserve remaining native westslope cutthroat trout populations as well as guide restoration efforts.

Hybridization between westslope cutthroat trout and other trout is another major cause in the decline of pure westslope cutthroat trout (Allendorf and Leary 1988; Allendorf et al. 2004). Pure westslope cutthroat trout with no sign of genetic introgression only occur in about 10% of currently occupied habitats (Shepard et al. 2003). Therefore, determining the thermal requirements of hybrids may help to explain the increasing prevalence of hybrids in nature.

Westslope cutthroat trout that still persist in their native range are typically confined to colder, headwater portions of streams whereas nonnative trout now occupy the larger, warmer downstream reaches formerly occupied by westslope cutthroat trout (Hanzel 1959; Sloat 2001). Because of their current confinement to headwater reaches, water temperature is considered a key element influencing the persistence of westslope cutthroat trout and competitive interactions with nonnative species (Paul and Post 2001). However, the thermal requirements of westslope cutthroat trout are largely unknown. Proper conservation and management of westslope cutthroat trout requires detailed knowledge of its temperature requirements. Particularly in light of global warming, thermal protection standards are needed to protect and restore populations of native, cold-water species, particularly salmonids (McCullough 1999).

Traditionally, the lethal thermal limits of aquatic ectotherms have been established by means of laboratory studies using two methods, the critical thermal maximum or minimum (CTM) method and the upper or lower incipient lethal temperature (ILT) method. The CTM method entails rapidly heating or cooling water until loss of equilibrium or death occurs, indicating the thermal limit of that fish species. The CTM method is advantageous in that measures of thermal tolerance can be simply and rapidly obtained. However, the unnaturally rapid rates of temperature change inherent in the method are unlikely to be experienced by an aquatic organism in nature (Becker and Genoway 1979) and thus its ecological relevance has been questioned (Becker and Genoway 1979; Selong et al. 2001). The ILT method entails transferring fish from an acclimation temperature directly to a constant test temperature and measuring time to death. The incipient lethal temperature is then defined as the temperature at which 50% of the population survives indefinitely, in most cases, based on a 7-d exposure period (Brett 1956; Jobling 1981). Although inclusion of exposure time in the ILT method is advantageous over the CTM method, the abrupt temperature change experienced by the fish is not analogous to water temperature changes in natural conditions (Becker and Genoway 1979; Selong et al. 2001).

The acclimated chronic exposure (ACE) method was developed as a thermal criteria method with enhanced application to conditions fish experience in nature (Zale 1984). The ACE method entails adjusting water temperature 1°C per day to allow for temperature acclimation among test fish until a predetermined test temperature is reached (Selong et al. 2001). Fish are then held at the constant test temperature for 60 days or until death, the length of the test period simulating the duration of seasonal maxima (or minima). Growth and survival are assessed over the 60-d test period to establish an optimum growth temperature and ultimate upper incipient lethal temperature (UUILT).

Thermal preference is an additional metric developed to assess thermal requirements of a species. Thermal preference is defined as the temperature individuals will select, given a wide range of equally available temperatures (Johnson and Kelsch 1998). The thermal preference of a species often corresponds to the species' thermal optimum for physiological functioning such as growth and aerobic metabolism (Jobling 1981; Bryan et al. 1984; Sauter et al. 2001), but can be influenced by a host of factors including age (Kwain and McCauley 1978), thermal history (Javaid and Anderson 1967; Konecki at al. 1995), and ration (Brett et al. 1969). For example, fish may select lower than optimal temperatures under conditions of low food availability (Brett et al. 1969; Welch et al. 1998). Mobile organisms typically express temperature preferences that optimize survival and reproduction, thus contributing to their evolutionary fitness (Reynolds 1977). Thermal preference information can help identify habitat suitable for a species in terms of water temperature. It can also help identify possible overlap in suitable thermal habitat by competitors or predators (Edsall and Cleland 2000).

Typically, thermal preference has been measured in the laboratory (Javaid and Anderson 1967; Cherry et al. 1975; McCauley et al. 1977; Kwain and McCauley 1978; Peterson et al. 1978; Edsall and Cleland 2000; Myrick et al. 2004) or using field observations relating presence of fish to water temperature (Brett 1971; Roper and Scarnecchia 1994; Bonneau and Scarnecchia 1996; Welch et al. 1998). Field observations of thermal preference are often difficult to interpret due to confounding variables such as food availability, competition and predation, and presence of thermal refugia. Therefore, exact determination of thermal preference is routinely done in the laboratory where preference can be examined under closely controlled conditions.

A method commonly used involves determining the acute thermal preference temperature by allowing a fish to acclimate to a certain temperature and then placing this fish in a thermal gradient for a short (2 h or less) time period. Acute preference temperatures typically represent the average temperature from multiple readings of temperatures at which fish are located (Sauter et al. 2001). This process is then repeated for several acclimation temperatures as acute thermal preferences are influenced by acclimation temperature (Sauter et al. 2001). The final preference temperature is derived from the regression of acclimation temperature and acute preferences and is the temperature at which the preferred temperature and acclimation temperature are equal (Fry 1947). The relationship between acclimation and preferred temperature varies among species from direct to inversely proportional (Kelsch and Neill 1990). Generally, the acute thermal preference temperature of salmonids increases with increasing acclimation temperature (Sauter et al. 2001).

Traditional thermal preference testing has used horizontal gradient (Javaid and Anderson 1967; Peterson et al. 1979), and vertical gradient chambers (Kwain and McCauley 1978; Edsall and Cleland 2000), as well as shuttle boxes that produce a constantly varying horizontal gradient controlled by the organisms' movement (McCauley et al. 1977; Konecki et al. 1995). Each test system has advantages and disadvantages because of the presence of confounding variables such as water depth, cover, and light intensity (Myrick et al. 2004). To eliminate these variables, an annular chamber with no corners or other cover, and uniform illumination and water depth, was developed for thermal preference studies of aquatic animals (Myrick et al. 2004). A modified version of this annular chamber was developed for use in this study.

Objectives

Our primary goal was to characterize the thermal biology of westslope cutthroat trout, specifically the optimum growth temperature, upper lethal level, and preferred temperature. Our secondary goal was to characterize the thermal biology of rainbow trout, with respect to the above metrics to compare the thermal biology of westslope cutthroat trout with rainbow trout, a nonnative competitor. We also planned to characterize the thermal biology of first-generation westslope cutthroat-rainbow trout hybrids. Lastly, we planned to determine the influence of water temperature on competitive interactions between westslope cutthroat trout and rainbow trout and brook trout, which are nonnative competitors displacing westslope cutthroat trout throughout their native range.

Our specific objectives were as follows:

1. To define the optimal growth temperature range of westslope cutthroat trout, rainbow trout, and their hybrids.

The optimum growth temperature has been established for numerous species but was completely lacking for westslope cutthroat trout and hybrids, and was lacking for rainbow trout using the methodology in this study. This information would allow for comparison of optimal temperatures between these three different species groups. Such a comparison would have implications as to whether water temperature plays a role in competitive interactions and as to why hybrids have been so successful and are now so prevalent in nature.

2. To define the upper lethal temperature tolerance level of westslope cutthroat trout, rainbow trout, and their hybrids.

The upper lethal limit as defined by either the critical thermal maximum or upper incipient lethal temperature has been defined for numerous species. However, this information was completely lacking for westslope cutthroat trout or hybrids, and had not been established for rainbow trout using the ACE method. This information would allow comparison between westslope cutthroat trout and rainbow trout, and help to identify suitable thermal habitat for westslope cutthroat trout reintroduction programs. Upper lethal temperature information for hybrids may lead to increased insight as to why hybrids have been so successful.

3. To determine the thermal preference of westslope cutthroat trout, rainbow trout, and their hybrids.

The thermal preference has been established for many species but is lacking for westslope cutthroat trout and hybrids, and for rainbow trout using the methodology in this study. The thermal preference in conjunction with the optimum growth temperature will be used to define the temperature range for optimal physiological functioning for the species.

4. To determine the influence of water temperature on competitive interactions between westslope cutthroat and rainbow trout and between westslope cutthroat and brook trout.

Direct quantification of the influence of water temperature on competitive interactions is scarce and is lacking for westslope cutthroat and rainbow and brook trout. Knowledge of the effect of nonnatives on the growth and survival of westslope cutthroat trout at increased temperatures can aid in management of areas where overlap of species occurs.

Methods

A thermal test facility housed at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (BFTC), used previously to determine the thermal biology of bull trout (Selong et al. 2001), was employed in our study to simultaneously assess fish growth and survival under varying thermal regimes for prolonged periods. Westslope cutthroat trout used in experiments were obtained either as eggs or fry from a wild stock at Rogers Lake, MT, or as fry from a wild stock maintained at the Westslope Trout Company, a private fish hatchery near Ronan, MT. All rainbow trout were obtained as eggs from the Fish Lake strain maintained at the U.S. Fish and Wildlife Service Ennis National Fish Hatchery (ENFH) near Ennis, MT. Hybrids were produced by fertilizing roughly 11,000 westslope cutthroat trout eggs from Rogers Lake with rainbow trout milt from the Fish Lake strain at ENFH. Brook trout were obtained as eggs from the Crystal Lakes Hatchery, a private fish hatchery near Eureka, MT.

The protocol for all experiments was similar to that outlined in Selong et al. (2001). A flow-through thermal testing system provided constant water flow, high dissolved oxygen concentrations, and metabolite flushing. Water supplied from cold (8°C) and warm (22°C) springs and three 40,000-BTU water heaters was mixed to achieve test temperatures from 8°C to 30°C. Water from the sources was passed through a de-gassing column, and mixed in 12 separate head tanks. From the head tanks, water was then supplied to 36, 75-L aluminum test tanks (120 by 35 by 25 cm) at a flow rate of 3 L/min. All connecting pipes and tank surfaces were covered with foam insulation to reduce atmospheric heat loss. Three replicates were tested for each of the 12 testing temperatures.

In accord with the ACE method, 50 fish were randomly selected, placed in each of 36 test tanks, and acclimatized to 14°C water for at least 14 days (standardization period) in each experiment. Fish were distributed so that the bulk weight of each tank was within ±10% of the mean of all tanks. After the standardization period, water temperature in each tank was raised or lowered 1.0°C per day until the desired test temperature was reached (acclimation period). Temperature adjustments were staggered so that all tanks reached the final treatment temperatures on the same day. After final treatment temperatures were reached, fish were held at a constant temperature (test period) for 60 days or until mortality levels in tanks experiencing chronic mortalities reached 50% in each replicate tank. On day 1, day 30, and day 60 of the test period, fish in each tank were counted and weighed in bulk to compute a starting, mid-point, and final weight per fish in each tank. Additionally, on day 60 or the end of the experiment, all remaining fish from each tank were sacrificed and length and weight data were recorded for each individual.

Fish were fed using an automatic belt feeder that supplied a constant feed ration over a 12-h period from about 0800 to 2000 hours. Fish were fed daily and feed was adjusted to maintain ration levels in excess of satiation. Tanks were cleaned daily and mortalities removed, weighed to the nearest tenth of a gram, and measured to the nearest millimeter. Temperature, dissolved oxygen concentration, and percent saturation were recorded daily in each head tank using a YSI meter and adjustments were made to ensure adequate dissolved oxygen concentration in each tank.

Temperature was recorded hourly with an Onset Optic StowAway® Temp logger placed in each test tank. All thermographs were calibrated after the final experiment and a correction factor was used to determine average temperatures experienced by fish in each tank over the 60-d test period. Photoperiod was monitored throughout the duration of the project with an Onset StowAway® Light Intensity Logger placed near the system.

Three experiments were conducted to determine the growth and survival of westslope cutthroat trout over the temperature range of 8°C to 30°C. However, only the results from the third experiment are reported here because in the first two experiments a fixed ration was provided that was later determined to be less than satiation consumption at some test temperatures. A reduced ration would lead to an optimum growth temperature lower than that expected compared to feeding in excess of satiation (Brett et al. 1969; Brett 1971). Therefore, in the third experiment rations were determined by measuring consumption and ensuring that feeding level exceeded satiation. Each week, uneaten feed and feces were removed from each tank, separated by sieving, and uneaten food material was dried and weighed to determine amount consumed. Feed levels were then adjusted to provide feed availability in excess of consumption at each test temperature.

Two experiments were conducted to determine the growth and survival of rainbow trout over the temperature range of 8°C to 28°C. However, only the results from the second experiment are reported here because in the first experiment a fixed ration was provided that was later determined to be less than satiation consumption at some test temperatures. Therefore, in the second experiment consumption was measured and satiation rations ensured by the method described above.

Data Analysis

Growth and survival data were analyzed to determine the optimum growth temperature and ultimate upper incipient lethal temperature for westslope cutthroat and rainbow trout. Relative growth rate (expressed as %) was calculated to characterize the change in wet weight of fish throughout the experiment [(Y2-Y1)/(Y1(t))*100, where Y1 is the initial weight in grams, Y2 in the final weight in grams, and t is the time period of the experiment] to account for initial size differences among test fish at the start of each experiment. Growth and temperature data for each species were used to develop a growth curve using quadratic regression in Sigma Plot 8.0. Regression line and confidence intervals (95%) for the regression line were constructed and the resulting parameter estimates used to determine the optimum growth temperature using the equation: Growth = a + b* temperature + c* $(\text{temperature})^2$. The optimal growth range for each species was determined as the range encompassing the peak growth between the lower and upper 95% confidence intervals.

Upper lethal temperature ranges for westslope cutthroat trout and rainbow trout were examined by comparing (1) daily survival rates over the 60-d test period; (2) total survival at the end of the experiment for each test temperature; and (3) observed LD50 values at each test temperature. Survival data for each species was analyzed using an exponential decay regression in Sigma Plot 8.0. Regression line and confidence intervals (95%) were constructed and the resulting parameter estimates used to determine the ultimate upper incipient lethal temperature (UUILT) and LD50 (lethal dose to 50% of the population) using the equations: ILT = $\log e^{(\text{days} / a)/ -b}$ and LD50 = $a^* e^{-(b^*\text{temperature})}$. Lethal temperature ranges were determined as the area between the lower and upper 95% confidence intervals corresponding to an LD50 at 60 days. For both species, survival at some temperatures never dropped below the 50% level at the end of the 60-d test period. Therefore, the LD50 (days) was arbitrarily projected to be 120 days (twice the length on the test period) at 18°C and 20°C for westslope cutthroat trout, and at 22°C and 24°C for rainbow trout. This was done to reflect fish at these temperatures that survived beyond 60 days, and should therefore be considered when determining the UUILT. These projected points were included in the regression analysis but not represented graphically.

Survival over the test period for each temperature was determined by plotting survival (%) by temperature using sigmoid regression. Regression line and confidence intervals (95%) were constructed and resulting parameters used to determine the temperature where the survival over 60 days was 50% using the equation: Survival = a / $1+e^{-((\text{temperature}-x0) / b)}$.

Results

Optimum growth temperature

The growth of juvenile (age-1) westslope cutthroat trout in 60-d experiments varied significantly over temperatures from 8°C to 20°C. Relative growth rate increased to a peak (2.36%) and declined steadily with increasing temperature. The optimum growth temperature estimated by regression analysis was 13.6°C with an optimal growth range, based on 95% confidence intervals, from 10.3°C to 17.0°C (Figure 1). The predicted lower and upper thermal limits for westslope cutthroat trout growth were 5.5°C and 21.8°C, respectively. These limits were determined as the points at which the regression line intersected the x-axis.

Rainbow trout showed the same peak growth rate (2.36%) as westslope cutthroat trout and an optimum growth temperature similar to westslope cutthroat trout. The optimum growth temperature for rainbow trout estimated by regression analysis was 13.1°C with an optimal growth range from 6.8°C to 18.2°C (Figure 2). The predicted lower and upper thermal limits for rainbow trout growth were 1.8°C and 24.3°C, respectively.

Growth of westslope cutthroat trout and rainbow trout was similar over the range of temperatures from 10°C to 16°C. However, rainbow trout had a wider predicted growth range and grew significantly faster than westslope cutthroat trout at temperatures less than 6.8°C and temperatures greater than 20.8°C, as indicated by non-overlapping 95% confidence intervals of the regression lines (Figure 3). The optimum growth temperature for westslope cutthroat trout differed by 0.5°C from that of rainbow trout (13.6°C and 13.1°C, respectively) yet the predicted maximum growth range for westslope cutthroat trout (10.3 – 17.0°C) was narrower than for rainbow trout (6.8-18.2°C). Relative growth rate at the optimum growth temperature for rainbow trout was slightly lower than that for westslope cutthroat trout (2.18% and 2.31%, respectively).

Upper Lethal Temperature

Westslope cutthroat trout were much less tolerant of high temperatures than rainbow trout. Few fish (< 20%) survived prolonged exposure to temperatures greater than 20°C (Figure 4). Survival was intermediate at 20°C (36%) and high (≥ 90%) across all other test temperatures (8, 10, 12, 13, 14, 15, 16, and 18°C). No fish survived to reach test temperatures of 28°C and 30°C (Figure 5). At 26°C, mortality was high (99%) during ramping, and few fish survived to reach the final test temperature; all fish perished by day three of the experiment. At 23°C and

24°C, mortality declined steadily from the start of the experiment, whereas at 21°C and 22°C, survival was initially high for about one week before declining sharply. At 20°C, survival was greater than 90% for 30 days, followed by a sharp decline that then stabilized until the end of the experiment. The temperature at which 50% survival occurred over 60 days, as predicted by regression analysis of the survival vs. temperature curve, was 19.6°C (95% CI, 19.1 - 19.9°C) (Figure 5). The ultimate upper incipient lethal temperature, derived from plotting LD50 for each temperature followed by exponential regression analysis, was 19.7°C (95% CI, 19.1 - 20.3°C) (Figure 6), indicating that both methods yielded a similar UUILT value.

In contrast, rainbow trout survival was high (> 82%) at test temperatures from 8°C to 20°C (Figure 5). Survival was intermediate at 24°C (≥ 41%) but declined steadily at 26°C before stabilizing at 2% for the remainder of the experiment (Figure 7). At 28°C, mortality was high (70%) during ramping and all fish perished by day three of the experiment. At 26°C, mortality declined steadily from the start of the experiment, whereas at 22°C and 24°C, survival was initially high for several weeks before declining. The temperature at which 50% survival occurred over 60 days, as predicted by regression analysis of the survival vs. temperature curve, was 24.3°C (95% CI, 24.0 -24.7°C) (Figure 5). The ultimate upper incipient lethal temperature, derived from plotting LD50 for each temperature followed by exponential regression analysis, was 24.2°C (95% CI, 22.9 – 25.4°C) (Figure 8,) indicating that both methods yielded a similar UUILT value. Survival of westslope cutthroat trout was significantly lower than that of and rainbow trout from 20°C to 24°C, as indicated by non-overlapping 95% confidence intervals of the regression lines (Figure 5 and Figure 9).

Thermal preference

The annular chamber for the thermal preference experiments has been constructed and the apparatus is currently being adjusted to obtain desired test temperatures. Thermal preference testing was originally planned for summer of 2004 but ongoing water supply problems at the BFTC precluded use of the chamber simultaneously with other thermal experiments. Hence, the preference studies will be completed after the end of other thermal tolerance and growth experiments, with a scheduled completion date in early 2005 assuming no further disruptions of water availability.

Westslope cutthroat-rainbow trout hybrids

No experiments with hybrids were possible because of extremely low hatching success. Within two days of fertilization, roughly one-third of all hybrid eggs had died. No such mortality with eggs from any other species or stock occurred during this project. Mortality continued at a high rate through hatching. Upon hatching, only a few hundred hybrids remained. These numbers were insufficient to run a full experimental trial, thus the optimum growth, upper lethal, and preferred temperatures of hybrids could not be determined. Poor survival of hybrid fry experienced in this study has been found in similar studies of first-generation westslope cutthroat-rainbow trout hybrids (Leary et al. 1995; Allendorf et al. 2004)

Brook trout and rainbow trout competition

No experiments were conducted to determine the influence of water temperature on competitive interactions between westslope cutthroat and brook trout and between westslope cutthroat and rainbow trout because of insufficient numbers of fish to conduct experiments. Westslope cutthroat trout obtained for use in these experiments suffered from a chronic *Hexamita salmonis* infection that persisted despite multiple and varied prophylactic treatments. *Hexamita salmonis* is a small, mobile protozoan with eight flagella found in the intestines of salmonids. Infection can result in poor growth and increased mortality, especially in small fish. The recommended treatment for *Hexamita salmonis* is 3% Epsom salt concentration added to the food and given for 3 days (Warren 1991; Winton 2001). Westslope cutthroat trout were first diagnosed with *Hexamita salmonis* by the U.S. Fish and Wildlife Service Fish Health Lab in Bozeman, MT, after mortalities began to increase in February 2004. Over the course of the next six months, fish were given the recommended treatment five times, which suppressed the disease for a short period, followed by another outbreak. Finally, fish were given a bath treatment with the antibiotic Metronidazole for three days in July. Mortality after treatment declined for several days, before increasing to pre-treatment levels. In addition, fish were diagnosed with *Ichthyobodo* (*Costia)* twice during this time, which likely contributed to the ongoing mortality. *Ichthyobodo* is a small (5 by 12 micrometers) protozoan that infects the gills and skin of fish, causing listlessness, drop in appetite, and mortality (Warren 1991; Winton 2001). *Ichthyobodo* was first diagnosed from routine skin scrapes in April and July of 2004. Fish were given the recommended treatment of 150-200 parts per million formalin bath for one hour, after both diagnoses. In August 2004 it was determined that these westslope cutthroat trout obtained for use in competition experiments were too compromised by disease to be used in future thermal studies and the fish were euthanized to prevent disease spread to other tanks at the BFTC.

Brook trout obtained for use in competition experiments began showing increased mortality in October 2004. Several routine skin scrapes revealed no external parasites. A sample taken to the USFWS Fish Health Lab revealed swollen gills, minor fin erosion, and small numbers of bacteria on fin edges, but no sign of any serious disease infection. However, brook trout mortality continued to increase throughout October greatly reducing numbers of available fish. It was deemed that because of the high mortality and potentially undiagnosed disease, fish were compromised and would not be used in future experiments. Therefore, because of insufficient numbers of fish resulting from disease, competition and thermal preference experiments with brook trout were not initiated.

Discussion

Optimum growth temperature

The optimum growth temperature for westslope cutthroat trout in this study was similar to that previously reported for another cutthroat trout subspecies and for bull trout. The optimum growth temperature and upper growth limit for westslope cutthroat trout (13.6°C and 21.8°C, respectively) were comparable to those of Lahontan cutthroat trout of 12°C to13°C, and 22°C to 23°C, respectively (Dickerson and Vinyard 1999; Meeuwig et al. 2004). The optimum growth temperature of westslope cutthroat trout corresponded closely with that of bull trout of 13.2°C (Selong et al. 2001). However, these optimum growth temperatures are below those reported for other salmonids such as Arctic charr (13.8°C; Lyytikäinen and Jobling 1998), brook trout (14.0°C; T.E. McMahon, unpublished data. Montana State University), brown trout (16.9°C; Ojanguren et al. 2001), chinook salmon (19°C; Brett et al. 1982), and sockeye salmon (15°C; Brett et al. 1969). Therefore, westslope cutthroat trout, along with Lahontan cutthroat trout and bull trout, have thermal growth optima in the lower range for salmonids. The optimal range reported in this study also coincides with the maximum scope for activity of cutthroat trout of 15°C (Dwyer and Kramer 1975) and the optimal temperature range predicted for cutthroat trout of 12°C to 15°C based on scope for activity analysis (Hickman and Raleigh 1982). These metrics generate a fundamental niche for westslope cutthroat trout, defined by Christie and Regier (1988) as -3 to +1°C of the optimum growth temperature, from 10.6°C to 14.6°C.

In contrast, the optimum growth temperature for rainbow trout in this study does not coincide with that previously reported for the species. The optimum growth temperature for rainbow trout in this study (13.1°C) was substantially lower than that previously reported for rainbow trout of 17.2°C over a 50-d test period (Hokanson et al. 1977). This difference is likely attributable to smaller fish size and different methods used by Hokanson et al. (1977), as typically, larger, older fish have optimum and preferred temperatures below those of their smaller, younger counterparts (Kwain and McCauley 1978; Selong et al. 2001; Meeuwig et al. 2004). The growth ranges reported in this study generate a fundamental niche for rainbow trout from 10.1°C to 14.1°C.

Whereas optimum growth temperatures for westslope cutthroat trout and rainbow trout were similar, rainbow trout were predicted to grow significantly faster than westslope cutthroat trout at temperatures below 6.8°C and above 20.8°C. Increased growth by rainbow trout at these temperatures could be the mechanism by which rainbow trout are out-competing cutthroat trout. Furthermore, the high degree of overlap between the fundamental niches of the two species indicates the potential for intense competition in areas where the species co-occur. Based on the information from this study, the two species should be equally competitive from 12°C to 16°C, but rainbow trout may gain a competitive advantage, in the form of increased growth, at temperatures above and below this range.

Upper lethal temperature

The advantage of using the ACE method to assess survival of fishes is that long-term effects of temperature can be determined. Survival of westslope cutthroat trout at 20°C was greater than 50% until day 34, at which point survival dropped rapidly to low levels. This indicates that at 20°C, there is a time threshold that westslope cutthroat trout can survive and once this threshold is surpassed, survival quickly declines below the critical level of 50% of the population. Traditional ILT experiments lasting only seven days lack the ability to determine these longer term thresholds, which may be critical for survival in nature as seasonal high temperatures often last longer than seven days.

Furthermore, survival of rainbow trout at 24°C over 60 days differed between experiments, likely because of differences in fish size. Rainbow trout in the experiment with decreased survival (47%) were larger and older than fish in the experiment with improved survival (97%). The case of larger, older fish being more sensitive to warmer temperatures than younger fish has been well recognized (Selong et al. 2001; Meeuwig et al. 2004). However, at seven days the survival of rainbow trout in these experiments was essentially the same, indicating again that a traditional seven-day experiment would not have revealed this survival differential.

The 60-d ultimate upper incipient lethal temperature for westslope cutthroat trout derived from exponential regression (LD50 vs. temperature) in this study is not directly comparable to that for most other salmonids. The majority of existing data on UUILT for salmonids are for seven-day periods, not sixty days as was used in this study. The predicted UUILT at seven days for westslope cutthroat trout from exponential regression in this study was 24.7°C. This 7-d UUILT for westslope cutthroat trout is near that reported for other salmonids such as Lahontan cutthroat trout (24 - 25°C; Dickerson and Vinyard 1999), Bonneville cutthroat trout (24.2°C; Johnstone and Rahel 2003), chinook salmon (25.1°C; Brett 1952), coho salmon (25.0°C; Brett 1952), sockeye salmon (24.4°C; Brett 1952), and dolly varden (24 - 25°C; Takami et al. 1997). The 60-d UUILT for westslope cutthroat trout (19.7°C) was similar to that for bull trout (20.9°C) using the same methodology (Selong et al. 2001) suggesting that westslope cutthroat trout have an upper lethal limit at the lower range of that expressed by salmonids.

However, the 60-d UUILT, from exponential regression (LD50 vs. temperature), for rainbow trout in this study (24.2°C) was considerably lower than the previously reported 7-d UUILT for rainbow trout of 26.2°C (Kaya 1978) and 25.6°C (Hokanson 1997). Such differences are likely due to differing methodologies in determining the UUILT for fishes. For comparison, the predicted 7-d UUILT, from exponential regression, for rainbow trout in this study was 31.1°C which is far greater than the UUILT previously reported for rainbow trout.

Based on the information from this study, westslope cutthroat and rainbow trout would have similar survival from 8°C to 18°C, with rainbow trout having greater survival at increased temperatures. The 7-d and 60-d UUILT for westslope cutthroat trout was below that for rainbow trout in this study. This survival differential may account for the increased occurrence of rainbow trout at lower elevations that cutthroat trout (Paul and Post 2001). If water temperatures increase from anthropogenic influence, the range of suitable habitat for westslope cutthroat trout will continue to be restricted.

Ecological Implications

Optimal ranges and upper limits for survival of westslope cutthroat and rainbow trout in this study closely correspond to temperatures associated with the abundance of these species in nature. Westslope cutthroat trout are associated with habitats having average daily stream temperatures less than 12°C and maximum daily stream temperatures less than 16°C (Sloat 2001), whereas rainbow trout are associated with habitat having average daily stream temperatures less than 13°C and maximum daily stream temperatures less than 18°C.

Water quality standards addressing water temperature can be implemented in streams that are critical to westslope cutthroat trout survival. This study indicates maximum average daily temperatures below 16°C would be adequate for maximum growth, whereas maximum daily stream temperatures below 20°C would be adequate to maintain survival of westslope cutthroat trout. The thermal requirements for westslope cutthroat trout found in this study, along with stream temperature modeling, could help identify streams for successful restoration, predict the occurrence of westslope cutthroat trout and areas where rainbow trout may have a competitive advantage over westslope cutthroat trout, and predict the potential impacts of land use practices on the distribution of westslope cutthroat trout.

The ACE Methodology

As noted, the ACE technique has some important advantages over ILT and CTM methods for determining thermal requirements for aquatic ectotherms. The short term nature of the latter methods, and the limited ability for test animals to properly acclimate, may limit the ecological relevance of these techniques compared to the ACE method. However, our studies with cutthroat trout reported here have revealed that the long term nature of the ACE method may, in turn, present some logistical difficulties. The length of the experiments (~90 d) limits the number that can be conducted to about four per year. Obtaining eggs from wild parentage stocks is also problematic as they may only be available during limited time periods unlike more domesticated stocks which have been selected for longer duration breeding. This necessitates holding of test fish for long periods between experiments, thus making it difficult to keep fish size constant among different experiments. However, in this study we found that of greater concern is that the long holding periods increase the likelihood for disease outbreaks. In our study, several planned experiments could not be completed because of this problem. If a disease outbreak occurs during a test year, this may delay running an experiment by a full year. Wild stocks also are likely to be less disease resistant than hatchery-bred stocks, further adding to the potential for disease outbreaks. The many advantages of the ACE method outweigh these potential disadvantages, but planning of future studies with wild salmonids should include steps to minimize these risks by isolating test fish and careful monitoring and treatment of disease.

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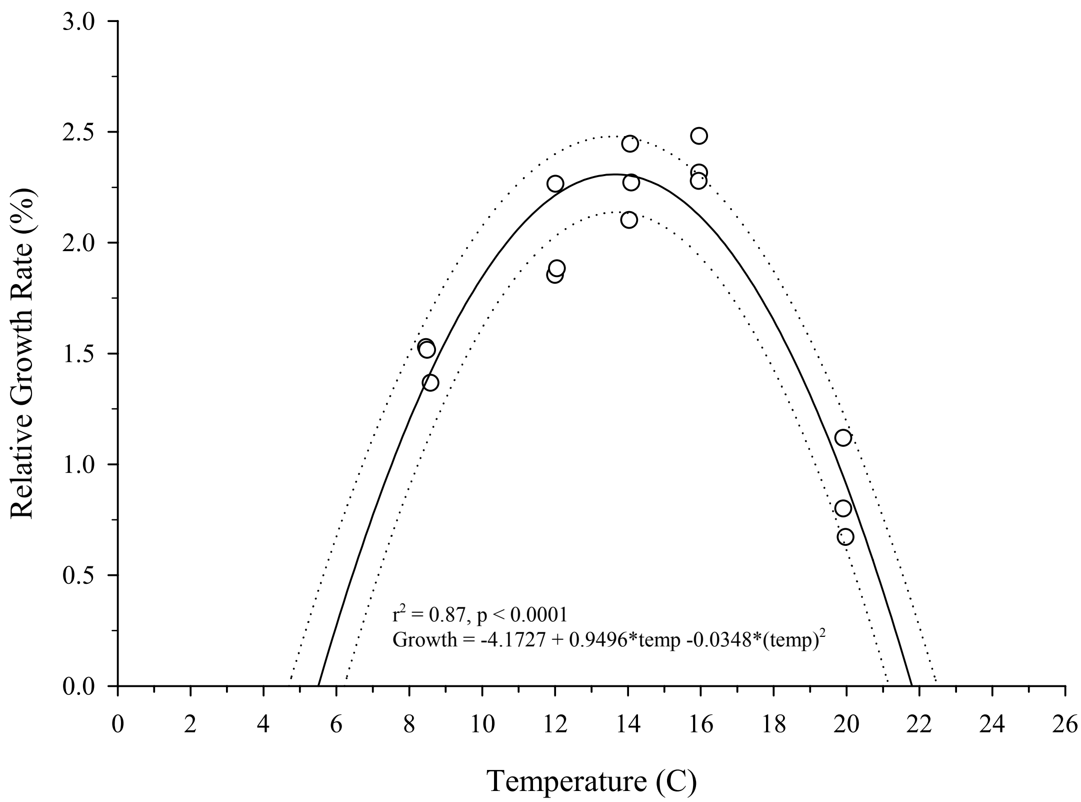

Figure 1. Growth of westslope cutthroat trout over 60 days in relation to temperature. Each circle represents the relative growth rate (%) per tank with three tanks tested at each temperature. Dotted lines indicate the 95% confidence interval of the regression line.

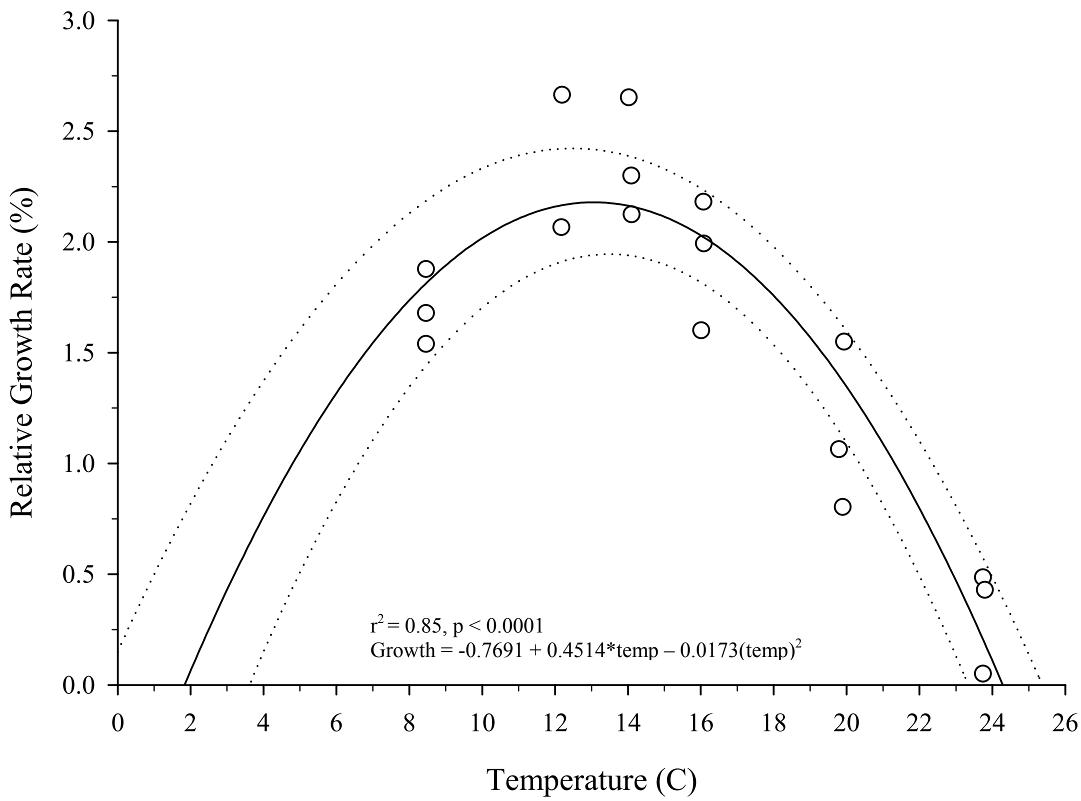

Figure 2. Growth of rainbow trout over 60 days in relation to temperature. Each circle represents the relative growth rate (%) per tank with three tanks tested at each temperature. Dotted lines indicate the 95% confidence interval of the regression line.

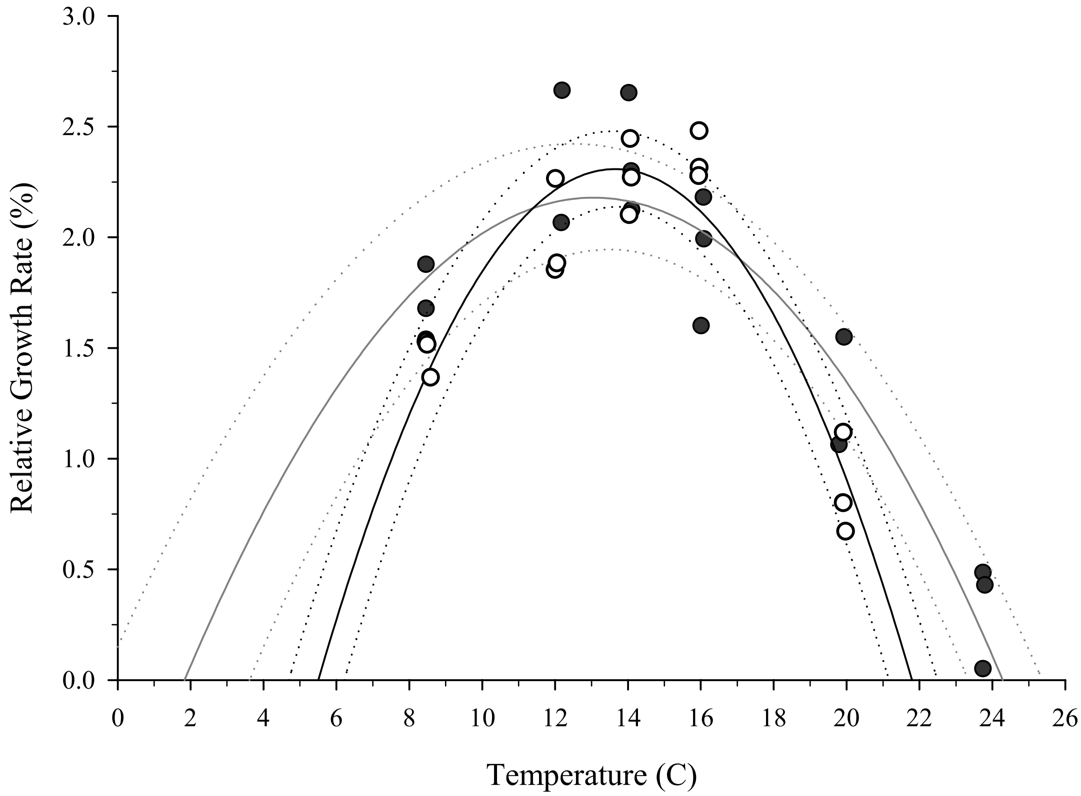

Figure 3. Growth of westslope cutthroat trout (white) and rainbow trout (black) over 60 days in relation to temperature. Each circle represents the relative growth rate (%) per tank with three tanks tested at each temperature. Dotted lines indicate the 95% confidence interval of the regression line.

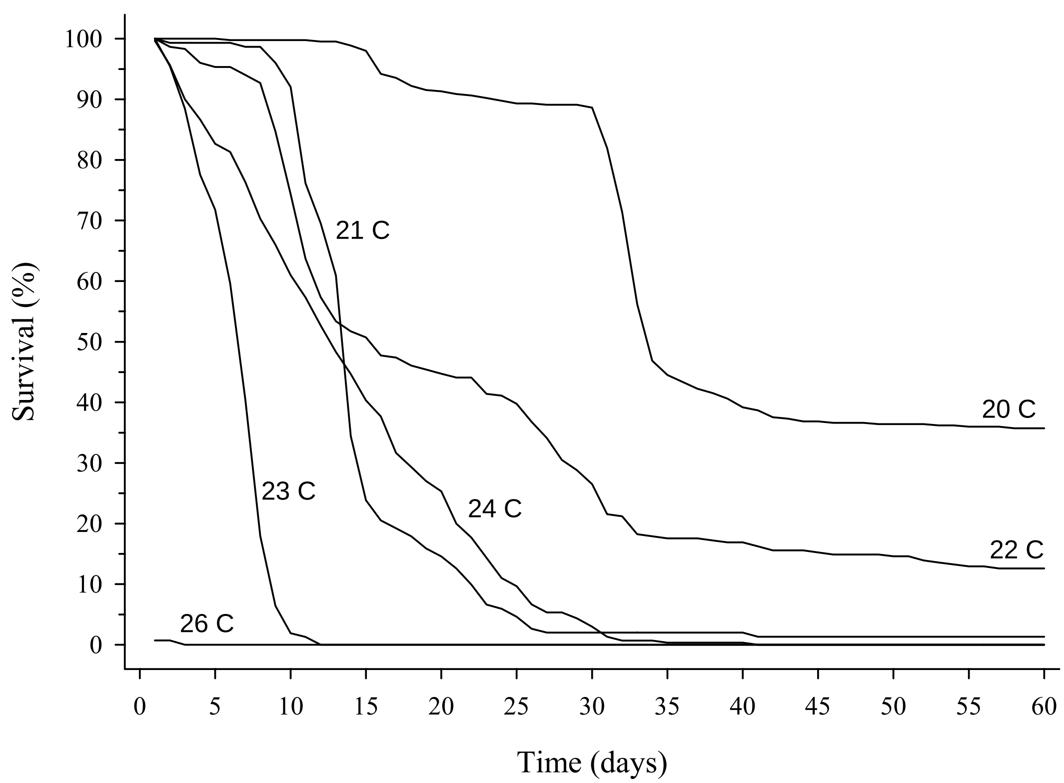

Figure 4. Survival (%) of age-1 westslope cutthroat trout during 60-d exposure to constant temperatures.

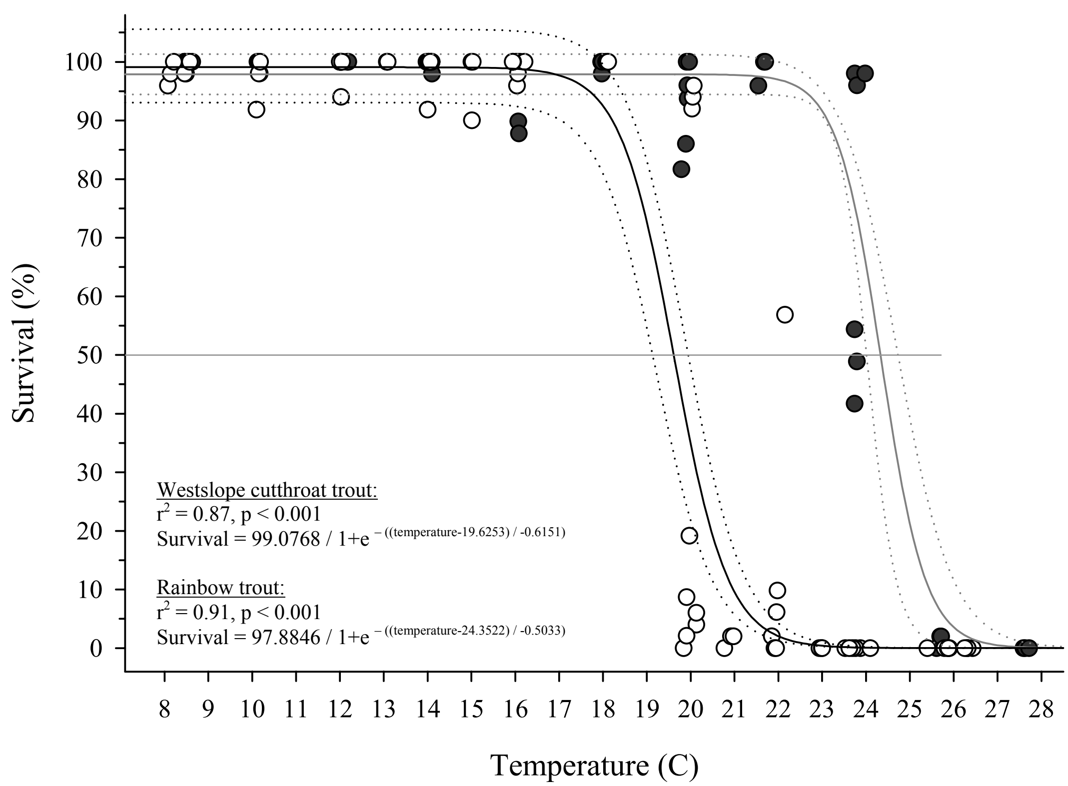

Figure 5. Survival (%) of age-1 westslope cutthroat trout (white) and rainbow trout (black) over 60 days in relation to temperature. Each point represents the percent survival in an individual tank at a given temperature. Dotted lines indicate the 95% confidence interval of the regression line.

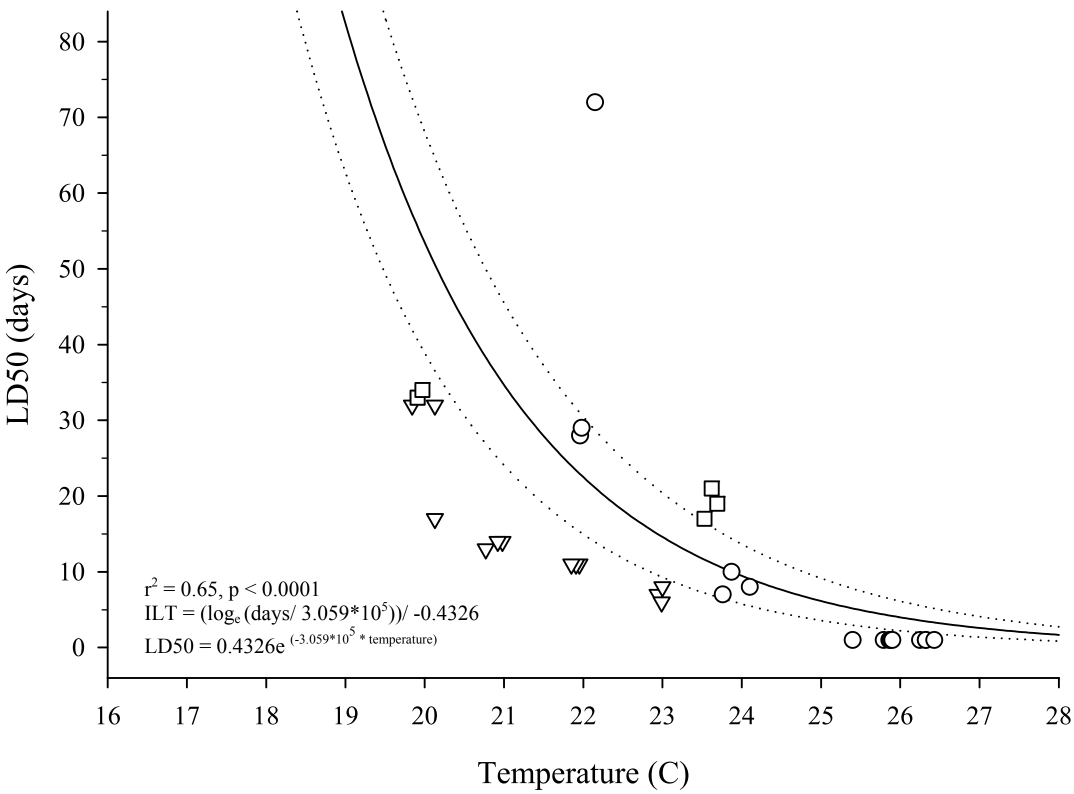

Figure 6. Survival of age-1 westslope cutthroat trout over 60 days in relation to temperature. Each point represents the median survival time (LD50) in an individual tank at a given temperature for each of three experiments; experiment one (circle), experiment two (triangle) and experiment three (square). The incipient lethal temperature given time of exposure is ILT. Dotted lines indicate the 95% confidence interval of the regression line.

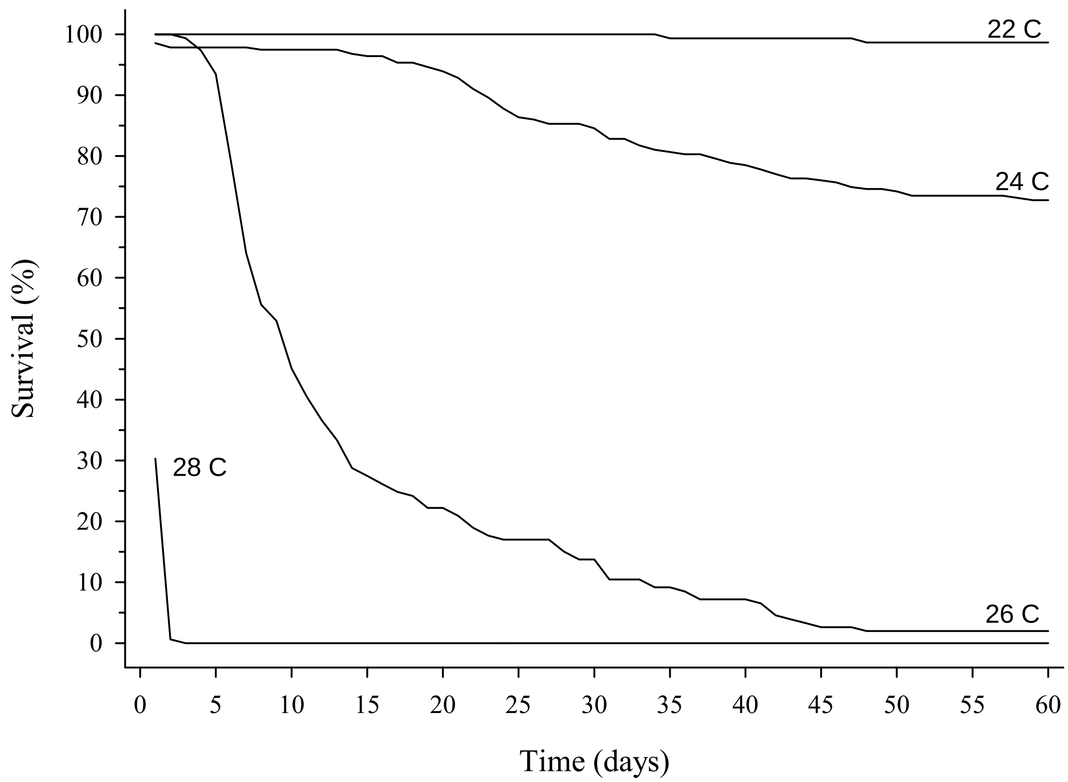

Figure 7. Survival (%) of age-1 rainbow trout during 60-d exposure to constant temperatures.

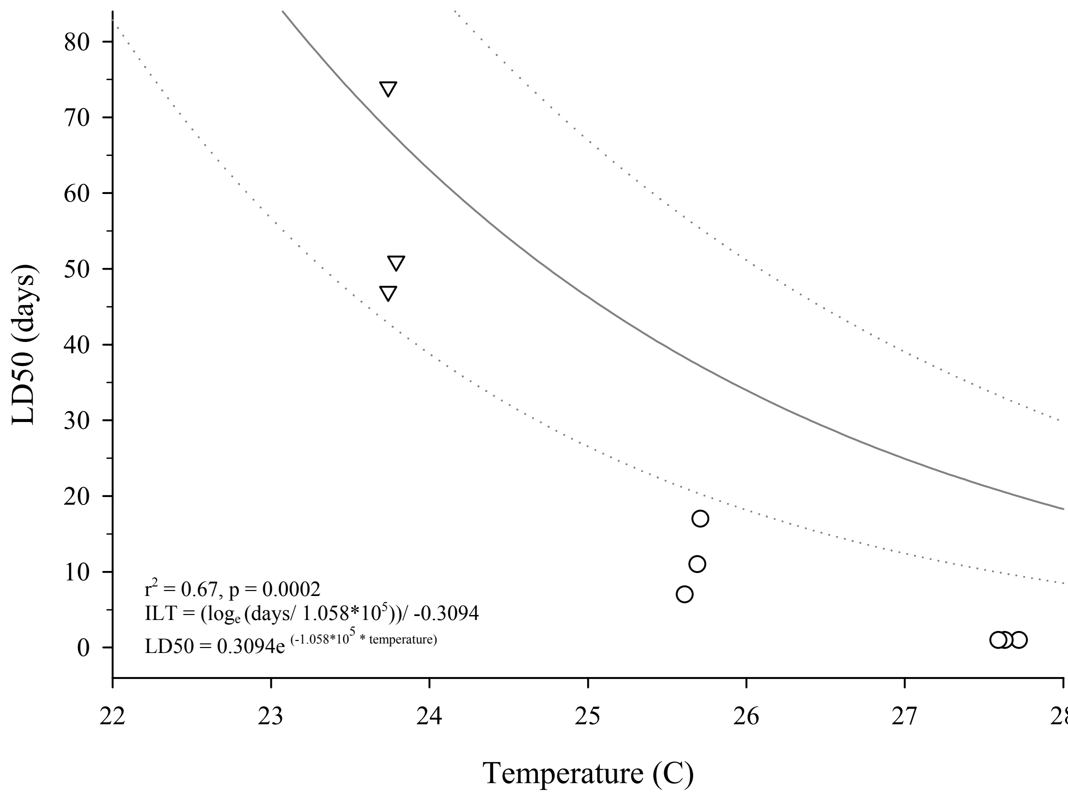

Figure 8. Survival of age-1 rainbow trout over 60 days in relation to temperature. Each point represents the median survival time (LD50) in an individual tank at a given temperature for each of two experiments; experiment one (circle) and experiment two (triangle). The incipient lethal temperature given time of exposure is ILT. Dotted lines indicate the 95% confidence interval of the regression line.

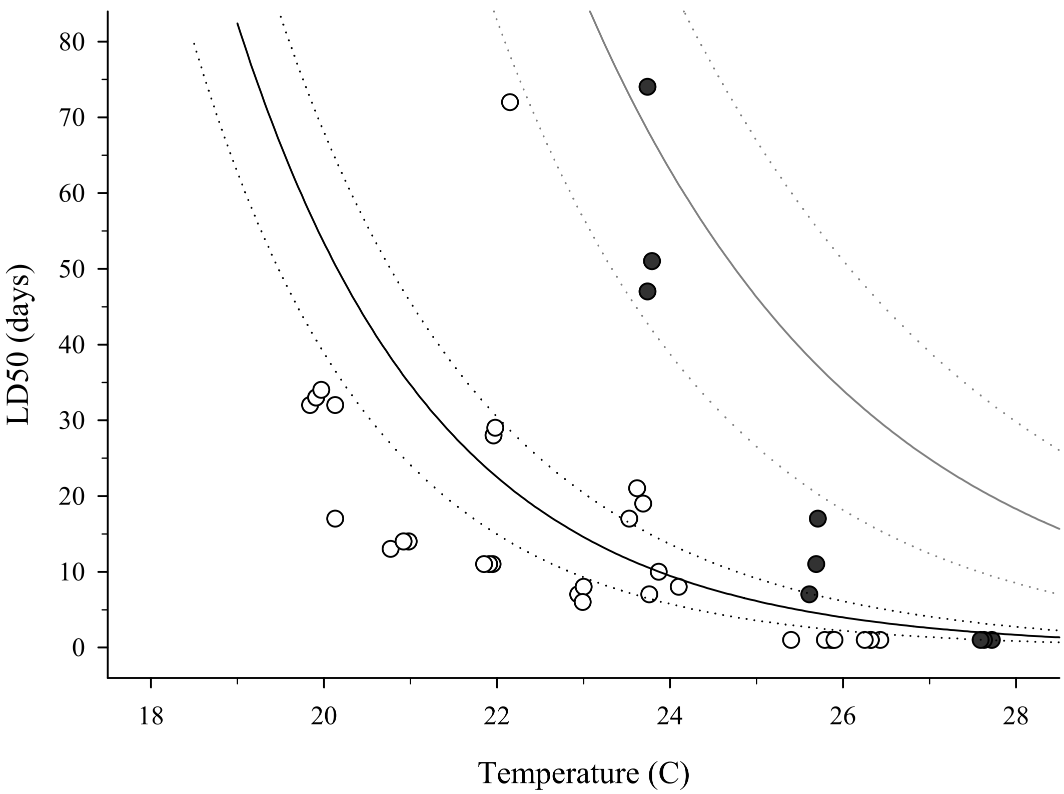

Figure 9. Survival of age-1 westslope cutthroat trout (white) and rainbow trout (black) over 60 days in relation to temperature. Each point represents the median survival time (LD50) in an individual tank at a given temperature. The incipient lethal temperature given time of exposure is ILT. Dotted lines indicate the 95% confidence interval of the regression line.