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[Article]

Upper Temperature Tolerance of Loach Minnow under Acute, Chronic, and Fluctuating Thermal Regimes

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Abstract.—We used four methods to estimate the upper lethal temperature of loach minnow *Rhinichthys cobitis*: the lethal thermal method (LTM), chronic lethal method (CLM), acclimated chronic exposure (ACE) method with static temperatures, and ACE method with diel temperature fluctuations. The upper lethal temperature of this species ranged between 32°C and 38°C, depending on the method and exposure time; however, temperatures as low as 28°C resulted in slowed growth compared with the control groups. In LTM trials, we increased temperatures 0.3°C/min and death occurred at 36.8 ± 0.2°C (mean ± SE) for fish (37–49 mm total length) acclimated to 30°C and at 36.4 ± 0.07°C for fish acclimated to 25°C. In CLM trials, temperatures were increased more slowly (1°C/d), allowing fish to acclimate. Mean temperature at death was 33.4 ± 0.1°C for fish 25–35 mm and 32.9 ± 0.4°C for fish 45–50 mm. In the ACE experiment with static temperatures, we exposed fish for 30 d to four constant temperatures. No fish (20–40 mm) survived beyond 30 d at 32°C and the 30-d temperature lethal to 50% of the test animals was 30.6°C. Growth at static 28°C and 30°C was slower than growth at 25°C, suggesting that fish were stressed at sublethal temperatures. In ACE trials with diel temperature fluctuations of 4, 6, and 10°C and a 32°C peak temperature, over 80% of fish (20–40 mm) survived 30 d. Although brief exposures to 32°C were not lethal, the growth of fish in the three fluctuating-temperature treatments was significantly less than the growth at the ambient temperature (25–29°C). To minimize thermal stress and buffer against temperature spikes, we recommend that loach minnow habitat be managed to avoid water temperatures above 28°C.

The loach minnow *Rhinichthys cobitis* is a small (<80 mm total length [TL]), benthic stream fish endemic to the Gila River basin in Arizona and New Mexico and the San Pedro River basin in Arizona and Sonora, Mexico (Minckley 1973; Propst et al. 1988). This species has suffered range reductions (>85%) in the last 75 years and was listed as threatened under the U.S. Endangered Species Act in 1986 (U.S. Fish and Wildlife Service 1986). The recovery plan for loach minnow recommends research that quantifies habitat requirements and analyzes the effect of habitat modification on life cycle completion, including tolerance to extreme temperatures (Marsh 1991). Temperature is a critical factor in the habitat requirements because it influences nearly all biochemical, physiological, and life history activities of fishes (Fry 1967; Brett 1971; Beitinger et al. 2000).

Small streams in the Gila River basin have historically experienced high summer temperatures. The highest temperatures recorded in small, flowing Arizona streams occurred in July and August and ranged from 24°C to 40.3°C with a 15–20°C daily fluctuation (Deacon and Minckley 1974; Deacon et al. 1987). Although native fish evolved under these temperature conditions, recent habitat degradation in small streams may have made this temperature range greater than before and perhaps beyond the range that native fish can tolerate physiologically. Surface flows in southwestern U.S. streams have notably diminished in the last 100 years due to groundwater pumping and diversion of water for irrigation, resulting in loss of riparian vegetation and stream channel drying (Minckley and Douglas 1991). Stream temperatures increase as a function of increasing heat energy or decreasing surface flows, so loss of riparian shading and diminished surface flows make southwestern U.S. streams vulnerable to increases in temperature range and in amplitude of diel temperature fluctuations (Poole and Berman 2001).

Loach minnow have been observed dying in Aravaipa Creek at water temperatures of 30.5°C (Deacon and Minckley 1974) and 34.5°C (July 2002; observation by Widmer, Carveth, and Simms). These mortalities were attributed to thermal stress, although

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other biotic and abiotic factors cannot be discounted. In the laboratory, other factors can be better controlled, making it possible to calculate an accurate lethal temperature.

Our objective was to determine the upper temperature tolerance of loach minnow using four standard methods: the lethal thermal method (LTM), acclimated chronic exposure (ACE) method with static temperatures, ACE with diel temperature fluctuations, and chronic lethal method (CLM).

Methods

Fish collection and holding.—We used loach minnow from Aravaipa Creek, Arizona, either wild caught or F_1 fish raised by Bubbling Ponds State Fish Hatchery, Arizona. Use of captive-bred fish reduced impact of collection on wild populations. We collected wild fish using a 1.6-mm mesh seine and transported them to the University of Arizona Environmental Research Laboratory in Tucson, Arizona, under guidelines from the Arizona Game and Fish Department. We mixed wild and F_1 fish at the laboratory and administered prophylactic treatments of formalin (Quickcure) for *Ichthyophthirius*. We also treated fish with antibiotics before all experiments, except ACE with static temperatures. The fish in this experiment were all F_1 fish and had no history of bacterial infection. We held fish in captivity at least three months before experiments, to eliminate any effects of antibiotics or capture stress, and fed them a finely ground dry food mixture containing freeze-dried bloodworms, sinking pellet food (45% protein), and tropical fish flakes (40% protein). We changed aquaria water (10–20% of total) 3–4 times per week with treated (Amquel or Stresscoat) municipal water to prevent build-up of nitrogenous waste. We used fish in experiments only once and used only healthy fish, with the exception of a LTM trial testing fish infected with yellow grub *Clinostomum complanatum*.

Lethal thermal method (LTM).—The LTM involves a linear change in water temperature from an acclimation temperature to one where death occurs and measures acute temperature tolerance. Death was defined as the cessation of opercular movement, and the temperature at death was the lethal thermal maximum (LTMax; Becker and Genoway 1979). The rate of temperature change we used for the LTM was fast enough that the fish were unable to acclimate to the changing temperatures, but slow enough that there was not a significant lag between the water temperature and fish body temperature. Becker and Genoway (1979) found 0.3°C/min satisfied these criteria for small-bodied fish. We chose the LTM over the critical thermal method (CTM; Cox 1974), because defining death as an endpoint was a less subjective test than using loss of equilibrium for loach minnow. Loss of equilibrium was difficult to detect in this benthic species, because fish would rest on the bottom of the beaker, moving only when prodded. The use of death as an endpoint is justified when typical CTM endpoints are not obvious (Beitinger et al. 2000), and is consistent with the other three methods in this paper.

We acclimated each of 12 loach minnow either infected with yellow grub (2–7 grubs externally visible) or not infected in 75-L glass aquaria at 25°C and 30°C (±0.5°C) maintained by 200-W Ebo-jager aquarium heaters for a minimum of 14 d. Fish with yellow grub did not survive 14 d at 30°C and were not tested. The 25°C and 30°C acclimation temperatures were common in natural loach minnow habitat. Large windows in the laboratory provided natural light cycles during acclimation. We conducted six trials with fish acclimated to 25°C ($N = 4$ for each trial, total $N = 24$) in February and March 2003 and three trials with fish acclimated to 30°C ($N = 3$–4 for each trial, total $N = 10$) in August 2003. We did not feed fish 24 h before testing.

For each trial, we randomly selected four fish from the acclimation tank and placed each in a separate 1-L beaker filled with water from the acclimation tank and equipped with a digital thermometer (Lifegard) and an air stone to mix and oxygenate the water. We placed the four beakers in a metal water bath (42 × 28 × 11 cm) with a powerhead (Rio 1100) to mix the water, and a metal grate to allow water to flow around all sides of the four beakers. We maintained the water bath at the acclimation temperature for 30 min to allow recovery from handling stress and then placed the water bath on a preheated hotplate (Fisher Scientific; 120 V, 5.4 A). We increased water temperature at 0.3°C/min by turning up heat settings at timed intervals. One person observed fish and a second recorded data and maintained hotplate settings. At the completion of the test, we measured fish (TL) and examined them for yellow grub before preserving them for the University of Arizona fish collection. We analyzed the effects of acclimation temperature, TL, and the presence of yellow grub on temperature at death with multiple linear regression (JMP version 4.0.4).

Acclimated chronic exposure (ACE) with static temperatures.—The ACE method (Zale 1984; Selong et al. 2001) involves a slow (1°C/d) increase from the acclimation temperature to the test temperatures and long exposure to static test temperatures (30+ d). We used this method to examine the effects of prolonged exposure to high temperatures on the survival and growth of loach minnow.

We conducted ACE tests from August to October

2003. We randomly assigned 13 fish of similar size (20–40 mm TL) and a temperature treatment of 25 (control), 28, 30, or 32°C, to each tank with three replicates of each treatment. To compare fish growth from each treatment, we calculated the average TL of the fish in each tank. The aluminum tanks (122 cm × 36 cm × 25 cm tall) were insulated with 5-cm-thick foam board, and filled with 72 L of water. Each tank contained a sponge filter, a 10-cm air stone, a powerhead (Rio 1100), and a 200-W aquarium heater (Ebo Jager). Powerheads created water current and maintained uniform water temperature. The sponge filter denitrified the tank water and provided fish with refuge from the current. We covered each tank with a foamboard lid, which had a screened window to allow light penetration. Timers maintained light cycles at an Arizona summer photoperiod (14 h light : 10 h dark). We fed fish to satiation with the dry food mixture daily, and provided live brine shrimp *Artemia* spp. three or more times per week to stimulate feeding and supplement the diet. We siphoned excess food and waste from tanks and replaced about 10% of the water with treated (Amquel) municipal water each day. Once a week, we measured pH, ammonia, and dissolved oxygen levels in the tanks.

Fish were acclimated to 25 ± 1°C for a minimum of14 d, and then tank temperatures were increased 1°C/d until test temperatures were reached. We maintained test temperatures for 30 d, which is the longest consecutive period that fish in the San Pedro River, Arizona, would experience temperature peaks above 30°C (J. Simms, unpublished thermograph data). We measured tank water temperatures 1–2 times/d to ensure that they remained within 1°C of the test temperature. Mortalities were recorded and preserved in 10% formalin.

At the end of the experiment, we removed and measured fish from all tanks on the same day so that fish growth (mean change in TL) was calculated over the same number of days for all treatments. This means that some fish were left in tanks at test temperatures 2–7 d past the completion of their 30-d exposure period. This extra exposure period equaled the amount of time necessary for the high-temperature treatments to complete the 30-d exposure period. The 30-d exposure period for the high-temperature treatments started and finished later than those for the low-temperature treatments, because it took longer to achieve test temperatures from the acclimation temperature based on an increase of 1°C/d. Only mortalities that occurred in the first 30 d of exposure to a test temperature were used in mortality analysis.

We used simple linear regression to analyze the effect of temperature on growth. Growth at 32°C was not calculated, because no fish survived the test period at this temperature. One tank at 32°C suffered equipment failure and data from this replicate were discarded. We plotted survival and mortality data with logistic regression for binomial counts and predicted the 30-d LT50, the temperature survived by 50% of fish after 30 d (Newman 1994).

Acclimated chronic exposure (ACE) with diel temperature fluctuations.—Natural streams undergo a diel temperature cycle, exposing fish to the highest temperatures for only a couple of hours each day (Sinokrot and Stefan 1993; Poole and Berman 2001). We examined the effect of temperature fluctuations on loach minnow growth and survival in the laboratory.

We conducted fluctuating-temperature tests in June–August 2004 in the tanks used in the previous ACE experiment but connected the tanks to a recirculating water system with computerized temperature control (Widmer et al., in press). Water in each tank was replaced each hour. Thermocouples (precision, ±0.5°C) recorded tank temperatures every 5 min, and we manually recorded tank temperatures twice daily using a digital thermometer (Lifegard). We calibrated the thermocouples and digital thermometer using an International Organization for Standardization–registered mercury thermometer. Other methods are the same as those used for the ACE with static temperatures experiment.

Temperature fluctuations were sinusoidal on a 24-h cycle, the highest temperature being reached at 1500 hours and the lowest at 0300 hours. All fluctuating treatments had the same upper temperature (32°C), but different lower temperatures (22, 26, 28°C; three replicates each). We chose 32°C as the upper temperature because it slightly exceeded the 30-d LT50 calculated from the ACE experiment with static temperatures and was lethal within 7 d under static conditions. We held three additional control tanks at ambient temperature (25–29°C) and three at static 32 ± 0.5°C. We randomly assigned 10 fish (20–40 mm) to each treatment tank.

We acclimated fish to 25 ± 0.5°C for a minimum of 14 d before starting temperature fluctuations. We then changed the temperature 1°C/d until the lowest temperature for each treatment was reached. From the lowest temperature, we increased the fluctuation amplitude 1°C/d until the desired test fluctuation was achieved and then maintained the test fluctuation for 30 d. Tank temperatures stayed within 0.5°C of the desired test temperature for the 30-d exposure, except for a 24-h period in the first week due to equipment failure. No fish died during the failure and the tank temperatures

remained below peak test temperatures (29–31°C). We recorded mortalities twice daily and preserved them in 95% ethanol. After all treatments had completed the 30-d exposure to test fluctuations, we removed the surviving fish from the tanks and measured TL.

We calculated percent survival for fish in each tank and used the Tukey–Kramer honestly significant difference (HSD) procedure ($\alpha = 0.05$) to compare survival among treatments. We calculated mean change in TL for each tank, except for tanks with no survivors (i.e., static 32°C), and used the Tukey–Kramer HSD procedure to compare growth among treatments.

Chronic lethal method (CLM).—The CLM involves a slow change in water temperature from an acclimation temperature until the death of the fish occurs. The rate of temperature change (1°C/d) is much slower than the rate use in the LTM and allows fish to acclimate during the experiment. The lethal temperature is the chronic lethal maximum (CLMax; Beitinger et al. 2000). We used this test to examine differences in thermal tolerance associated with size, because body temperature does not lag behind water temperature as it can with the LTM.

We conducted the CLM experiment in the same aquarium system as the ACE with diel temperature fluctuations. We grouped fish into two size classes (25–35 mm and 40–50 mm) and assigned 20 fish to each of four tanks, two replicates per size-class. Fish were acclimated to 30 ± 0.5°C for a minimum of 14 d and then we increased the temperature 1°C/d until all fish had died. Due to a calibration problem, water temperatures were 30.7, 31.7, 32.7, and 33.7°C during the temperature increase, rather than 31, 32, 33, and 34°C. We recorded the number of mortalities and the water temperature twice daily. We used an independent *t*-test to compare temperatures at death between size-classes.

Results

Lethal Thermal Method

In the LTM trials, death occurred at 36.8 ± 0.2°C (mean ± SE) for fish (37–49 mm TL) acclimated to 30°C and at 36.4 ± 0.07°C for fish acclimated to 25°C. We found some evidence that acclimation temperature, length, and infection status affected temperature tolerance of loach minnow (Table 1). The lethal thermal maximum increased 0.14°C (95% confidence interval [CI], 0.04–0.23; $F_{1,21} = 8.90$, $P = 0.007$) for every 1°C increase in acclimation temperature and 0.09°C (95% CI, 0.01–0.17; $F_{1,21} = 5.35$; $P = 0.032$) for every 1-mm increase in TL (multiple linear regression: $R^2 = 0.56$). Before accounting for differences in TL, LTMax was not significantly different ($P = 0.087$) between acclimation temperatures. For fish infected with yellow grub, LTMax was 0.39°C (95% CI, 0.22–0.57) lower than for uninfected fish after accounting for differences in TL ($R^2 = 0.72$; $F_{1,20} = 21.44$; $P = 0.0001$), although the severity of yellow grub infection (2–7 yellow grubs) did not affect LTMax ($P = 0.32$).

Acclimated Chronic Exposure with Static Temperatures

The 30-d LT50 for loach minnow in ACE tests with static temperatures was 30.6°C (95% CI, 30.3–31.09; likelihood ratio test: $\chi^2 = 94.06$; $P < 0.0001$; Figure 1). The temperature in ACE trials that resulted in death of all fish in the tanks was 32°C. This was 2.4°C lower than the LTMax. No fish survived more than 6 d at 32°C and mortality started on day 1 of exposure (Figure 2). Of the six fish that died at 30°C, four were in one tank. No fish died at 28°C. All fish at 25°C survived except one, which we failed to detect and presumed dead on day 30. Dead fish decomposed quickly at test temperatures and could be obscured by items in the tanks. We omitted the mortality at 25°C from the logistic regression of survival data used to predict the LT50 ($R^2 = 0.76$; Hosmer and Lemeshow goodness-of-fit test: $\chi^2 = 4.60$; $P = 0.10$), because the regression fit poorly when the mortality at 25°C was included ($R^2 = 0.60$; $\chi^2 = 519.16$; $P < 0.0001$).

Loach minnow growth and temperature were inversely related; fish that survived temperature treatments at 28°C and 30°C exhibited less growth than those at 25°C. The mean change in TL during the experiment was 1.27 mm (95% CI, 0.65–1.89 mm) less for every 1°C increase in temperature (simple linear regression; $F_{1,7} = 23.16$; $P = 0.0019$) (Figure 3).

Acclimated Chronic Exposure with Diel Temperature Fluctuations

Temperature fluctuations that included exposure to 32°C for approximately 1.5 h/d were less harmful to loach minnow than exposure to static 32°C. No fish died during the acclimation or temperature ramping period, and all fish survived exposure to the ambient temperature (25–29°C). More than 80% of the fish survived the three fluctuating-temperature treatments; the highest rate of mortality (16.7%) occurred in the treatment with the highest mean temperature (Table 2). No fish survived the static 32°C treatment; mortality started on day 8 of exposure and all fish died by day 17. No differences in growth existed among the three fluctuating-temperature treatments (Figure 4), but all showed significantly less growth than fish at ambient

TABLE 1.—Temperature at death (i.e., the mean lethal thermal maximum [LTMax]) for loach minnow acclimated to two temperatures and subjected to a dynamic temperature change of 0.3°C/min. A subset of the fish were also infected with yellow grubs.

Acclimation temperature (°C)	Yellow grub	Number of fish	Number of trials	Total length (mm)	Weight (g)	LTMax (°C)
25	No	13	3	42 (40–44)	0.68 (0.58–0.79)	36.4 (36.3–36.6)
25	Yes	11	3	40 (37–43)	0.70 (0.52–0.88)	36.0 (35.8–36.2)
30	No	10	3	38 (36–40)	0.50 (0.43–0.56)	36.8 (36.3–37.3)

temperature (Tukey–Kramer HSD procedure: $q = 3.20$, $P < 0.05$).

Chronic Lethal Method

The CLMax was intermediate to the LTMax and the lethal temperature recorded in static ACE trials. The mean CLMax was 33.4°C (95% CI, 32.4–34.27) for fish 25–35 mm and 32.9°C (95% CI, 31.9–33.8) for fish 40–50 mm. The difference in lethal temperature between the size-classes was not significant (independent *t*-test: $t = 1.70$, df = 2, $P = 0.23$). One fish from the 40–50-mm size-class died during acclimation and was excluded from analyses.

Discussion

The relationships among the calculated lethal temperatures from different thermal tolerance methods provide insight on how laboratory-derived data may be applied in natural settings. For loach minnow, ACE lethal temperature was less than the CLMax, which in turn was less than the LTMax. The upper temperature tolerance of loach minnow exposed to dynamic temperature increases was consistently higher than tolerance of loach minnow exposed to static temperatures, agreeing with the results using other species (Fry 1967; Lohr et al. 1996; Currie et al. 1998; Selong et al. 2001). Therefore, the harmful effects of high temperature increase with exposure time. A temperatures of about 33°C was lethal to loach minnow within 24 h with the CLM and 32°C was lethal within 1 week under ACE with static temperatures. The physiological cost to an individual, when exposed to a stressor, changes over time (Selye 1973), so loach minnow may have died at temperatures lower than 32°C if static temperature conditions had persisted for longer than 30 d. Loach minnow grew more slowly at 28 and 30°C than at 25°C, which suggests they were stressed at sublethal temperatures. We found little variation in lethal temperature of loach minnow with acclimation temperature, size, or yellow grub infection; the differences were probably not biologically significant.

The mortality data most relevant to natural stream conditions are those determined under fluctuating temperatures. Rapid temperature-increases used in LTM trials, and static temperatures used in the CLM and the static ACE trials, rarely occur in nature, so the lethal temperatures determined by these methods may not correspond well to lethal temperatures in natural environments. Exposure to peak temperatures generally occurs for just a few hours during a normal diel

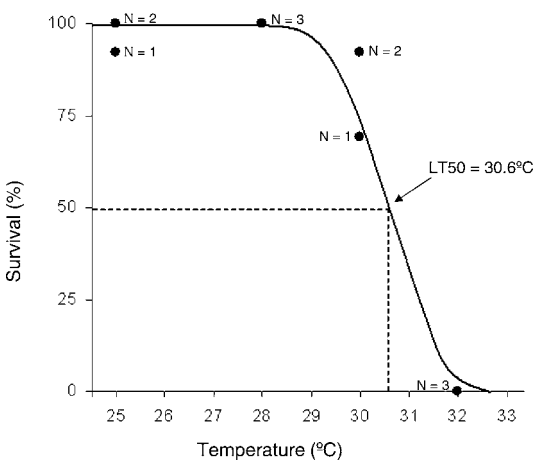

FIGURE 1.—Percent survival of loach minnow in each tank($N = 12$) after 30-d exposure to one of four constant temperatures: 25, 28, 30, and 32°C, with three replicates each and 13 fish per tank. The 30-d LT50 is the predicted temperature at which 50% of the fish would survive after 30 d.

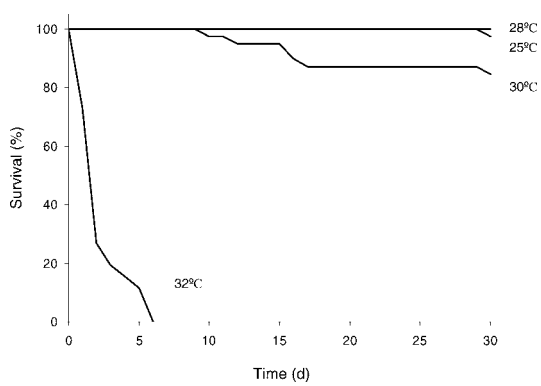

FIGURE 2.—Percent survival of loach minnow ($N = 39$) during a 30-d exposure to one of four static temperatures: 25, 28, 30, and 32°C.

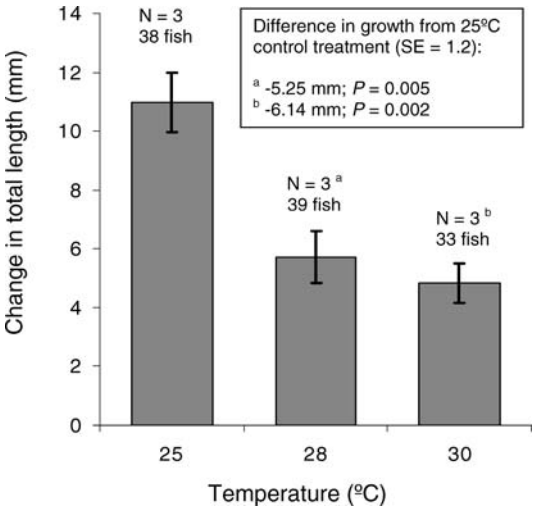

FIGURE 3.—Change in total length (average of tank means ± SE) of loach minnow exposed to 30 d of constant temperatures of 25, 28, and 30°C, with three replicates each.

temperature fluctuation. Most loach minnow were able to tolerate repeated brief exposures to 32°C for 30 d, even though 32°C was lethal under static temperature conditions. However, loach minnow exposed to temperature fluctuations including 32°C grew slower than fish exposed to constant lower temperatures. Thus, even if temperature fluctuations reduce stress and mortality associated with exposure to thermal extremes, they are not stress-free conditions. Temperature fluctuations appear to have similar effects on salmonids. Bonneville cutthroat trout *Oncorhynchus clarkii utah* (Johnstone and Rahel 2003) and Lahontan cutthroat trout *Oncorhynchus clarkii henshawii* (Dickerson and Vinyard 1999) survived fluctuations up to 26°C for 7 d even though lower temperatures were lethal under static conditions. Although the desert-dwelling loach minnow and salmonids may have little in common ecologically, physiological and behavioral response patterns are similar for many fish species when exposed to thermal extremes (Lutterschmidt and Hutchison 1997).

TABLE 2.— Survival of loach minnow exposed to 30 d of diel temperature fluctuations. Treatments with significant differences in mean 30-d survival rates are assigned different letters (Tukey–Kramer honestly significant difference test: $q = 3.29$, $\alpha = 0.05$).

Temperature (°C)	Survival (%)
22–32	96.7 z
26–32	96.7 z
28–32	83.3 y
32	0 x
Ambient (25–29)	100 z

Fluctuations may increase temperature tolerance because fish become heat-hardened during the brief exposures to high temperatures. Heat hardening, a temporary increase in thermal tolerance following heat shock, has been noted in animals exposed to near-lethal temperatures during CTM trials (Hutchison 1961; Hutchison and Maness 1979; Maness and Hutchison 1980). Furthermore, the rate of thermal acclimation is faster when fish are exposed to cyclic temperatures with a natural periodicity than when exposed to static temperatures (Heath 1963; Lowe and Heath 1969). Heat shock proteins are also thought to contribute to thermal tolerance under variable temperature conditions. These proteins are produced in response to periods of high sublethal stress and are thought to aid in cellular recovery as well as protect cellular function during subsequent exposures to the stressor (Coleman et al. 1995; Iwama et al. 1999). Although the same conditions can produce heat hardening and induce heat shock protein production, the mechanisms behind these responses may be different (Easton et al. 2005).

The maximum temperatures fishes can tolerate are often far higher than those that are optimal. Brett (1971) examined 25 different measures of physiological performance for sockeye salmon *Oncorhynchus nerka*, such as heart rate, selected temperature, and growth rate and found that most of these functioned optimally at 15°C. This was much lower than the upper

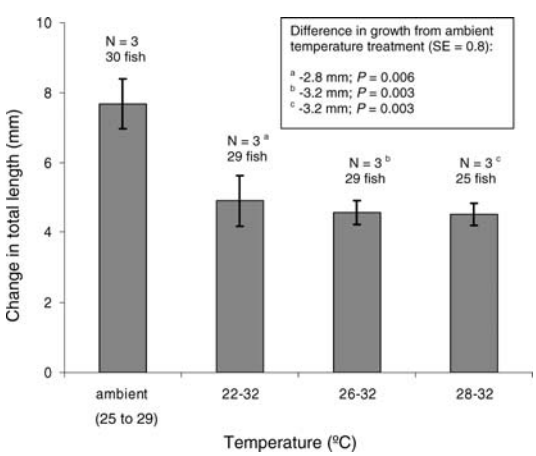

FIGURE 4.—Change in total length (average of tank means ± SE) of loach minnow exposed to 30 d of three fluctuating-temperature treatments and the ambient temperature, with three replicates each. The number of fish that survived 30 d of temperature treatment and were used in growth determination is indicated.

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lethal tolerance of 24°C for sockeye salmon. Stream management decisions based on conservative measures of temperature tolerance would help protect fish against unexpected temperature spikes and cumulative effects of other stressors (e.g., disease, changes in water level, interspecific competition) (Elliot 1981; Wedemeyer and McLeay 1981; McCullough 1999). Survival of 100% of loach minnow exposed to static 28°C during the ACE test suggests that a temperature regime with peak water temperatures below 28°C would cause little mortality. Exposure to static 28°C caused slowed growth, which indicates that fish were stressed at this temperature under static conditions. However, short periods of exposure to 28°C during a natural diel temperature fluctuation would be less stressful.

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References

Becker, C. D., and R. G. Genoway. 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. Environmental Biology of Fishes 4:245–256.

Beitinger, T. L., W. A. Bennett, and R. W. McCauley. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environmental Biology of Fishes 58:237–275.

Brett, J. R. 1971. Energetic responses of salmon to temperature: a study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). American Zoology 11:99–113.

Coleman, J. S., S. A. Heckathorn, and R. L. Hallberg. 1995. Heat shock proteins and thermotolerance: linking molecular and ecological perspectives. Trends in Ecology and Evolution 10:305–306.

Cox, D. K. 1974. Effects of three heating rates on the critical thermal maximum of bluegill. Pages 158–163 *in* J. W. Gibbons and R. R. Sharitz, editors. Thermal ecology. National Technical Information Service, Springfield, Virginia.

Currie, R. J., W. A. Bennett, and T. L. Beitinger. 1998. Critical thermal minima and maxima of three freshwater game species acclimated to constant temperatures. Environmental Biology of Fishes 51:187–200.

Deacon, J. E., and W. L. Minckley. 1974. Desert fishes. Pages 385–488 *in* G. W. Brown, Jr., editor. Desert biology. Academic Press, New York.

Deacon, J. E., P. B. Schumann, and E. L. Stuenkel. 1987. Thermal tolerances and preferences of fishes of the Virgin River system (Utah, Arizona, Nevada). Great Basin Naturalist 47:538–546.

Dickerson, B. R., and G. L. Vinyard. 1999. Effects of high chronic temperatures and diel temperature cycles on the survival and growth of Lahontan cutthroat trout. Transactions of the American Fisheries Society 128:516–521.

Easton, D. P., P. S. Rutledge, and J. R. Spotila. 2005. Heat shock protein induction and induced thermal tolerance are independent in adult salamanders. Journal of Experimental Zoology 241:263–267.

Elliot, J. M. 1981. Some aspects of thermal stress in freshwater teleosts. Pages 209–249 *in* A. D. Pickering, editor. Stress and fish. Academic Press, New York.

Fry, F. E. J. 1967. Reponses of vertebrate poikilotherms to temperature. Pages 375–409 *in* A. H. Rose, editor. Thermobiology. Academic Press, New York.

Heath, W. G. 1963. Thermoperiodism in sea-run cutthroat trout (*Salmo clarki clarki*). Science 142:486–488.

Hutchison, V. H. 1961. Critical thermal maximum in salamanders. Physiological Zoology 34:92–125.

Hutchison, V. H., and J. D. Maness. 1979. The role of behavior in temperature acclimation and tolerance in ectotherms. American Zoologist 19:367–384.

Iwama, G. K., M. M. Vijayan, R. B. Forsyth, and P. A. Ackerman. 1999. Heat shock proteins and physiological stress in fish. American Zoologist 39:901–909.

Johnstone, H. C., and F. J. Rahel. 2003. Assessing temperature tolerance on Bonneville cutthroat trout based on constant and cycling thermal regimes. Transactions of the American Fisheries Society 132:92–99.

Lohr, S. C., P. A. Byorth, C. M. Kaya, and W. P. Dwyer. 1996. High-temperature tolerances of fluvial arctic grayling and comparisons with summer river temperatures of the Big Hole River, Montana. Transactions of the American Fisheries Society 125:933–939.

Lowe, C. H., and W. G. Heath. 1969. Behavioral and physiological responses to temperature in the desert pupfish, *Cyprinodon macularius*. Physiological Zoology 42:53–59.

Lutterschmidt, W. I., and V. H. Hutchison. 1997. The critical thermal maximum: history and critique. Canadian Journal of Zoology 5:225–264.

Maness, J. D., and V. E. Hutchison. 1980. Acute adjustment of thermal tolerance in vertebrate ectotherms following exposure to critical thermal maxima. Journal of Thermal Biology 5:225–233.

Marsh, P. C. 1991. Loach minnow, *Tiaroga cobitis*, recovery

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plan. U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

McCullough, D. A. 1999. A review and synthesis of effects of alterations to the water temperature regime on freshwater life stages of salmonids, with special reference to Chinook salmon. U.S. Environmental Protection Agency, EPA Report 910-R-99-010, Seattle.

Minckley, W. L. 1973. Fishes of Arizona. Arizona Game and Fish Department, Phoenix.

Minckley, W. L., and M. E. Douglas. 1991. Discovery and extinction of western fishes: a blink of the eye in geologic time. Pages 7–17 *in* W. L. Minckley and J. E. Deacon, editors. Battle against extinction: native fish management in the American West. University of Arizona Press, Tucson.

Newman, M. C. 1994. Quantitative methods in aquatic ecotoxicology. CRC Press, Boca Raton, Florida.

Poole, G. C., and C. H. Berman. 2001. An ecological perspective on instream temperature: natural heat dynamics and mechanisms of human-caused degradation. Environmental Management 27:787–802.

Propst, D. L., K. R. Bestgen, and C. W. Painter. 1988. Distribution, status, biology, and conservation of the loach minnow, *Tiaroga cobitis* Girard, in New Mexico. New Mexico Department of Game and Fish, Santa Fe.

Selong, J. H., T. E. McMahon, A. V. Zale, and F. T. Barrows. 2001. Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerance in fishes. Transactions of the American Fisheries Society 130:1026–1037.

Selye, H. 1973. The evolution of the stress concept. American Scientist 61:692–699.

Sinokrot, G. A., and H. G. Stefan. 1993. Stream temperature dynamics: measurements and modeling. Water Resources Research 29 (28 October 1986):2299–2312.

U.S. Fish and Wildlife Service. 1986. Endangered and threatened wildlife and plants; determination of threatened status for the loach minnow; final rule. Federal Register 51:39468–39478.

Wedemeyer, G. A., and D. J. McLeay. 1981. Methods for determining the tolerance of fishes to environmental stressors. Pages 247–275 *in* A. D. Pickering, editor. Stress and fish. Academic Press, London.

Widmer, A. M., C. J. Carveth, J. W. Keffler, and S. A. Bonar. In press. Design of a computerized temperature-controlled recirculating aquaria system. Aquacultural Engineering.

Zale, A. V. 1984. Applied aspects of the thermal biology, ecology, and life history of the blue tilapia, *Tilapia aurea* (Pisces: Cichlidae). Doctoral dissertation. University of Florida, Gainesville.