Water Pollution Studies

Federal Aid Project F-234-R21

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Job Progress Report

Colorado Parks & Wildlife

Aquatic Wildlife Research Section

Fort Collins, Colorado

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State: Colorado Study No. F-243-R21

Title: Water Pollution Studies

Period Covered: July 1, 2013 to June 30, 2014

Project Objective: To develop quantitative chemical and toxicological data on the effects of pollutants and water quality on aquatic life, investigate water quality problems in the field, and provide expertise in aquatic chemistry and aquatic toxicology.

STUDY PLAN A: LABORATORY STUDIES

Brief Description: Conduct laboratory-based experiments to measure effects of contaminants and water quality on aquatic organisms.

2013-2014 Job A.1. Bear Creek Greenback Cutthroat Trout Fitness

Job A.1. Objective: Compare fitness of Bear Creek greenback cutthroat trout with fitness of Rio Grande River cutthroat trout and Bear Creek-Rio Grande River crosses.

ACCOMPLISHMENTS

Fitness of Bear Creek Cutthroat Trout

INTRODUCTION

Recent molecular work on Colorado's native cutthroat trout has revealed the existence of a unique lineage native to the South Platte basin (Bear Creek, El Paso County, Colorado; Metcalf et al. 2007, Metcalf et al. 2012) thought to be the only true representative of our state fish, the greenback cutthroat trout. Ironically, the same widespread stocking efforts that jeopardized many native trout populations across the west served to preserve this South Platte native outside its aboriginal range in a fishless stream above a natural barrier for 130 years (Kennedy 2010, Rogers 2012). It is likely that this population experienced two genetic bottlenecks when founding the initial population, and while establishing the captive broodstock. It is not surprising that this population displays the least genetic diversity of any examined so far in the state.

The Greenback Cutthroat Trout Recovery Team, an inter-agency consortium of researchers and managers, is anxious to replicate this population elsewhere across its former range to help provide long-term security for this lineage. However the ability of this strain to survive and prosper in the wild is understudied. Informal observations from laboratories culturing the broodstock suggest these fish are particularly finicky, are rife with physical deformities, and display poor growth. Large repatriation projects are planned, but apparent

inbreeding depression may complicate those efforts.

The Colorado Parks and Wildlife Aquatic Toxicology Laboratory (CPWATL) planned to conduct numerous studies that compare fitness of Bear Creek greenback cutthroat trout with fitness of Rio Grande River cutthroat trout and Bear Creek-Rio Grande River crosses. Comparisons to Rio Grande River cutthroats were not possible. Carr Creek Colorado River Cutthroats were available and gravid.

This study explored basic fitness measures in a controlled setting, allowing us to compare performance of the Bear Creek fish with a more genetically diverse Carr Creek cutthroat trout and will priovide insight into the level of inbreeding depression that may have occurred.

METHODS

Crosses were made at the Leadville National Fish Hatchery, Leadville, CO, USA. Sixteen families were created with one of four female Bear Creek or four female Carr Creek cutthroat trout, each being fertilized with extended pooled milt from either four Bear Creek or four Carr Creek male cutthroat trout yielding four families of pure Bear Creek fish, four families of pure Carr Creek fish, and eight families of hybrids. Fertilized eggs were transported to the Colorado Parks and Wildlife Aquatic Toxicology Lab from 24 June 2013 to 11 June 2014 where they were raised under blind common garden conditions.

Egg hatch success, survival and growth of fry were assessed throughout the experiment. A sub-sample of fry from each replicate was exposed to high temperature and low dissolved oxygen conditions using standardized experimental methods. Susceptibility to aqueous zinc exposure was not assessed due to the limited number of organisms. All mortalities (including eggs) were preserved for genetic analysis and were provided to Colorado Parks & Wildlife (CPW) and University of Colorado researchers to tie developmental success and environmental fitness observed in the CPWATL to genetic observations in the field. A portion of each family was sent on to Colorado State University's Foothills Research Campus for subsequent investigations on fluctuating asymmetry displayed by these fish. Remaining hybrids have been returned to Leadville National Fish Hatchery for rearing to adult size so that F2 crosses can be made, whose progeny will allow even greater insight into alleles that are responsible for the deformities seen. Data analysis is ongoing.

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2013-2014 Job A.2. Interaction between Gill Lice Infestation and Sensitivity to Stressors

Job A.2. Objective: Study the effect of gill lice infestation on responses of mountain whitefish, kokanee salmon, cutthroat trout and rainbow trout to temperature, hypoxia and chemical stressors.

ACCOMPLISHMENTS

Mountain whitefish (*Prosopium williamsoni*) were spawned on 10 October 2013. The eggs were hatched, and organisms reared in the CPWATL Kokanee salmon (*Oncorhynchus nerka*), obtained in 2012, are being raised to sufficient size for exposure to gill lice (*Salmincola californica*).

Water treatment methods and biosecurity practices were devised to ensure gill lice would not contaminate laboratory space. Infrastructure and treatment tanks for experiments were fabricated and modified to conduct experiments that allow for control of physical and chemical conditions needed to support salmon and gill lice life cycles while preventing contamination of laboratory space.

Research and method development surrounding the biology of infecting fish with gill lice found laboratory conditions are not conducive to maintaining the gill lice life cycle. Young age classes were found to be resistant to gill lice by both informal experiments and natural occurrence.

Gill lice infestations have frequently been noted near Handcart Creek, Colorado (Steven Brinkman, personal communication), a drainage with significant aqueous metal concentrations from acid-rock drainage. This suggests metal stress might also increase susceptibility to gill lice. Because salmonids are most sensitive to metal pollution at young (30 days post swim-up) age classes and are most susceptible to gill lice infestation at much older age classes, the study of interactions between these stressors is complicated and will require experiments that are numerous years in duration. Due to mortality in both salmon and whitefish cultures, an insignificant number of organisms are available to assess interactions between stress from toxicants and stress form gill lice parasites. The remaining organisms from these cultures were provided to CPW and Colorado State University (CSU) researchers exposing salmonid fish species to gill lice. Their preliminary results suggest organisms previously stressed by high temperatures and/or low dissolved oxygen were more susceptible to infestation.

2013-2014 Job A.3. Effects of Application of the Pesticide Permethrin for Mosquito Control on Fishes

Job A.3. Objective: Conduct *in situ* exposures of cutthroat and/or rainbow trout fry to Gunnison River water following aerial application of permethrin.

ACCOMPLISHMENTS

Due to municipalities on the Gunnison River basin altering management plans, permethrin applications will not be repeated as in 2012 when anglers reported co-occurrence of stonefly mortalities, a major food source for trout. *In Situ* experiments and observations were not possible. Application of pesticides to mitigate mosquito and mountain pine beetle populations may pose a threat to aquatic ecosystems and this will continue to be a priority for Colorado Parks and Wildlife (CPW) toxicological research. To conduct both laboratory and field research improved analytical capabilities and personal safety equipment are needed. Method development for assessment of permethrin, piperonyl butoxide (a synergist frequently formulated with permethrin) and carbamates is ongoing (March 2013-present). Laboratory ventilation systems that prevent exposure of workers to organic pesticides have been fabricated and installed in the Colorado Parks and Wildlife Aquatic Toxicology Laboratory (CPWATL), Fort Collins, CO. Fabrication of treatment vessels and toxicant diluter systems that mimic natural environments and avoid materials that adsorb organic toxicants is ongoing. Experiments are planned for the fall of 2014.

2013-2014 Job A.4. Control of Blue-green Algal Blooms

Job A.4. Objective: Study effects of nutrient enrichment on blue-green algae and toxins.

ACCOMPLISHMENTS

Blue-green algae (cyanobacteria) are not utilized by fish and is associated with poor drinking water quality. Green algae compete with cyanobacteria and are utilized by food webs that support sport fish. Experiments examining the effects of nutrients (e.g. ammonium nitrate) and toxicants on the ratio of blue-green algae to green algae proved difficult on the scale proposed in the 2013-2014 narrative. Method development for assessment of community structure by pulse amplitude modulated fluorimetry is ongoing. This technique has the potential to assess relative concentrations of algae utilized and not utilized by fish. *In situ* experiments of larger size will need to be conducted to address this research objective in the years to come.

STUDY PLAN B: TECHNICAL ASSISTANCE

Brief Description: Conduct toxicity experiments and provide technical assistance as requested from regulators to be incorporated into policy; conduct water chemistry analysis and training for CPW and other agencies.

2008-2014 Job B.1. Water Quality Assistance to Colorado Parks and Wildlife Personnel and Other State and Federal Agencies

Job B.1. Objective: To provide technical assistance and expertise, consultation, evaluation and training in aquatic toxicology and aquatic chemistry to Colorado Parks and Wildlife (CPW) and other state and federal personnel as requested. Assist in the investigation of fish kills. Conduct short or long term experiments to produce toxicity data, or develop site-specific field studies, when such data in the literature are lacking or inadequate. Collect and analyze water and/or fish tissues to assess water quality problems. Analyze rotenone in water samples as part of CPW reclamation projects. Publish results of experiments and water quality investigations in peer-reviewed journals. Ultimately, these activities will assist regulatory agencies in the development, implementation, and enforcement of water quality standards needed to protect or enhance the aquatic resources of Colorado.

ACCOMPLISHMENTS

On-site assessment of rotenone concentrations for reclamation projects in Colorado

Reclamation projects at Square Top Lakes (fall 2013) and Zimmerman Lake (fall 2013 and summer 2014) utilized rotenone pesticide to remove invasive fish before reintroduction of native fish. Field assessment of rotenone concentrations were needed to ensure target concentrations had been met and ensure rotenone had been neutralized afterwards. The unique analytical capabilities of the CPWATL Mobile Environmental Toxicology and Analytical Laboratory (METAL) were employed to provide this information on site.

On site assessment of rotenone concentrations for reclamation project in Arizona

Removal of the Red Shiner on the Virgin River of Arizona, Utah and Nevada has been ongoing. A large stretch of this river, scheduled for reclamation the summer of 2014, had unique qualities that made the river extremely challenging. Flow is heavily regulated for agricultural and municipal use leading to a variable hydrograph. Throughout the Arizona strip (region north of the Grand Canyon) the Virgin River's flow recedes to subsurface flow and resurfaces numerous times throughout the 35 km reach. This reduces the amount of the river that needs to be treated with rotenone but adds tremendous uncertainty in the speed the toxicant will be traveling and in the volume of water that needs to be treated.

As part of an inter-agency effort to improve Colorado River watersheds, CPW made the unique analytical capabilities of our Mobile Environmental Toxicology and Analytical Laboratory (METAL) available to reclamation efforts from 15 June 2014 to the final assessment

on 20 June 2014. The real time analysis of rotenone in water samples was able to provide managers with concentrations in 10-25 minutes at the incident command post. The nearest agency lab capable of producing these results had a 10-24 hour turnaround time.

Throughout the reclamation projects CPW's METAL provided concentrations of field and drinking water samples. Lower than expected rotenone observations were blamed on matrix interferences or decay of rotenone in stock containers. Both had the potential to reduce bioavailable pesticide in the river system. Several impromptu experiments were run to validate or discredit these possibilities. A sub-sample of stock solutions being used for reclamation operations were assessed to be within 2% of the value posted on labels. Spiked samples throughout the week validated that organic material was not biasing the concentrations reported by the CPW's High Performance Liquid Chromatograph (HPLC). Ultimately lower than expected concentrations were blamed on timing because of reduced flows; the plume of rotenone from the dripping stations traveled far slower than expected. This was accounted for in subsequent days.

Because bioassays for this experiment were done using only nominal concentrations long before CPW staff arrived at the site, CPW recreated the bioassay with limited replication to ensure target concentrations. Observed concentrations were similar and lethal to the target species.

Rapid turnaround of results afforded by CPW's unique analytical capabilities allowed staff and security to leave 48 hours earlier than using Arizona Game and Fish laboratory facilities in Phoenix.

Technical support to Colorado State University - Investigations of thermal tolerance

Technical support was provided to Colorado State University's Biology Department where thermal tolerance of various aquatic insects is being assessed. These results will aid regulators in efforts to devise protective temperature standards for Colorado.

Technical Support to Colorado State University - Indirect effects of Fe and aqueous metal toxicants

Aquatic insects are the primary source of food for cold water sport fish in Colorado Mountain Streams. Understanding macroinvertebrate community response to pollution is key to protecting the ecosystems that support trout fisheries. Analyses of iron, copper and zinc were provided to Colorado State University (CSU) Fish, Wildlife and Conservation Biology department researchers that are examining indirect effects of iron pollution on aquatic insects. Results and publications are pending. Data are currently being analyzed and will be reported next segment.

Technical support to CPW hatcheries – Temperature effects on trout spawning success

To address high rates of pick-off (mortality) of eggs in the CPW hatchery system an experiment was conducted on Hofer (German) strain rainbow trout (*Oncorhynchus mykiss GR strain*) crossed with Harrison Lake strain rainbow trout (*Oncorhynchus mykiss HA strain*). Crossed females and males were spawned and their eggs were hardened in 5 different combinations of water temperatures. Raceway (0.6-2.2 º C) and well water (6.1-6.8 º C) temperatures often differ significantly at the CPW's Poudre River Hatchery especially in winter months when raceway waters are near freezing. To determine if temperature was a source of egg mortality the following treatments were assigned: spawning and hardening in raceway water (R-R), spawning and hardening in well water (W-W) and spawning and hardening in an equal mixture of well and raceway water (50%-50%). To ensure differences in water chemistry were not affecting results, well water was chilled to raceway water temperatures and a treatment group was spawned and hardened in chilled well water (CW-CW). This group was compared to the R-R treatment levels. Importance of spawning temperature and hardening temperature was assessed by exposing eggs in the 5th treatment level to chilled well water during spawning and unchilled well water during hardening (CW-W). To reduce variability associated with genetics and fecundity of parents, the eggs of one female and milt of three males (family) was split into 5 containers, each receiving one of the 5 treatment levels. Photo analysis of embryos and statistical analysis is ongoing. Results will be published in the 2015 progress report.

Technical support to Colorado State University - Aquatic macroinvertebrate culturing

Aquatic insect communities comprise the majority of food for trout species in Colorado Mountain Streams. These aquatic insects respond to pollution differently than do fish species. Lab space and infrastructure was provided to CSU's Fish, Wildlife and Conservation Biology Department to assist with experiments exposing aquatic insects to metal mixtures. This study will help examine indirect and direct effects of iron on aquatic systems, and improve mixed-metal toxicity models. Data are currently being analyzed. Results will be available in the 2015 progress report.

Technical support to State and Federal Water Quality Regulators - Aquatic macroinvertebrate culturing

Professional opinions and consulting was provided to State Water and Federal Agencies (USEPA, CDPHE, CPW) on numerous issues that directly affect sport fish health and habitat, not limited to opinions of state-wide water quality criteria changes, review and comment on site specific alterations to water quality standards, suggestions of regulation strategies for unusual toxicants and professional opinion of Fe, ammonia, selenium, nutrients, Cu, and Zn regulation. Sport fish populations will only be sustainable if water quality standards are protective of the species and ecosystem functions that sustain these fisheries. Providing advice and counsel to co-operating agencies helps ensure water quality standards will promote sport fish populations in the future.

Technical support to State and Federal Water Quality Regulators – Development of temperature standards protective of trout species

Differences in high temperature tolerance in three Colorado Cutthroat Trout strains.

INTRODUCTION

Variation in environmental conditions is pervasive in natural systems. At some point many organisms will have to survive through a period when environmental conditions will approach the limits of their tolerance. Surviving these extremes is a requirement for the persistence of a species. Recent global climate trends indicate that the duration and magnitude of extreme weather events are increasing. The goal of this study was to determine what effects extreme temperatures may have on the Colorado River cutthroat trout (*Oncorynchus clarkii pleuriticus*).

Temperature is an environmental characteristic that fundamentally affects fishes (Beitinger et al. 2000), and is currently receiving much attention due to the fact that global temperatures appear to be increasing. Global climate change is well supported and accepted by a majority of the scientific community (Oreskes 2004). Within the next century temperatures in the western US are expected to increase between 2.1°C and 5.7°C (IPCC 2007). An increase of this magnitude would certainly affect Colorado fish species, especially stenothermal coldwater species such as Colorado River cutthroat trout. One effect that warming is predicted to have is to shift trout ranges from larger low elevation streams to smaller high elevation streams (Wenger et al. 2011). Many populations of cutthroat trout in Colorado are already highly isolated in the upper reaches of rivers, leaving few temperature refuges if temperatures in the state do sharply increase. Isolation of these populations will likely become more profound if temperatures drastically increase, compounding one of the largest obstacles that the species faces.

One possible response that cutthroat trout may have to these increased temperatures is adaptation. Adapting to higher temperatures could allow cutthroat trout to continue to occupy their realized niche even if temperatures increase. There are many questions about how quickly and to what degree trout can adapt and evolve in response to warmer temperatures. If they are too slow to keep up with global climate change then they will be subjected to range constrictions, but it would stand to reason that adaptation might allow these fish to expand their realized niche.

METHODS

Acquisition of test organisms

Lake Nanita and Trapper Creek cutthroat strains were obtained as eyed eggs from the Colorado Parks and Wildlife Glenwood Springs Hatchery on June 13, 2012. These eggs were packed with ice in a cooler and driven to the Colorado Parks and Wildlife Aquatic Toxicology Laboratory in Fort Collins. Navajo River eggs were obtained from the Colorado Parks and Wildlife Durango Hatchery on July 2, 2012. The Durango hatchery shipped the eggs on June 27, 2012. Eggs from each strain were placed into separate labeled aquaria. Except for the Navajo River eggs, each aquarium was kept at 13.5°C until eggs hatched and embryos achieved swimup.

Navajo River eggs were kept at 6°C until swimup and then maintained at 13.5°C in order to delay their development to conform to laboratory limitations. At one week post swim-up, forty two fish from each population were removed from the holding tanks and used in the growth experiment.

Critical Thermal Maxima - CTM

The critical thermal maximum tests were conducted in rectangular glass tanks (18 x 9 x 12 cm). Individual fry were transferred to the tank which contained 1.75 L of water at the acclimation temperature. A temperature controller/programmer (B-series Love Controls Division) controlled a submersible aquarium heater which heated the water at a rate of 0.3°C/min, as recommended by Becker and Genoway (1979). Aeration of the tank maintained saturated dissolved oxygen levels and ensured that temperature was homogeneous throughout the tank. Water temperatures were increased until sustained (≥10s) loss of equilibrium (LOE) was observed in the fish being tested. LOE was defined as failure to maintain a dorsal-ventral vertical orientation. This endpoint was used because a fish exhibiting LOE in nature would be unable to escape excessive high temperatures and would most likely perish (Beitinger & Bennett, 2000).

Once an organism lost equilibrium, the temperature of the water was recorded and the fish was removed from the experimental apparatus and placed into a small recovery container with water at the acclimation temperature. Each fish was monitored for twenty minutes in order to ensure that it survived the test and regained equilibrium. At the end of the recovery period fish were euthanized using MS-222 and were weighed.

Growth Experiment

The growth experiment apparatus consisted of forty two ~1.5L tanks which were grouped into seven blocks of six tanks, each block ultimately maintained at a different temperature. At the start of the experiment all tanks were held at the fish's acclimation temperature and were then increased to target temperatures at 1°C/day during the following week. Within each block, Lake Nanita and Trappers Creek strains of cutthroat trout were randomly assigned to three of the six tanks resulting in twenty-one of the containers holding Lake Nanita strain cutthroat trout and twenty-one holding Trapper Creeks strain. Two fish were initially added to each container to buffer against any mortality observed during the temperature adjustment week, however no mortality was observed. The target temperatures for each block were 6.0°C, 8.5°C, 11.0°C, 13.5°C, 16.0°C, 18.5°C, and 21.0°C respectively. Once target temperatures were reached, one fish was culled leaving one fish per container. The remaining fish was subsequently weighed thus marking day 0 of the experiment. Weights were then taken every 7 days until the final day of the experiment's conclusion 21 days after day 0. Fish were fed to satiation twice a day in order to achieve the maximum growth rates possible for each fish at each temperature. Feed consisted of newly hatched brine shrimp, frozen cyclop-eeze (Argent, Redmond, WA USA), and a 3:1 mixture of trout chow starter and freeze-dried cyclop-eeze.

Upper Incipient Lethal Temperature (UILT)

Ten fry (0.266 g mean weight) were randomly selected and distributed into one of thirty 7.6 L glass aquaria. Water for each tank was supplied by one of five aerated temperature controlled head tanks at a rate of 50 ml/min. Treatment temperatures were 21.5ºC, 23.0ºC, 24.5ºC, 26.0ºC, and 27.5°C and were replicated six times (three replicates per strain). Laboratory limitations made it impractical to test all three strains at the same time, therefore Lake Nanita and Trapper's Creek strains were tested concurrently while Navajo River strain was tested immediately after the first two strains. The initial temperature for each head tank was 20°C, the same as the acclimation temperature of the test organisms. Temperatures were then adjusted upwards at a rate of 1°C/d until target temperatures were reached. Temperature adjustments were programmed so that all treatments reached their target temperatures on the same day. Fish were fed to satiation twice a day. Tanks were siphoned every day in order to reduce waste accumulation and to maintain cleanliness. Any deceased fish were removed and the time and fish weight were recorded. The test was terminated seven days after target temperatures were reached, at which point all remaining fish were euthanized, counted, and weighed.

Temperature Preference

Temperature preference trials were carried out in an annular chamber similar in design to that suggested by Myrick et al. (2004). This design included a series of three concentric rings. The outermost ring was divided into 16 sections which allowed for the input of water at different temperatures and allowed water to overflow from three holes drilled into the top portion of each section of the ring. The water overflowed from the outermost ring into a middle ring where a temperature gradient was present and where fish were placed for the trial. Finally, water could flow from the middle ring to the innermost ring, where water depth could be manipulated by three standpipes, two of which allowed the water to recirculate and the third of which drained water from the system. Capturing some of the water made the system partially recirculating which allowed less water to be used while running the apparatus.

The first step for each trial allowed the temperature gradient to establish and then temperatures were recorded around the gradient. Temperatures were taken in the middle ring at positions corresponding to 1 of 32 numbered sections. Once temperatures were taken and the gradient was characterized an individual fish was placed into the middle ring. Once in the apparatus the fish was given one hour for acclimation and recovery from handling stress. A curtain was placed around the entire apparatus in order to reduce fish reactions to movement around the temperature preference chamber. At the end of the acclimation period a video monitoring system which viewed the entire ring was activated and the fish's movement was recorded for one hour. At the conclusion of one hour the temperature gradient was flipped using two three way valves, reversing the side of the chamber that received warm and cold water. The gradient switching process took approximately 30 minutes. At the conclusion of the switch the fish was given another hour to select its preferred temperature. This process of gradient switching allowed for greater confidence that the fish was selecting its position based on temperature and not due to an artifact of the laboratory. Fish would generally have to move in order to find the same temperature in the first gradient and the second.

The process described above resulted in two hours of video of each fish for analysis. Video analyses involved recording the position of the fish every minute for each hour long portion of the trial. If a fish failed to make a selection the data was discarded and data from a different fish was used.

RESULTS

Of the three strains tested Lake Nanita and Trapper's Creek exhibited the same critical thermal maximums (CTMs) with temperatures of 29.8°C (±0.4, n=31 and ± 0.4, n=24 respectively) while Navajo Lake strain exhibited a lower CTM of 29.1°C (±0.4, n=21; Figure 1). The CTMs of Trappers Creek and Lake Nanita strains were not significantly different while Navajo River strain was significantly lower than the other two strains tested (Figure 1).

In the 7-day UILT test, survival varied among temperature treatments and among the different strains (Figure 2). In the lowest treatment (21.0°C) there was little difference between the strains, with Lake Nanita incurring the least mortality at 6.67%, Navajo River incurring the most mortality at 13.33%, and Trapper's Creek incurring the median mortality with 10%. The upper two temperature treatments (26°C and 27.5°C) caused 100% mortality in all three strains. The highest treatment caused complete failure within two days of reaching target temperatures (one day for Navajo River strain). The second highest temperature treatment caused complete failure of the Navajo River strain within three days, Lake Nanita strain within six days, and Trapper's Creek strain within seven days. UILTs decreased rapidly as the test progressed. The final 7-day UILT for the strains was 24.8ºC, 23.9ºC, and 23.7°C for Trapper's Creek, Navajo River, and Lake Nanita strains respectively.

The three strains all showed different temperature preferences. The combined mean temperature preference for each of the three strains was 15.6°C (standard error =0.3), 14.9°C (std. err. =0.2), and 13.9°C (std. err. =0.8) for Trapper's Creek, Navajo River, and Lake Nanita respectively (Table 1). The mean size of the test fish did vary between strains with Trapper's Creek being largest (3.94 g), followed by Lake Nanita (3.26 g), and Navajo River (3.26 g).

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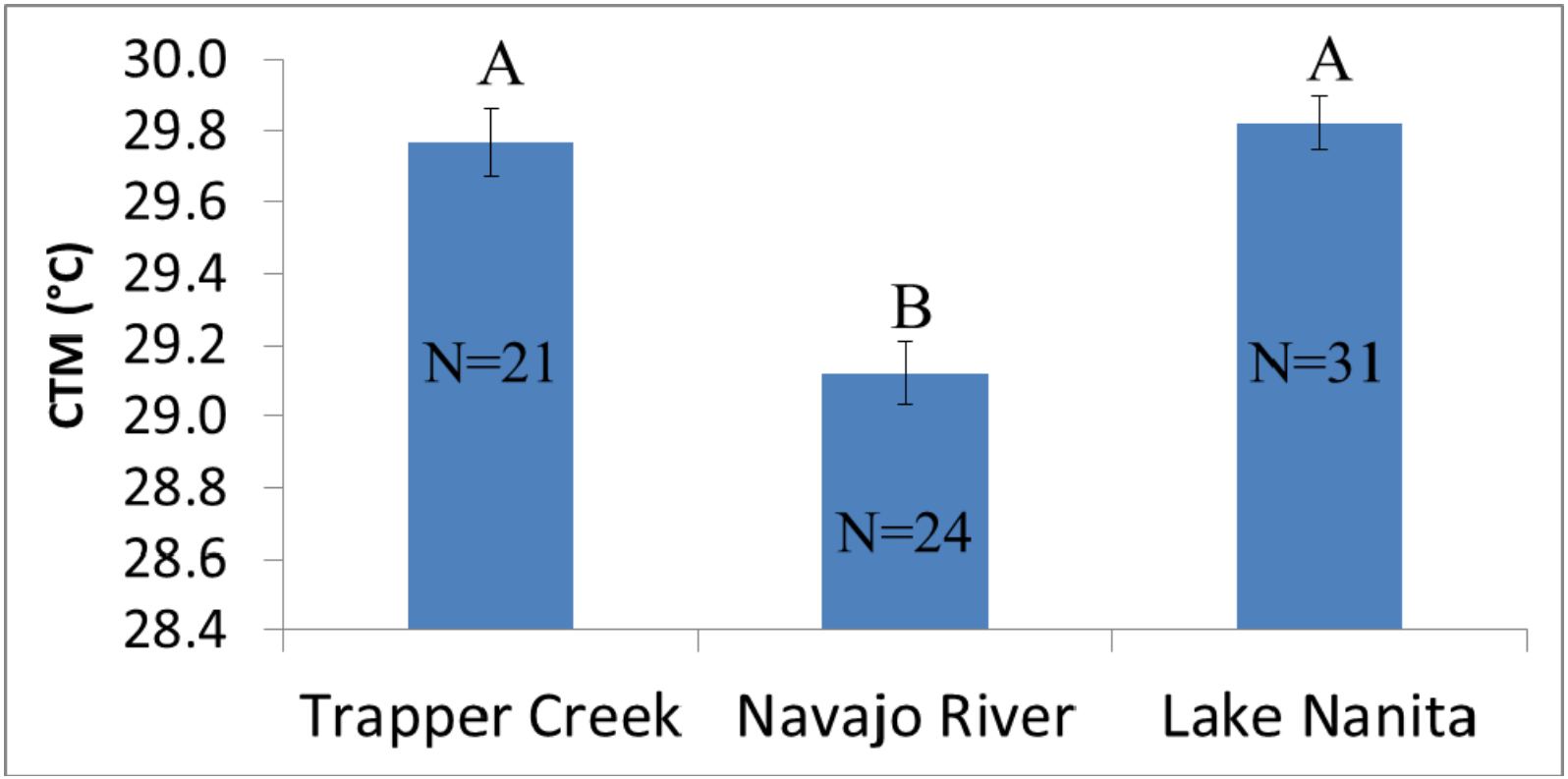

Figure 1 – Average Critical Thermal Maxima for Trapper Creek, Navajo River and Lake Nanita strains of Colorado Cutthroat trout.

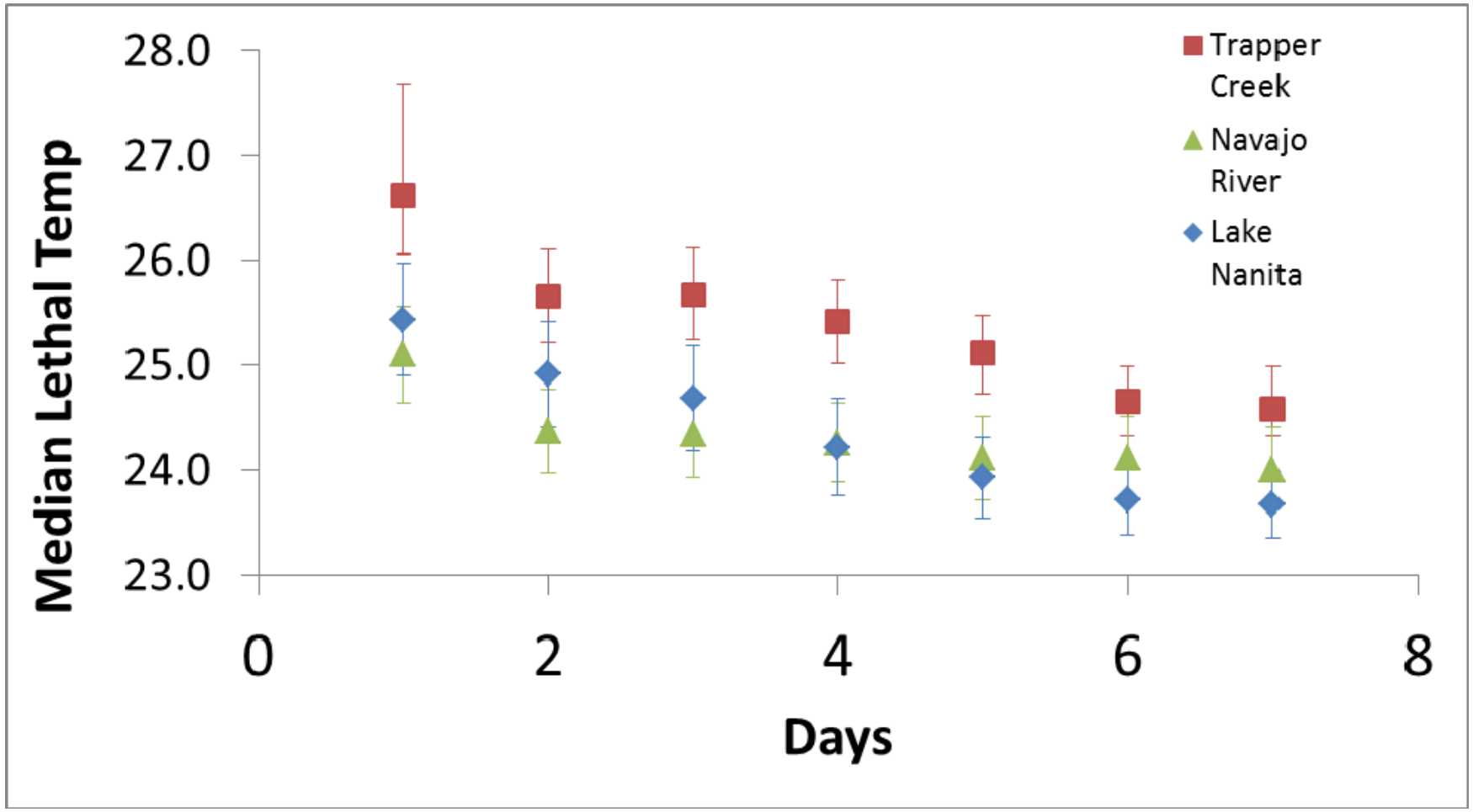

Figure 2- Median Lethal Temperature observed for Trapper Creek, Navajo River and Lake Nanita strains of Colorado Cutthroat trout.

Strain	Mean Preferred Temp	Standard Error	Mean Weight	n
Trapper Creek	15.6°C	0.3	3.94 g	6
Navajo River	14.9°C	0.2	3.01 g	6
Lake Nanita	13.9°C	0.8	3.26 g	6

Table 1 – Preferred temperatures for Trapper Creek, Navajo River and Lake Nanita strains of Colorado Cutthroat trout.

Accomplishments initiated under previous Federal Aid projects F243R from 2008 to 2013 that were not detailed in the 2013-2014 Grant Narrative, but conducted or completed under this segment.

2008-2012 Job A.3. Toxicity of Metals to Fish

Job A.3. Objective: Continued characterization of metal toxicity on sport fish.

2012-2013 Job A.5. Testing and Validation of the Biotic Ligand Model

Determine the ability of the Biotic Ligand Model to estimate acute and chronic toxicity effects of metals on aquatic organisms exposed under multiple water quality conditions.

ACCOMPLISHMENTS

Effects of copper on survival and olfaction of fathead minnow and rainbow trout in low hardness water

A series of tests were conducted to evaluate the effect of low water hardness on toxicity of copper to fathead minnows (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*). Standard acute copper toxicity tests were conducted with fathead minnows and rainbow trout to measure direct effects in the form of lethality. Additional tests were conducted to measure effects of low sublethal copper concentrations on olfaction of rainbow trout. Recent research demonstrated that low sublethal copper concentrations reduce or eliminate the ability of salmonids to detect alarm cues (Sandahl et al. 2007, Kennedy et al. 2012, McIntyre et al. 2012). Juvenile rainbow trout were exposed to low levels of copper and an alarm cue was introduced to the exposure chambers. Responses of the individuals were monitored using a video system. Videos and data are currently being assessed. Results will be reported in the 2015 progress report. Because much of Colorado's mountain streams exhibit low DOC and low hardness this study has potential to assess if Colorado's biotic ligand model based Cu standards are protective.

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Effects of Cu and Zn on brook trout and cutthroat trout under fluctuating and static temperature regimes

INTRODUCTION

Anticipated climate changes are expected to have profound effects on structure and function of aquatic ecosystems. Cold-water trout species are especially vulnerable to a warming climate (Ficke et al. 2008). Considerable research has evaluated potential responses of trout species to rising stream temperatures and reduced habitat due to anticipated climate changes (Rahel et al. 2008, Williams et al. 2009, Wenger et al. 2011, Roberts et al. 2013). Temperature directly influences distribution of trout species but also interacts with other stressors that may increase risk to populations. Increasing water temperatures have the potential to increase toxicity of contaminants (Ficke et al. 2008). Less considered is the possibility that contaminants may adversely affect thermal tolerance. To examine this, toxicity tests with copper and zinc were conducted at high and low temperatures using two cold-water salmonids-brook trout and cutthroat trout. The effect of zinc and copper exposure on thermal tolerance of brook trout was also measured.

MATERIALS and METHODS

Organisms

Brook trout eggs were collected from wild adults during spawning runs from Trapper's Lake (Garfield County, Colorado). Eggs from six females were stripped into a dry bowl and fertilized with milt from two males per female. Fertilization rates were enhanced by maintaining anhydrous conditions during the process, with conception occurring in the ovarian fluid bath. Eggs were water hardened for an additional 30 minutes in water from the brood source before transport in 1 gallon coolers to the Colorado Parks and Wildlife Aquatic Toxicology Laboratory in Fort Collins, CO. Upon arrival, eggs were treated with 1600 mg/L formalin to control fungus (Piper et al. 1982) and incubated in egg incubation trays that received Fort Collins dechlorinated municipal tap water (hardness near 50 mg/L as $CaCO_3$, temperature 10°C, pH 7.4) at a rate of 200 mls/min. Unfertilized or fungus infected eggs were carefully removed daily. Upon swimup, fry were fed commercial soft moist trout chow starter.

Colorado River cutthroat trout embryos of the Lake Nanita lineage were obtained as eyed eggs from the Colorado Parks and Wildlife Glenwood Springs fish hatchery. Cutthroat trout eggs and fry were treated as described for brook trout except incubation and rearing temperature was 12°C.

Toxicity tests

Copper and zinc toxicity tests were conducted with brook trout at low (10°C) and high (20°C) temperatures. Low temperature toxicity tests were conducted with 30 d post swimup fry reared at 10°C. For the high temperature toxicity tests, brook trout fry were acclimated from their rearing temperature (10°C) to 20°C by adjusting water temperature at 1°C/day until holding temperature of 20°C was achieved which was then maintained for 7 days prior to the toxicity tests. Copper tests were conducted with cutthroat trout fry at low (12°C) and high temperatures (20°C). Acclimation of cutthroat trout fry from the rearing temperature of 12°C to the higher temperature (20°C) was the same as for brook trout. In addition to conducting a copper test with cutthroat trout fry at a static temperature of 20ºC, a test was conducted in which the water temperature was fluctuated ± 5°C on a diel cycle between a low of 15°C at 6:00 AM to a high of 25°C at 6:00 PM. Water temperatures were controlled using heaters and chillers that controlled a water temperature bath via a programmable temperature controller (Love Series 16B, Love Controls, Michigan City, IN, USA). Temperature loggers (Hobo) placed in six randomly selected exposure chambers measured water temperature every 5 minutes.

Toxicity tests were conducted following guidance provided by ASTM method E729 (ASTM 1997). Dechlorinated Fort Collins municipal tap water supplied continuous-flow diluters (Benoit et al. 1982) constructed of teflon, polyethylene and polypropylene components. The diluter delivered five exposure levels with a 50% dilution ratio and an exposure control. A flow splitter allocated each concentration equally among four replicate exposure chambers at a rate of 40 mLs/min each. Exposure solutions were delivered using food-grade vinyl tubing. Exposure chambers consisted of polypropylene containers with a capacity of 2.8 L. Test solutions overflowed from exposure chambers into temperature-controlled water baths. For the fluctuating temperature test, the water bath temperature was controlled using a temperature controller/programmer (B-series Love Controls Division) and a series of heat exchangers, water chillers and water pumps. The temperature controller was programmed to fluctuate the temperature of the water bath on a diel cycle between 15°C at 6:00AM to 25°C at 6:00 PM. The controllers were programmed to change the temperature at a constant linear rate resulting in a sawtooth profile (Figure 1).

Semi-opaque lids covered the exposure chambers and limited light exposure from dim fluorescent lighting (16h/8h photoperiod) and prevented organisms from escaping. Chemical stock solutions were prepared by dissolving calculated amounts of analytical reagent grade toxicant ($CuSO_4 \cdot 5H_20$ Mallincrodt or $ZnSO_4 \cdot 7H_2O$ JT Baker) in deionized water. Chemical stock solutions were delivered to the diluter via peristaltic pump at approximately 2.0 mLs/min. New stock solutions were prepared as needed during the toxicity tests. Diluters and toxicant flow rates were monitored daily to ensure proper operation. Biomass loading in test chambers was less than 29% of maximum recommended rates (ASTM 1997). At the start of each test, individuals were distributed one at a time to each exposure chamber and the process was repeated until ten fry were distributed to each chamber. Fry were fasted for 24 hours before the start of the acute test and were not fed during the exposure. Mortality data were collected multiple times daily. Mortality was operationally defined as the failure to respond to repeated prodding with a fish net. Dead fry were removed from the test chambers, blotted dry with a paper towel, weighed and recorded. Ninety six hour median lethal concentrations (LC50) were

estimated using the Trimmed Spearman-Karber technique (Hamilton et al. 1977, Hamilton 1978) with automatic trim.

Water quality characteristics of exposure waters were measured at 0, 48 and 96 hours. Alkalinity was determined titrimetrically according to Standard Methods (APHA 1998). Dissolved oxygen, conductivity and pH were measured using electronic meters calibrated prior to each use. Water temperature was recorded hourly by a temperature logger (HOBO) placed in a randomly selected aquarium. Water samples for metal analyses were collected weekly from each exposure level with surviving fry. Exposure water was passed through a 0.45 µm filter (Acrodisc), collected in 2 oz HDPE bottles (Nalgene) and immediately preserved with high purity nitric acid (JT Baker) to pH < 2. Copper, zinc, sodium, potassium, calcium and magnesium concentrations were measured using a Thermo Jarrell Ash ICP (IRIS) spectrometer calibrated prior to each use and the calibration verified using a NIST traceable QAQC standard (High Purity Standards, Charleston SC). Water samples for chloride and sulfate analyses were analyzed with a Flow Injection Analyzer (QuikChem 8000, Lachat Instruments, Loveland, CO, USA) using EPA methods 325.1 and 375.4, respectively. Sample splits and spikes were collected at each sampling event. Water samples for dissolved organic carbon (DOC) were gravity-filtered through pre-combusted 47 mm glass fiber filters (1.0 µm size particle retention) (Gelman Sciences Inc., Ann Arbor, MI, USA) using a stainless steel filter holder into pre-cleaned amber glass bottles (VWR Trace Clean) and submitted to a commercial laboratory for analysis.

Critical Thermal Maxima

Critical thermal maxima (CTMs) were measured on fry surviving the toxicity tests using methodology recommended by Becker and Genoway (1979). The critical thermal maximum tests were conducted in rectangular glass tanks (18 x 9 x 12 cm). Individual fry were transferred to the tank containing 1.75 L of water at the acclimation temperature. A temperature controller/programmer (B-series Love Controls Division) controlled a submersible aquarium heater which heated the water at a rate of 0.3 °C/min. Aeration of the tank maintained saturated dissolved oxygen levels and ensured a homogeneous temperature throughout the tank. Water temperatures were increased until sustained (≥10s) loss of equilibrium (LOE) was observed in the fish being tested. LOE was defined as failure to maintain a dorsal-ventral vertical orientation. This endpoint was used because a fish exhibiting LOE in nature would be unable to escape excessive high temperatures and would most likely perish (Beitinger et al. 2000).

Once a fish lost equilibrium, the temperature of the water was recorded and the fish was removed from the experimental apparatus and placed into a small recovery container containing water at the acclimation temperature. Each fish was monitored for twenty minutes in order to ensure that it survived the test and regained equilibrium. At the end of the recovery period fish were euthanized using MS-222 and were weighed.

RESULTS

Brook trout-Cu

Toxicity of copper to brook trout was greater at 20°C than at 10°C (Figure 2). At 20°C, copper toxicity was manifested quickly with a large majority of mortality occurring in the initial 24 hours of exposure. As a result, the median lethal concentration (LC50) of copper at 20°C decreased only slightly with duration of exposure. In contrast, copper toxicity at 10°C progressed more slowly resulting in a more pronounced decrease of the LC50 with time. Critical thermal maxima (CTMs) of brook trout fry acclimated to 20°C were about 2°C higher than fry acclimated to 10°C (Figure 3). Exposure to copper decreased CTMs at both temperatures. Exposure to copper concentrations ≥20 µg/L at 10ºC significantly decreased CTMs. At 20°C, CTMs were significantly decreased by exposure to 40 µg/L copper.

Brook trout-Zn

Toxicity of zinc to brook trout fry was not significantly affected by temperature (Figure 4). The LC50 of zinc at both temperatures decreased with duration of exposure in a manner similar to copper at 10°C. CTMs of brook trout fry acclimated to 20°C were not significantly affected by zinc exposure (Figure 5). However, fry acclimated to 10°C had significantly reduced CTMs after exposure to zinc concentrations of 1000 µg/L.

Cutthroat trout-Cu

As with brook trout fry exposed to copper, toxicity was less at the lower static temperature (12°C) than at the higher static temperature (20°C) (Figure 6). The trend of LC50s with time also bore a resemblance to the pattern observed with brook trout. Specifically, mortality at the static low temperature was delayed and the LC50 exhibited a rapid decrease as duration of exposure increased, whereas onset of mortality at the high temperature occurred shortly after initiation of exposure and within a small window of time resulting in LC50s decreasing much more gradually with duration of exposure.

The fluctuating temperature regime was intended to fluctuate the water temperature in the exposure tanks on a diel cycle between a low of 15°C at 6:00 AM and increasing linearly with time to a high temperature of 25°C at 6:00 PM before decreasing again to 15°C at 6:00 AM the next day. The average temperature was intended to be 20°C. This pattern was for the most part followed. Water temperatures recorded by temperature loggers placed in six randomly selected tanks closely followed the intended cycle (Figure 1). The average minimum temperature was 14.90°C (SD 0.09°C), the average maximum temperature was 24.64°C (SD 0.20°C). The average temperature of the fluctuating temperature test (mean 19.74°C; SD 0.11°C) was very close to the average temperature of the 20°C static test (mean 19.66°C; SD 0.56°C). Despite the similarity of the average temperatures between the fluctuating and static tests, the copper LC50s of the fluctuating test were significantly lower than those of the static test at all durations (Figure 5).

DISCUSSION

A few general conclusions may be derived from the above tests. Water temperature may greatly affect toxicity of some metals (e.g. copper) but toxicity of other metals may be unaffected (e.g. zinc). Temperature significantly affects the kinetics of toxicity; at higher temperatures, mortality of both brook trout and cutthroat trout occurred at lower copper concentrations and after much shorter durations compared to lower temperatures. Toxicity of copper is increased in fluctuating temperature regimes compared to static regimes, even though the average temperature may be the same. Finally, exposure to copper and zinc reduces thermal tolerance of brook trout.

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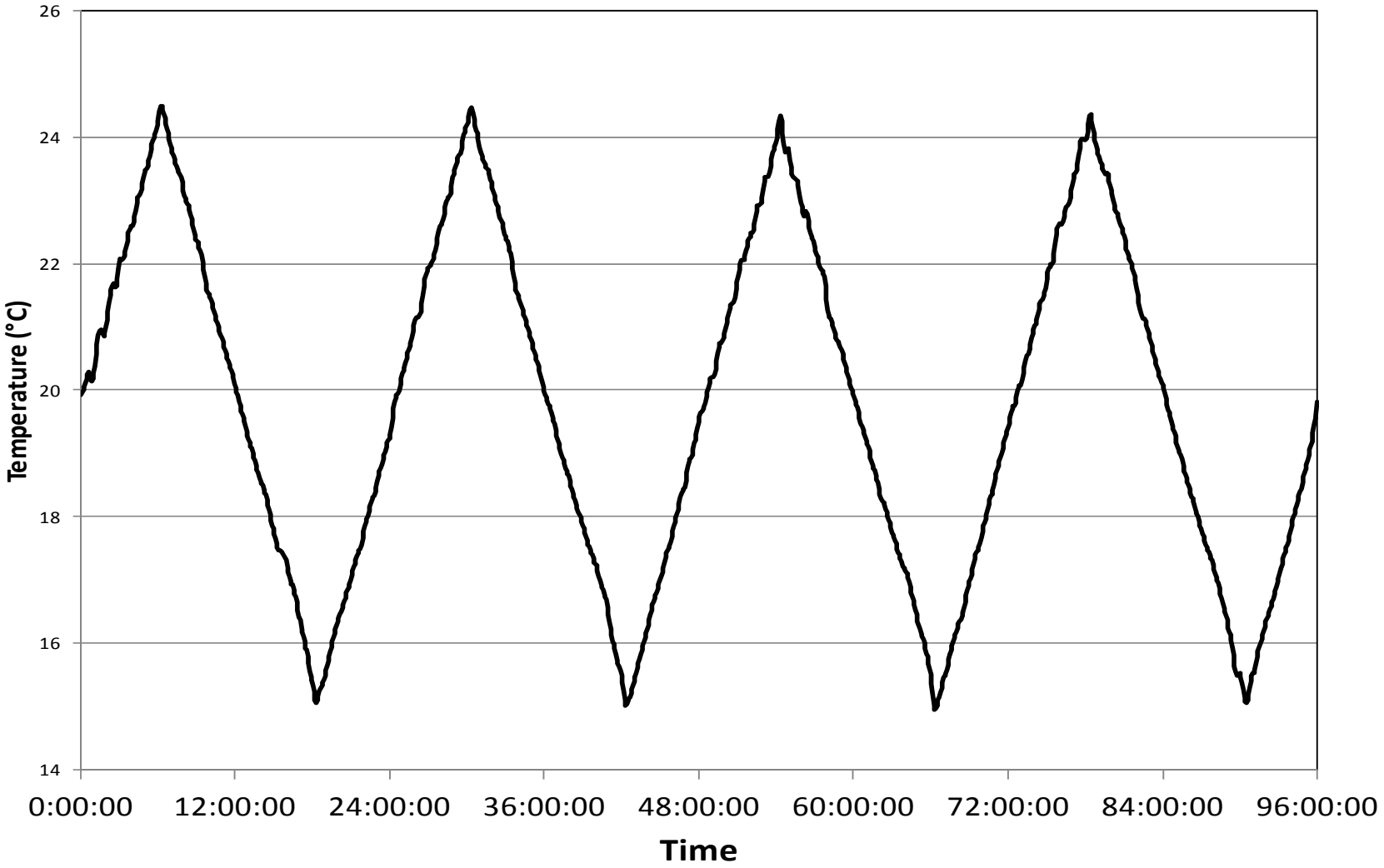

Figure 1. Average measured temperature (°C) of exposure chambers of copper toxicity test conducted during fluctuating temperatures.

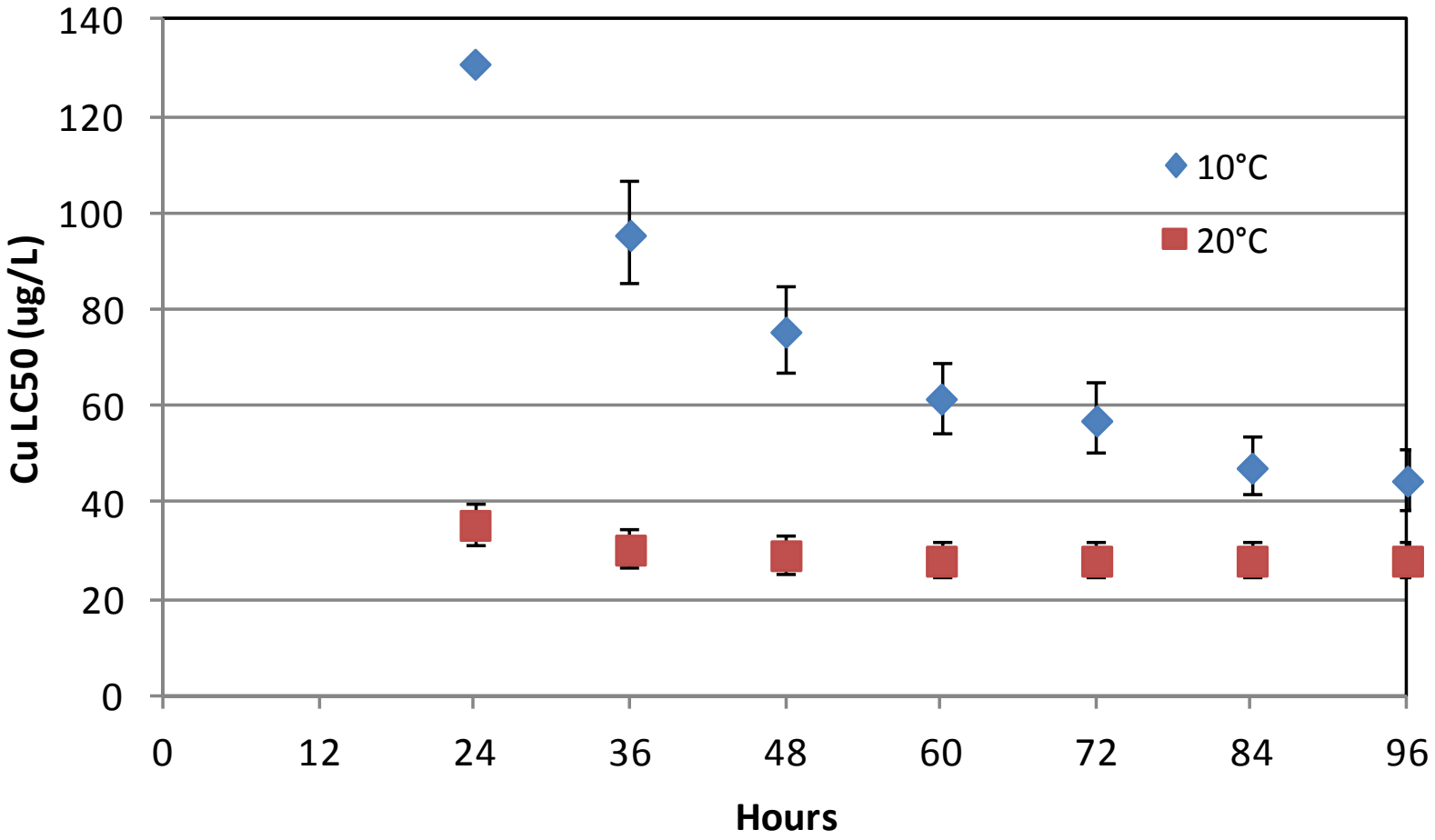

Figure 2. Median lethal concentration (LC50) of copper to brook trout at 10°C and 20°C as a function of time. Error bars represent 95% confidence limits.

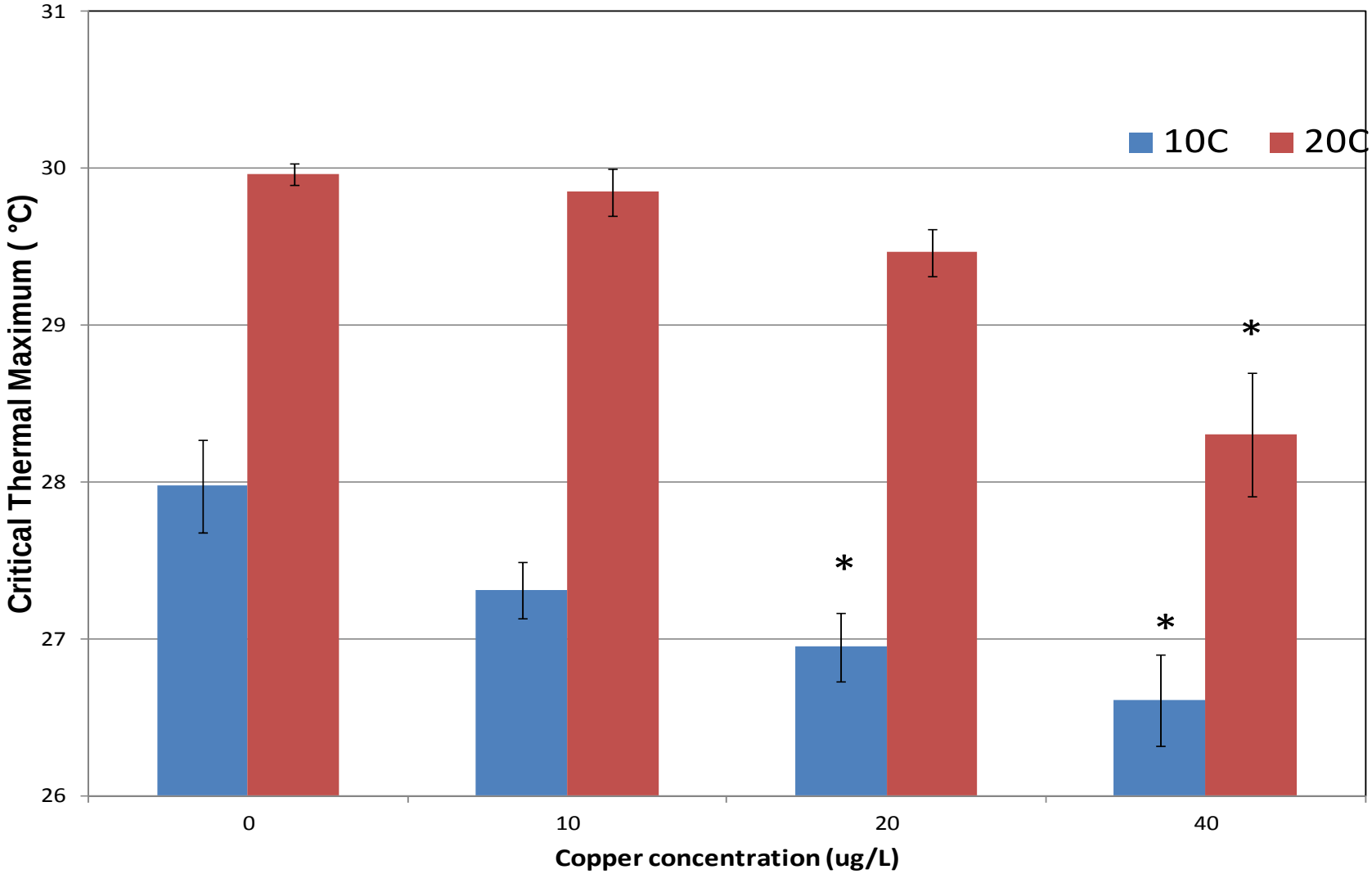

Figure 3. Critical thermal maxima of brook trout acclimated to 10°C and 20°C and exposed to 0, 10, 20 and 40 µg/L of copper for 96 hours. Asterisk indicates significant difference from control.

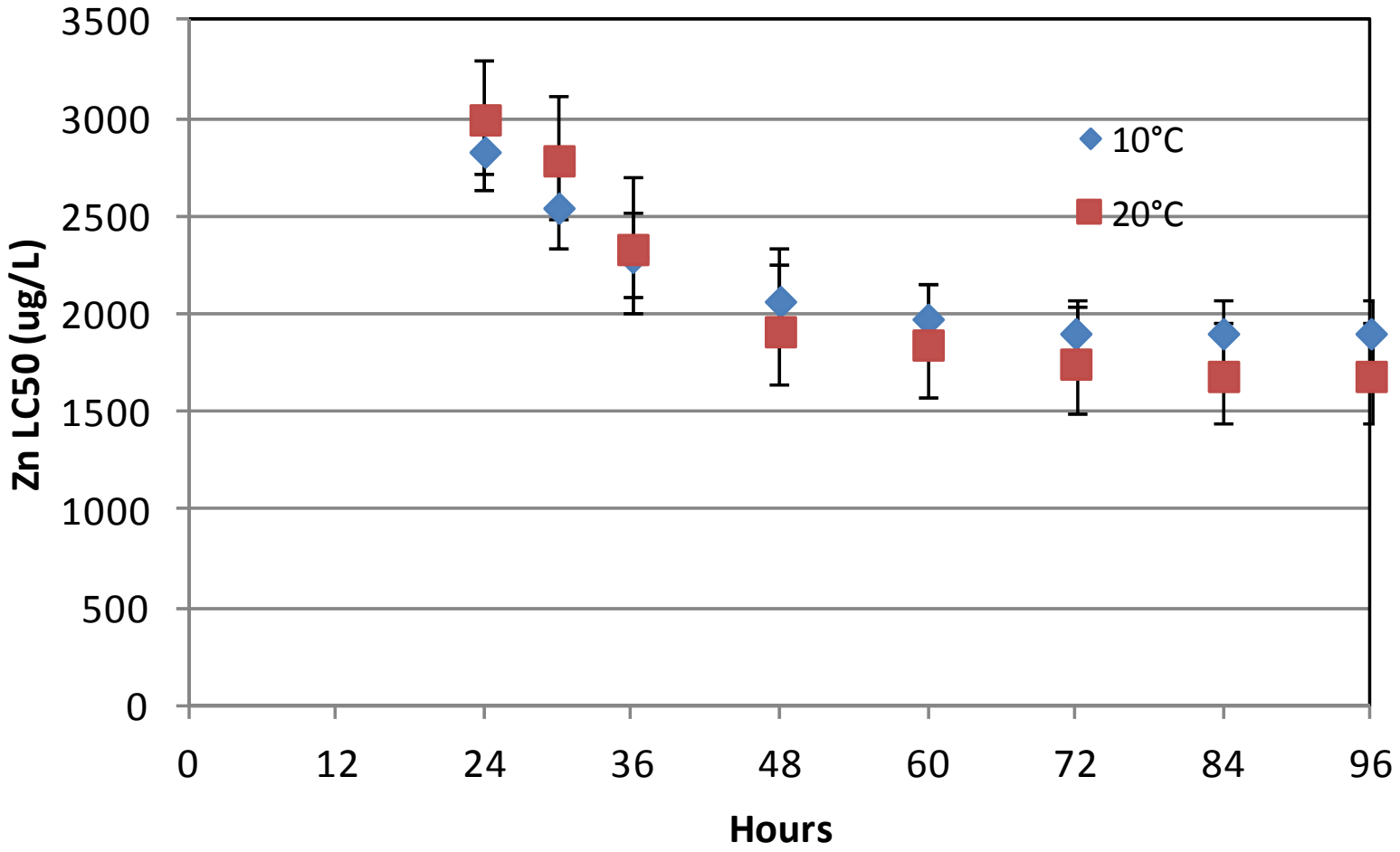

Figure 4. Median lethal concentration (LC50) of zinc to brook trout at 10°C and 20°C as a function of time. Error bars represent 95% confidence limits.

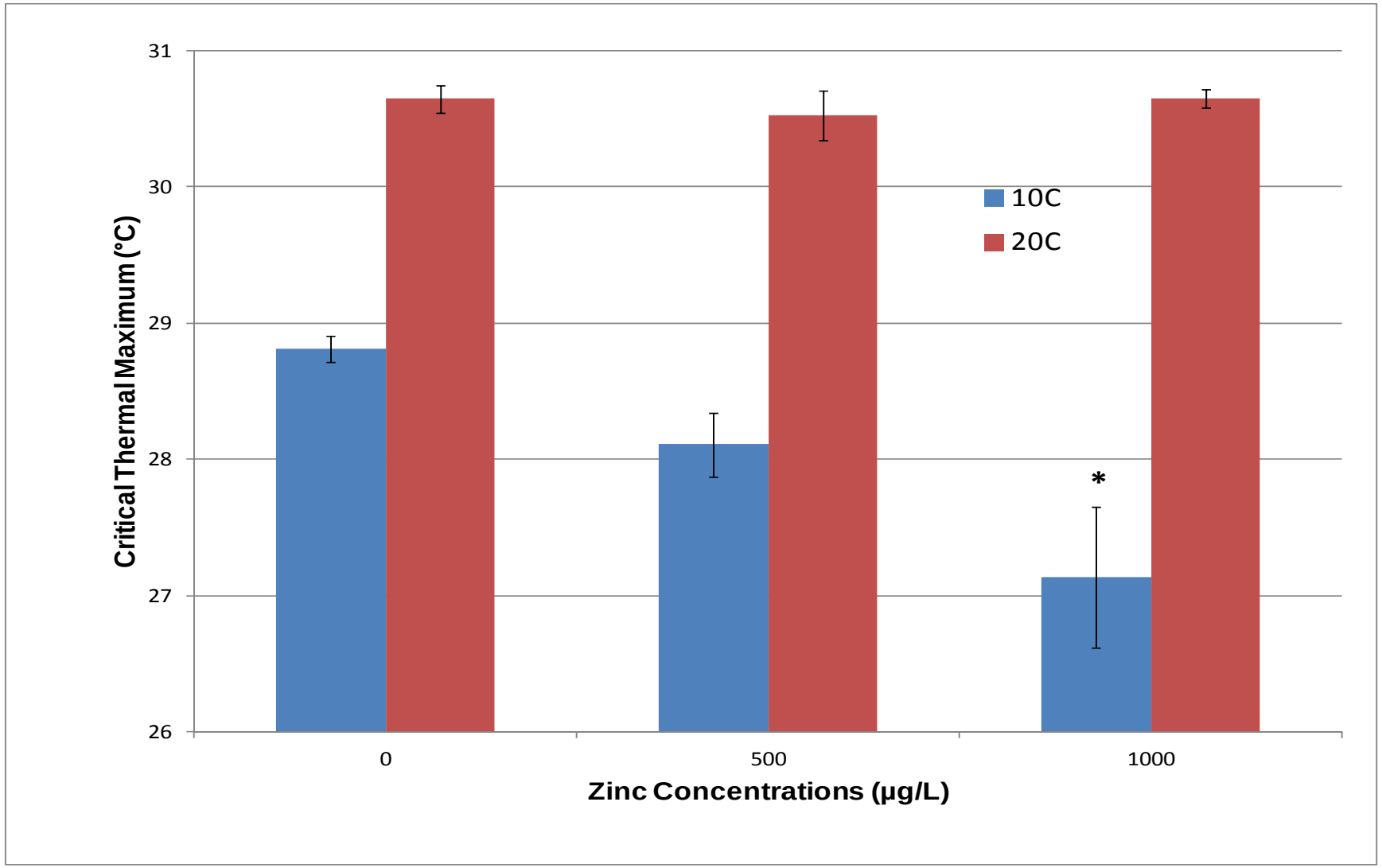

Figure 5. Critical thermal maxima of brook trout acclimated to 10°C and 20°C and exposed to 0, 500 and 1,000 µg/L of zinc for 96 hours. Asterisk indicates significant difference from control.

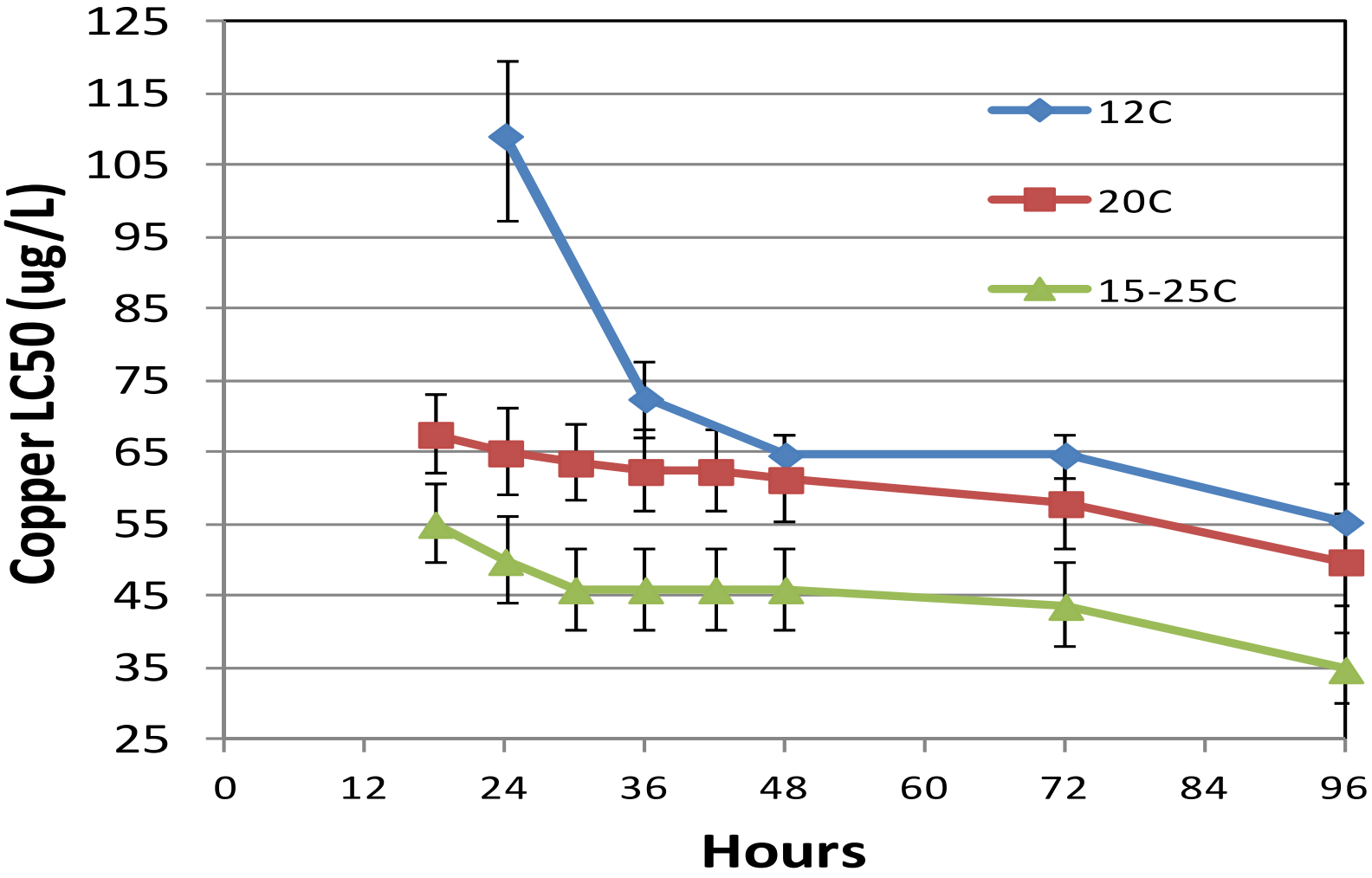

Figure 6. Median lethal concentration (LC50) of copper to cutthroat trout at static temperatures 12°C and 20°C fluctuating temperatures between 15-25°C as a function of time. Error bars represent 95% confidence limits.