

Water Pollution Studies

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Federal Aid in Fish and Wildlife Restoration

Job Progress Report

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State: Colorado

Study No. F243R

Title: Water Pollution Studies

Period Covered: July 1, 2008 to June 30, 2009

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

STUDY PLAN A: LABORATORY TOXICITY STUDIES

Brief Description: Conduct laboratory-based experiments to test effects of contaminants on aquatic organisms.

Job A.1. Reproductive Toxicity of Endocrine Disrupting Compounds

Job Objective: Determine whether exposure to hormonally active agents results in feminization of rainbow trout, fathead minnows and/or other aquatic organisms. Effects of feminization on reproduction and fecundity will be measured. Concentrations of endocrine disrupting compounds that result in significant feminization will be compared to concentrations observed in wastewater treatment plant effluents and in Colorado streams.

Job A.2. Reproductive Toxicity of Atrazine Herbicide

Job Objective: Measure fecundity and biomarkers of feminization of red shiners exposed to a range of atrazine. Relate concentrations that result in impairment in the laboratory with concentrations observed in Colorado eastern plains streams.

Job A.3. Toxicity of Metals to Fish

Job Objective: Measure acute (96 hour) and chronic (60 day) effects of zinc, copper and/or cadmium exposure on hatching, survival and growth of different life stages of mottled sculpin, longnose dace and/or other species. Results from these experiments will compare toxicity thresholds to USEPA metal criteria to ensure that these species are protected.

Job A.4. Effects of Dietary Exposure of Metals to Fish

Job Objective: Measure the effect of zinc, copper, cadmium and/or selenium from dietary sources on survival and growth of fish in the laboratory. Evaluate the sensitivity of dietary-exposed organisms to waterborne exposure. Relate dietary levels that cause diminished performance in the laboratory with levels found in dietary sources in metal impacted areas such as the upper Arkansas River, Clear Creek and the Eagle River.

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

Job Objective: 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.

STUDY PLAN B: TECHNICAL ASSISTANCE

Brief Description: Conducts toxicological experiments as requested from regulators to be incorporated into policy; conducts water chemistry analysis and training for CDOW and other agencies.

Job B.1. Water Quality Assistance to Division of Wildlife Personnel and Other State and Federal Agencies

Job Objectives: To provide technical assistance and expertise, consultation, evaluation and training in aquatic toxicology and aquatic chemistry to Division of Wildlife and other state and federal personnel as requested. 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. 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

Job A.1. Reproductive Toxicity of Endocrine Disrupting Compounds

The project continued to provide equipment and support for onsite bioassays conducted by the University of Colorado. The studies' objectives were to detect and quantify estrogenic activity in the city of Boulder wastewater treatment plant effluent.

Job A.2. Reproductive Toxicity of Atrazine Herbicide

No activities during this segment.

Job A.3. Toxicity of Metals to Fish

Mountain whitefish eggs were collected and reared in the laboratory. Several toxicity tests were conducted in the late winter to evaluate the effect of temperature and developmental stage on the acute toxicity of copper. The ICP spectrometer used to analyze water samples that were collected during the test for copper and major cations is in need of repair and has been scheduled for replacement. Water samples will be analyzed on the new instrument and the results of the toxicity tests reported next segment.

A static-renewal bioassay was conducted to assess toxicity of Snake River water to rainbow, brown, and brook trout. Snake River water upstream of Dillon CO was collected in early spring prior to runoff and transported to the toxicity laboratory. Mixtures of Snake River water and laboratory water were prepared and the toxicities of the mixtures to the different trout species measured. Water samples will be analyzed on the new ICP instrument and the results of the toxicity tests reported next segment.

Job A.4. Effects of Dietary Exposure of Metals to Fish

Factors affecting bioaccumulation of mercury in sport fish in Colorado reservoirs

This ongoing study is being conducted by Jesse Lepak (Post Doctoral Fellow) and Dr. Brett Johnson in the Department of Fish, Wildlife and Conservation Biology at Colorado State University.

Mercury (Hg) testing by the Colorado Department of Public Health and Environment (CDPHE) has uncovered a growing number of Colorado waters that contain fish with Hg concentrations that exceed 0.3 ppm, the USEPA (2001) fish tissue residue criterion for the protection of human health. In 2008, Colorado Division of Wildlife (CDOW) funded a three-year investigation to address the issue of Hg contamination in Colorado reservoirs. The goal of this research is to characterize the relative importance of factors influencing Hg bioaccumulation in reservoirs and evaluate the efficacy of fishery management

strategies to reduce Hg concentrations in sport fish. We selected to study four Colorado reservoirs based on their attributes (e.g., similar size, fish assemblages and available data). Carter and Horsetooth reservoirs were selected to represent contaminated food webs, both having Hg consumption advisories for walleye (*Sander vitreus*). Chatfield and Union reservoirs also contain walleye and were selected to represent food webs without fish consumption advisories. We collected zooplankton (pelagic), chironomids (profundal), crayfish (littoral), prey fish and walleye from each reservoir to characterize Hg bioaccumulation in food webs. We also characterized abiotic factors thought to influence Hg dynamics in reservoirs including water level fluctuation, water temperature, secchi depth, conductivity and water chemistry (e.g., DO, P, Al). Preliminary findings suggest that we can evaluate the importance of several factors (e.g., reservoir productivity, hypoxia, walleye growth and water level fluctuations) that have been found to influence Hg bioaccumulation in eastern North America. We have already encountered some unexpected results that have yielded valuable and new perspectives on Hg cycling. Furthermore, our findings provide a better understanding of Hg bioaccumulation that will be used to design fishery management strategies aimed at remediating Hg contamination in reservoirs.

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

Rainbow trout fingerlings were exposed to a range of concentrations of the stable zinc isotope ^{67}Zn . Accumulation of the stable isotope by the gills was measured in low and high water hardness over a range of time intervals between 45 minutes and 72 hours. Acute toxicity tests were conducted concurrently so that a median lethal accumulation value (LA50) could be calculated at each water hardness. Results of the study were recently published in *Environmental Toxicology and Chemistry* 28(6):1233-1243. The abstract of the study is presented below.

Portions of the study described above were repeated using brook trout. The objective was to determine whether the increased zinc tolerance of brook trout relative to rainbow trout can be attributed to different gill binding properties or due to an inherent ability to withstand higher levels of zinc on the gills. Additional tests and exposures will be conducted later this summer.

Job B.1. Water Quality Assistance to Division of Wildlife Personnel and Other State and Federal Agencies.

An experiment was conducted to study the effect of temperature on mountain whitefish hatching success, survival and growth. Results of the study are reported below.

An experiment was conducted to measure tolerance of boreal toad tadpoles to elevated pH. Results are reported below.

Pete Cadmus (MS student) and Dr. Will Clements from Department of Fish, Wildlife and Conservation Biology, Colorado State University continue to collaborate with CDOW to determine the dietary effects of metals on aquatic invertebrates. A method to measure subcellular compartmentalization of zinc was modified and adapted for mayfly nymphs. Although laboratory toxicity tests have shown that mayflies are highly tolerant to aqueous Zn exposure, field biomonitoring studies have shown marked decreases in mayfly abundance at relatively low concentrations of metals. To investigate possible causes of this discrepancy, we examined the role dietary exposure to Zn in a series of laboratory toxicity tests. Two species of grazing mayflies (*Ameletus* sp. and *Rhithrogena* sp.) were collected from unpolluted streams (Cache La Poudre at the Narrows) and exposed for 7 days to sublethal levels of Zn. Experimental treatments included three levels of aqueous exposure and three levels of dietary exposure. We measured total accumulation of Zn as well as Zn associated with several sub-cellular fractions including exoskeleton, cell fragments, heat-labile cytosolic proteins and metallothionein-like proteins. In general, dietary exposure increased total Zn concentration in mayflies compared to the aqueous only treatments. We compared these metal concentrations to those in organisms collected from the Arkansas River, a metal-contaminated stream in Colorado. Despite much greater aqueous concentrations of Zn in the laboratory experiments, Zn bound to heat-labile cytosolic proteins was consistently greater in mayflies collected from the field. The disproportionately large amount of Zn associated with heat-labile proteins in organisms collected from the Arkansas River may help explain the discrepancy between results of laboratory toxicity tests and field biomonitoring studies.

Water samples for metals analysis were collected from the Arkansas River upstream of Leadville to Salida. Livers and kidneys of brown trout were also collected from several sites for analysis of metals. Analysis of tissue digests is ongoing and will be reported next segment.

DOW participated as Party Status in several Water Quality Control Commission Rulemaking and Administrative Action Hearings, including the Rulemaking Hearing for The South Platte Basin and Control Regulations for several reservoirs in the Front Range. We also presented a summary of our toxicity testing protocols and findings to the Water Quality Control Commission. We continue to serve on BTAG (Biological Technical Assistance Group) committees for the Arkansas River mine site and for the Standard Mine on Coal Creek near Crested Butte, where we provide expertise and data. We represent

DOW on CDPHE's Technical Advisory Committee for mercury contamination in fish tissues. Mercury action limits are being set and protocols for notifying the public of potential health hazards are being developed. We assisted DOW biologists in coordinating their fish collection with CDPHE chemical analysts to assess risks to anglers at numerous reservoirs around the State. DOW also presented our role in the mercury issues to the Air Toxics Stakeholder Forum in Pueblo (May 2009).

DOW worked with the US FWS, BLM, CDPHE, EPA and the Attorney General's Office on other water quality issues, including Natural Resource Damage Claims for the upper Arkansas River and the Rocky Mountain Arsenal superfund sites. DOW wrote several letters of support for academic researchers and agencies who are seeking nationally-sponsored funding to conduct experiments with heavy metals and/or endocrine disruptors.

AN ENRICHED STABLE-ISOTOPE APPROACH TO DETERMINE THE GILL-ZINC BINDING PROPERTIES OF JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) DURING ACUTE ZINC EXPOSURES IN HARD AND SOFT WATERS

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ABSTRACT

The objective of the present study was to employ an enriched stable-isotope approach to characterize Zn uptake in the gills of rainbow trout during acute Zn exposures in hard (~140 mg/L CaCO₃) and soft waters (~30 mg/L CaCO₃). Juvenile rainbow trout were acclimated to test hardnesses, and exposed for up to 72 h in static exposures to a range of Zn concentrations in hard (0-1000 µg/L Zn) and soft waters (0-250 µg/L Zn). To facilitate detection of new gill Zn from endogenous gill Zn, the exposure media was significantly enriched with ⁶⁷Zn stable isotope (89.60% vs. 4.1% natural abundance). Additionally, acute Zn toxicity thresholds (96-h LC50s) were experimentally determined through traditional, flow-through toxicity tests in hard (580 µg/L Zn) and soft waters (110 µg/L Zn). Following short-term exposures (≤3 h), significant differences in gill-Zn accumulation between hard and soft water treatments were observed at the three common concentrations (75, 150, and 250 µg/L), with soft water gills accumulating more Zn than hard water gills. Short-term gill-Zn accumulation at hard and soft water LC50 concentrations (45-minute LA50s) was similar (0.27 µg/g ww and 0.20 µg/g ww respectively). Finally, comparison of experimental gill-Zn accumulation with accumulation predicted by the biotic ligand model (“BLM”) demonstrated that model output reflected short-term (<1 h) experimental gill-Zn accumulation, and predicted observed accumulation differences between hard and soft waters. Our results indicate that significant differences exist in short-term gill-Zn accumulation following acclimation and exposure to different water hardnesses, but that the relationship between short-term Zn accumulation and eventual acute toxicity appears to remain consistent.

Effect of temperature on hatching, survival and growth of mountain whitefish (*Prosopium williamsoni*)

Stephen F. Brinkman and Harry J. Crockett

INTRODUCTION

Mountain whitefish (*Prosopium williamsoni*) are one of two salmonids native to Colorado. Mountain whitefish populations have declined precipitously in the Yampa River starting in the late 1990s. A statewide drought coincided with the decline leading to a suspicion that elevated temperatures may have been a contributing factor. Indeed, elevated temperatures have been linked to heavy mortality of mountain whitefish eggs (Rajagopal 1979, CDOW unpublished data). The objective of this study was to measure mountain whitefish embryo, larvae and fry survival and growth at different temperatures.

MATERIAL and METHODS

Organisms

Eggs from four females and two partially spent females were collected near the mouth of Fish Creek in Steamboat Springs CO on 10/21/2008. Eggs were fertilized in the field and immediately transported in a cooler to the Aquatic Toxicology Laboratory in Ft. Collins CO. Temperature of the water during spawning was 7°C. Approximately 10,350 eggs were collected with a size of approximately 650 eggs/oz. Upon arrival, eggs were treated with 1600 ppm formalin for 15 minutes before placement in an egg incubation tray with flowing water (4.5°C dechlorinated Ft Collins municipal tap water). Hatched larvae and fry were fed <24hr brine shrimp naupalii (Argent Chemical Laboratories, Redmond WA) *ad lib* three times a day (once a day on weekends and holidays) supplemented with a 50:50 mixture of freeze-dried brine shrimp and bloodworms (Hikari) sieved through a 500µm screen. Tanks were siphoned daily to remove feces and uneaten food.

Egg Incubation

Twenty mountain whitefish eggs eight days post-fertilization were randomly distributed into egg incubation cups constructed from 2.54 cm² x 75 mm acrylic with 100 µm nylon mesh screen affixed to the end with aquarium-grade silicone adhesive. Egg incubation cups were suspended in 2L glass tanks (18.5 x 9 x 12 cm) and received 30 mls per minute from one of four stainless steel head tanks, each initially at 4.5°C. After allocation of the eggs, aquarium heaters were used to adjust temperature of the water in the head tank to a target temperature of 4, 6, 8, or 10°C. Temperature in each egg incubation cup was measured daily. Mortality and hatching of eggs were monitored and recorded daily. Dead eggs were carefully removed with a glass tube and preserved in 10% formalin. Hatched fry were released from the egg cup into the glass tank and fed <24hr brine shrimp naupalii (Argent Chemical Laboratories, Redmond WA) *ad lib* three times a day (once a day on weekends and holidays). Fry were maintained at their respective

temperatures until they had experienced a total of 550 °C–days (660 °C–days for fry in the 10°C) post-fertilization. Surviving fry were terminally anesthetized with MS222, blotted dry with a paper towel and measured for length (mm) and weight (0.001g).

Fry Growth

Whitefish embryos for the fry growth study were maintained at 4.5°C until hatch. After hatch, the water temperature was increased to 10°C. Fry were 9 days post-hatch at the start of the fry growth study. Twenty fry were randomly distributed into 2 L glass tanks (18.5 x 9 x 12 cm). Each glass tank received 30mls/min from one of four stainless steel head tanks. The temperature of the head tanks were adjusted from a starting temperature of 10°C to target temperatures of 4, 8, 12, 16°C at a rate of 1°C/day. Five fry were subsampled at 0, 11, 22, and 33 days after the tanks had attained the target temperature. Subsampled fry were terminally anesthetized with MS222, blotted dry with a paper towel and lengths and weights measured. Fry were <24hr brine shrimp naupaliii (Argent Chemical Laboratories, Redmond WA) *ad lib* three times a day (once a day on weekends and holidays) supplemented with a 50:50 mixture of freeze-dried brine shrimp and bloodworms (Hikari, Hayward CA) sieved through a 500µm screen. Temperatures and mortality were monitored daily.

Statistics

ANOVA was used to determine differences in hatching success, fry survival, overall survival, and growth among the different temperatures. Means were compared using Tukey's test ($\alpha=0.05$). Hatch success and survival data were arcsine-square root transformed prior to ANOVA.

RESULTS

Egg Incubation

Temperatures during the egg incubation study varied considerably due to poor temperature control provided by the aquarium heaters (Figure 1). Temperature variation was greatest at 10 and 8°C. Temperatures in the 4 and 6°C treatments were relatively constant but were about 1 and 2°C warmer than target temperatures, respectively. After 30 days, temperature controllers were installed (TS2 Temperature Digital Controller, Love Controls Division) which reduced the temperature variation somewhat. Temperature in the glass tanks was 1-2°C warmer than the water flowing through the egg incubation cups. As a result, measured temperatures increased following release of the hatched fry from the incubation cups into the tanks.

Mountain whitefish embryos hatched between 365 and 495 °C–days, though most hatching occurred near 400 °C–days. There was no difference in °C–days to hatch at the different temperatures (Figure 2). Hatching success was $\geq 95\%$ in the 4 and 6°C treatments, 80-95% at 8°C, and less than 50% at 10°C (Figure 3). Survival of fry from hatch to termination of the egg incubation portion of the study was also near 100% at 4 and 6°C, intermediate at 8° (68-100%) and low at 10°C (0-28%). Overall survival (embryo to 550 °C –days) was again high at 4 and

6°C, intermediate at 8°C (65-95%) and low at 10°C ($\leq 10\%$).

Fry Growth

Temperatures in the fry growth study were more constant compared to the egg incubation study (Figure 4). Measured temperatures were near target temperatures except the 4°C treatment which was about 1.5°C warmer than the target. A total of nine mortalities occurred during the fry growth study. Two mortalities occurred during tank cleaning, four losses occurred due to fry jumping out of the tanks, and three were unexplained. The unexplained mortalities did not appear to be related to temperature; two occurred at 12°C and one at 4°C. Fry grew rapidly on a diet of *ad lib* brine shrimp (Figure 5). Growth rate increased as temperature increased.

DISCUSSION

In Colorado, mountain whitefish have declined in the Yampa River but not the White River. Other Rocky Mountain State wildlife agencies have reported declines in some rivers but not others. Determining the cause(s) of the decline is hindered by a general lack of data on historic population density and poor understanding of the life-history of this native salmonid. We undertook this study to better understand the thermal requirements of mountain whitefish embryos and the effect of temperature on hatch, survival and growth.

Hatch success of mountain whitefish eggs was significantly reduced when incubated at temperatures 9-12°C (Figure 3). Most of the fry that hatched at 10°C died shortly thereafter. Similarly, Rajagopal (1979) reported a complete loss of eggs incubated at 12°C and 15°C and more than 97% mortality of eggs at 11°C. The few fry that hatched at 11°C died within a week.

The deleterious effects of elevated temperatures to whitefish eggs and embryos was not observed in the fry growth test that started with 9d post-hatch fry. No temperature related mortality was observed at temperatures up to 16°C, the highest temperature tested. Growth rates increased with increasing temperature (Figure 5). Stalnaker and Gresswell (1974) reported increased growth rates as temperatures increased from 6°C to 9°C to 12°C.

It is interesting that fry that were incubated at 9-12°C experienced high mortality rates at 14 °C while fry incubated at 4.5°C survived and thrived at 16°C. This result suggests that elevated temperatures during egg incubation somehow led to later mortality in post-hatch fry. Rajagopal (1979) noted that mountain whitefish eggs incubated at higher temperatures had a higher rate of deformities in fry that managed to hatch.

The results of the present and previous studies demonstrated that mountain whitefish eggs required cold temperatures for successful incubation. However after hatch, fry tolerated and grew faster at temperatures that were lethal to eggs. The necessity for cold temperatures for egg incubation and warmer temperatures for fry growth is consistent with observations that whitefish move upstream into colder water during spawning but that fry are most often found downstream where water temperatures are warmer (Baxter 2002, CDOW unpublished data).

Acknowledgements-We would like to thank Lindsay Ciepiela and Michael Brown for assistance with data collection and care of whitefish fry during the test.

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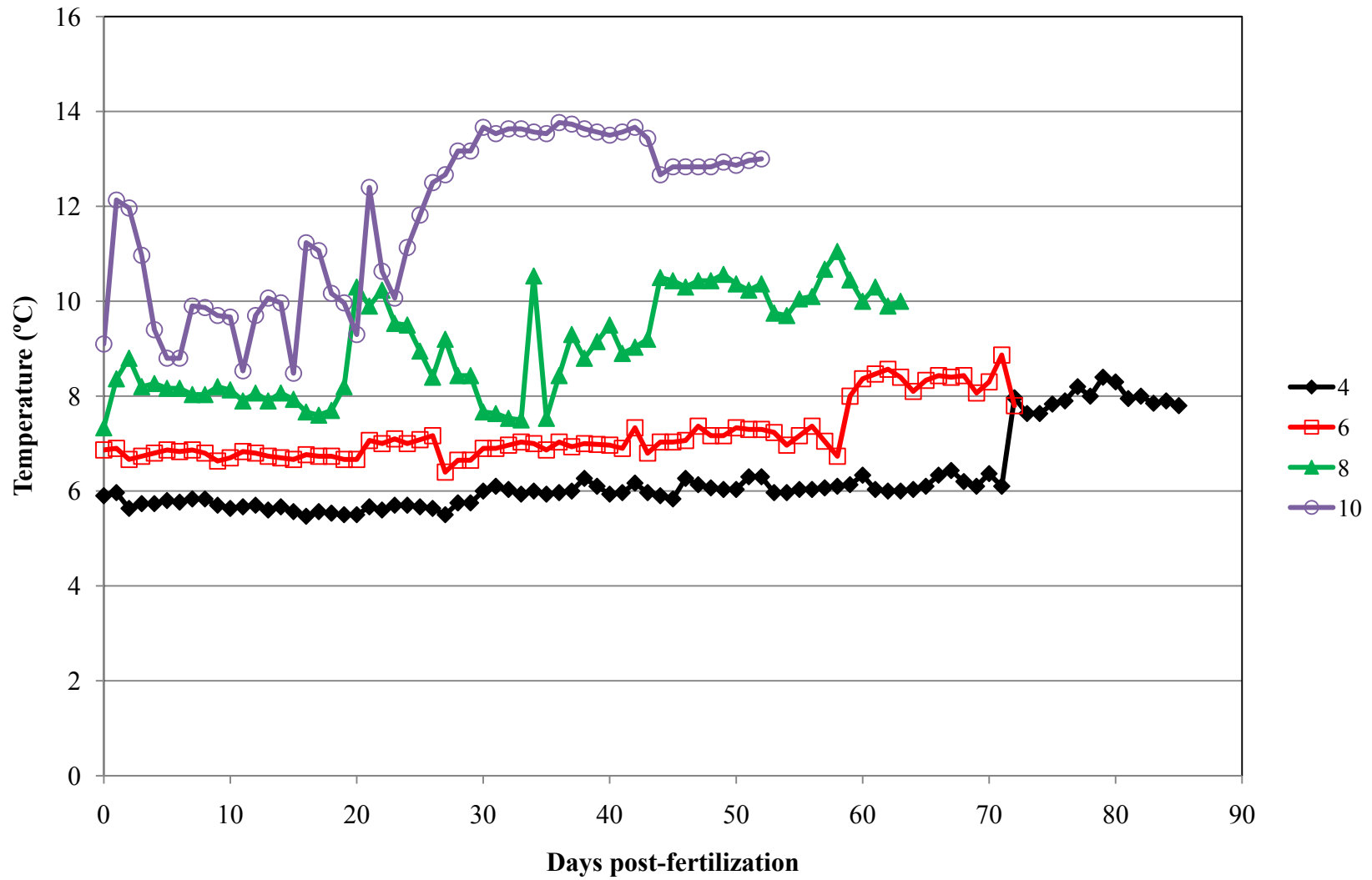


Figure 1. Water temperatures mountain whitefish eggs in the 4, 6, 8, and 10°C treatments during the egg incubation study.

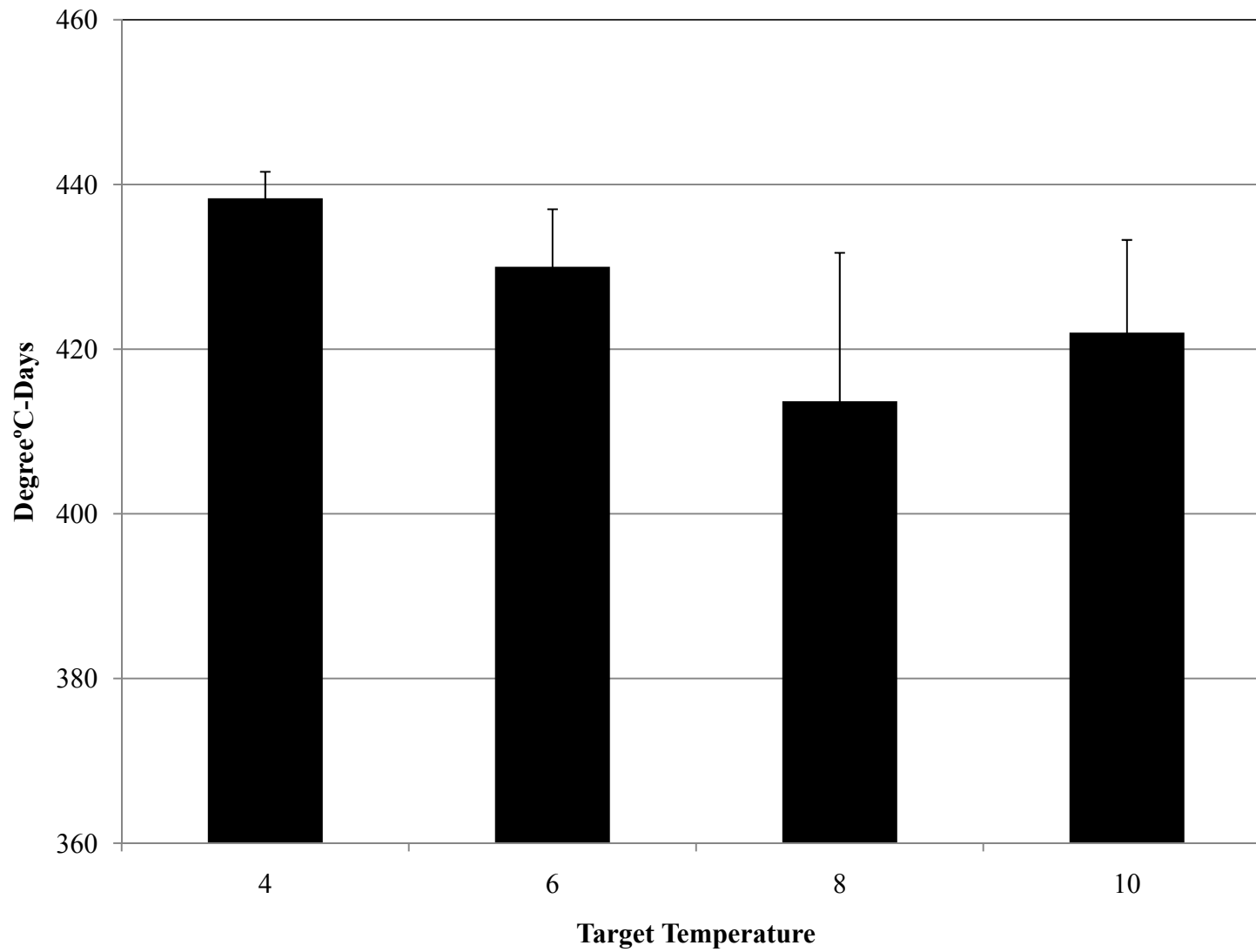


Figure 2. Mean degree°C-days to hatch for mountain whitefish eggs at different temperatures.

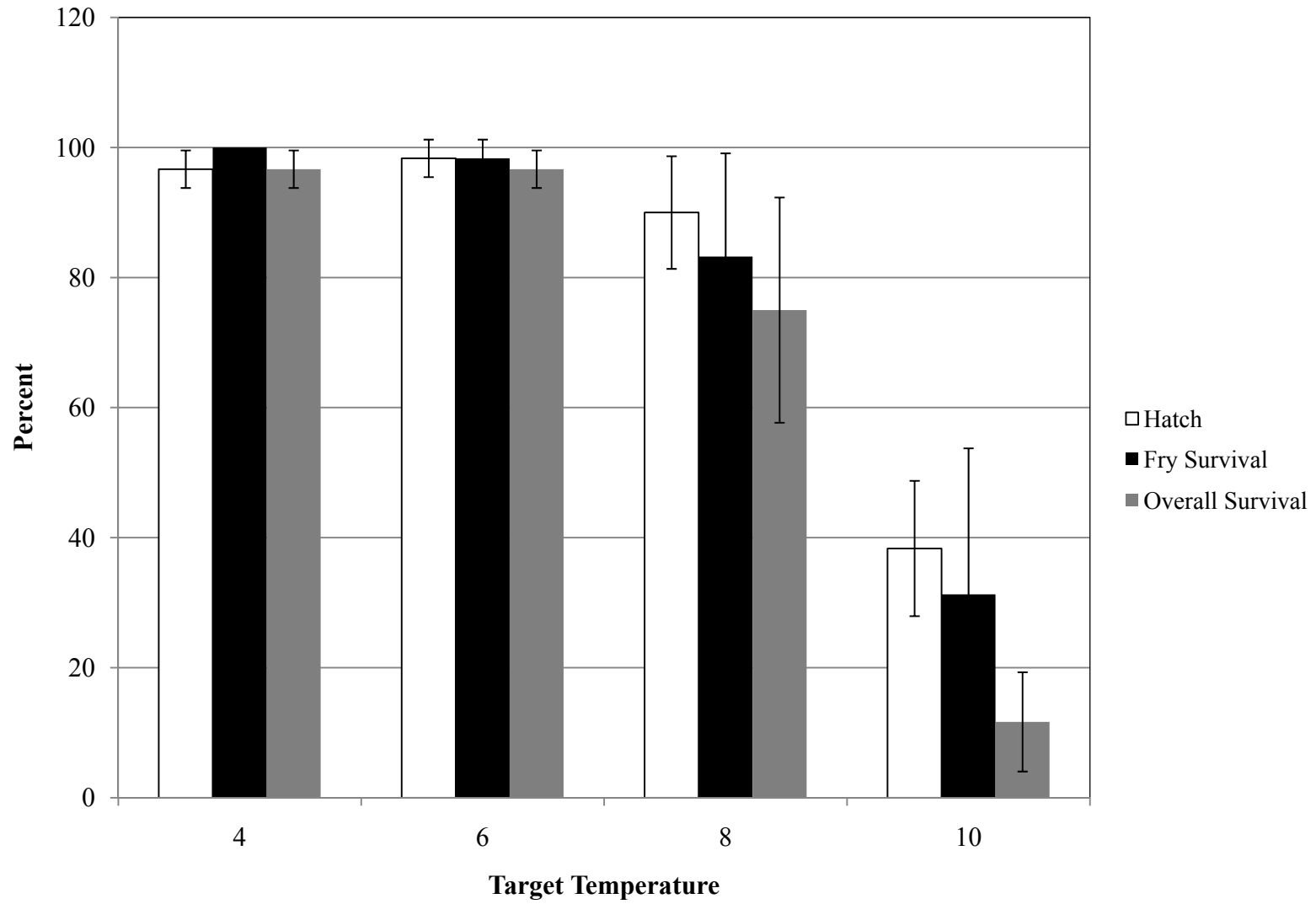


Figure 3. Hatch, fry survival and overall survival (%) of mountain whitefish at different temperatures.

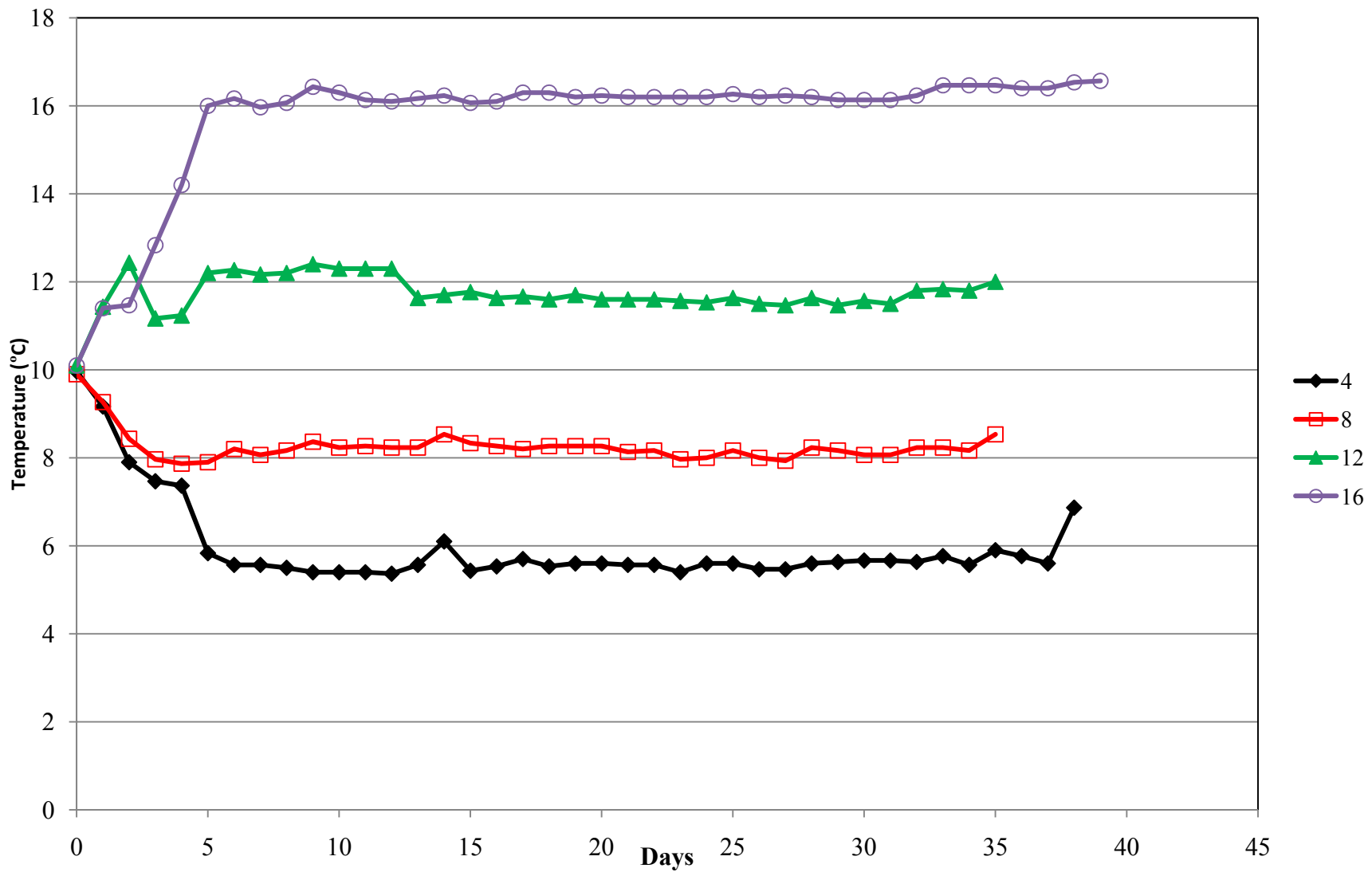


Figure 4. Measured daily temperature during mountain whitefish fry growth study.

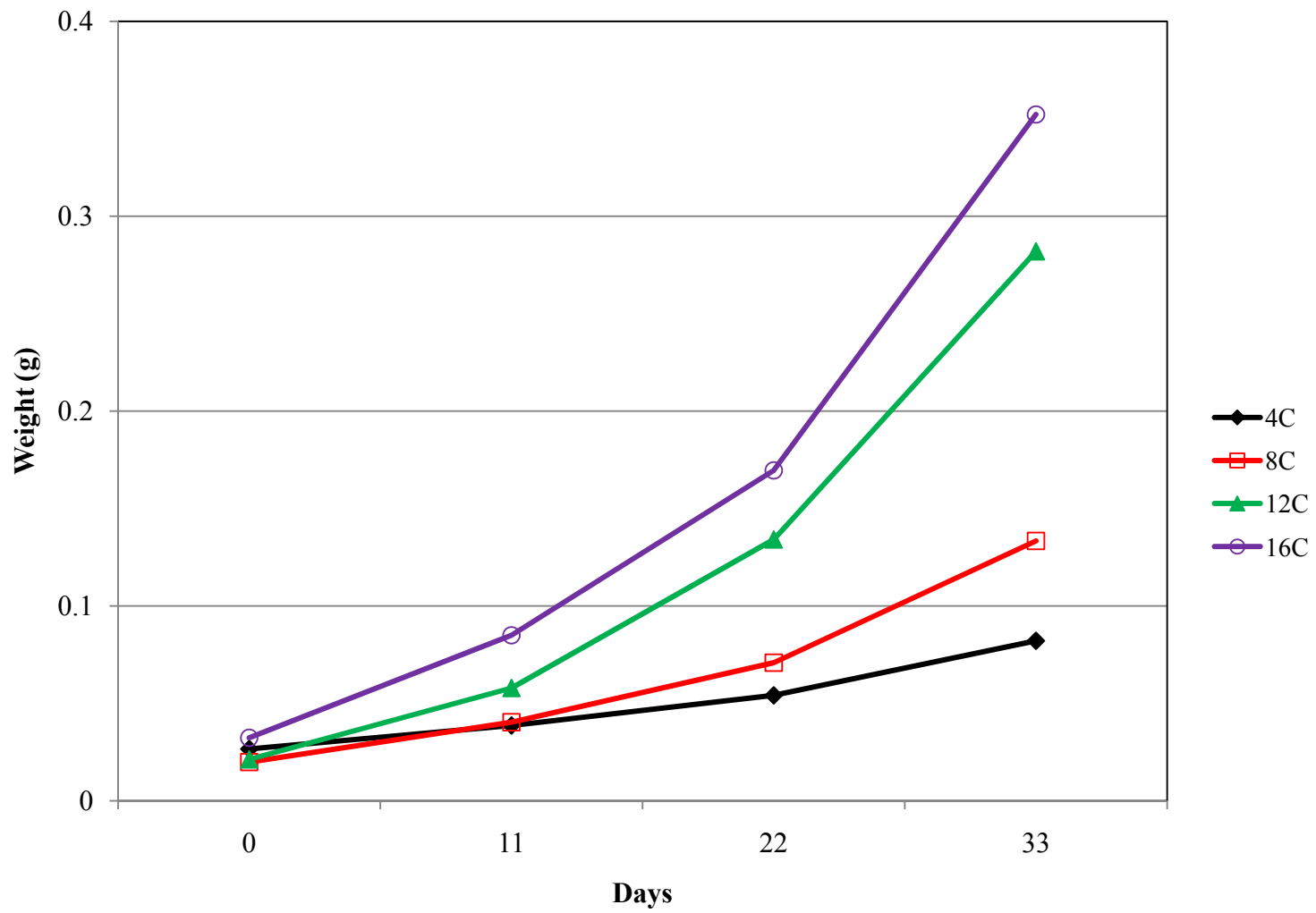


Figure 5. Growth of mountain whitefish fry at different temperatures.

Effect of elevated pH on boreal toad tadpoles (*Bufo boreas*)

INTRODUCTION

Boreal toad populations in Colorado have declined significantly. The cause of the decline has been attributed infections of Chytrid fungus. To assist with reintroductions of boreal toads, they are bred and reared at the Colorado Native Aquatic Species Restoration Facility (NASRF). Toadlets frequently experience poor survival and retention of tags at the facility is poor compared with toadlets tagged in the wild. The pH of the water at the facility is somewhat elevated and greater than historic breeding ponds in Colorado. The objective of this study was to determine whether elevated pH of water used at the facility impacts tadpole survival.

MATERIAL and METHODS

Four hundred boreal toad tadpoles were received from NASRF and maintained in 200 L aquarium containing a mixture of dechlorinated Fort Collins municipal tap water and onsite well water at 15°C. The conductivity of the water mixture closely approximated conductivity of water at NASRF although the pH of laboratory water was 7.6 compared to 8.5 for the water at NASRF. Water was adjusted from 15°C to test temperature 20°C over a period of ten days. Tadpoles were fed *ad libitum* a diet developed by NASRF which consisted of algae wafers, Mazuri amphibian food, and a blended mixture of frozen collard greens, mustard greens, yellow squash, and zucchini. Tadpoles were allowed to acclimate to laboratory conditions for 14 days during which the mortality was 1.5%. The pH tolerance test exposed tadpoles to six levels of pH (8.00, 8.25, 8.50, 8.75, 9.00, and 9.25) with four replicates each. Exposure solutions were prepared in five gallon polycarbonate carboys by the addition of 0.1M NaOH. Solutions were aerated overnight and adjusted the next day, as necessary. At the start of the test, ten tadpoles were randomly allocated to polypropylene containers containing 2.7 L of exposure solution. Test containers were gently aerated with airstones. Test containers were placed in a recirculating water bath to maintain temperature at 20°C. Photoperiod was 16:8 light:dark. Tadpoles were transferred to fresh exposure solutions each day. Daily measurements of pH were made in each container using a Thermo Orion 635 pH meter. At the end of the test, tadpoles were euthanized with MS222 and their Gosner development stage (Gosner 1960) and wet weight measured. ANOVA was used to detect differences in weights and development of surviving tadpoles.

RESULTS and DISCUSSION

Measured pH was about 0.1 S.U. less than target values but was constant over the duration of the 7 d test (Table 1). No mortality was observed in any containers (Table 2). Exposure to elevated pH for seven days did not significantly affect wet weight ($p=0.11$) or Gosner stage ($p=0.12$).

A water quality survey of known, historic or suspected boreal toad breeding pond conducted 1997-2001 found that pH ranged from 3.2-8.66. A large majority of measured

pH values were 6.5-8.0 (Figure 6). There are little data on the tolerance of pH by boreal tadpoles. One study exposed boreal toad larvae to solutions at pHs 3.1, 4.0, 5.0, 6.0, 6.5, and 7.0 to measure the effect of acid mine drainage (Porter and Hakanson 1976). Other studies on the effects of pH on amphibians have focused on low pH and the effects of acid rain. The results of this study did not detect a significant adverse effect on boreal toad tadpoles exposures to pHs as high as 9.16 for 7 days.

Acknowledgements-We thank Elaine Davinroy and Dave Schnoor of NASRF for providing boreal toad tadpoles and for advice on their care.

Table 1. Mean measured pH and standard deviation of exposure solutions used in boreal toad tadpole pH tolerance test.

Target pH	8.00	8.25	8.50	8.75	9.00	9.25
Mean pH	7.88	8.16	8.41	8.65	8.87	9.16
Std. Dev.	0.004	0.009	0.007	0.007	0.010	0.006

Table 2. Survival, mean weight at test termination, and Gosner development stage of boreal toad tadpoles at the end of the pH tolerance test. Standard deviations are in parentheses.

Target pH	8.00	8.25	8.50	8.75	9.00	9.25
Survival (%)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
Weight at termination (g)	0.305 (0.028)	0.323 (0.022)	0.299 (0.021)	0.297 (0.009)	0.308 (0.029)	0.269 (0.037)
Gosner stage	32.9 (0.14)	32.8 (0.29)	32.7 (0.27)	32.6 (0.17)	32.5 (0.21)	32.6 (0.06)

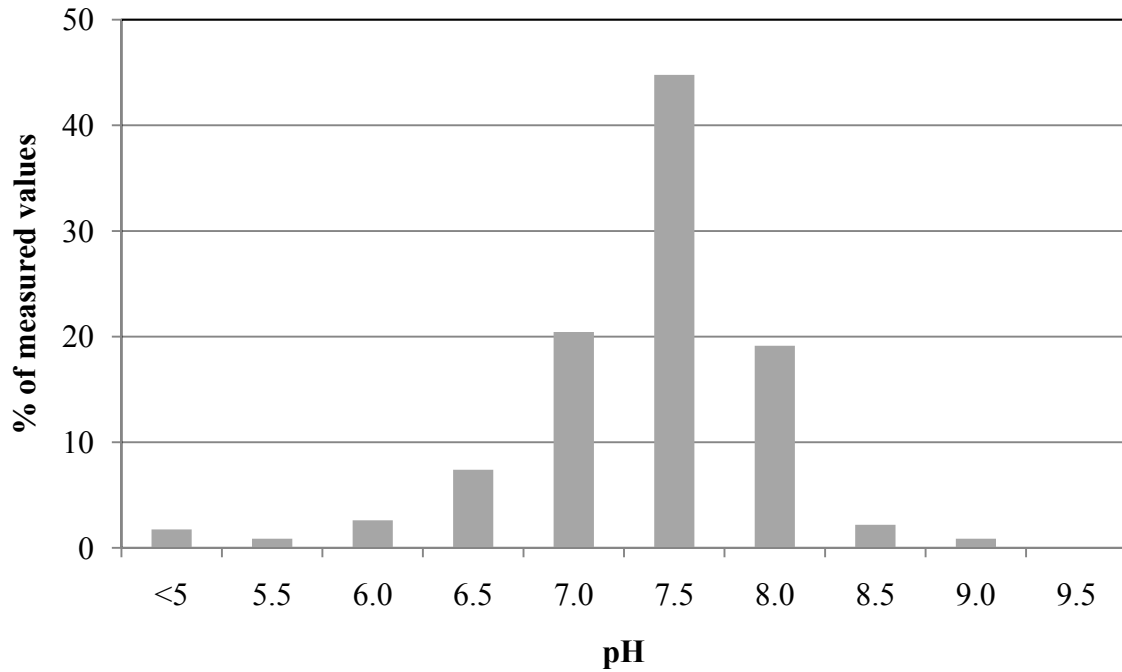


Figure 6. Percent frequency of measured pH values at historic and suspected boreal toad breeding ponds 1997-2001.

LITERATURE CITED

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