TOLERANCE OF LARVAL AND JUVENILE LOST RIVER AND SHORTNOSE SUCKERS TO HIGH pH, AMMONIA CONCENTRATION, AND TEMPERATURE, AND TO LOW DISSOLVED OXYGEN CONCENTRATION

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Summary

During April-October 1994, short-term (96-hr-long) laboratory tests were conducted to determine the tolerance of larval and juvenile Lost River (Deltistes luxatus) and shortnose (Chasmistes brevirostris) suckers to high pH, ammonia concentration, and water temperature, and to low dissolved oxygen concentration. The objectives were two-fold: (i) to verify earlier results (obtained in 1992-93) for larval and juvenile Lost River suckers and juvenile shortnose suckers by repeating the tests; and (ii) to generate heretofore unavailable results for larval shortnose suckers by conducting replicated tests. With two exceptions, we achieved the objectives of this study. We did not complete tests on the tolerance of larval Lost River suckers to high unionized ammonia concentration and temperature because sufficient numbers of test animals were not available.

For larval Lost River suckers, estimates of 96-hr LC_{50} values (95% confidence intervals in parentheses) were as follows: pH, 10.45 (10.43-10.48); and dissolved oxygen, 2.1 mg/L (2.0-2.2 mg/L) or 25.8% saturation (24.9-26.8% saturation). For juvenile Lost River suckers, estimates of 96-hr LC_{50} values (95% confidence intervals in parentheses) were as follows: pH, 9.92 (9.87-9.96); unionized ammonia, 0.750 mg/L (0.599-0.944 mg/L); temperature, 31.2°C (30.8-31.5 °C); and dissolved oxygen, 1.4 mg/L (1.0-2.0 mg/L) or 19.1% saturation (13.9-26.2% saturation).

For larval shortnose suckers, estimates of 96-hr LC_{50} values (95% confidence intervals in parentheses) were as follows (variable, first replicate, second replicate): pH, 10.33 (10.01-10.66), 10.46 (10.12-10.83); unionized ammonia, 0.750 mg/L (0.730-0.770 mg/L), 1.40 mg/L

(1.24-l .68 mg/L); temperature, 3 1.9°C (3 1 .9 -32.0°C), 3 1.4°C (3 1.2-3 1.6°C); and dissolved oxygen, 2.3 mg/L (2.2-2.4 mg/L) or 30.3% saturation (28.9-3 1.6% saturation), 1.7 mg/L (1.6- 1.8 mg/L) or 22.3% saturation (21 .O-23.6% saturation). For juvenile shortnose suckers, estimates of 96-hr LC_{50} values (95% confidence intervals in parentheses) were as follows: pH, 9.85 (9.76-9.95); un-ionized ammonia, 0.956 mg/L (0.320-2.46 mg/L); temperature, 3 1.2 °C (30.8-3 1.6"C); and dissolved oxygen, 1.2 mg/L (0.7-l .7 mg/L) or 14.7% saturation (7.6-22.5% saturation).

In general, our 96-hr LC_{50} estimates for larval and juvenile Lost River suckers and juvenile shortnose suckers were similar to previously reported results. Compared to field measurements of pH, ammonia, temperature, and dissolved oxygen, our laboratory data suggest that ambient summertime water quality conditions in Upper Klamath Lake can be acutely lethal to suckers.

Introduction

The Lost River sucker (Deltistes luxatus) and the shortnose sucker (Chasmistes brevirostris) are endemic to the Klamath Basin of northern California and southern Oregon. Recent studies indicate that populations of these suckers have declined precipitously, with only 11,860 Lost River and 2,650 shortnose suckers counted during field surveys of spawning adults in 1984 and 1985 (Bienz and Ziller 1987). On July 18, 1988, the U.S. Fish and Wildlife Service listed these two suckers as endangered species.

Upper Klamath Lake is the primary refuge for Lost River and shortnose suckers (Buettner and Scoppettone 1990). In recent years, however, water quality in the lake has deteriorated as agricultural activities increased in the Klamath Basin. During August 1986, mass mortalities of Lost River suckers and smaller numbers of shortnose suckers were attributed to unsuitable water quality (Scoppettone 1986). Although the precise cause of the die-offs is unknown, high pH, low dissolved oxygen concentrations, high water temperatures, and high concentrations of ammonia were possible contributing factors.

Summertime water temperatures in Upper Klamath Lake often reach 30°C near the surface, and temperatures of 22-24°C are common in the upper few feet of water (Bortleson and Fretwell 1990). During this same period, dissolved oxygen concentrations are often photosynthetically supersaturated in the upper part of the water column, but less than 2.0 mg/L may occur near the bottom. Concentrations of dissolved oxygen can drop as low as 0.2 mg/L throughout the water column in response to a combination of high temperature, algal senescence, and stagnant water (no mixing of the water column by the wind). During periods of peak algal

productivity, pH values commonly vary from 9 to 10, and may even exceed 10.7 (Jacob Kann, Klamath Tribe, Chiloquin, OR, unpublished data). Water-column concentrations of total ammonia have varied from 0.08 mg/L to 1.2 mg/L in July and August (Bortleson and Fretwell 1993). At 25 °C and pH 9, these concentrations of total ammonia are toxic to salmonids and other sensitive cold-water fish (Bortleson and Fretwell 1993).

Falter and Cech (199 1) reported that the critical pH maximum for juvenile (l-3 g) shortnose suckers average 9.55 (standard deviation, 0.43). Although the critical maximum (determined by gradually increasing or decreasing a variable away from acclimation until loss of equilibrium or some other physical disorganization response occurs) is useful for comparing the relative tolerance of different life stages or species of fishes, this measurement can overestimate the tolerance level (or lethal level) because most organisms do not immediately lose equilibrium or die when exposed to environmental conditions that can eventually cause death (Becker and Genoway 1979). The critical thermal maximum for juvenile shortnose suckers was 32.7°C (min-max, 32.1-33.3 °C), whereas the critical oxygen minimum for this same species was 0.69 mg/L (min-max, 0.44-0.98 mg/L; estimated from values reported in torr) (Castleberry and Cech 1993).

The Midwest Science Center (formerly the National Fisheries Contaminant Research Center) conducted a series of laboratory tests in 1992-l 993 to better define the tolerance limits of Lost River suckers (larvae and juveniles) and shortnose suckers (juveniles only) to high pH, ammonia concentration, and temperature, and to low dissolved oxygen concentration. These studies yielded the following 96-hr LC_{50} values (all life stages and species combined): pH, 9.84-10.68; unionized ammonia, 0.34-0.70 mg/L; temperature, 29.4-3 1.8 °C; and dissolved oxygen, 1 S-2.1 mg/L (Monda and Saiki 1993, 1994). However, Monda and Saiki (1993, 1994) strongly

cautioned against using their data as “definitive measures” of the tolerance of Lost River and shortnose suckers until several limitations or uncertainties in the data were assessed. Foremost among the uncertainties was that none of the tests had been repeated. Therefore, no measure of test-to-test variation was available.

The objectives of this study are two-fold: (i) to verify the earlier findings of Monda and Saiki (1993, 1994) by repeating all laboratory tests with larval and juvenile Lost River suckers and juvenile shortnose suckers; and (ii) to generate heretofore unavailable results for the larval life stage of shortnose suckers by conducting additional laboratory tests. When combined with data from a proposed field (in-situ) study of fish survival in Upper Klamath Lake (see Appendix C of Monda and Saiki 1993), results from this and earlier laboratory studies will help to identify water quality variables in Upper Klamath Lake that could be remediated as part of restoration efforts for the suckers. In addition, the results may be useful for identifying sites in the Klamath Basin where hatchery-reared suckers can be successfully reintroduced.

Methods

Lost River and shortnose suckers used in this study were obtained from the Klamath Tribe’s Braymill Fish Hatchery near Chiloquin, Oregon. These fish were reared from eggs and sperm collected from wild adults. Adult fishes were captured using trammel or dip nets during spawning runs in the Williamson River and at Sucker and Ouxy springs along the northeastern shore of Upper Klamath Lake.

Eggs and sperm were obtained at approximately 9-day intervals during March-June 1994. After collecting a small quantity of eggs or sperm, the adults were released. The eggs were then fertilized by gently mixing them with milt in small plastic buckets. Fertilized eggs were transported in the buckets, which were placed in plastic coolers, to the Braymill Fish Hatchery where they were incubated in upwelling incubator jars. After 10- 13 days of incubation, the larvae were transferred to 300-gallon round fiberglass tanks. Spawns were kept in separate tanks and assigned unique identification numbers so that fish used in each test had a known age and were full siblings.

Incubator jars and fish tanks were supplied with water from a recirculating system that included a biofilter and an ultraviolet sterilization unit. Water for the recirculating system was pumped from the Williamson River, then chlorinated and dechlorinated prior to use.

Early lots of larvae from both species of suckers exhibited extreme hypersensitivity to handling. Even when handled gently, large numbers of larvae went into rigor (became stiff or rigid) and died within 10-20 seconds. In the hatchery, mass mortalities occurred among larvae about 35 days post-spawn even though a disease agent was not evident. Preserved specimens (in 10% formalin) were examined by the U.S. Fish and Wildlife Service's California-Nevada Fish Health Center (Anderson, California) and by Mississippi State University's College of Veterinary Medicine Fish Health Laboratory (Starkville, Mississippi), but no consistent diagnosis was reached. Larvae were fed the same ration (90% krill flakes and 10% Spirulina, dry weight basis) used by Monda and Saiki (1994) in earlier tests. Although this ration was supplemented with other fish-food preparations, there was no improvement in larval survival. However, when live

brine shrimp nauplii were added to the krill:Spirulina diet, survival and average size of larvae increased and the larvae were no longer hypersensitive to handling.

Fish used in juvenile tests came from some of the same lots as those used in larval tests. These juveniles were reared in the round fiberglass tanks in which they had been placed shortly after hatching-out as larvae.

Fish used in larval and juvenile tests were transported from the Braymill Fish Hatchery to our laboratory in Klamath Falls, Oregon, in 48-quart insulated ice chests aerated with compressed oxygen. Larvae were transported to the laboratory when they were 30 days old (post spawn), whereas juveniles were transported when 91-106 days old.

Larvae and juveniles were acclimated to laboratory conditions (water temperature, 20°C; photoperiod, 16-hr light:8hr dark) in a round 250-L fiberglass tank for at least 4 days prior to use in tests. The tank received approximately two changes of reconstituted water per day from a gravity-fed flow-through system.

Reconstituted water was prepared in batches of 500-l 800 L by adding reagent-grade sodium bicarbonate ($NaHCO_3$), calcium sulfate dihydrate ($CaSO_4*2H_2O$), hydrated magnesium sulfate ($MgSO_4*7H_2O$), and potassium chloride (KCl) to deionized water (city tapwater water treated by charcoal filtration and reverse osmosis). Sodium hydroxide (5M NaOH) was used to adjust the pH of reconstituted water to about 8.0. This recipe was identical to that used by Monda and Saiki (1993, 1994), and was intended to mimic springtime water quality conditions in Upper Klamath Lake.

Target values for selected water quality variables in the reconstituted water were as follows: pH, 8.0±0.8; calcium hardness, 18.3±1.8 mg/L as $CaCO_3$; total hardness, 34.7±2.4

mg/L as $CaCO_3$; total alkalinity, 38k3.8 mg/L as $CaCO_3$; specific conductance, 200±40 µS/cm @ 25 °C; total ammonia, <0.01 mg/L; sulfates, 40±4 mg/L; and chlorides, 2.W0.2 mg/L. If water quality variables did not meet these criteria, the variables were adjusted by adding appropriate amounts of deionized water or chemicals.

During the 4-day acclimation period, larvae were fed three times daily whereas juveniles were fed once daily, both with the dry ration (90% krill: 10% Snirulina) used by the Braymill Fish Hatchery. After tests were initiated, larvae were fed twice daily with the dry ration whereas juveniles were not fed.

Larval tests were initiated when fish were 35 days old, whereas juvenile tests were initiated when fish were 96-l 11 days old.

Experimental Design

Our tests were designed specifically to assess the individual effects of four water quality variables--pH, un-ionized ammonia, water temperature, and dissolved oxygen--on larval and juvenile survival during a 96-hr-long exposure period. Except when manipulated upwards or downwards as part of a test, target levels for the four variables were as follows: temperature, 20 °C; pH, 8.0; dissolved oxygen, 100% saturation; and unionized ammonia, <0.05 mg/L.

Each test consisted of five levels of the specified variable and one control. Although test chambers were duplicated, the water (test medium) came from a common source (i.e., each pair of duplicate test chambers contained treatments that were not independent from each other). Thus, data from duplicate treatments were pooled when computing the LC,, value.

Instead of operating one diluter as in Monda and Saiki (1993, 1994), two diluters were operated simultaneously to decrease the time necessary for completing all tests. At any given time, different water quality variables were tested in each diluter.

Each treatment and control was intended to contain 40 larval fish or 10 juvenile fish. Larval fish were added to test chambers in four groups of 10 fish. Individual larvae comprising each group were captured from the holding tank (control conditions) with a fine-mesh aquarium net, placed into a clean l-pint plastic container filled with water from the holding tank, then fish and water were poured into the test chamber. The same procedure was used with juveniles except that fish were added to the test chamber in three groups of 3 fish and one group of 1 fish.

Treatments were checked for mortalities 1 hr after the test was started and at 24-hr intervals thereafter. Larvae or juveniles were considered dead if they showed no response to physical contact (i.e., prodding with a glass rod).

Each treatment or control was contained within a 25-L glass aquarium (test chamber). Test chambers were randomly placed in water baths so that each water bath accommodated one complete set of duplicate treatments and controls. To maintain a constant temperature of 20°C water baths were equipped with Little Giant@ submersible pumps (for water circulation) and Visitherm® thermostatically controlled immersion heaters.

Each test chamber received periodic deliveries of water from a proportional diluter (Mount and Brungs 1967), with displaced water overflowing the test chamber through a screened outlet. The diluter cycled at 15-min intervals, thus delivering about two changes of water per day to each test chamber. Prior to entering the test chamber, water from the diluter was

temporarily stored in a 4-L head tank. The head tank was partially immersed in the water bath to help equilibrate incoming water to thermal conditions in the test chamber.

For tests with un-ionized ammonia and pH, a Micromedic® precision metering pump injected stock solutions of ammonium chloride (NH_4Cl) or NaOH into the proportional diluter. The diluter automatically created and dispensed five concentrations (treatments) of test solution and a control to individual test chambers. Each of the five treatments declined progressively by a factor of 50% through dilution of stock solutions with reconstituted water.

For temperature and dissolved oxygen tests, proportional diluters were used only to deliver reconstituted water to the test chambers. A series of five progressively higher water temperatures was achieved by placing thermostatically controlled aquarium heaters into chambers separated by 2.5-cm-thick Styrofoam@ sheets within the water baths. Five progressively lower dissolved oxygen concentrations were achieved by bubbling appropriate amounts of nitrogen gas, compressed air, or both, through water in the head tanks. The tops of test chambers were modified for dissolved oxygen tests by attaching plexiglass covers to reduce the influence of atmospheric oxygen.

All tests were terminated 96 hr after initiation. Surviving fish were euthanized by overdosing with MS-222. In larval tests, fish from controls were preserved in 10% formalin. In juvenile tests, standard length (mm) and weight (g) of fish from controls were recorded prior to preservation in 10% formalin. (Note: To convert from standard length [SL] to total length [TL], use the following equations [species, conversion equation, sample size, correlation coefficient] : Lost River juveniles, TL=l.712540+1.200345*SL, 50, 0.9897; shortnose juveniles, TL=8.402538+1.042301 *SL, 50, 0.8734.)

pH Tests. A 2M stock solution of NaOH was made by dissolving NaOH pellets in deionized water. The intake tube of a Micromedic® precision metering pump was placed in the solution. With each diluter cycle, the Micromedic® pump delivered a fixed volume of NaOH stock solution to a compartment where it mixed with reconstituted water. From this compartment, the NaOH solution flowed by gravity into five other compartments where it underwent further dilution with reconstituted water to yield five progressively lower concentrations of NaOH Target levels for pH approximated those used by Monda and Saiki (1993, 1994): 8.0 (control), 9.0, 9.5, 10.0, 10.5, and 11 .O. If pH levels in test chambers fluctuated during the test, the Micromedic® pump was adjusted accordingly to increase or decrease the volume of NaOH stock solution injected into the diluter.

Ammonia Tests. A 2M stock solution of NH_4Cl was prepared by dissolving reagent-grade NH_4Cl salts in deionized water. Treatment levels of unionized ammonia were made by following the general procedure described for pH tests. A 0.25M solution of NaOH was also injected into the diluter to keep pH within the target level (about pH 8.0). Target concentrations of unionized ammonia-nitrogen (mg NH,-N/L) approximated those used by Monda and Saiki (1993, 1994): <0.01 (control), 0.146, 0.295, 0.594, 1.03, and 2.60.

Temnerature Tests. For temperature tests, six equally spaced compartments were constructed within the long axis of each rectangular-shaped waterbath. The compartments were formed by inserting snuggly fitting sheets of 25-mm-thick Styrofoam@ into wooden brackets attached to inside walls of the waterbaths. Each compartment was large enough to hold a 4-L head tank, a test chamber, and a drainage standpipe. Aquarium heaters (Visitherm®) were used to maintain desired levels of water temperature in each compartment. Target levels for

temperature (°C) approximated those used by Monda and Saiki (1993, 1994): 20 (control), 25, 27, 29, 31, and 33.

Dissolved Oxveen Tests. Desired amounts of dissolved oxygen were produced in test chambers by bubbling appropriate proportions of compressed air:compressed nitrogen into the 4-L head tanks. Target concentrations of dissolved oxygen (mg/L) were similar to those used by Monda and Saiki (1993, 1994): 9.07 (control), 4.54, 3.63, 2.72, 1.81, and 0.9.

Water Quality Measurements

The following water quality variables were measured twice daily in each test chamber: pH; total ammonia; temperature; dissolved oxygen; and specific conductance. Hydrogen-ion concentration (pH) was measured with a Cole-Parmer® Digisense™ pH meter; total ammonia concentration, with an Orion@ portable ion/pH meter and an Orion@ model 95- 12 ammonia electrode; water temperature, with a mercury thermometer; dissolved oxygen concentrations, with a YSI® model 57 oxygen meter; and specific conductance, with a Cole-Parmer® model 9100-00 conductivity meter. The fraction of total ammonia in unionized form was calculated from equations in Emerson et al. (1975).

Other water quality variables were measured from pooled water samples of each treatment at the beginning and end of each test. The variables and analytical procedures were as follows: total and calcium hardness, by titration with a HACH® model HAC-DT Total-and-Calcium Hardness kit; sulfates, by the HACH® SulfaVer 4 method; total alkalinity, by potentiometric titration (APHA et al. 1981); and chlorides, by HACH® mercuric nitrate buret

titration or HACH® silver chloride buret titration. (Note: During this study, the method for measuring chlorides was changed from mercuric nitrate buret titration to silver nitrate buret titration to avoid producing mercuric chloride, a hazardous waste.) Water quality measurements in individual test chambers were discontinued after fish mortalities reached 100%.

Data Analysis

Arithmetic means and standard deviations for water quality and fish mortality data were calculated with SAS® System software (SAS Institute Inc. 1987).

The 96-hr LC_{50} values were calculated with two versions of a computer program written by the U.S. Environmental Protection Agency (Environmental Monitoring and Support Laboratory, Cincinnati, Ohio). The early version of the program ("LC5O.BAS") computed LC_{50} values and 95% confidence limits by using binomial, moving average, and probit procedures, whereas the later version ("US EPA Toxicity Data Analysis Software") computed similar statistics by using probit and trimmed Spearman-Karber procedures (binomial and moving average procedures were omitted).

Statistical methods for computing LC,, values were chosen after considering assumptions of the various methods (Gelber et al. 1985). The binomial method was used if none of the treatments resulted in partial mortalities. If partial mortalities occurred in at least one treatment, then either the binomial or the trimmed Spearman-Karber methods were used. If two or more treatments had partial mortalities, the probit method was also used. If two or more methods were suitable for estimating the LC_{50} value, we used subjective criteria to select the "best" method.

For example, we favored the method that computed an LC,, value closely approximating a graphical interpolation of the LC_{50} value or that computed the narrowest 95% confidence interval (however, the 95% confidence interval should not extend below 0% mortality or above 100% mortality).

Results

Larval tests were conducted from April 18 to July 7, 1994, whereas juvenile tests were conducted from August 22 to October 7, 1994. A total of 18 tests were completed (two with Lost River larvae, four with Lost River juveniles, eight with shortnose larvae, and four with shortnose juveniles).

Other than hypersensitivity to handling exhibited in early lots of larvae, there were no overt symptoms of diseases, parasites, or other biological problems among larval and juvenile fish used in tests. To reduce handling stress, we hurried the transfer of larvae and juveniles from the acclimation tank into test chambers. However, this action created counting errors when dispensing fish into individual test chambers (our goal was 40 larvae or 10 juveniles per test chamber).

Except for tests with ammonia, mortalities in the most stressful treatments occurred shortly after a given test was initiated. If fish survived the first hour of testing, they usually were still alive when the test was terminated. Tests with ammonia were exceptional because mortalities occurred continuously during the 96-hr testing period.

Shortages of reconstituted water occurred during a few tests (our ability to formulate and store adequate volumes of reconstituted water was occasionally exceeded when operating two diluters). Water shortages usually lasted l-3 hr, the length of time needed to formulate a new batch of reconstituted water. When reconstituted water was once again available, the test chambers were thoroughly flushed by temporarily reducing the interval between diluter cycles. Interruptions in the supply of reconstituted water did not measurably affect treatment levels or other water quality variables.

pH Tests

Immediately after larval and juvenile Lost River and shortnose suckers were placed in test chambers containing the highest pH treatments, they swam about rapidly for a few minutes, then lost equilibrium (turned belly up) and died soon thereafter. Dead and dying fish secreted copious amounts of mucus. If fish were not removed shortly after death in the highest treatments, their body tissues began to dissolve or liquefy.

Hydrogen ion concentrations in test chambers increased over time particularly among higher treatments, necessitating an increase in the amount of NaOH injected into the diluter to maintain target levels of pH Moreover, concentrations of calcium hardness and total hardness decreased with time in the test chambers; these effects were most pronounced in higher pH treatments, where a whitish precipitate (possibly calcium carbonate, $CaCO_3$) occurred in both the diluter and the test chambers).

In general, as treatment levels of pH increased, the concentrations of un-ionized ammonia and total alkalinity increased whereas the concentrations of calcium hardness and total hardness decreased. Moreover, the values for specific conductance increased. Other water quality variables were not influenced by pH manipulations.

Lost River Larvae. The Spearman-Karber method was used to calculate a 96-hr LC,, value of 10.45 (95% C.I., 10.43-10.48) for pH (Table 1). Survival of Lost River larvae exhibited a strong dose response to progressively higher levels of pH (Table 2a). Although mortalities were negligible between pH 7.86 and 10.26, very high mortalities occurred at pH 10.72 and above. At pH 10.72 and 10.80, complete mortalities occurred within 5 hr whereas, at pH 11.25-1 1.28, complete mortalities occurred within one hr.

Mean values for water quality variables in test chambers varied as follows: pH, 7.86-11.28; unionized ammonia, 0.001-0.027 mg/L; temperature, 19.5-20.5 °C; dissolved oxygen concentration, 7.8-8.2 mg/L; and specific conductance, 164-800 µS/cm @ 25 °C (Table 2b). Also total alkalinity, 42-2 19 mg/L as $CaCO_3$; total hardness, 32-35 mg/L as $CaCO_3$; calcium hardness, 16-l 9 mg/L as $CaCO_3$; sulfates, 35.1-40.2 mg/L; and chlorides, 2.8-3.5 mg/L (Table 2c).

Lost River Juveniles. The Spearman-Karber method was used to calculate a 96-hr LC_{50} value of 9.92 (95% C.I., 9.87-9.96) for pH (Table 1). All fish survived in pH as high as 9.80 (Table 3a). Mean SL of juvenile fish used in the test was 28±1.3 mm (about 35 mm TL), whereas mean weight was 0.280±0.038 g.

Mean values for water quality variables in test chambers varied as follows: pH, 7.84-1 1 .01 ; unionized ammonia, 0.002-0.041 mg/L; temperature, 20.0-20.4 °C; dissolved oxygen

concentration, 8.0-8.4 mg/L; and specific conductance, 138-530 µS/cm @ 25°C (Table 3b). Also total alkalinity, 37- 160 mg/L as $CaCO_3$; total hardness, 24-34 mg/L as $CaCO_3$; calcium hardness, 14-18 mg/L as $CaCO_3$; sulfates, 35.2-41.2 mg/L; and chlorides, 2.0-2.8 mg/L (Table 3c).

Shortnose Larvae. Two pH tests were conducted with shortnose larvae. In the first test, the 96-hr LC_{50} value calculated by the binomial method was 10.33 (95% C.I., 10.01-10.66) (Table 1). All fish survived at pH levels as high as 10.01, whereas no fish survived at pH 10.66 (Table 4a). In the second test, the 96-hr LC,, value calculated with the binomial method was 10.46 (95% C.I., 10.12-10.83) (Table 1). Complete survival occurred at pH levels as high as 10.16, whereas no survival occurred at pH 10.8 1 (Table 5a).

During the first test, mean values for water quality variables in test chambers varied as follows: pH, 7.8 **1** - 11.15; unionized ammonia, 0.002-0.206 mg/L; temperature, 19.8-20.8 °C; dissolved oxygen concentration, 7.6-8.0 mg/L; and specific conductance, 157-745 @/cm @ 25 °C (Table 4b). Also total alkalinity, 39-203 mg/L as $CaCO_3$; total hardness, 20-35 mg/L as $CaCO_3$; calcium hardness, 12-19 mg/L as $CaCO_3$; sulfates, 32.3-50.3 mg/L; and chlorides, 3.0-4.0 mg/L (Table 4c).

During the second test, mean values for water quality variables in test chambers varied as follows: pH, 7.92-l 1.33; unionized ammonia, 0.003-o. 145 mg/L; temperature, 19.8-22.1 °C; dissolved oxygen concentration, 7.5-8.1 mg/L; and specific conductance, 143-740 µS/cm @ 25°C (Table 5b). Also total alkalinity, 39-220 mg/L as $CaCO_3$; total hardness, 34-37 mg/L as $CaCO_3$; calcium hardness, 12-19 mg/L as $CaCO_3$; sulfates, 33.7-40.8 mg/L; and chlorides, 2.8-3.0 mg/L (Table 5c).

Shortnose Juveniles. The Spearman-Karber method was used to calculate a 96-hr LC_{50} value of 9.85 (95% C.I., 9.76-9.95) for pH (Table 1). All fish survived at pH levels as high as 9.5 1, 90% survival occurred at pH 10.25, and no fish survived at pH levels of 10.38 or higher (Table 6a). Fish used in this test averaged 4W2.3 mm SL (about 50 mm TL) and 1.114±0.244 g in weight.

Mean values for water quality variables in test chambers varied as follows: pH, 7.86-1 1.40; unionized ammonia, 0.002-0.057 mg/L; temperature, 19.9-20.3 °C; dissolved oxygen concentration, 7.7-7.8 mg/L; and specific conductance, 155-785 µS/cm @ 25 °C (Table 6b). Also total alkalinity, 42-227 mg/L as $CaCO_3$; total hardness, 33-37 mg/L as $CaCO_3$; calcium hardness, 16-l 9 mg/L as $CaCO_3$; sulfates, 26.4-35.1 mg/L; and chlorides, 3.0-3.3 mg/L (Table 6c).

Ammonia Tests

Larval and juvenile Lost River and shortnose suckers exposed to unionized ammonia concentrations exhibited strong dose responses. In the highest treatments, complete mortalities generally occurred within 1 hr. By comparison, complete mortalities in the second highest treatments did not occur until 24-48 hr after tests were initiated. At still lower treatments, mortalities were either incomplete or did not occur even after 96 hr.

Unionized ammonia concentrations generally decreased over time particularly at higher treatment levels. To compensate for temporal variations (detected during twice daily

measurements of ammonia concentrations), the volume of NH_4Cl injected into diluters was gradually increased during the test.

As treatment concentrations of unionized ammonia increased, pH, specific conductance, and chloride concentrations also increased. To a much lesser extent, the concentrations of unionized ammonia were also associated with concentrations of total alkalinity, total hardness, and calcium hardness.

Lost River Larvae. This test was not conducted because sufficient numbers of Lost River larvae were not available.

Lost River Juveniles. The probit method was used to calculate a 96-hr $LC_{,,}$ value of 0.750 mg/L (95% C.I., 0.599-0.944 mg/L) for unionized ammonia (Table 1). Complete survival occurred at unionized ammonia concentrations as high as 0.274 mg/L (Table 7a). Fish experience 10% mortality at concentrations of 0.3 15-0.625 mg/L, and 25% mortality at 0.715 mg/L. No fish survived at concentrations of 1.60 mg/L and higher. Fish used in the test averaged 40±1.9 mm SL (about 50 mm TL) and 0.803±0.141 g in weight.

Mean values for water quality variables in test chambers varied as follows: pH, 7.72-7.92; unionized ammonia, 0.002-l .64 mg/L; temperature, 19.0-20.2 °C; dissolved oxygen concentration, 7.3-8.0 mg/L; and specific conductance, 15 l-780 µS/cm @ 25 °C (Table 7b). Also total alkalinity, 40-52 mg/L as $CaCO_3$; total hardness, 34-36 mg/L as $CaCO_3$; calcium hardness, 19-20 mg/L as $CaCO_3$; sulfates, 32.3-39.1 mg/L; and chlorides, 3.0-153.0 mg/L (Table 7c).

Shortnose Larvae. Two ammonia tests were conducted with shortnose larvae. In the first test, the Spearman-Karber method was used to calculate a 96-hr LC_{50} value of 0.750 mg/L (95%

C.I., 0.730-0.770 mg/L) for unionized ammonia (Table 1). All fish survived at concentrations as high as 0.460 mg/L, although 5% mortality occurred in another treatment at this concentration (Table 8a). Complete mortality occurred at concentrations of 1.07 mg/L or higher.

In the second test, the probit method was used to calculate a 96-hr $LC_{,}$ value of 1.40 mg/L (95% C.I., 1.24-1.68 mg/L) (Table 1). All fish survived at unionized ammonia concentrations as high as 0.52 mg/L (Table 9a). A mortality of 2.5% occurred at 0.56 mg/L, 12.5% at 0.94 mg/L, and 25% at 1.11 mg/L. Complete mortality occurred at concentrations of 3.35 mg/L or higher.

During the first test, mean values for water quality variables in test chambers varied as follows: pH, 7.86-8.18; unionized ammonia, 0.002-3.20 mg/L; temperature, 19.3-20.6°C; dissolved oxygen concentration, 7.7-7.9 mg/L; and specific conductance, 164-720 µS/cm @ 25 °C (Table 8b). Also total alkalinity, 41-54 mg/L as $CaCO_3$; total hardness, 34-38 mg/L as $CaCO_3$; calcium hardness, 18-22 mg/L as $CaCO_3$; sulfates, 37.5-39.0 mg/L; and chlorides, 3.0-132.0 mg/L (Table 8c).

During the second test, mean values for water quality variables in test chambers varied as follows: pH, 7.98-8.27; unionized ammonia, 0.002-4.46 mg/L; temperature, 18.0-20.0°C; dissolved oxygen concentration, 7.8-8.0 mg/L; and specific conductance, 144-735 µS/cm @ 25 °C (Table 9b). Also total alkalinity, 40-52 mg/L as $CaCO_3$; total hardness, 34-38 mg/L as $CaCO_3$; calcium hardness, 18-22 mg/L as $CaCO_3$; sulfates, 42.8-47.8 mg/L; and chlorides, 3.0-156.0 mg/L (Table 9c).

Shortnose Juveniles. The moving average method was used to calculate a 96-hr $LC_{,}$ value of 0.956 mg/L (95% C.I., 0.32-2.46 mg/L) for unionized ammonia (Table 1). With one

exception, complete survival occurred at concentrations as high as 0.296 mg/L (Table 1 Oa). For unknown reasons, 10% mortality (1 of 10 fish) occurred at 0.071 mg/L. A mortality of 20% occurred at 0.708 mg/L, and 40% mortality occurred at 0.745 mg/L. Complete mortality occurred at 2.24 mg/L or higher concentrations. Fish used in the test averaged 40±2.3 mm SL (about 50 mm TL) and 1.999±0.55 1 g in weight.

Mean values for water quality variables in test chambers varied as follows: pH, 7.50-8.18; unionized ammonia, 0.002-2.67 mg/L; temperature, 20.0-20.3 °C; dissolved oxygen concentration, 7.8-8.0 mg/L; and specific conductance, 144-630 µS/cm @ 25 °C (Table 10b). Also total alkalinity, 38-47 mg/L as $CaCO_3$; total hardness, 34-35 mg/L as $CaCO_3$; calcium hardness, 18-20 mg/L as $CaCO_3$; sulfates, 35.1-3 8.6 mg/L; and chlorides, 3.5-173.5 mg/L (Table 1 0c).

Temperature Tests

In general, high mortalities occurred rapidly (usually within 1 hr after tests were initiated) in the highest lethal temperature treatments, whereas mortalities occurred later (1 or more days after tests were initiated) in lower lethal temperature treatments. In all tests, the difference between nonlethal and lethal temperatures was small (1.6-4.4 °C).

As the temperature of treatments progressively increased, pH and specific conductance also increased whereas dissolved oxygen concentrations decreased.

Lost River Larvae. This test was not conducted because sufficient numbers of Lost River larvae were not available.

Lost River Juveniles. The trimmed Spearman-Karber method was used to calculate a 96-hr LC_{50} value of 3 1.2 °C (95% C.I., 30.8-3 1.5 °C) for water temperature (Table 1). Complete survival occurred at 28.1 °C or lower, whereas partial mortalities (0.9-10%) occurred at 28.4-30.5 °C (Table 1 la). All fish died at temperatures of 32.5 °C or higher. Fish used in this test averaged 40±1.9 mm SL (about 50 mm TL) and 0.863±0.199 g in weight.

Mean values for water quality variables in test chambers varied as follows: pH, 7.7 1-7.93; unionized ammonia, 0.002-0.20 mg/L; temperature, 19.6-33 .0°C; dissolved oxygen concentration, 5.9-8.0 mg/L; and specific conductance, 14 l-l 71 µS/cm @ 25 °C (Table 11 b). Also total alkalinity, 38-40 mg/L as $CaCO_3$; total hardness, 34-35 mg/L as $CaCO_3$; calcium hardness, 18-20 mg/L as $CaCO_3$; sulfates, 31.3-39.6 mg/L; and chlorides, 2.5-3.5 mg/L (Table 1 lc).

Shortnose Larvae. Two temperature tests were conducted with shortnose larvae. In the first test, the Spear-man-Karber method was used to calculate a 96-hr LC,, value of 3 1.9°C (95% C.I., 3 1.9-32.0°C) (Table 1). Complete survival occurred at temperatures as high as 30.7°C, with 2.4% mortality occurring at 3 1.2°C (Table 12a). All fish died at temperatures of 32.3 °C or higher.

In the second test, the binomial method was used to calculate a 96-hr LC_{50} value of 3 1.9°C (95% C.I., 29.0-33.5 °C) (Table 1). Complete survival occurred at water temperatures as high as 29.5 °C, with 2.5% mortality occurring at 3 1.4°C (Table 13a). All fish died at temperatures of 32.1 °C or higher.

During the first test, mean values for water quality variables in test chambers varied as follows: pH, 7.87-8.12; un-ionized ammonia, 0.001-0.008 mg/L; temperature, 20.9-33.7°C;

dissolved oxygen concentration, 5.9-7.5 mg/L; and specific conductance, 159-170 µS/cm @ 25 °C (Table 12b). Also total alkalinity, 4 l-43 mg/L as $CaCO_3$; total hardness, 34-37 mg/L as $CaCO_3$; calcium hardness, 18-19 mg/L as $CaCO_3$; sulfates, 29.2-37.4 mg/L; and chlorides, 2.5-3.3 mg/L (Table 12c).

During the second test, mean values for water quality variables in test chambers varied as follows: pH, 7.96-8.20; unionized ammonia, 0.002-0.013 mg/L; temperature, 19.8-33.0°C; dissolved oxygen concentration, 6.1-7.9 mg/L; and specific conductance, 138-l 54 µS/cm @ 25 °C (Table 13b). Also, total alkalinity, 40-40 mg/L as $CaCO_3$; total hardness, 34-37 mg/L as $CaCO_3$; calcium hardness, 17-21 mg/L as $CaCO_3$; sulfates, 39.8-44.0 mg/L; and chlorides, 2.8-3.3 mg/L (Table 13c).

Shortnose Juveniles. The trimmed Spearman-Karber method was used to calculate a 96-hr $LC_{,,}$ value of 3 1.2°C (95% C.I., 30.8-3 1.6°C) (Table 1). All fish survived at temperatures as high as 30.8°C, whereas 60% mortality occurred at 30.9°C (Table 14a). Complete mortality occurred at 32.5°C and higher. Fish used in this test averaged 33±2.8 mm SL (about 43 mm TL) and 0.540±0.146 g in weight.

Mean values for water quality variables in test chambers varied as follows: pH, 7.73-8.04; unionized ammonia, 0.002-0.03 1 mg/L; temperature, 19.7-33.1 °C; dissolved oxygen concentration, 5.9-8.0 mg/L; and specific conductance, 139-170 µS/cm @ 25 °C (Table 14b). Also total alkalinity, 38-39 mg/L as $CaCO_3$; total hardness, 30-34 mg/L as $CaCO_3$; calcium hardness, 18-20 mg/L as $CaCO_3$; sulfates, 29.9-38.9 mg/L; and chlorides, 2.0-2.5 mg/L (Table 14c).

Dissolved Oxygen Tests

Larval and juvenile Lost River and shortnose suckers responded rapidly to lethal concentrations of dissolved oxygen, with the lowest concentrations generally causing 100% mortality within a few hours. Fish stressed by low dissolved oxygen concentrations spent most of the time near the water surface. Dying fish had difficulty swimming, and exhibited a gasping behavior while struggling to stay near the surface. By comparison, fish in other treatments and the control swam throughout the water column without exhibiting the gasping behavior.

Although dissolved oxygen concentrations fluctuated somewhat over time in the treatments, other water quality variables remained at target levels.

Lost River Larvae. The probit method was used to calculate a 96-hr LC_{50} value of 2.1 mg/L (95% C.I., 2.0-2.2 mg/L) for dissolved oxygen concentrations (Table 1) or 25.8% saturation (95% C.I., 24.9-26.8% saturation). Although 2.5% mortality occurred at a dissolved oxygen concentration of 2.7 mg/L, fish survival was generally very high at concentrations as low as 2.5 mg/L (Table 15a). Mortalities increased to 82.5-100% at 1.8 mg/L, and no fish survived at lower concentrations. In treatments containing <1 .O mg/L of dissolved oxygen, complete mortality occurred within 3 hr.

Mean values for water quality variables in test chambers varied as follows: pH, 7.58-8.20; unionized ammonia, 0.001-0.006 mg/L; temperature, 19.3-21.3 °C; dissolved oxygen concentration, 0.7-7.6 mg/L; and specific conductance, 163-165 µS/cm @ 25 °C (Table 15b). Also total alkalinity, 39-42 mg/L as $CaCO_3$; total hardness, 34-36 mg/L as $CaCO_3$; calcium

hardness, 18-20 mg/L as $CaCO_3$; sulfates, 34.2-38.1 mg/L; and chlorides, 2.5-3.5 mg/L (Table 15c).

Lost River Juveniles. The binomial method was used to calculate a 96-hr LC,, value of 1.4 mg/L (95% C.I., 1.0-2.0 mg/L) (Table 1) or 19.1% saturation (95% C.I., 13.9-26.2% saturation). All fish survived at dissolved oxygen concentrations as low as 1.9 mg/L, whereas no fish survived at 1.3 mg/L and lower (Table 16a). Fish used in this test averaged 4 1±2.2 mm SL (about 5 1 mm TL) and 0.864±0.173 g in weight.

Mean values for water quality variables in test chambers varied as follows: pH, 7.59-8.30; unionized ammonia, 0.001-0.012 mg/L; temperature, 19.8-20.6°C; dissolved oxygen concentration, 0.7-7.7 mg/L; and specific conductance, 146-152 µS/cm @ 25°C (Table 16b). Also total alkalinity, 37-39 mg/L as $CaCO_3$; total hardness, 34-35 mg/L as $CaCO_3$; calcium hardness, 18-20 mg/L as $CaCO_3$; sulfates, 27.6-37.7 mg/L; and chlorides, 1.8-2.5 mg/L (Table 16c).

Shortnose Larvae. Two dissolved oxygen tests were conducted with shortnose larvae. In the first test, the probit method was used to calculate a 96-hr LC,, value of 2.3 mg/L (95% C.I., 2.2-2.4 mg/L) (Table 1) or 30.3% saturation (95% C.I., 28.9-3 1.6% saturation). All fish survived at dissolved oxygen concentrations as low as 4.3 mg/L, whereas mortalities of 2.4-74.4% occurred at concentrations of 2.2-3.7 mg/L (Table 17a). Complete mortality occurred at dissolved oxygen concentrations of 1.6 mg/L and lower. In the second test, the moving average method was used to calculate a 96-hr LC_{50} value of 1.7 mg/L (95% C.I., 1.6-l .8 mg/L) (Table 1) or 22.3% saturation (95% C.I., 21.0-23.6% saturation). Complete survival occurred at dissolved oxygen concentrations as low as 2.6 mg/L (Table 18a). Mortality varied from 56.8% to 100% at

dissolved oxygen concentrations of 0.8-l .7 mg/L, with all fish dying at a concentration of 0.7 mg/L (the lowest concentration tested). (Note: Towards the end of the second test, fish in Treatment 4b were exposed to mercury from a broken thermometer. However, no mortalities occurred in this treatment. Thus, data from this treatment were included when computing the 96-hr LC_{50} value.)

During the first test, mean values for water quality variables in test chambers varied as follows: pH, 7.70-8.77; un-ionized ammonia, 0.001-0.018 mg/L; temperature, 20.4-2 1.7 °C; dissolved oxygen concentration, 0.6-7.3 mg/L; and specific conductance, 155- 165 µS/cm @ 25 °C (Table 17b). Also total alkalinity, 37-42 mg/L as $CaCO_3$; total hardness, 20-34 mg/L as $CaCO_3$; calcium hardness, 12-19 mg/L as $CaCO_3$; sulfates, 38.8-42.5 mg/L; and chlorides, 3.0-3.5 mg/L (Table 17c).

During the second test, mean values for water quality variables in test chambers varied as follows: pH, 7.47-8.46; un-ionized ammonia, 0.001-0.015 mg/L; temperature, 20.0-2 1.4 °C; dissolved oxygen concentration, 0.7-7.9 mg/L; and specific conductance, 140-149 µS/cm @ 25°C (Table 18b). Also total alkalinity, 39-41 mg/L as $CaCO_3$; total hardness, 34-36 mg/L as $CaCO_3$; calcium hardness, 19-20 mg/L as $CaCO_3$; sulfates, 3 1.2-40.5 mg/L; and chlorides, 3 .0-3.3 mg/L (Table 18c).

Shortnose Juveniles. The binomial method was used to calculate a 96-hr LC_{50} value of 1.2 mg/L (95% C.I., 0.7-1.7 mg/L) (Table 1) or 14.7% saturation (95% C.I., 7.6-22.5% saturation). No mortalities occurred at dissolved oxygen concentrations as low as 2.6 mg/L, whereas 10% mortality occurred at 1.6-l .7 mg/L (Table 19a). All fish died at dissolved oxygen

concentrations of 0.7 mg/L and lower. Fish used in the test averaged 40±2.6 mm SL (about 50 mm TL) and 1.152±0.200 g in weight.

Mean values for water quality variables in test chambers varied as follows: pH, 7.48 7.80; unionized ammonia, 0.00 l-0.01 5 mg/L; temperature, 18.8-20.0 °C; dissolved oxygen concentration, 0.6-7.7 mg/L; and specific conductance, 145-154 µS/cm @ 25 °C (Table 19b). Also total alkalinity, 36-42 mg/L as $CaCO_3$; total hardness, 36-38 mg/L as $CaCO_3$; calcium hardness, 18-20 mg/L as $CaCO_3$; sulfates, 35.6-39.8 mg/L; and chlorides, 2.5-3.0 mg/L (Table 1 9c).

Discussion

Water quality variables fluctuate daily and seasonally in Upper Klamath Lake and, to some extent, throughout the Upper Klamath Basin. Especially during summer months, variables such as pH, unionized ammonia, dissolved oxygen, and temperature could approach or exceed toxic thresholds for fish, resulting in fish kills or sublethal effects. In addition, when fish are stressed by one or more of these variables, their ability to tolerate the remaining variables could be affected. Our study was an attempt to better define the toxic thresholds of these water quality variables by repeating earlier tests with Lost River larvae and juveniles and shortnose juveniles (Monda and Saiki 1993, 1994), and by generating heretofore unavailable data for shortnose larvae.

The earlier studies by Monda and Saiki (1993, 1994) on pH tolerance yielded the following results (species and life stage, 96-hr LC_{50}, 95% confidence limits): Lost River larvae,

9.84, 9.77-9.94; Lost River juveniles, 10.68, 10.52-l 1.03; and shortnose juveniles, 10.50, 9.82-11 .O. For un-ionized ammonia, the results were as follows: Lost River larvae, 0.54 mg/L, 0.43-0.85 mg/L; Lost River juveniles, 0.70 mg/L, 0.34-0.82 mg/L; and shortnose juveniles, 0.34 mg/L, 0.14-0.73 mg/L. For temperature, the results were as follows: Lost River larvae, 3 1.8°C, 30.5-33.6°C; Lost River juveniles, 30.0°C, 29.9-30.3°C; and shortnose juveniles, 29.4°C, 27.8-34.1 °C. Finally, for dissolved oxygen concentrations, the results were as follows: Lost River larvae, 2.1 mg/L, 2.0-2.3 mg/L; Lost River juveniles, 1.8 mg/L, 1.6-l .9 mg/L; and shortnose juveniles, 1.5 mg/L, 0.6-2.4 mg/L. In all but a few instances, our data agreed with these earlier findings (see Table 1).

The results reported by Monda and Saiki (1993, 1994) differed significantly (i.e., 95% confidence intervals did not overlap) from our data for the following variables: pH (Lost River larvae and juveniles only); unionized ammonia (shortnose larvae only); temperature (Lost River juveniles only); and dissolved oxygen (shortnose larvae only). Reasons for the variations are not well understood. One possibility is that test results were affected by the quality of test animals. Monda and Saiki (1993, 1994) mentioned that their fish (especially juveniles) exhibited evidence of nutritional deficiency and disease. During our tests with larvae, we also noted symptoms of nutritional deficiency that were seemingly corrected by supplementing artificial diets with live brine shrimp nauplii. Another possible source of variation concerns the ages of test animals. During tests by Monda and Saiki (1993), juveniles varied from 5-months-old to 1 O-months-old whereas our juveniles were less than 4-months-old.

As judged by combined data from Monda and Saiki (1993, 1994) and the present study, the tolerance of larval and juvenile Lost River and shortnose suckers to high pH and high un-

ionized ammonia concentrations did not exhibit consistent patterns (Figure 1). On average, larvae of Lost River and shortnose suckers were slightly more tolerant than juveniles to high temperatures whereas juveniles were more tolerant than larvae to low dissolved oxygen concentrations (Figure 1). However, the differences were not statistically significant ($P > 0.05$).

One difficulty with using 96-hr LC,, values to estimate lethal thresholds relates to the sudden transfer of test animals from acclimation conditions to test conditions. Test animals may die from shock following abrupt changes in water quality conditions even though survival might have occurred if the changes were less dramatic (Warren 1971; Sprague 1985; Murray and Ziebell 1984). If shock from suddenly transferring test animals to new water quality conditions strongly influences survival, the 96-hr LC_{50} tests may underestimate the actual tolerance limits. Future studies might attempt to reduce the effects of shock by acclimating test animals to water quality conditions near the lethal levels.

Coleman et al. (1988) and Kann and Smith (1993) documented summertime pH values exceeding 10.0 over large areas of Upper Klamath Lake. Moreover, these elevated pH values can persist for 2-3 weeks or longer (U.S. Bureau of Reclamation, Klamath Project Office, Klamath Falls, OR, unpublished data). Jacob Kann (unpublished data) also measured total ammonia concentrations during summer months that exceeded 0.6 mg/L. At 20°C and pH 9.5 (conditions prevailing at the time that ammonia concentrations were measured), 0.6 mg/L of total ammonia contains about 0.33 mg/L of unionized ammonia. Coleman et al. (1988) and J. Kann (unpublished data) reported maximum summertime water temperatures as high as 29°C in Upper Klamath Lake. Finally, Coleman et al. (1988) reported that dissolved oxygen concentrations in Upper Klamath Lake often fluctuates widely in response to weather conditions and algal

productivity cycles. Oxygen concentrations less than 4.5 mg/L can persist lake-wide for several weeks to a month, and concentrations less than 0.5 mg/L can occur for more than six hours either throughout the water column in isolated areas of the lake or near bottom when the lake is thermally stratified (Coleman et al. 1988; Kann and Smith 1993).

According to Monda and Saiki (1993, 1994) and results from the present study (Table 1), Lost River and shortnose suckers could experience high mortality if exposed to one or more of the following conditions: pH, ≥ 9.84; unionized ammonia concentration, ≥0.34 mg/L; water temperature, ≥29.4°C; and dissolved oxygen concentration, ≤2.3 mg/L. Collectively, these results suggest that summertime water quality conditions in Upper Klamath Lake can approach or exceed the tolerance limits of these fishes.

Conclusions and Research Needs

Monda and Saiki (1993, 1994) reported that water quality conditions in Upper Klamath Lake can be acutely lethal to Lost River and shortnose suckers. Our results corroborated those earlier findings. Mortality of larvae and juveniles from exposure to lethal water quality during late spring and summer might explain why the numbers of adult suckers in Upper Klamath Lake are low even though these adults spawn successfully in tributaries such as the Williamson and Sprague rivers (and possibly the Wood River and Crooked Creek) and in Sucker Springs on the eastern shore of the lake (Stubbs and White 1993). Moreover, stressful water quality conditions that curtail successful recruitment of early life stages could explain the long-term decline of sucker populations throughout the Klamath Basin.

Although our results were similar to earlier findings by Monda and Saiki (1993, 1994), the estimated lethal thresholds of selected water quality variables to Lost River and shortnose suckers are still preliminary because several limitations or uncertainties remain. First, we used test animals that were the progeny of matings from relatively few wild-caught fish. The data generated from these test animals might not adequately reflect species-wide responses to experimental conditions. Second, our results were based on test animals acclimated to presumably optimal water quality conditions whereas, according to Warren (1971), fish are highly adaptable and can often tolerate much harsher conditions if allowed to gradually acclimate to the stressful limits. Third, we did not repeat two tests involving unionized ammonia and temperature with Lost River larvae and, therefore, we have no measure of variation for these two water quality variables.

We recommend that unionized ammonia and temperature tests with Lost River larvae be repeated. By repeating these tests, our confidence in the data should improve because more rigorous statistical evaluations will be possible.

We also recommend that all four variables (pH, unionized ammonia, temperature, and dissolved oxygen) be tested with fish acclimated to suboptimal (sublethal) water quality conditions that closely approach the lethal levels. Such tests would allow estimation of lethal levels where confounding effects from shock (caused by abrupt transfer of test animals from acclimation conditions to test conditions) are greatly reduced.

Additional recommendations were previously mentioned by Monda and Saiki (1993, 1994). For example, completion of multiple variable tests initiated by Monda and Saiki (1993, 1994) would allow construction of a predictive model that estimates the probability of fish

survival under variable water quality conditions. Also, laboratory tests of longer duration (28 days or more) in which water quality variables are intentionally fluctuated to mimic diurnal and other cycles (e.g., phytoplankton blooms and "crashes") would allow measurement of sublethal effects such as reductions in growth and physiological or biochemical changes (e.g., blood chemistry and hormonal changes, lipid storage and utilization, histopathological changes in organs, reductions in swimming performance). These long-term tests might include behavioral measurements such as avoidance or attraction to stressful conditions, altered feeding efficiency, and changes in predator avoidance. Finally, field studies should be implemented to validate laboratory results. Field validation tests are necessary because the response of fish to stressors under laboratory conditions may not resemble their response under field conditions (Boudou and Ribeyre 1989).

References

APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Federation. 198 1. Standard methods for the examination of water and wastewater, 15th edition, 1980. American Public Health Association, Washington, DC. 1134 pages.

Becker, C.D., and R.G. Genoway. 1979. Evaluation of critical thermal maxima for determining thermal tolerance of freshwater fish. Environmental Biology of Fish 4:245-256.

Beinz, C.S., and J.S. Ziller. 1987. Status of three lacustrine sucker species (Catostomidae). Klamath Tribe, Chiloquin, OR. Unpublished manuscript.

Bortleson, G.C., and M.O. Fretwell. 1993. A review of possible causes of nutrient enrichment and decline of endangered sucker populations in Upper Klamath Lake, Oregon. U.S. Geological Survey, Water Resources Investigations Report 93-4087.

Boudou, A., and F. Ribeyre. 1989. Aquatic ecotoxicology: Fundamental concepts and methodologies. Vol. II. CRC Press, Inc., Boca Raton, FL. 666 pp.

Buettner, M., and G. Scoppettone. 1990. Life history and status of Catostomids on Upper Klamath Lake, Oregon. U.S. Fish and Wildlife Service, National Fisheries Research Center-Seattle, Reno Field Station, Reno, NV. Completion report. 108 pp.

Castleberry, D.T., and J.J. Cech. 1993. Critical thermal maxima and oxygen minima of five fishes from the Upper Klamath Basin. Calif. Fish and Game 78: 145-152.

Coleman, M.E., J. Kann, and G.G. Scoppettone. 1988. Life history and ecological investigations of catostomids from the Upper Klamath Basin, Oregon. Draft annual report to the U.S. Fish and Wildlife Service, National Fisheries Research Center, Seattle, WA. 113 pp.

Emerson, K., R.C. Russo, R.E. Lund, and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: effects of pH and temperature. Journal of the Fisheries Research Board of Canada 32:2379-2383.

Falter, M.A., and J.J. Cech. 1991. Maximum pH tolerance of three Klamath Basin fishes. Copeia 4:1109-1111.

Gelber, R.D., P.T. Lavin, C.R. Mehta, and D.A. Schoenfeld. 1985. Chapter 5; statistical analysis. Pages 1 10-123 in G.R. Rand and S.R. Petrocelli, eds. Fundamentals of aquatic toxicology. Taylor and Francis (Hemisphere Publishing Corporation), Bristol, PA.

Kann, J., and V.H. Smith. 1993. Chlorophyll as a predictor of elevated pH in a hypertrophic lake: Estimating the probability of exceeding critical values for fish success. Klamath Tribes Research Report KT-93-02, The Klamath Tribes, Chiloquin, OR. 22 pp.

Monda, D., and M.K. Saiki. 1993. Tolerance of juvenile Lost River and shortnose suckers to high pH, ammonia concentration, and temperature and to low dissolved oxygen concentration. Final report prepared by the U.S. Fish and Wildlife Service for the U.S. Bureau of Reclamation, Denver, CO, and Klamath Falls, OR.

Monda, D., and M.K. Saiki. 1994. Tolerance of larval Lost River sucker to high pH, ammonia concentration, and temperature, and to low dissolved oxygen concentration. Final report prepared by the U.S. Fish and Wildlife Service for the U.S. Bureau of Reclamation, Denver, CO and Klamath Falls, OR.

Mount, D.I., and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Research 1:2 l-29.

Murray, C.A., and C.D. Ziebell. 1984. Acclimation of rainbow trout to high pH to prevent stocking mortality in summer. Progressive Fish-Culturist 46: 176- 179.

SAS Institute Inc. 1987. SAS/STAT user's guide, release 6.03 edition. Cary, NC. 1028 pages.

Scoppettone, G.G. 1986. Upper Klamath Lake, Oregon, catostomid research. U.S. Fish and Wildlife Service, National Fisheries Research Center-Seattle, Reno Field Station, Reno, NV. Unpublished report. 14 pp.

Sprague, J.B. 1985. Chapter 6; Factors that modify toxicity. Pages 124-163 in G.R. Rand and S.R. Petrocelli, eds. Fundamentals of aquatic toxicology. Taylor and Francis (Hemisphere Publishing Corporation), Bristol, PA.

Stubbs, K., and R. White. 1993. Lost River (Deltistes luxatus) and shortnose (Chasmistes brevirostris) sucker recovery plan. U.S. Fish and Wildlife Service, Portland, OR. 95 pp.

Warren, C.E. 1971. Biology and water pollution control. W.B. Saunders Company, Philadelphia, PA. 434 pp.