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

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Summary

Short-term (96 h) laboratory tests were conducted between April 26 and July 17, 1993, to determine the tolerance of larval Lost River sucker, Deltistes luxatus, to high pH, un-ionized ammonia concentration, and temperature, and to low dissolved oxygen concentration. In addition to assessing the lethal levels of the four water-quality variables in single-variable tests, we measured the toxicity of un-ionized ammonia under sublethal levels of the other three variables in a series of multiple-variable tests. The 96-h LC_{50} values (95% confidence intervals in parentheses) for single-variable tests were as follows: pH, 9.84 (9.77-9.94); un-ionized ammonia (as NH_3-N) concentration, 0.54 mg/L (0.43-0.85 mg/L); dissolved oxygen concentration, 2.1 mg/L (2.0-2.3 mg/L) [dissolved oxygen saturation, 27% (26-29%)]; and temperature, 31.8°C (30.5-33.6°C). The 96-h LC_{50} values for un-ionized ammonia concentration in multiple-variable tests varied from 0.46 mg/L to 0.89 mg/L, with overlapping confidence intervals. With one exception, results of these tests with larvae were similar to those of tests with juveniles conducted in 1992. The exception occurred in the single-variable test with pH, where larvae were more sensitive than juveniles. However, these studies must be viewed as preliminary because none of the single-variable and multiple-variable tests were repeated.

Introduction

Over the past 100 years, increased use of fertilizers and pesticides for agriculture, expansion of livestock grazing operations, an altered hydrologic regime from construction and operation of dams and canals, and other human activities and natural events have combined to greatly alter water-quality conditions in Upper Klamath Lake and other surface waters in the Upper Klamath Basin of southern Oregon and northern California. These altered conditions are believed to threaten long-term survival of the Lost River sucker (Deltistes luxatus) and the shortnose sucker (Chasmistes brevirostris), both of which occur naturally in the Upper Basin (Coleman et al., 1988; Buettner and Scoppettone, 1990; Kann, 1993; Kann and Smith, 1993; Stubbs and White, 1993). Unfavorable water quality may have caused a massive die-off of suckers and other fishes in Upper Klamath Lake during August 1986 (Scoppettone, 1986). On July 18, 1988, Lost River and shortnose suckers were listed as endangered by the U.S. Fish and Wildlife Service (1988).

Water-quality variables of greatest concern in the Upper Klamath Basin are pH, ammonia, temperature, and dissolved oxygen. In 1992, we used juvenile (5-10 months old) Lost River and shortnose suckers to determine the lethal levels of these individual variables, whereas juvenile Lost River sucker was used to determine the lethal levels of selected combinations of these variables (Monda and Saiki, 1993). As judged by our results,

ambient summertime pH, un-ionized ammonia concentration, and dissolved oxygen concentration reported by Coleman et al. (1988) and Kann (1993) in Upper Klamath Lake approached or exceeded lethal levels for juvenile suckers.

The purpose of this laboratory study was to determine if ambient water-quality conditions in Upper Klamath Lake are lethal to larvae of the Lost River sucker during spring and early summer (the time period in which larvae occur in the lake). According to Stubbs and White (1993), larval Lost River sucker spend relatively little time in streams where they are spawned. Although larval suckers may leave spawning sites in the Sprague and Williamson rivers for Upper Klamath Lake between mid-April and early July, peak movements usually occur in May or June (Stubbs and White, 1993). Because larval fish are still undergoing rapid physiological development and growth, this life stage is usually more sensitive than older life stages (e.g., juveniles spawned the previous year) to stressful conditions (Rand and Petrocelli, 1985; Sprague, 1990).

Results from this study will contribute to our knowledge of the ecological requirements of the Lost River sucker. In addition, the results will be useful for identifying the physicochemical variables in need of remediation as part of restoration efforts mandated for this fish by the federal Endangered Species Act (i.e., to help develop water-quality goals for the Klamath Basin), and to predict the likelihood of success

of proposed restoration efforts (Kann and Smith, 1993; Stubbs and White, 1993).

Methods

Larvae of Lost River sucker were supplied by the Klamath Tribe's Braymill Fish Hatchery, Chiloquin, Oregon. Adult fish were captured from Upper Klamath Lake at Sucker Springs at 9-day intervals during March-April 1992, and artificially spawned. At the hatchery, fertilized eggs were incubated in upwelling incubator jars until just before swim-up (about 10-13 days after fertilization). Larvae were then transferred to 300-gal round fiber glass tanks. After reaching the swim-up stage, larvae were fed a ration composed of 90% krill and 10% Spirulina (air-dry biomass).

The incubator jars and tanks were components of a recirculating water system where the water was mechanically filtered and irradiated with ultraviolet light. Water for the recirculating system was obtained from the Sprague River. Before using this water, it was chlorinated for 24 hours (>10 mg/L of free chlorine), then dechlorinated using sodium thiosulfate (L. Dunsmoor, Klamath Tribe, Chiloquin, Oregon, personal communication).

When larvae were 30 days old, they were transported from the hatchery to our laboratory in Klamath Falls, Oregon, in a 48-qt insulated ice chest. Compressed oxygen was used to aerate the

water in the ice chest during the 45-min trip. Upon arrival at the laboratory, larvae were transferred into a 250-L fiber glass holding tank and acclimated for four days in reconstituted water (i.e., artificial water formulated to mimic the springtime water quality in Upper Klamath Lake) before being used in tests.

Our intent was to initiate single-variable and multiple-variable tests with larvae that were 35 days old. In addition, we wanted the larvae for each test to come from matings of different pairs of adults. These goals were achieved in single-variable tests. However, an unexpected episode of lethal water quality at the hatchery caused the loss of three of four separate batches of larvae that we had planned to use in multiple-variable tests. This resulted in dissimilar ages of larvae for the following multiple-variable tests (age of larvae given in parentheses): 1a and 1b (35 days old); 2a and 2b (71 days old); 3a and 3b (80 days old); and 4a and 4b (86 days old). Moreover, all larvae used in multiple-variable tests were offspring from the one-time spawning of a single pair of adult fish.

There were no outbreaks of disease among larvae in the hatchery or in our laboratory that required treatment with therapeutic chemicals. Although rarely found, larvae exhibiting obvious abnormalities were discarded when selecting fish for our tests. Overall, fish used in the tests were vigorous and healthy.

During acclimation and testing, we originally planned to standardize the feeding of larvae at four times per day with the

krill-*Spirulina* ration. However, because larvae were considerably larger in multiple-variable tests 3a, 3b, 4a, and 4b, we deviated from this schedule by feeding the fish only twice daily.

Reconstituted water was made by adding reagent-grade sodium bicarbonate ($NaHCO_3$), calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$), hydrated magnesium sulfate ($MgSO_4 \cdot 5H_2O$ or $MgSO_4 \cdot 7H_2O$), and potassium chloride (KCl) to deionized city tapwater. Sodium hydroxide (NaOH) was used to adjust the pH of reconstituted water to 8.0. The concentrations of major ions in reconstituted water were determined from water samples that we collected in Upper Klamath Lake during Spring 1992 (Monda and Saiki, unpublished data). The water-quality measurements of our samples--pH, about 8.0; dissolved oxygen saturation, 100%; temperature, 18-22°C; calcium hardness, 20-25 mg/L as $CaCO_3$; magnesium hardness, 15-20 mg/L as $CaCO_3$; total alkalinity, 30-50 mg/L as $CaCO_3$; conductivity, 140-200 µS/cm @ 25°C; total ammonia, <0.1 mg/L; sulfate, 30-40 mg/L; and chloride, 2-3 mg/L--were assumed to be suitable for high survival of larval suckers.

Single-variable Tests

Single-variable tests were conducted to determine the tolerance limits of larvae to high pH, ammonia concentration, and temperature, and to low dissolved oxygen concentration. Each test consisted of five paired treatments and one paired control

(for a total of 10 treatments and two controls). (Note: Although each pair of treatments or controls were targeted to exhibit identical physicochemical conditions, our measurements indicated that slight differences usually existed.) Each treatment or control consisted of a separate 25-L glass aquarium (test chamber) containing 40 fish. Test chambers were held in two waterbaths (each waterbath contained one of the pairs of treatments and control) equipped with recirculating heater-chiller units to maintain proper temperature.

A proportional diluter (50% dilutions; Mount and Brungs, 1967) and precision metering pump (Micromedic®) were used to deliver reconstituted water to test chambers during all tests and to create treatment concentrations for pH, ammonia, and multiple-variable tests. The diluter was adjusted to deliver two volume exchanges (50 L) per day to each test chamber. Water flowed from the diluter into 4-L head tanks situated in the waterbaths, then into the test chambers; this allowed sufficient time for incoming water to equilibrate to the desired temperature. The uppermost 2.5-cm of one wall of each test chamber was constructed from 0.5 mm stainless steel screen to allow excess water to drain away. Artificial lighting was maintained on a 16-h light:8-h dark photoperiod. Details specific to each test are outlined below.

<u>pH Test</u>. A concentrated stock solution made by dissolving NaOH pellets in deionized water was injected by the precision pump into the diluter to obtain ten progressively higher levels of pH (the desired pH in controls was 8.0). Temperature was

maintained at 20°C ± 1°C, dissolved oxygen saturation at 100%, and un-ionized ammonia concentration at <0.05 mg/L.

Ammonia Test. A concentrated stock solution made by dissolving ammonium chloride salts in deionized water was injected into the diluter to obtain ten progressively higher concentrations of un-ionized ammonia (the concentration in controls was <0.05 mg/L). Temperature was maintained at 20°C ± 1°C, dissolved oxygen saturation at 100%, and pH at 8.0.

Temperature Test. Aquarium heaters (Visitherm® 200 watt) were used to manipulate water temperature in the test chambers. Each waterbath was divided into six compartments, each compartment containing one test chamber, by inserting sheets of 2.5-cm-thick styrofoam that fit snugly against the sides and bottom of the waterbath. One or two heaters were placed into each compartment, and temperature was adjusted to the desired level. There were ten levels of progressively higher temperature, plus two controls in which temperature was maintained at 20°C ± 1°C (acclimation temperature). The diluter was used only to deliver reconstituted water to each test chamber. Dissolved oxygen saturation was maintained at 100%, pH at 8.0, and un-ionized ammonia concentration at <0.05 mg/L.

Dissolved Oxygen Test. Dissolved oxygen saturation was manipulated by bubbling compressed nitrogen gas (N_2) through water in the 4-L head tanks and in each test chamber. By using different ratios of N_2 to air (mixed in desired ratios by Airco Gas and Gear, Woodland, CA), ten progressively lower

concentrations of dissolved oxygen were obtained. Aquarium aerators were used to keep dissolved oxygen in the controls at 100% saturation. The diluter was used only to deliver reconstituted water to the test chambers. Temperature was maintained at 20°C ± 1°C, pH at 8.0, and un-ionized ammonia concentration at <0.05 mg/L.

Multiple-variable Tests

Multiple-variable tests were conducted to determine if the toxicity of one variable (un-ionized ammonia) increased as larvae were exposed to sublethal stresses from as many as three other variables (pH, temperature, and dissolved oxygen).

Testing procedures were similar to those used in the single-variable tests, except that pH was adjusted across treatments (rather than within treatments) by adding NaOH during the formulation of reconstituted water. Also, due to a shortage of larvae in tests 4a and 4b, we used only 23 fish per treatment instead of the usual 40 fish.

Four multiple-variable tests were conducted, each with two target levels of pH (9.5 or 9.8), two target levels of dissolved oxygen saturation (50% or 100%), two target levels of temperature (20°C or 25°C), and five levels of un-ionized ammonia within each pH, dissolved oxygen, and temperature regime (Table 1). We did not standardize our target concentrations of un-ionized ammonia among tests; instead, we varied the concentrations so that one or

more of the highest treatments in each test would result in complete mortality of test animals.

Water Quality Measurements

During single-variable and multiple-variable tests, the following water quality variables were measured in test chambers at least once daily: temperature (°C); dissolved oxygen (mg/L); pH; total ammonia (NH_3 + NH_4^+ expressed as mg NH_3/L); conductivity (μS/cm @ 25°C); total and bicarbonate alkalinity (mg/L as $CaCO_3$); total, calcium, and magnesium hardness (mg/L as $CaCO_3$); chloride (mg/L); and sulfate (mg/L). Water temperature was measured with a VWR® ASTM-certified mercury thermometer; dissolved oxygen, with a YSI® model 57 oxygen meter; pH, with a Cole-Parmer® model 5994 Digisense™ pH meter; total ammonia, with an Orion® model 270 portable pH/ion meter and ROSS™ model 9512 ammonia electrode; conductivity, with a Cole-Parmer® model 19100-00 analog conductivity meter; total and bicarbonate alkalinity, by potentiometric titration (American Public Health Association, 1981); total hardness, by titration with a HACH® model HAC-DT Total-and-Calcium Hardness kit; chloride, by the HACH® mercuric nitrate buret titration method; and sulfate, by the HACH® SulfaVer™ 4 method.

The frequency of measurement for each water-quality variable depended on the test being conducted (e.g., if the test variable was ammonia, then total ammonia was measured no less than twice

daily). The fraction of total ammonia in un-ionized form as NH_3 was calculated from equations in Emerson et al. (1975) and converted to mg NH_3-N/L.

Data Analysis

In single-variable and multiple-variable tests, the response variable was the percent mortality occurring in each test chamber after 96 h of exposure. Estimates of the 96-h LC_{50} value and its 95% confidence interval were calculated by using the binomial method, the moving average method, or the Spearman-Karber method (Stephan, 1977; Gelber et al., 1985). The LC_{50} value is the concentration at which 50% of test organisms are expected to die within the time-frame of the test. The estimated 95% confidence interval is the interval that has a 95% chance of containing the true LC_{50} value.

A screening process was used to select methods for calculating LC_{50} values. We first determined if the data met the assumptions of one or more of the three methods (i.e., binomial, moving average, and Spearman-Karber). For example, when none of the treatments within a given test resulted in partial mortalities, the binomial method was used to estimate the LC_{50} value. If partial mortalities occurred in at least one treatment, then either the binomial method or the moving average method could be used. Finally, if two or more treatments had partial mortalities, then the Spearman-Karber method could be

used along with the binomial and moving average methods. If more than one method could be used to calculate the LC_{50} value, we used a subjective procedure to select the "best" method. For example, if we had to choose from two or more methods, we favored the method that yielded an LC_{50} value closest to the treatment level with observed mortalities approaching 50% and where the 95% confidence intervals coincided with treatments yielding mortalities greater than 0% but less than 100%.

In order to better visualize the distribution of data points from each test, mortality or survival data were plotted against treatment levels (treatment level on X-axis, percent mortality or percent survival on Y-axis). In addition, the data were fitted to the logistic distribution model

$$Y = 1/(1 + e^{-(\alpha + \beta X)})$$

where Y = expected mortality (%), α = intercept parameter, β = slope parameter, and X = treatment level. The logistic distribution is similar to a normal distribution, but has heavier "tails", and more flexibility to accurately describe treatment-response data than a normal distribution model. The parameters, α and β, were estimated with a regression procedure known as "iteratively re-weighted least squares".

After the parameters of each logistic distribution model (i.e., the curve for expected mortality) had been computed, a chi-square (χ^2) goodness-of-fit test was used to determine if the

model was valid by comparing observed mortality against expected mortality. We rejected the null hypothesis (H_o: data fits the logistic model) only if $\underline{P} \leq 0.1$. (Note: By selecting $\underline{P} \leq 0.1$ rather than $\underline{P} \leq 0.05$, we slightly increased the probability of committing a type 1 error [i.e., rejecting H_o when H_o was true]; however, we also decreased the probability of committing a type 2 error [i.e., failing to reject H_o when H_o was false], a precaution recommended for experiments of this type [E. Callahan, National Fisheries Contaminant Research Center, Columbia, Missouri, personal communication]).

The computer software (NEWLC50) for calculating LC_{50} values and their 95% confidence intervals was written and distributed by the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. Equations used to develop the software were obtained from Stephan (1977). Curve-plotting and goodness-of-fit tests were performed by using SAS® (1987) software.

Results

Testing was initiated on April 26, 1993, and completed on July 17, 1993. The results of these tests are summarized in Table 2 and described below in detail.

Single-variable Tests

pH Test. The moving-average method was used to calculate an LC_{50} value of 9.84 (95% C.I. = 9.77-9.94) (Table 2, Figure 1). Observed data did not fit the logistic distribution model (χ^2 = 63, P <0.01). All fish in the four highest treatments (pH 10.74-11.22) died within one hour. Mortality was 75% in pH 9.98, and 30% in pH 10.01. Mortality was low (8% or less) at pH ≤9.52. The behavior of dying fish was similar to that described by Monda and Saiki (1993) for juvenile suckers exposed to high pH: convulsions, erratic swimming, and excessive production of mucous. In addition, we observed visible eye damage (ruptured or bleeding eyes) in many dying larvae. Larvae surviving to 96 h appeared healthy; however, those exposed to pH 9.98 and pH 10.01 did not react to sudden movements (e.g., passing a hand rapidly over the water surface), suggesting that they were severely stressed. (Note: In the hatchery, larvae that failed to respond to sudden hand movements almost always died shortly thereafter [L. Dunsmoor, personal communication]).

Mean values of water quality variables in test chambers varied as follows: pH, 7.81-11.22; un-ionized ammonia, <0.05 mg/L; dissolved oxygen saturation, 100%; and temperature, 19.3-20.2°C (Table 3).

Ammonia Test. The binomial method was used to calculate an LC_{50} value of 0.54 mg/L (95% C.I. = 0.43-0.85 mg/L) for un-ionized ammonia (Table 2, Figure 2). Observed data did not fit

the logistic distribution model (χ^2 = 31, $\underline{P}$ <0.01). All larvae in the two highest treatments (both contained 1.98 mg NH_3-N/L) died within 8 h, whereas those in the next two lower treatments (0.93 mg NH_3-N/L and 0.85 mg NH_3-N/L) died within 48 h. In treatments containing 0.09-0.43 mg NH_3-N/L, mortalities varied from 3% to 16%. By comparison, the controls (<0.01 mg NH_3-N/L) sustained mortalities of 5-10%. At the end of the test, survivors were seemingly in good condition, but larvae in treatments containing 0.17-0.43 mg NH_3-N/L were unresponsive to sudden movements. Unlike the hyperactivity observed in juveniles exposed to ammonia (Monda and Saiki 1993), dying larvae in our test were not hyperactive. Instead, the larvae gradually became listless (comatose) and, except for brief bursts of rapid swimming, settled to the bottom of the test chamber where they remained motionless.

During the acclimation period, a total of 99 larvae died with nearly 92% of these fish succumbing during the first day. However, larvae used in the test were healthy and vigorous. Also, no unusual problems occurred during the rearing of this batch of larvae in the hatchery (L. Dunsmoor, personal communication). Although these observations suggest that larvae were stressed prior to or during the acclimation period (perhaps as they were transported from the hatchery to our laboratory), we do not recall handling this batch of larvae any differently than other batches.

Mean values of water quality variables in test chambers varied as follows: pH, 7.73-8.00; un-ionized ammonia, 0.003-1.98 mg/L; dissolved oxygen saturation, 97-100%; and temperature, 20.0-20.3°C (Table 4).

Dissolved Oxygen Test. The binomial method was used to calculate an LC_{50} value of 2.1 mg/L (95% C.I. = 2.0-2.3 mg/L) for dissolved oxygen (Table 2, Figure 3). The LC_{50} value as percent saturation was 27% (95% C.I. = 26-29%). Observed data fit the logistic distribution model (χ^2 = 9.18, P = 0.24).

A large increase in toxicity occurred within a narrow range of dissolved oxygen; only 5% of larvae survived at 2.0 mg/L, whereas survival increased to 88% at 2.3 mg/L (Figure 3). Above 2.3 mg/L, survival was 98-100%, whereas below 2.0 mg/L survival was ≤5%.

Larvae in treatments of ≤2.3 mg/L spent much of the time near the water surface, possibly seeking higher oxygen concentrations. Dying larvae had difficulty swimming, and struggled to stay near the surface. Larvae in treatments above 2.3 mg/L swam throughout the water column, and were seemingly healthy after 96 h. Surviving larvae in treatments containing ≤2.3 mg/L did not react to sudden movements, suggesting that they were stressed.

Mean values of water quality variables in test chambers varied as follows: pH, 7.70-8.26; un-ionized ammonia, ≤0.006 mg/L; dissolved oxygen saturation, 12-101% (1.5-7.9 mg/L); and temperature, 19.8-20.4°C (Table 5).

Temperature Test. The binomial method was used to calculate an LC_{50} value of 31.8°C (95% C.I. = 30.5-33.6°C) for temperature (Table 2, Figure 4). Observed data did not fit the logistic distribution model (χ^2 = 46, P >0.99).

Mortality was 100% within 2 h at temperatures of 33.6-33.7°C, but was ≤5% even after 96 h at 20.1-30.5°C (Figure 4). We did not notice any differences in behavior among treatments. At the end of the test, all surviving larvae reacted to sudden movements and were seemingly healthy.

Mean values of water quality variables in test chambers varied as follows: pH, 7.92-8.37; un-ionized ammonia, ≤0.006 mg/L; dissolved oxygen saturation, 100%; and temperature, 20.1-33.7°C (Table 6).

Multiple-variable Tests

Test 1a. The binomial method was used to calculate an LC_{50} value of 0.48 mg/L (95% C.I. = 0.31-0.58 mg/L) for un-ionized ammonia (Table 2, Figure 5). Observed data fit the logistic distribution model (χ^2 = 0.02, P >0.99). All larvae in 2.35 mg/L and 5.27 mg/L died within 3 h, and all larvae in 1.20 mg/L died within 22 h. After 96 h, mortality was 76% in 0.58 mg/L and 2.5% in 0.31 mg/L. There were no mortalities in the control (0.06 mg/L). After 96 h, larvae in 0.31 mg/L and 0.58 mg/L failed to react to sudden movements, suggesting that they were stressed.

Behavior of dying fish was similar to that described earlier for the single-variable test with ammonia.

Mean values of water quality variables in test chambers varied as follows: pH, 9.50-9.57; un-ionized ammonia, 0.06-5.27 mg/L; dissolved oxygen saturation, 100%; and temperature, 24.0-24.9°C (Table 7).

Test 1b. The binomial method was used to calculate an LC_{50} value of 0.51 mg/L (95% C.I. = 0.35-0.63 mg/L) for un-ionized ammonia (Table 2, Figure 5). Observed data fit the logistic distribution model ($\chi^2 = 0.00$, $P = 1.00$). Complete mortality occurred in 4.04 mg/L within 3 h, in 2.34 mg/L within 22 h, and in 1.25 mg/L within 25 h. Mortality after 96 h was 88% in 0.63 mg/L. No mortalities occurred at 0.35 mg/L, or in the control (0.06 mg/L). Larvae surviving the 96-h exposures to 0.35 mg/L and 0.63 mg/L failed to react to sudden movement, suggesting that they were stressed. Behavior of dying fish was similar to that described earlier for the single-variable test with ammonia.

Mean values of water quality variables in test chambers varied as follows: pH, 9.27-9.60 (excluding pH 9.27, which coincided with the highest treatment of un-ionized ammonia, the variation in pH was 9.51-9.60); un-ionized ammonia, 0.06-4.04 mg/L; dissolved oxygen saturation, 100%; and temperature, 19.8-20.2°C (Table 7).

Test 2a. The binomial method was used to calculate an LC_{50} value of 0.46 mg/L (95% C.I. = 0.21-0.66 mg/L) for un-ionized ammonia (Table 2, figure 6). Observed data fit the logistic

distribution model (χ^2 = 2.06, $\underline{P}$ = 0.73). Complete mortality occurred in 2.23 mg/L within 4 h, and in 0.87 mg/L within 11 h. By the third day, larvae in 0.42 mg/L and 0.66 mg/L failed to react to sudden movements, suggesting that they were stressed. After 96 h, mortality was 90% in 0.66 mg/L, 39% in 0.43 mg/L, and 2.5% in 0.21 mg/L and in the control (0.07 mg/L). Behavior of dying fish was similar to that described earlier for the single-variable test with ammonia.

Mean values of water quality variables in test chambers varied as follows: pH, 9.13-9.50 (excluding pH 9.50, which coincided with the highest treatment of un-ionized ammonia, the variation in pH was 9.13-9.27); un-ionized ammonia, 0.07-2.23 mg/L; dissolved oxygen saturation, 52-60% (3.7-4.4 mg/L); and temperature, 24.8-25.1°C (Table 8).

(Note: Prior to successfully completing test 2a, two earlier attempts were aborted because larvae in controls and all treatments experienced high mortality. The mortalities began within minutes after fish were transferred from the holding tank into test chambers. After 1.5-2 hours, fish in all test chambers were dead. Subsequent efforts to determine the source of mortality indicated that the unadulterated test water [i.e., before addition of ammonia] was toxic because fish exposed to this water quickly lost equilibrium but recovered within minutes when transferred back into water from the holding tank. The unadulterated test water had the following water-quality

characteristics: pH, 9.5-9.7; dissolved oxygen concentration, about 3 mg/L; and water temperature, about 25°C.)

Test 2b. The moving-average method was used to calculate an LC_{50} value of 0.50 mg/L (95% C.I. = 0.42-0.67) for un-ionized ammonia (Table 2, Figure 6). Observed data did not fit the logistic distribution model ($\chi^2 = 14$, $P < 0.01$). Complete mortality occurred in 2.52 mg/L within 4 h, in 1.06 mg/L within 34 h, and in 0.57 mg/L within 96 h. After 96 h, mortality was 51% in 0.51 mg/L and 2.5% in 0.22 mg/L. No mortality occurred in the control (0.07 mg/L). All larvae surviving the 96-h test reacted to sudden movements. Behavior of dying fish was similar to that described earlier for the single-variable test with ammonia.

Mean values of water quality variables in test chambers varied as follows: pH, 9.15-9.56 (excluding pH 9.56, which coincided with the highest concentration of un-ionized ammonia, the variation in pH was 9.15-9.30); un-ionized ammonia, 0.07-2.52 mg/L; dissolved oxygen saturation, 56-64% (4.4-5.0 mg/L); and temperature, 20.0-20.3°C (Table 8).

(Note: Prior to successfully completing test 2b, which was conducted concurrently with test 2a, two earlier runs were aborted because the unadulterated test water was toxic to larvae. The unadulterated test water had the following water-quality characteristics: pH, 9.5-9.7; dissolved oxygen concentration, about 3 mg/L; and water temperature, about 20°C. See results from test 2a for additional details.)

Test 3a. The Spearman-Karber method was used to calculate an LC_{50} value of 0.89 mg/L (95% C.I. = 0.78-1.01 mg/L) for un-ionized ammonia (Table 2, Figure 7). Observed data did not fit the logistic distribution model (χ^2 = 10.8, P = 0.03). Complete mortality occurred in 3.79 mg/L within 2.5 h, and in 1.70 mg/L within 10 h. After 96 h, mortality was 18% in 0.75 mg/L, 2.5% in 0.55 mg/L, and 5% in 0.23 mg/L and in the control (0.07 mg/L). Larvae surviving the test reacted to sudden movements. However, after 72 h, larvae in 0.55 mg/L and 0.75 mg/L were very excitable and, when netting fish that survived 96 h in these treatments, they seemingly tired more quickly (they were easier to catch) than larvae in other treatments. Moreover, some individuals from these treatments died during the attempt to net them. Behavior of dying fish was similar to that described earlier for the single-variable test with ammonia.

Mean values of water quality variables in test chambers varied as follows: pH, 9.76-9.91; un-ionized ammonia, 0.07-3.79 mg/L; dissolved oxygen saturation, 94-100%; and temperature, 24.5-24.7°C (Table 9).

Test 3b. The Spearman-Karber method was used to calculate an LC_{50} value of 0.71 mg/L (95% C.I. = 0.62-0.81 mg/L) for un-ionized ammonia (Table 2, Figure 7). Observed data fit the logistic distribution model (χ^2 = 3.5, P = 0.48). Complete mortality occurred in 3.88 mg/L within 2.5 h, and in 1.82 mg/L within 10 h. After 96 h, mortality was 68% in 0.80 mg/L, 5% in 0.54 mg/L, and 2.5% in 0.24 mg/L. There was no mortality in the

control (0.07 mg/L). The behavior of survivors was similar to those in Test 3a (i.e., larvae reacted to movement, but those in intermediate treatments--0.54 mg/L and 0.80 mg/L--were excitable and tired quickly when netted at the end of the test). Behavior of dying fish was similar to that described earlier for the single-variable test with ammonia.

Mean values of water quality variables in test chambers varied as follows: pH, 9.79-9.88; un-ionized ammonia, 0.07-3.88 mg/L; dissolved oxygen saturation, 96-100%; and temperature, 20.2-20.4°C (Table 9).

Test 4a. The binomial method was used to calculate an LC_{50} value of 0.67 mg/L (95% C.I. = 0.41-0.90 mg/L) for un-ionized ammonia (Table 2, Figure 8). Observed data fit the logistic distribution model ($\chi^2 = 4.9$, $P = 0.30$). All larvae died in 2.34 mg/L within 2 h. After 96 h, mortality was 74% in 0.90 mg/L, 13% in 0.41 mg/L, 4.3% in 0.29 mg/L, 0% in 0.14 mg/L, and 8.7% in the control (0.05 mg/L). Larvae surviving the 96-h exposures in all treatments reacted to sudden movements. However, over 50% of larvae in 0.29 mg/L and 0.41 mg/L, and all larvae in 0.90 mg/L did not feed after the first day of testing. Larvae in all treatments spent most of their time near the surface, swimming in the current created by the bubbling N_2 gas (used to adjust dissolved oxygen concentrations in the test chambers). After 96 h, larvae in 0.90 mg/L were very passive and easily netted, whereas larvae in lower concentrations were livelier. Dead and dying fish exhibited symptoms (eye damage and excessive mucous)

that resembled problems described previously in the single-variable test with pH.

Mean values of water quality variables in test chambers varied as follows: pH, 9.73-9.86; un-ionized ammonia, 0.05-2.34 mg/L; dissolved oxygen saturation, 55-66% saturation (3.9-4.7 mg/L); and temperature, 24.8-25.3°C (Table 10).

Test 4b. The binomial method was used to calculate an LC_{50} value of 0.70 mg/L (95% C.I. = 0.46-1.08 mg/L) for un-ionized ammonia (Table 2, Figure 8). Observed data did not fit the logistic distribution model ($\chi^2 = 13$, $P < 0.01$). Complete mortality occurred in 2.62 mg/L within 2 h, and in 1.08 mg/L within 21 h. After 96 h, mortality was 0% in 0.33 mg/L and in 0.46 mg/L, and 4.3% in 0.14 mg/L and in the control (0.06 mg/L). The behavior of larvae in treatments ≤0.46 mg/L resembled that of larvae in Test 4a for treatments ≤0.41 mg/L. In addition, dead and dying fish exhibited eye damage and excessive mucous.

Mean values of water quality variables in test chambers varied as follows: pH, 9.74-9.86; un-ionized ammonia, 0.06-2.62 mg/L; dissolved oxygen saturation, 56-69% (4.4-5.4 mg/L); and temperature, 20.3-20.6°C (Table 10).

Discussion

Our study attempted to identify the lethal thresholds of pH, ammonia, dissolved oxygen, and temperature, both individually and in selected combinations, for the larval life stage of Lost River

sucker. These water-quality variables fluctuate daily and seasonally in Upper Klamath Lake (Kann, 1993; Kann and Smith, 1993) and, to some extent, throughout the entire Upper Klamath Basin (Stubbs and White, 1993). Especially during late spring and summer months, variables such as pH, ammonia, and dissolved oxygen could approach or exceed lethal thresholds for larvae, resulting in die-offs 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.

The single-variable test with pH yielded a 96-h LC_{50} value of 9.84, which is lower than values (10.30 to 10.68) reported by Monda and Saiki (1993) for juvenile (6-7 months old) Lost River sucker. Although larvae in the controls of our multiple-variable tests 3a, 3b, 4a, and 4b tolerated pH levels as high as 9.80 with no significant mortality, these fish were older (80-86 days) than the 35-day-old fish used in the single-variable test. In general, young organisms are more susceptible than adult organisms to chemical agents possibly due to poorly developed detoxification mechanisms or to differences in rates of excretion of toxic chemicals (Rand and Petrocelli, 1985). Our results represent the first evidence that age-related variations may occur in the tolerance of Lost River sucker to environmental stressors.

During the single-variable test with pH, we observed signs of stress in dying larvae (e.g., eye damage, excessive mucous secretion) that had previously been reported in laboratory

studies with other fish species (Daye and Garside, 1976). Eye damage was also reported in adult Lost River sucker captured from Upper Klamath lake (M. Buettner, U.S. Bureau of Reclamation, Klamath Falls, Oregon, personal communication).

Additional comparisons between results of 96-h LC_{50} tests with larvae (present study) and juveniles (Monda and Saiki, 1993) yielded minor differences in the lethality of un-ionized ammonia (larvae, 0.54 mg/L; juveniles, 0.70 mg/L), dissolved oxygen (larvae, 2.1 mg/L; juveniles, 1.8 mg/L), and temperature (larvae, 31.8°C; juveniles, 30.0°C). These results suggest that, except for pH, age-related differences between larvae and juveniles do not greatly influence their tolerance to water-quality variables.

The variations in LC_{50} values for un-ionized ammonia that we obtained in our larval multiple-variable tests (0.46-0.89 mg NH_3-N/L) were similar to those reported by Monda and Saiki (1993) for juveniles (0.45-0.88 mg NH_3-N/L). Sublethal water-quality conditions in larval and juvenile multiple-variable tests were similar for temperature and dissolved oxygen concentration, but differed for pH (larval tests, 9.8-9.9; juvenile tests, 9.5-9.7). Our results and those of Monda and Saiki (1993) indicated that exposure of larval and juvenile suckers to sublethal levels of pH, temperature, and dissolved oxygen did not greatly alter the toxicity of un-ionized ammonia (Table 2). However, other investigators (e.g., Alabaster et al., 1979; Thurston et al., 1981a, 1981b; Thurston and Russo, 1983; Broderius et al., 1985; Nimmo et al., 1989; Bergerhouse, 1992) reported that high pH,

high temperature, and low dissolved oxygen concentrations were among several water-quality variables that exacerbated the toxicity of un-ionized ammonia to fish. Two possible reasons for the discrepancy between our results and the findings of other investigators were previously discussed by Monda and Saiki (1993): (i) the levels of pH, temperature, and dissolved oxygen used in our multiple-variable tests were not sufficiently stressful to affect the tolerance of Lost River sucker to un-ionized ammonia; and (ii) our results were confounded by variations in the age (physiological development) of fish used in the tests. Larvae used in tests 1a, 1b, 2a, and 2b (pH 9.5) were relatively young (35-71 days old), whereas larvae used in tests 3a, 3b, 4a, and 4b (pH 9.8) were older (80-86 days old) (Table 1).

Conclusions and Recommendations

Monda and Saiki (1993) reported that water-quality conditions in Upper Klamath Lake can be acutely lethal to the juvenile life stage of Lost River sucker. Our results with larvae corroborated these earlier findings. Because larvae have poorly developed organ systems (which affects their ability to tolerate environmental stressors) and are less mobile than juveniles or adults, they could be especially vulnerable to poor water quality. 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).

Monda and Saiki (1993) cautioned that their test results with juveniles should be viewed as preliminary information because of several limitations or uncertainties. Our results with larvae are also preliminary because some limitations or uncertainties mentioned by Monda and Saiki (1993) apply to our tests. First, we used larvae that were the progeny of matings from only a few wild-caught fish. The data generated with these larvae might not adequately reflect species-wide responses to the experimental conditions. Second, our experimental results were based on larvae 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 any of the single-variable and multiple-variable tests and therefore have no measure of test-to-test variability.

We recommend that single-variable and multiple-variable tests be repeated, and that additional levels of pH, dissolved oxygen, and temperature be included as part of the multiple-variable tests. By conducting more tests, our confidence in the data could improve because more rigorous statistical evaluations

will be possible. Multiple-variable tests should cover the breadth of water quality measurements encountered during the late spring and summer in Upper Klamath Lake so that a predictive model can be constructed to estimate the probability of fish survival under various water-quality conditions. The predictive model would be especially useful for identifying the physicochemical variables in need of remediation as part of restoration efforts mandated by the federal Endangered Species Act and to estimate the likelihood of success of proposed restoration efforts (Kann and Smith, 1993; Stubbs and White, 1993).

We also recommend laboratory studies of longer duration (i.e., 28 days or longer) in which water-quality variables are intentionally fluctuated in order to better mimic diurnal and other cycles (e.g., phytoplankton blooms and "crashes") occurring in the field. These long-term studies should include measurements of sublethal effects on growth, behavior (e.g., avoidance or attraction to stressful conditions, altered feeding efficiency, changes in predator avoidance), and physiological or biochemical processes (e.g., blood chemistry and hormonal changes, lipid storage and utilization, swimming performance), some of which help fish adapt to stressful conditions. A better understanding of how sublethal effects are influencing long-term survival of larvae and juveniles could yield insights into why so few individuals from these early life stages reach adulthood.

Finally, we recommend that field studies be implemented to validate the 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). For this reason, regulatory agencies (e.g., U.S. Environmental Protection Agency) often require on-site or in-situ studies in addition to laboratory studies when developing water quality standards (Gersich and Hopkins, 1989; Mayes et al., 1986; Nimmo et al., 1989).

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Table 1. Target water quality conditions for the multiple-variable tests.

Test[a]	pH	Dissolved oxygen saturation	Temperature	Un-ionized ammonia
1a	9.5	100%	25°C	variable
1b	9.5	100%	20°C	variable
2a	9.5	50%	25°C	variable
2b	9.5	50%	20°C	variable
3a	9.8	100%	25°C	variable
3b	9.8	100%	20°C	variable
4a	9.8	50%	25°C	variable
4b	9.8	50%	20°C	variable

[a]Each pair of tests (e.g., 1a and 1b) were run simultaneously.

Table 3. Summary of 96-h LC_{50} values (95% confidence intervals in parentheses) for single-variable (SV) and multiple-variable (MV) tests. "Logistic distribution" refers to whether or not the observed data fit the hypothesized logistic distribution model ($P \leq 0.10$; see Methods for explanation).

Test type	Test	96-h LC_{50} (95% C.I.)	Logistic distribution
SV	pH	9.84 (9.77-9.94)	No
SV	Un-ionized ammonia (mg NH_3-N/L)	0.54 (0.43-0.85)	No
SV	Dissolved oxygen (mg/L)	2.1 (2.0 - 2.3)	Yes
SV	Temperature (°C)	31.8 (30.5-33.6)	No
MV	1a (mg NH_3-N/L)	0.48 (0.31-0.58)	Yes
MV	1b (mg NH_3-N/L)	0.51 (0.35-0.63)	Yes
MV	2a (mg NH_3-N/L)	0.46 (0.21-0.66)	Yes
MV	2b (mg NH_3-N/L)	0.50 (0.42-0.67)	No
MV	3a (mg NH_3-N/L)	0.89 (0.78-1.01)	No
MV	3b (mg NH_3-N/L)	0.71 (0.62-0.81)	Yes
MV	4a (mg NH_3-N/L)	0.67 (0.41-0.90)	Yes
MV	4b (mg NH_3-N/L)	0.70 (0.46-1.08)	No

Table 3. Water quality data for the single-variable test with pH. Except as noted, values are means based on N = 5 (standard deviations are given beneath the means). Ammonia, alkalinity (total), hardness (total), chloride, and sulfate were measured in samples composited from "a" and "b" treatments.

	Treatment											
Parameter	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b
pH[a]	7.85	7.81	9.09	9.13	9.48	9.52	9.98	10.01	10.74	10.76	11.21	11.22
	0.20	0.24	0.15	0.16	0.14	0.15	0.14	0.15	--[b]	--	--	--
Temperature[a]	20.2	20.2	20.1	20.1	20.1	20.1	20.1	20.2	19.3	19.5	19.0	19.4
(°C)	0.6	0.4	0.6	0.5	0.6	0.5	0.6	0.4	--	--	--	--
Dissolved O_2[c]	7.9	7.9	7.9	8.0	8.0	8.0	8.0	7.9	8.1	8.2	8.2	8.2
(mg/L)	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.3	--	--	--	--
Conductivity[d]	130	132	145	146	162	162	202	203	313	316	576	582
(μS/cm)	1	1	1	1	0	2	1	1	--	--	--	--
Un-ionized NH_3-N	0.003		0.030		0.045		0.051		0.034		0.035	
(mg/L)	0.0009		0.014		0.023		0.027		--		--	
Alkalinity	40		50		59		78		118		220	
(mg/L as $CaCO_3$)	2		1		1		1		--		--	
Hardness	38		38		37		36		26		18	
(mg/L as $CaCO_3$)	2		1		2		0		--		--	
Chloride	2.9		2.8		2.9		2.8		2.5		3.0	
(mg/L)	0.2		0.3		0.2		0.3		--		--	
Sulfate	36		36		40		36		38		39	
(mg/L)	2		3		4		5		--		--	

[a]N = 13.

[b]N = 1; measurements were discontinued after all fish died.
[c]N = 9.
[d]N = 4.

Table 4. Water quality data for the single-variable test with un-ionized ammonia. E cept as noted, values are means based on N = 5 (standard deviations are given beneath the means). Alkalinity (total), hardness (total), chloride, and sulfate were measured in samples composited from "a" and "b" treatments.

	Treatment											
Parameter	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b
Un-ionized NH_3-N[a]	0.003	0.004	0.092	0.096	0.17	0.21	0.37	0.43	0.93	0.85	1.98	.98
(mg/L)	0.001	0.002	0.022	0.026	0.04	0.03	0.07	0.06	0.17	0.20	0.21	.39
Temperature[b]	20.1	20.1	20.0	20.1	20.0	20.1	20.0	20.1	20.0	20.0	20.0	2 .3
(°C)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.8	.4
pH[c]	7.73	7.85	7.85	7.85	7.84	7.87	7.88	7.89	7.99	7.97	8.02	.00
	0.20	0.16	0.14	0.14	0.14	0.13	0.12	0.11	0.07	0.06	0.06	.08
Dissolved O_2[d]	7.7	7.8	7.8	7.7	7.8	7.8	7.6	7.9	7.8	7.8	7.8	.9
(mg/L)	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.1	--[e]	.2
Conductivity[f]	132	130	153	151	176	177	221	223	310	319	405	40
(µS/cm)	4	1	2	4	2	2	4	3	4	4	--	-
Alkalinity[g]	40		41		43		44		46		51	
(mg/L as $CaCO_3$)	1		1		1		1		1		--	
Hardness[g]	37		37		37		37		38		36	
(mg/L as $CaCO_3$)	1		1		1		1		0		--	
Chloride[g]	4.0		12.0		19.0		34.0		67.0		136.0	
(mg/L)	0.0		1.0		1.0		2.0		4.0		--	
Sulfate[g]	30		34		31		33		36		30	
(mg/L)	3		6		4		3		2		--	

[a]N = 9 for treatments 1a-3a and 4a, N = 4 for treatments 3b and 5a, N = 5 for treatment 4b, and N = 2 for treatments 5b, 6a, and 6b.

[b]N = 13 for treatments 1a-4b, N = 5 and N = 7 for treatments 5a and 5b, and N = 2 for treatments 6a and 6b.
[c]N = 9 for treatments 1a-4b, N = 4 and N = 5 for treatments 5a and 5b, and N = 2 for treatments 6a and 6b.
[d]N = 9 for treatments 1a-4b, N = 4 and N = 5 for treatments 5a and 5b, and N = 2 for treatment 6b.
[e]N = 1.
[f]N = 5 for treatments 1a-4b, N = 2 and N = 3 for treatments 5a and 5b.
[g]N = 3 for treatment 5.

Table 5. Water quality data for the single-variable test with dissolved oxygen. Exc ɔt as noted, values are means based on N = 5 (standard deviations are given beneath the means). Ammonia, alkalinity (total), hardness (total), chloride, and sulfate were measured in samples composited from "a" and "b" treatments.

	Treatment											
Parameter	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	ɔb
Dissolved O_2[a]	7.9	7.9	3.8	3.8	3.0	2.9	2.3	2.3	2.0	1.9	1.6	1 5
(mg/L)	0.3	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.1	–[b]
Temperature[c]	19.9	19.8	20.0	19.8	19.9	19.8	19.8	19.8	20.0	19.8	19.9	2(4
(°C)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.2	(6
pH[d]	7.70	7.73	8.17	8.23	8.09	8.20	8.12	8.24	8.18	8.26	8.25	ε 15
	0.14	0.14	0.19	0.17	0.21	0.16	0.20	0.17	0.15	0.14	0.14	--
Conductivity	126	125	125	125	125	125	125	126	125	125	125	132
(μS/cm)	12	12	12	12	12	12	12	12	12	12	12	--
Un-ionized NH_3-N	0.002		0.006		0.005		0.006		0.005		0.00ɜ	
(mg/L)	0.001		0.002		0.002		0.002		0.001		--	
Alkalinity	40		41		41		41		41		41	
(mg/L as $CaCO_3$)	1		1		1		1		1		1	
Hardness	38		37		37		38		38		37	
(mg/L as $CaCO_3$)	1		1		1		1		1		1	
Chloride	3.3		2.8		2.9		2.9		3.0		2.7	
(mg/L)	0.3		0.3		0.2		0.2		0.0		0.3	
Sulfate	29		33		35		33		31		33	
(mg/L)	5		4		4		4		3		3	

[a]N = 13.

[b]N = 1; measurements were discontinued after all fish died.
[c]N = 13 for treatments 1a-6a, and N = 2 for treatment 6b.
[d]N = 9.

Table 6. Water quality data for the single-variable test with temperature. Except as noted, values are means based on N = 5 (standard deviations are given beneath the means). Ammonia, alkalinity (total), hardness (total), chloride, and sulfate were measured in samples composited from "a" and "b" treatments.

Parameter	Treatment											
	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b
Temperature[a] (°C)	20.1 0.5	20.1 0.5	25.9 1.6	25.4 1.3	27.5 1.0	27.0 1.1	28.8 0.7	28.2 0.8	30.5 0.4	30.4 0.4	33.6 --[b]	33.7 --
pH[c]	7.92 0.17	7.92 0.16	7.93 0.23	7.95 0.21	7.95 0.20	8.00 0.16	8.01 0.17	7.95 0.18	7.94 0.21	8.00 0.17	8.35 --	8.37 --
Dissolved O_2[c] (mg/L)	7.7 0.3	7.9 0.2	7.0 0.2	7.1 0.2	6.8 0.3	6.9 0.3	6.8 0.3	6.8 0.3	6.4 0.3	6.5 0.3	6.7 --	6.7 --
Conductivity[d] (µS/cm)	132 1	132 2	133 4	132 2	135 2	134 2	135 1	133 2	136 2	135 1	138 --	138 --
Un-ionized NH_3-N (mg/L)	0.003 0.001		0.004 0.002		0.005 0.002		0.005 0.001		0.006 0.002		0.004 --	
Alkalinity (mg/L as $CaCO_3$)	40 2		41 0		42 1		42 1		42 1		43 --	
Hardness (mg/L as $CaCO_3$)	39 2		39 2		38 1		38 0		38 1		40 --	
Chloride (mg/L)	3.6 0.1		3.4 0.1		3.3 0.1		3.3 0.1		3.2 0.1		3.5 --	
Sulfate (mg/L)	36 8		33 7		34 7		34 8		33 7		39 --	

[a]N = 15.

[b]N = 1; measurements were discontinued after all fish died.
[c]N = 9.
[d]N = 4.

Table 7. Water quality data for multiple-variable tests 1a and 1b. Except as noted, values are means based on N = 5 (standard deviations are given beneath the means). Alkalinity (total), hardness (total), chloride, and sulfates were measured in samples composited from "a" and "b" treatments (i.e., values for 1a = 1b, values for 2a = 2b, etc.).

Parameter	Treatment											
	1a	2a	3a	4a	5a	6a	1b	2b	3b	4b	5b	6b
Un-ionized NH_3-N[a]	0.061	0.31	0.58	1.20	2.35	5.27	0.063	0.35	0.63	1.25	2.34	4.04
(mg/L)	0.036	0.03	0.05	0.04	0.04	0.05	0.037	0.03	0.05	0.16	0.21	0.42
pH[a]	9.55	9.57	9.55	9.57	9.50	9.51	9.59	9.60	9.58	9.57	9.51	9.27
	0.09	0.08	0.07	0.05	0.05	0.03	0.09	0.08	0.10	0.12	0.11	0.11
Temperature[b]	24.9	24.9	24.9	24.8	24.3	24.0	19.8	19.8	19.8	20.0	19.9	20.2
(°C)	0.4	0.5	0.4	0.5	0.3	0.2	0.3	0.3	0.3	0.5	0.5	0.7
Dissolved O_2[c]	7.2	7.2	7.2	7.6	7.7	7.8	8.0	7.9	8.0	8.2	8.1	8.1
(mg/L)	0.4	0.4	0.3	0.1	0.1	0.0	0.4	0.4	0.3	0.2	0.1	0.1
Conductivity[d]	164	167	171	179	191	231	161	165	169	174	188	229
(μS/cm)	2	2	5	4	--[e]	--	1	2	2	1	4	--
Alkalinity[f]	62	63	63	64	66	74	62	63	63	64	66	74
(mg/L as $CaCO_3$)	4	3	2	2	2	--	4	3	2	2	2	--
Hardness[f]	36	36	36	36	36	36	36	36	36	36	36	36
(mg/L as $CaCO_3$)	1	0	0	0	0	--	1	0	0	0	0	--
Chloride[f]	3.6	5.1	7.0	10.5	17.3	31.5	3.6	5.1	7.0	10.5	17.3	31.5
(mg/L)	0.2	0.2	0.0	0.0	0.4	--	0.2	0.2	0.0	0.0	0.4	--
Sulfate[f]	36	34	35	33	30	31	36	34	35	33	30	31
(mg/L)	3	4	5	3	3	--	3	4	5	3	3	--

[a]N = 9 for treatments 1-3a and 1-3b, N = 3 for treatments 4a, 4b, and 5b, and N = 2 for treatments 5a, 6a, and 6b.

[b]N = 13 for treatments 1-3a and 1-3b, N = 4 for treatments 4a, 4b, and 5b, and N = 2 for treatments 5a, 6a, and 6b.
[c]N = 9 for treatments 1-3a, N = 8 for treatments 1-3b, N = 3 for treatments 4a, 4b, and 5b, and N = 2 for treatments 5a, 6a, and 6b.
[d]N = 3 for treatments 1-3a and 1-3b, and N = 2 for treatments 4a, 4b, and 5b.
[e]N = 1.
[f]N = 2 for treatments 4a, 4b, 5a, and 5b.

Table 8. Water quality data for multiple-variable tests 2a and 2b. Except as noted, values are means based on N = 5 (standard deviations are given beneath the means). Alkalinity (total), hardness (total), chloride, and sulfates were measured in samples composited from "a" and "b" treatments (i.e., values for 1a = 1b, values for 2a = 2b, etc.).

Parameter	Treatment											
	1a	2a	3a	4a	5a	6a	1b	2b	3b	4b	5b	6b
Un-ionized NH_3-N[a]	0.065	0.21	0.43	0.66	0.87	2.23	0.070	0.22	0.51	0.57	1.06	2.52
(mg/L)	0.031	0.07	0.14	0.20	0.18	--[b]	0.034	0.08	0.18	0.16	0.37	--
pH[a]	9.23	9.22	9.13	9.20	9.27	9.50	9.15	9.20	9.24	9.26	9.30	9.56
	0.20	0.20	0.20	0.20	0.30	--	0.21	0.20	0.21	0.22	0.25	--
Temperature[c]	25.1	25.1	25.0	25.1	25.1	24.8	20.3	20.3	20.2	20.2	20.1	20.0
(°C)	0.4	0.4	0.4	0.4	0.5	0.1	0.2	0.3	0.2	0.2	0.3	0.3
Dissolved O_2[a]	3.9	4.4	4.3	4.2	4.1	3.7	4.4	4.4	4.4	4.4	4.7	5.0
(mg/L)	0.4	0.5	0.6	0.6	0.5	--	0.4	0.5	0.4	0.4	0.3	--
Conductivity[d]	216	219	226	230	230	260	207	214	222	218	233	260
(μS/cm)	7	9	10	12	--	--	7	7	12	4	4	--
Alkalinity[e]	58	58	60	61	61	65	58	58	60	61	61	65
(mg/L as $CaCO_3$)	3	3	3	3	2	--	3	3	3	3	2	--
Hardness[e]	39	38	39	38	39	36	39	38	39	38	39	36
(mg/L as $CaCO_3$)	2	2	2	3	1	--	2	2	2	3	1	--
Chloride[e]	4.4	5.1	7.7	9.2	12.0	17.5	4.4	5.1	7.7	9.2	12.0	17.5
(mg/L)	0.7	0.7	1.3	1.5	2.8	--	0.7	0.7	1.3	1.5	2.8	--
Sulfate[e]	33	37	35	37	44	50	33	37	35	37	44	50
(mg/L)	3	4	3	3	9	--	3	4	3	3	9	--

[a]N = 9 for treatments 1-4a and 1-3b, N = 4 for treatment 4b, and N = 2 and N = 3 for treatments 5a and 5b.

[b]N = 1; measurements were discontinued after all fish died.
[c]N = 11 for treatments 1-4a and 1-3b, N = 5 for treatment 4b, N = 3 and N = 4 for treatments 5a and 5b, and N = 2 for treatments 6a and 6b.
[d]N = 2 for treatments 4b and 5b.
[e]N = 2 for treatments 5a and 5b.

Table 9. Water quality data for multiple-variable tests 3a and 3b. Except as noted, values are means based on N = 5 (standard deviations are given beneath the means). Alkalinity (total), hardness (total), chloride, and sulfate were measured in samples composited from "a" and "b" treatments (i.e., values for 1a = 1b, values for 2a = 2b, etc.).

Parameter	Treatment											
	1a	2a	3a	4a	5a	6a	1b	2b	3b	4b	5b	6b
Un-ionized NH_3-N[a]	0.072	0.23	0.55	0.75	1.69	3.79	0.065	0.24	0.54	0.80	1.82	3.88
(mg/L)	0.035	0.037	0.05	0.05	0.11	0.43	0.031	0.03	0.03	0.07	0.18	0.27
pH[a]	9.76	9.76	9.77	9.80	9.89	9.91	9.79	9.80	9.81	9.83	9.86	9.88
	0.10	0.10	0.09	0.09	0.01	0.01	0.11	0.09	0.10	0.11	0.11	0.10
Temperature[b]	24.7	24.7	24.6	24.6	24.5	24.5	20.3	20.3	20.2	20.3	20.3	20.4
(°C)	1.0	1.0	1.0	1.0	0.2	0.2	0.5	0.6	0.4	0.4	0.1	0.1
Dissolved O_2[a]	6.9	7.0	6.7	6.9	7.8	7.8	7.6	7.5	7.5	7.7	8.1	8.1
(mg/L)	0.7	0.6	0.8	0.7	0.1	0.1	0.5	0.5	0.5	0.4	0.4	0.3
Conductivity	238	241	247	251	270	305	234	238	245	250	270	305
(μS/cm)	4	4	3	2	--[c]	--	2	3	5	0	--	--
Alkalinity	65	66	68	69	71	81	65	66	68	69	71	81
(mg/L as $CaCO_3$)	3	2	2	1	--	--	3	2	2	1	--	--
Hardness	37	37	36	37	40	38	37	37	36	37	40	38
(mg/L as $CaCO_3$)	1	2	2	2	--	--	1	2	2	2	--	--
Chloride	4.3	4.9	6.4	7.5	10.5	18.5	4.3	4.9	6.4	7.5	10.5	18.5
(mg/L)	0.8	0.5	0.4	0.4	--	--	0.8	0.5	0.4	0.4	--	--
Sulfate	36	36	34	37	38	47	36	36	34	37	38	47
(mg/L)	5	3	4	3	--	--	5	3	4	3	--	--

[a]N = 9 for treatments 1-4a and 1-4b, and N = 2 for treatments 5a, 5b, 6a and 6b.

[b]N = 10 for treatments 1-4a and 1-4b, and N = 2 for treatments 5a, 5b, 6a, and 6b.
[c]N = 1; measurements were discontinued after all fish died.

Table 10. Water quality data for multiple-variable tests 4a and 4b. Except as noted, values are means based on N = 5 (standard deviations are given beneath the means). Alkalinity (total), hardness (total), chloride, and sulfate were measured in samples composited from "a" and "b" treatments (i.e., values for 1a = 1b, values for 2a = 2b, etc.).

Parameter	Treatment											
	1a	2a	3a	4a	5a	6a	1b	2b	3b	4b	5b	6b
Un-ionized NH_3-N[a]	0.050	0.14	0.29	0.41	0.90	2.34	0.057	0.14	0.33	0.46	1.08	2.62
(mg/L)	0.023	0.02	0.02	0.03	0.13	--[b]	0.032	0.02	0.03	0.05	0.17	--
pH[a]	9.75	9.73	9.76	9.78	9.79	9.86	9.74	9.78	9.80	9.81	9.85	9.86
	0.10	0.10	0.09	0.10	0.10	--	0.10	0.10	0.10	0.11	0.08	--
Temperature[c]	24.9	24.9	24.8	24.8	24.9	25.3	20.4	20.4	20.4	20.3	20.4	20.6
(°C)	0.4	0.5	0.5	0.5	0.5	--	0.2	0.2	0.2	0.2	0.2	--
Dissolved O_2[c]	3.9	4.1	4.2	4.3	4.3	4.7	4.4	4.4	4.5	4.5	5.3	5.4
(mg/L)	0.6	0.4	0.5	0.6	0.5	--	0.6	0.2	0.4	0.2	0.6	--
Conductivity[d]	235	238	242	247	256	285	232	237	242	244	250	285
(µS/cm)	8	8	7	7	4	--	7	7	7	7	0	--
Alkalinity	65	67	67	69	72	72	65	67	67	69	72	72
(mg/L as $CaCO_3$)	5	5	5	5	4	--	5	5	5	5	4	--
Hardness	36	36	36	35	34	36	36	36	36	35	34	36
(mg/L as $CaCO_3$)	1	0	0	1	1	--	1	0	0	1	1	--
Chloride	4.7	5.1	6.0	6.8	9.3	15.0	4.7	5.1	6.0	6.8	9.3	15.0
(mg/L)	0.6	0.4	0.5	0.4	0.8	--	0.6	0.4	0.5	0.4	0.8	--
Sulfate	36	39	38	40	40	36	36	39	38	40	40	36
(mg/L)	3	5	5	4	3	--	3	5	5	4	3	--

[a]N = 9 for treatments 1-5a and 1-4b, and N = 3 for treatment 5b.
[b]N = 1; measurements were discontinued after all fish died.

[c]N = 10 for treatments 1-5a and 1-4b, and N = 4 for treatment 5b.
[d]N = 2 for treatment 5b.

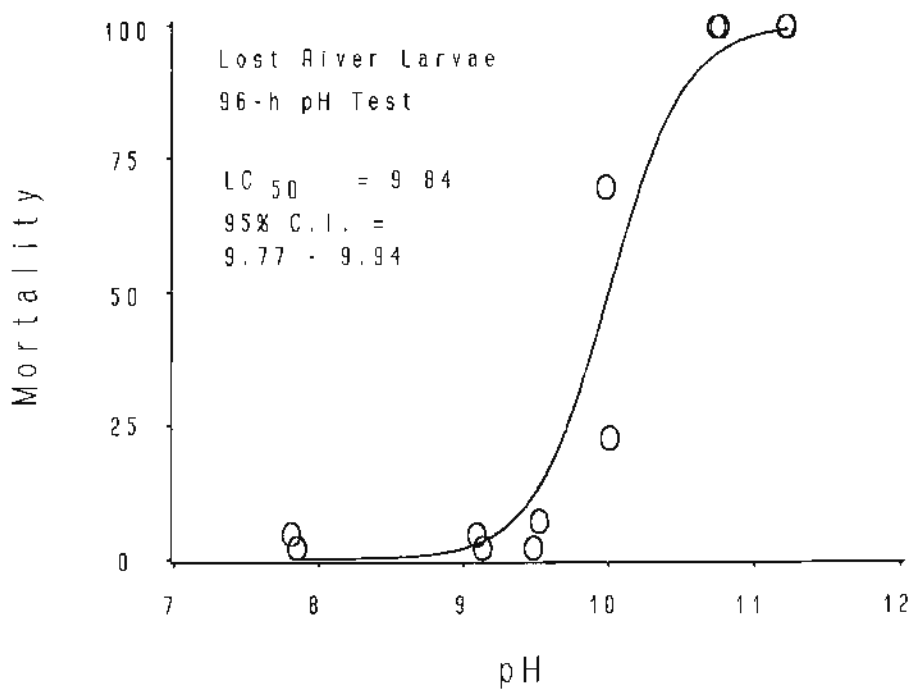

Figure 1. Observed (O) mortality for the single-variable test with pH.

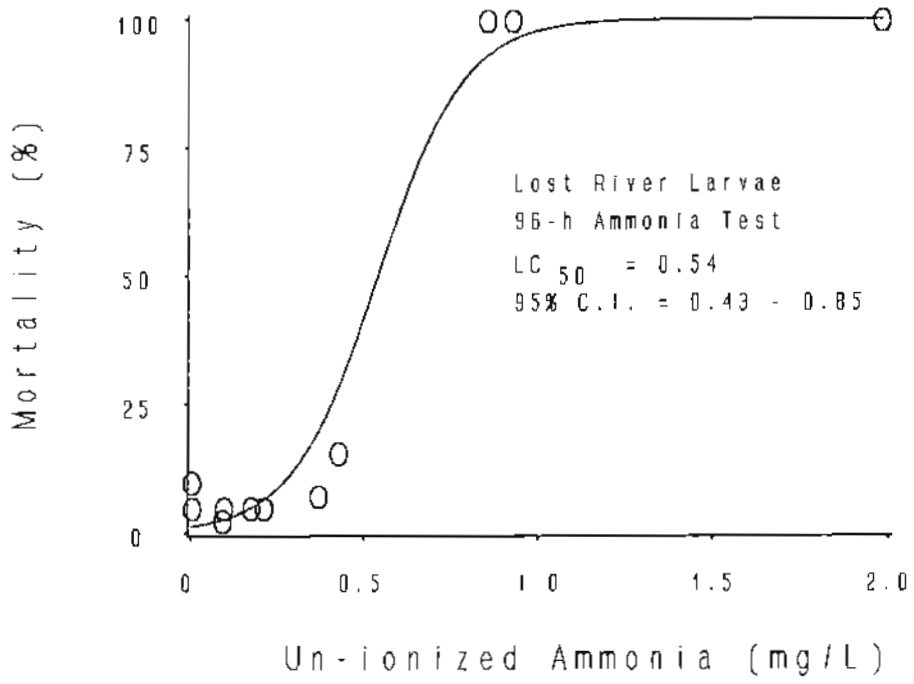

Figure 2. Observed (O) mortality for the single-variable test with un-ionized ammonia.

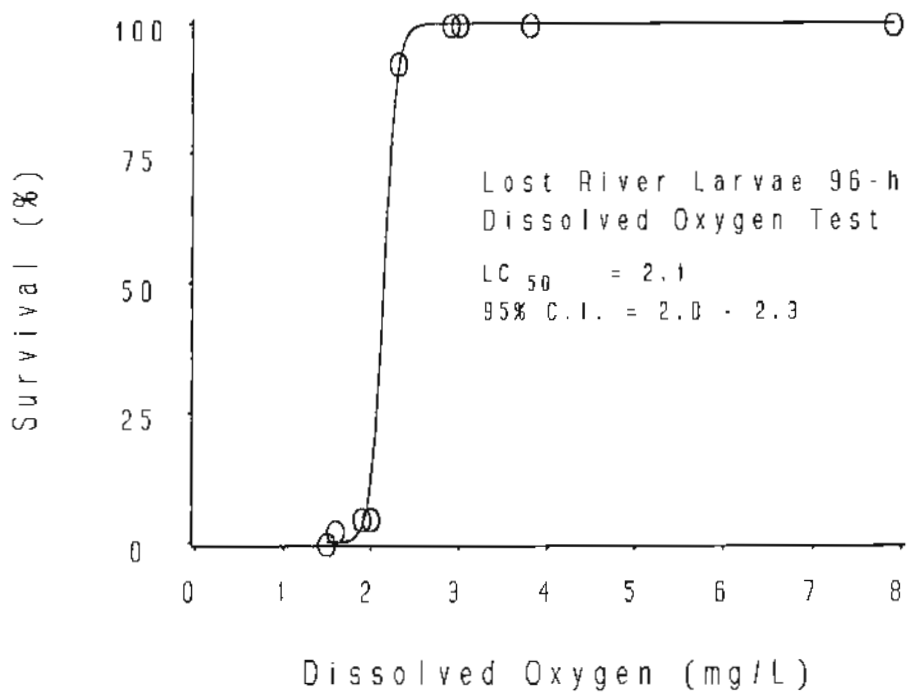

Figure 3. Observed (O) survival for the single-variable test with dissolved oxygen.

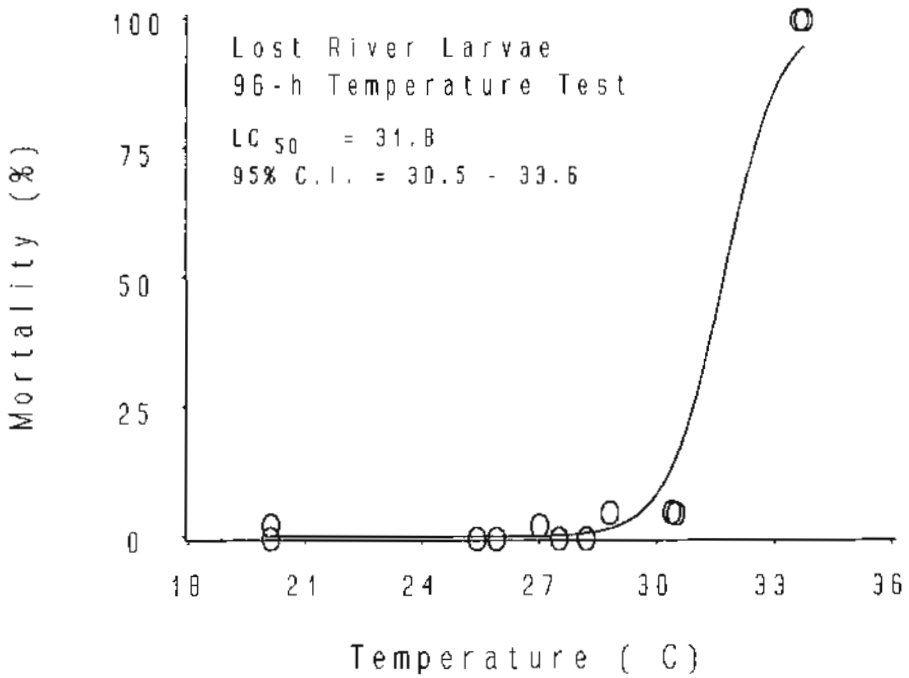

Figure 4. Observed (O) mortality for the single-variable test with temperature.

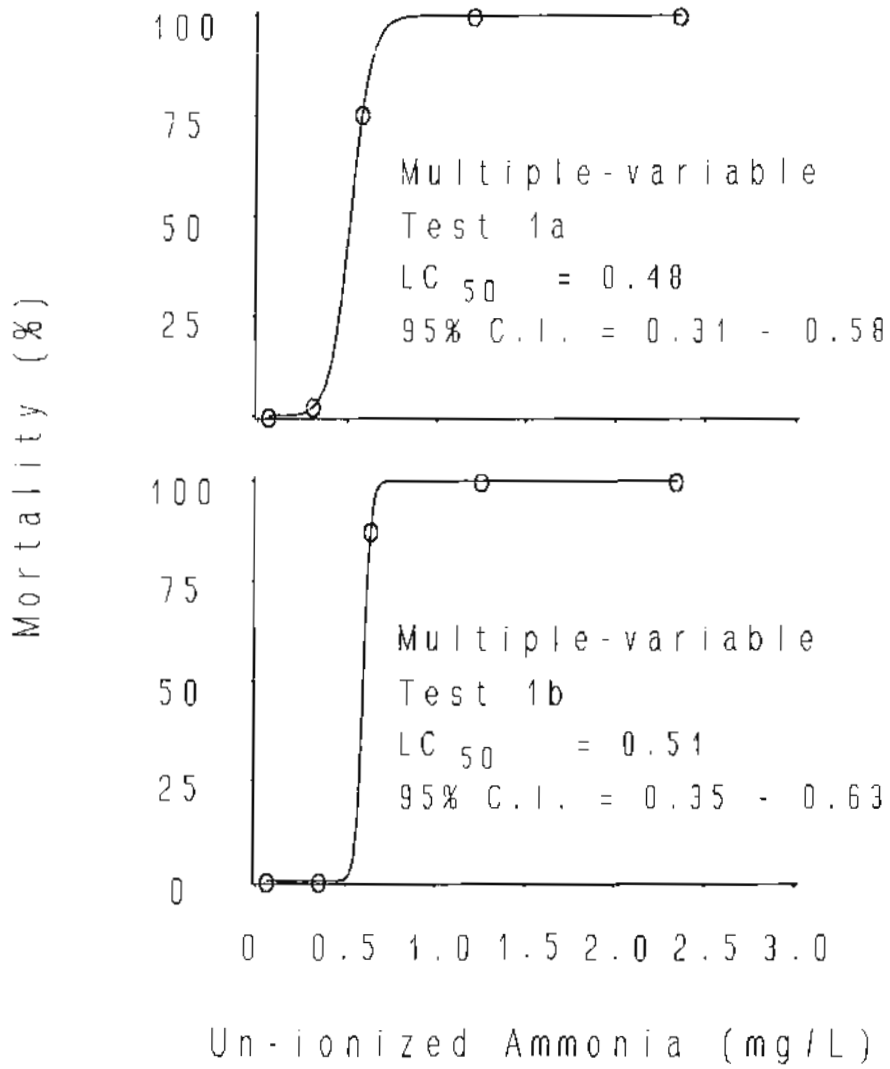

Figure 5. Observed (O) mortality for multiple-variable tests 1a and 1b. For test 1a, pH = 9.50-9.57, temperature = 24.0-24.9°C, and dissolved oxygen saturation = 100%. For test 1b, pH = 9.27-9.60, temperature = 19.8-20.2°C, and dissolved oxygen saturation = 100%.

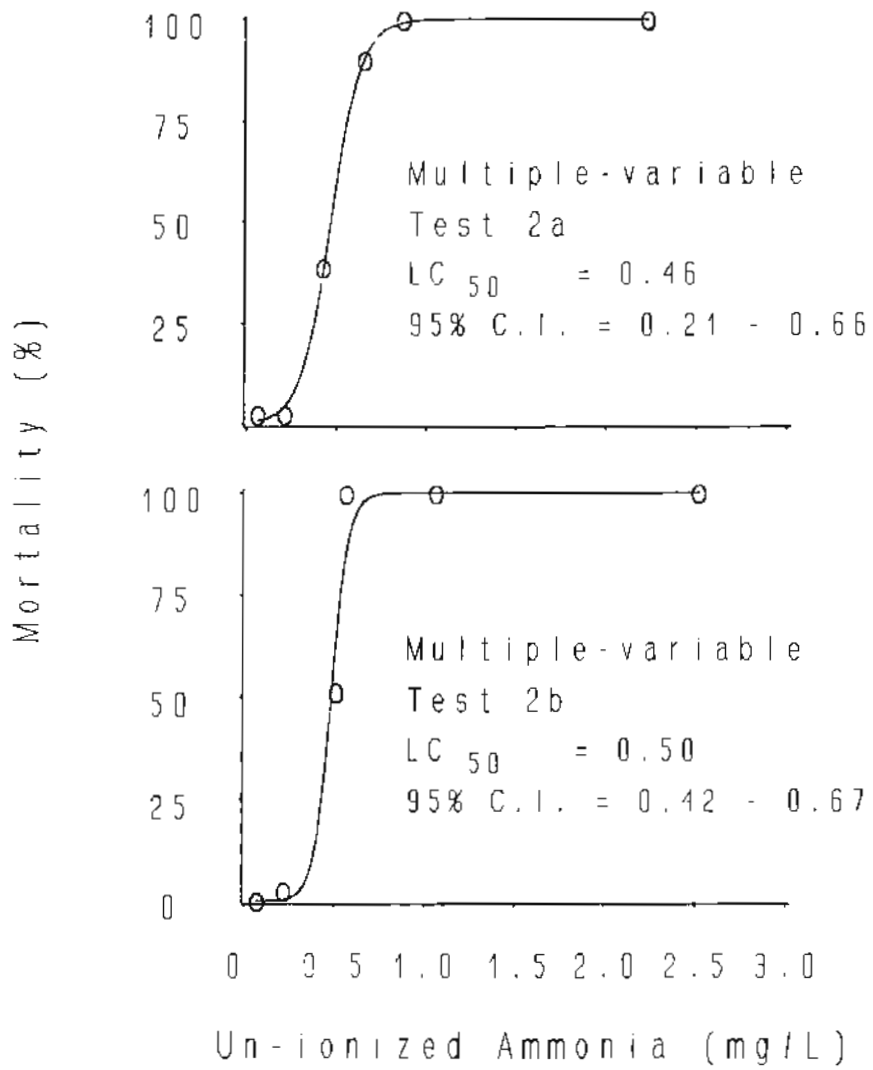

Figure 6. Observed (O) mortality for multiple-variable tests 2a and 2b. For test 2a, pH = 9.13-9.50, temperature = 24.8-25.1°C, and dissolved oxygen saturation = 52-60%. For test 2b, pH = 9.15-9.56, temperature = 20.0-20.3°C, and dissolved oxygen saturation = 56-64%.

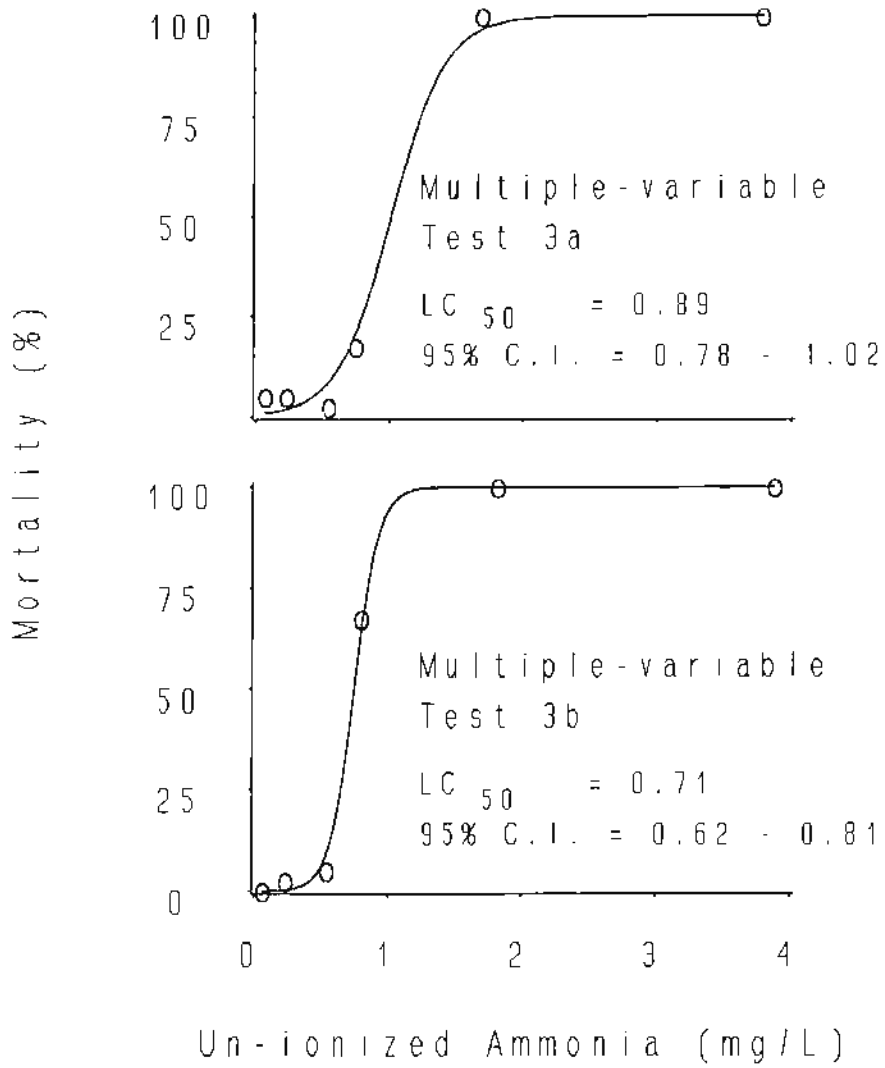

Figure 7. Observed (O) mortality for multiple-variable tests 3a and 3b. For test 3a, pH = 9.76-9.91, temperature = 24.5-24.7°C, and dissolved oxygen saturation ≥94%. For test 3b, pH = 9.79-9.88, temperature = 20.2-20.4°C, and dissolved oxygen saturation ≥96%.

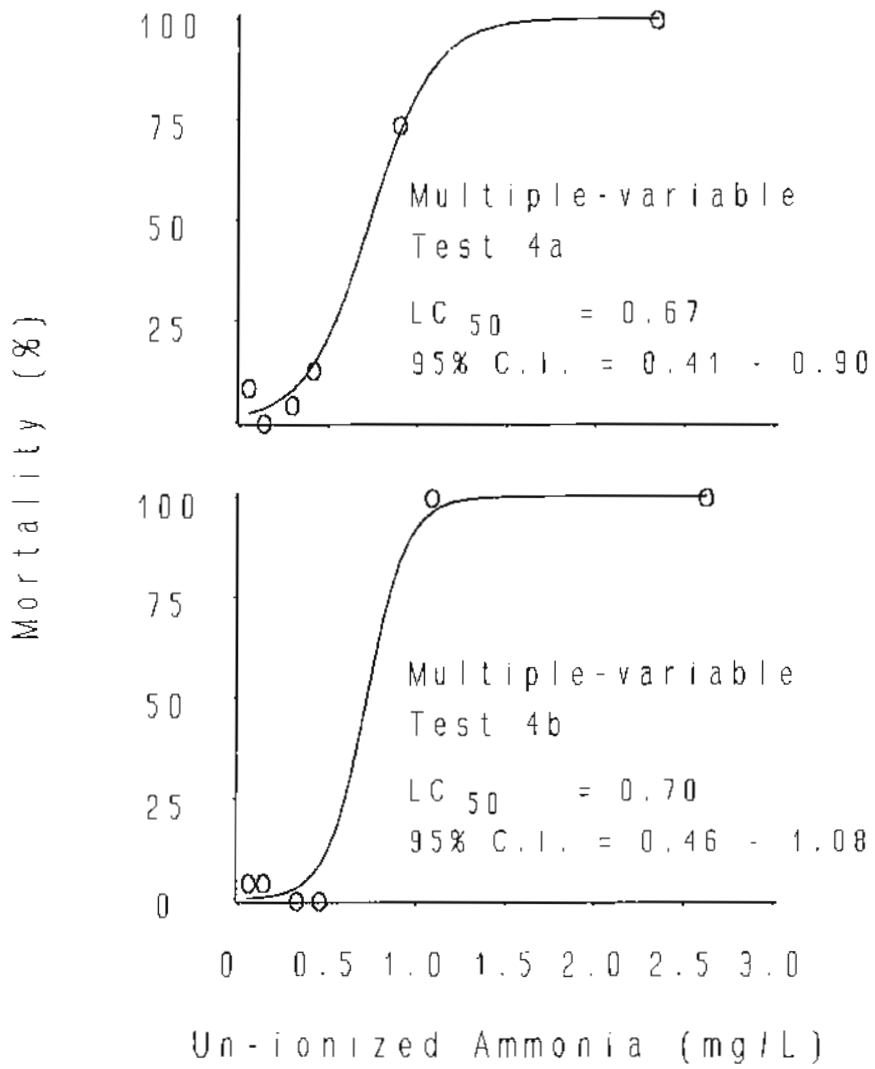

Figure 8. Observed (O) mortality for multiple-variable tests 4a and 4b. For test 4a, pH = 9.73-9.86, temperature = 24.8-25.3°C, and dissolved oxygen saturation = 55-66%. For test 4b, pH = 9.74-9.86, temperature = 20.3-20.6°C, and dissolved oxygen saturation = 56-69%.

Appendix A: Treatment-response data in single-variable and multiple-variable tests.

Table A-1. Comparison of observed and expected mortality in the single-variable test with pH. N = 40 unless otherwise noted.

Treatment	pH	Mortality (%)		Comment
		Observed	Expected	
1a[a] (control)	7.81	5.1	0	$\chi^2 = 63$
1b (control)	7.85	2.5	0	$P < 0.01$
2a	9.09	5	2.9	
2b	9.13	2.5	3.4	
3a	9.48	2.5	11.9	
3b	9.52	7.5	13.7	
4a	9.98	70	48.1	
4b[a]	10.01	23.1	51	
5a	10.74	100	94.5	
5b	10.76	100	94.9	
6a	11.21	100	99.1	
6b	11.22	100	99.1	

[a]N = 39.

Table A-2. Comparison of observed and expected mortality in the single-variable test with un-ionized ammonia. N = 40 unless otherwise noted.

Treatment	Un-ionized Ammonia (mg NH_3-N/L)	Mortality (%) Observed	Mortality (%) Expected	Comment
1a (control)	0.003	10	1.3	$\chi^2 = 31$
1b (control)	0.004	5	1.3	$P < 0.01$
2a[a]	0.092	2.6	2.6	
2b	0.096	5	2.6	
3a	0.174	5	4.9	
3b	0.211	5	6.5	
4a	0.367	7.5	19.8	
4b[b]	0.426	15.8	28.6	
5a	0.854	100	92.9	
5b[a]	0.926	100	95.9	
6a[c]	1.981	100	100	
6b[a]	1.982	100	100	

[a]N = 39.
[b]N = 38.
[c]N = 41.

Table A-3. Comparison of observed and expected survival in the single-variable test with dissolved oxygen. N = 40 unless otherwise noted.

Treatment	D.O. (mg/L)	Survival (%)		Comment
		Observed	Expected	
1a, 1b (control)	7.9	100	100	$\chi^2 = 9.18$
2a, 2b	3.8	100	100	$P = 0.24$
3a[a]	3.0	100	100	
3b	2.9	100	100	
4a, 4b	2.3	92.5	90.6	
5a[b]	2.0	4.9	12.4	
5b[b]	1.9	4.9	3.4	
6a	1.6	2.5	0.1	
6b	1.5	0	0	

[a]N = 39.
[b]N = 41.

Table A-4. Comparison of observed and expected mortality in the single-variable test with temperature. N = 40 unless otherwise noted.

Treatment	Temperature (°C)	Mortality (%)		Comment
		Observed	Expected	
1a (control)	20.1	0	0	$\chi^2 = 46$
1b (control)	20.1	2.5	0	$P < 0.01$
2a	25.4	0	0	
2b[a]	25.9	0	0	
3a	27.0	2.5	0.1	
3b	27.5	0	0.3	
4a	28.2	0	0.7	
4b	28.8	5	1.6	
5a[b]	30.4	5.1	13.6	
5b[a]	30.5	4.9	15.4	
6a	33.6	100	93.8	
6b	33.7	100	94.5	

[a]N = 41.
[b]N = 39.

Table A-5. Comparison of observed and expected mortality in multiple-variable tests 1a and 1b. Approximate test conditions were as follows (variable, treatment a, treatment b): pH, 9.53, and 9.54; temperature, 24.5°C, and 20.0°C; and dissolved oxygen saturation, 100%, and 100%. N = 40 unless otherwise noted.

Treatment	Un-ionized ammonia (mg NH_3-N/L)	Mortality (%)		Comment
		Observed	Expected	
1a (control)	0.061	0	0	$\chi^2 = 0.02$
2a	0.309	2.5	2.4	$\underline{P} > 0.99$
3a[a]	0.577	75.6	75.6	
4a	1.203	100	100	
5a	2.353	100	100	
6a	5.270	100	100	
1b (control)	0.063	0	0	$\chi^2 = 0.00$
2b	0.353	0	0	$\underline{P} = 1.00$
3b	0.633	87.5	87.5	
4b	1.252	100	100	
5b	2.340	100	100	
6b	4.040	100	100	

[a]N = 41.

Table A-6. Comparison of observed and expected mortality in multiple-variable tests 2a and 2b. Approximate test conditions were as follows (variable, treatment a, treatment b): pH, 9.20, and 9.23; temperature. 25.0°C, and 20.1°C; and dissolved oxygen saturation, 56%, and 60%. N = 40 unless otherwise noted.

Treatment	Un-ionized ammonia (mg NH_3-N/L)	Mortality (%)		Comment
		Observed	Expected	
1a (control)	0.065	2.5	0.8	$\chi^2 = 2.06$
2a	0.214	2.5	4.7	$\underline{P} = 0.73$
3a[a]	0.426	38.9	38.3	
4a[b]	0.655	90	90.6	
5a	0.871	100	99.2	
6a	2.230	100	100	
1b (control)	0.070	0	0	$\chi^2 = 14$
2b	0.218	2.5	0.4	$\underline{P} < 0.01$
3b[c]	0.509	51.3	64.4	
4b[d]	0.566	100	85.8	
5b	1.063	100	100	
6b	2.520	100	100	

[a]N = 36.
[b]N = 50.
[c]N = 39.
[d]N = 30.

Table A-7. Comparison of observed and expected mortality in multiple-variable tests 3a and 3b. Approximate test conditions were as follows (variable, treatment a, treatment b): pH, 9.84, and 9.83; temperature, 24.6°C, and 20.3°C; and dissolved oxygen saturation, 94%, and 96%. N = 40 unless otherwise noted.

Treatment	Un-ionized ammonia (mg NH_3-N/L)	Mortality (%)		Comment
		Observed	Expected	
1a (control)	0.072	5	1	$\chi^2 = 10.8$
2a	0.233	5	2	$P = 0.03$
3a	0.550	2.5	9	
4a	0.751	17.5	21.3	
5a	1.695	100	96.8	
6a	3.787	100	100	
1b (control)	0.065	0	0	$\chi^2 = 3.5$
2b	0.236	2.5	0.4	$P = 0.48$
3b[a]	0.544	5.1	9.8	
4b	0.802	67.5	65.1	
5b	1.823	100	100	
6b	3.883	100	100	

[a] N = 39

Table A-8. Comparison of observed and expected mortality in multiple-variable tests 4a and 4b. Approximate test conditions were as follows (variable, treatment a, treatment b): pH, 9.80, and 9.80; temperature, 25.0°C, and 20.4°C; and dissolved oxygen saturation, 60%, and 62%. N = 23.

Treatment	Un-ionized ammonia (mg NH_3-N/L)	Mortality (%)		Comment
		Observed	Expected	
1a (control)	0.049	8.7	2.2	$\chi^2 = 4.9$
2a	0.135	0	3.4	$\underline{P} = 0.30$
3a	0.294	4.3	8	
4a	0.414	13	14.5	
5a	0.898	73.9	71.9	
6a	2.342	100	100	
1b (control)	0.057	4.3	0.3	$\chi^2 = 13$
2b	0.143	4.3	0.6	$\underline{P} < 0.01$
3b	0.329	0	3	
4b	0.457	0	8.7	
5b	1.083	100	96.1	
6b	2.624	100	100	