Refining Ammonia Treatments for *Prymnesium Parvum* Control in Striped Bass Fingerling Production Ponds

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ABSTRACT

Texas state fish hatcheries use un-ionized ammonia nitrogen (NH_3-N) treatments of 0.14-0.25 mg/L to control *Prymnesium parvum* in *Morone* spp. fingerling production ponds. Originating from a published study of ammonia tolerance of 4- to 6-d-old sunshine bass (female white bass *M. chrysops* x male striped bass *M. saxatilis*), this practice is currently applied to the production of striped bass and palmetto bass (female striped bass x male white bass) up to 38 d old. Because fingerling production has been inconsistent and the treatments require frequent reapplications to maintain control of *P. parvum*, we conducted this study to answer two questions: 1) are the treatments, developed for sunshine bass, appropriate for striped bass culture, and 2) are the treatments developed for 4- to 6-d-old fish appropriate for older fish, and if yes, can the maximum treatment be increased as the fish grow, so that frequency of treatments can be reduced? Striped bass (4-, 10-, 20-, and 28-d old) were exposed to NH_3-N concentrations up to 1.2 mg/L for 96 h indoors. Ammonia concentrations and fish mortalities were monitored at 24-h intervals, their relationship was modeled using logistic regression, and the ammonia concentrations 90% of the fish survived during the various exposure periods (maximum concentrations) were estimated. The 4- to 6-d-old striped bass survived ammonia concentrations similar to those of sunshine bass of the same age group. The relationship between ammonia toxicity and striped bass age was nonlinear. We found 20-d-old fish to be the least tolerant of NH_3-N, followed by 4-, and then 10-d-old fish. The 28-d-old fish was the most tolerant of ammonia toxicity. The maximum ammonia concentrations varied with fish age. Thus, the current practice of treating for concentrations between 0.14 and 0.25 mg NH_3-N/L throughout production of phase-1 fish is inappropriate. The current practice of treating with concentrations between 0.14 and 0.25 mg NH_3-N/L can be suitable for culture of striped bass fry and fingerlings, with the exception of fish in the 22-24 d age range. Further, the upper limit of concentration may be increased up to 0.37 and 0.40 mg NH_3-N/L for 10- to 12-d-old and 28- to 30-d-old striped bass, respectively. However, the concentration must be lowered to 0.1 mg NH_3-N/L for 22- to 24-d-old striped bass. Future studies should investigate whether increasing the maximum treatment, when appropriate, can reduce the frequency of ammonia applications during phase-1 striped bass production.

INTRODUCTION

The haptophyte *Prymnesium parvum* forms toxic blooms that cause massive and extensive mortalities in cultured fishes in many parts of the world including the USA (Guo et al. 1996). Since 2001-2002, this microalga has become a persistent threat to fish production at two Texas Parks and Wildlife Department (TPWD) fish hatcheries. At these hatcheries, *P. parvum* causes massive mortalities among all species and life stages of fish unless strategies are used to control cell densities, toxicity, or both (Barkoh et al. 2010). Currently, these facilities use potassium permanganate to mitigate ichthyotoxicity and un-ionized ammonia nitrogen (NH_3-N) to control cell densities. Because NH_3-N is also harmful to fish, conservative concentrations of 0.14-0.25 mg/L (treatments) are used to control *P. parvum* cell densities in *Morone* spp. fingerling production ponds (Barkoh et al. 2004; 2010). Ammonium sulfate is used as the source of NH_3-N because it is the most appropriate chemical option available for *P. parvum* control where primary and secondary production are necessary for successful production of fingerlings (Sarig 1971; Guo et al. 1996; Barkoh et al. 2003).

Although NH_3-N can be effective against *P. parvum* in phase-1 (38-45 mm TL) *Morone* spp. production ponds, there are occasional fish kills in some ponds. Further, the narrow range of treatments requires frequent (2-3 times weekly) reapplications of ammonium sulfate to maintain control of *P. parvum*. Treatments are based on water temperature, pH, total dissolved solids (TDS), and total ammonia nitrogen (TAN) levels and require frequent monitoring in production ponds. Also, *P. parvum* cell density and ichthyotoxicity data are collected as part of the treatment decision-making process. These requirements make the current control protocol labor-intensive and, with a small margin for error due to the narrow range of the treatments, missteps can result in fish kills. Consequently, issues were raised that required investigation to attempt to improve the current TPWD treatment protocol.

The first issue concerns the appropriateness of the treatments (0.14-0.25 mg NH_3-N /L) for other moronids (e.g., striped bass, *Morone saxatilis* and palmetto bass, female striped bass x male white bass *M. chrysops*) when these treatments were developed for sunshine bass (female white bass x male striped bass) (Barkoh et al. 2004). Because responses to environmental stressors differ among striped bass and their hybrids (Harrell 1990; Kelly and Kohler 1999; Myers and Kohler 2000), it was thought that these fish at similar ages may have different sensitivities to ammonia toxicity. Previous studies have reported that sensitivity to ammonia toxicity varies by fish species (Ball 1967; Boudreaux et al. 2007). Thus, if ammonia sensitivity varies among striped bass and their hybrids then occasional fish kills or low survival in production ponds could be explained when the other relevant variables (e.g., water quality variables such DO and pH) were suitable for *Morone* spp. culture.

Based on reports that sensitivity to ammonia toxicity varies with fish size or life stage (Rice and Stokes 1975; Rice and Bailey 1980; Solbé and Shurben 1989) the second issue concerns the appropriateness of the treatments for all sizes during phase-1 striped bass production when the treatments were developed for 4- to 6-d-old fish (Barkoh et al. 2004). The rationale was if older fish ($\geq$ 10 mm) tolerate NH_3-N levels greater than the current 0.25 mg NH_3-N/L maximum treatment then higher rates of ammonium sulfate can be applied as the fish grow and thereby

reduce the frequency of reapplication. This thinking assumes that ammonia decline rates are similar for all concentrations within a defined treatment range.

The 0.14 mg NH_3-N/L treatment is the minimum concentration that kills approximately 90% of *P. parvum* in 48 h whereas 0.25 mg NH_3-N/L is the concentration that approximately 90% of 4-d-old sunshine bass survives in 48 h (48-h maximum tolerable concentration; MTC) (Barkoh et al. 2003, 2004). Thus, to answer the question "Are the current treatments suitable for striped bass culture?" we estimated the 48-h MTC of NH_3-N for 4-d-old striped bass for comparison with that reported for sunshine bass (Barkoh et al. 2004, 2010). Further, we estimated the 48-, 72-, and 96-h MTCs of NH_3-N for striped bass of ages 10, 20, and 28 d old to answer the question "Are the treatments appropriate for striped bass older than 6-d old; and if yes, can the 0.25 mg NH_3-N/L treatment be increased as the fish grow?" We used these life stages of striped bass because palmetto bass of similar ages were found to exhibit different sensitivities to ammonia and pH (Bergerhouse 1993). The 24-h MTC and no-effect-levels (NOEL; concentration a fish survives in a given period) of NH_3-N for striped bass also were estimated to increase flexibility and effectiveness of the NH_3-N treatments to control *P. parvum* while minimizing the risk of ammonia toxicity to the fish. Lastly, the median lethal concentrations (LC_{50}) of NH_3-N for striped bass were estimated to fill gaps in the available information.

MATERIALS AND METHODS

Experimental conditions.—This study was conducted indoors at the Dundee State Fish Hatchery (DSFH) near Wichita Falls, Texas using water from Lake Diversion, the water source for the hatchery. This water had calcium, sodium, magnesium, chlorides, and TDS concentrations of 169, 568, 55, 1,407, and 2,190 mg/L, respectively; total alkalinity and total hardness of 99 and 649 mg/L as $CaCO_3$, respectively; salinity of 4 ppt; and conductivity of 4,575 µmhos/cm. The experimental water was UV- and ozone-treated to remove *P. parvum* cells and toxins (Smith 2005; Barkoh et al. 2010), hereafter referred to as water. The water temperature was maintained close to the historical mid-April average outdoor pond water temperature of 20°C by using a room air conditioning system. Lighting was adjusted to simulate daylight between 0700-2000 hours. Twenty 6-L McDonald egg-hatching jars were cleaned with soap and water and thoroughly rinsed with water; four jars were randomly assigned to each of four treatments and a control, and randomly arranged on an egg incubation rack. Each jar was filled with water (5.4 L) just before each trial began. Before the jars were filled, microscopic examination and bioassay of the water verified absence of *P. parvum* cells and ichthyotoxicity (Ulitzur and Shilo 1964; Sarig 1971; Green et al. 1982; Larsen 1999).

Experimental fish.—Striped bass of ages 4, 10, 20, and 28 d were tested; 1 d was the day eggs hatched. Eggs for these fish were from one spawn, hatched in McDonald jars, and the fry reared in 75-L plastic vats to the target ages. Vats had flow-through water at rates of 3-4 L/min, and each was aerated with compressed air through an aeration ring around the base of a standpipe. Aeration maintained dissolved oxygen levels (DO) of 6 mg/L or greater (Brown and Gratzek 1980; Piper et al. 1982; Nicholson et al. 1990) in each vat. The 10-d-old and older fish were reared from 4-d-old fish at a stocking rate of 2,000 fish/vat using routine culture practices. Twice each day, these fish were offered zooplankton and a commercial diet (Nelson and Sons,

Inc., Murray, UT) consisting of a salmon ration starter, followed by number-1 granules (50% protein) and number-4 crumbles (32% protein) as the fish grew. When each target age was attained, the fish were collected with fine-mesh dipnets and counted into the McDonald jars at 20 fish/jar for ammonia-exposure treatments. Mean total lengths were 7, 12, 19, and 26 mm for the 4-, 10-, 20-, and 28-d-old fish, respectively.

Ammonia treatments.—We selected the ammonia treatment concentrations based on known tolerance values for *Morone* spp. (Barkoh at al. 2004). Treatment concentrations were 0 (i.e., ambient; control), 0.2, 0.4, 0.6, and 0.8 mg NH_3-N/L for the 4- and 10-d-old fish; 0, 0.4, 0.6, 0.8, and 1.0 mg NH_3-N/L for the 20-d-old fish; and 0, 0.6, 0.8, 1.0, and 1.2 mg NH_3-N/L for the 28-d-old fish. Ammonium sulfate was used as source of NH_3-N because it is routinely used to control *P. parvum* in fish culture ponds at Texas state fish hatcheries (Kurten et al. 2007; Barkoh et al. 2010). The quantity of ammonium sulfate required to generate each treatment NH_3-N concentration in 5.5 L of water was estimated graphically. Various amounts of ammonium sulfate were weighed on a Mettler electronic balance (Mettler-Toledo, Inc., Columbus, OH) to the nearest milligram; each was dissolved in 100 mL of water and thoroughly mixed with 5.4 L of water in a McDonald jar. The TAN, pH, and temperature in each jar were measured (see water quality below) and used along with the TDS of the water to calculate the corresponding NH_3-N concentration (Colt 2001). A graph of the quantities of ammonium sulfate verses the corresponding NH_3-N levels was used to back-estimate the required quantity of ammonium sulfate for each NH_3-N treatment level of the experiment. We assumed a linear relationship between ammonium sulfate and NH_3-N and no significant changes in pH, temperature, and TDS of the water.

For each trial, ammonium sulfate treatment solutions (100 mL each) were prepared individually for the treatment replicates. Test fish were counted into the McDonald jars before ammonia treatments to simulate the application of ammonium sulfate to fish production ponds to control *P. parvum*. Treatment solutions were transferred drop-wise, over a 30- to 40-min period, via small-bulb pipettes into the appropriate McDonald jars. The fish were exposed to the treatment and control solutions for 96 h. We used 96-h exposure treatments to complement earlier studies on moronids (e.g., Oppenborn and Goudie 1993; Harcke and Daniels 1999). Jars were examined for dead fish at 24, 48, 72, and 96 h after application of treatments. Dead fish were removed from jars and counted. At the end of 96 h, the remaining dead and live fish in each jar also were counted. Fish were considered dead if they displayed opaque body coloration or were unresponsive to touch. The test was conducted separately for each age fish. All tests were static exposures of treatment and control solutions to the fish. Jars were not aerated, and test fish were not fed during the ammonia exposure treatments.

Water quality.—Temperature, pH, and DO in each jar were measured within 2 h of fish transfer and treatment applications to ensure treatment concentrations were attained and relevant water quality variables (temperature, pH, and DO) were within ranges suitable for striped bass culture. Temperature, pH, and DO were measured with a YSI 650 MDS handheld meter fitted with a YSI 600 XL multiprobe sensor (Yellow Springs Instruments, Yellow Springs, Ohio). These same variables also were measured when water samples (about 120 mL each) were taken for TAN measurements at 24, 48, 72, and 96 h after the treatments were initiated. Total ammonia nitrogen was measured with a Denver Instruments Model 250 meter equipped with an

Accumet ammonia ion-selective electrode (Denver Instruments, Denver, Colorado). Un-ionized ammonia concentrations were calculated from TAN values with an equation that compensates for temperature, pH, and TDS (Colt 2001).

Data analysis.—Because some of the fish in the controls died, we adjusted the percent mortality data for the treatments by the control mortality values (Schneider-Orelli 1947) before statistical analysis. We conducted exploratory analysis using a locally-weighted regression scatter plot smoothing (LOESS) procedure (Neter et al. 1996) to better understand the shape of the dose-response curve. To estimate MTCs, the relationship between fish mortality and NH_3-N was modeled using logistic regression (SAS Institute 2008; Allison 1995; Hosmer and Lemeshow 1989; Appendix 1A-C). The NOEL values were estimated using an ANOVA approach that modeled mortality as a function of NH_3-N treatment level. Because the mortality data were not normally distributed, we transformed the data into ranks and used a one-way ANOVA on the ranks, followed by a Dunnett's test to determine which treatment differed from the control (Zar 1984). We defined the NOEL as the mean NH_3-N concentration of the highest treatment level where fish mortality did not significantly differ from that of the control. Differences in water quality variables or fish mortality among treatments and control were determined by ANOVA, followed by Tukey's test (SAS Institute 2008). Effects or differences were considered significant at P –values less than or equal to 0.05.

RESULTS

Water quality.—Concentrations of TDS were 2,212; 2,220; 2,180; and 2,190 mg/L for the 4-, 10-, 20-, and 28-d-old fish tests, respectively. Temperature, DO, and pH did not significantly differ among treatments during each 4-d experiment (Table 1), and all were within ranges deemed suitable for the culture of striped bass (Harrell et al. 1990). Across age groups, these water quality variables did not statistically differ at any treatment level. Only NH_3-N concentration significantly differed among treatments. The concentrations of NH_3-N declined during each 4-d experiment, and the percent decline increased with treatment concentration (Table 2). For the same treatment levels, percent declines were extremely high for the 28-d-old fish compared to the others and the reason is unclear.

4-d-old fish.—Mortalities were low in the controls throughout the 4-d experiment, less than 15% for the 0.2-mg NH_3-N/L treatment, and higher than 50% for the three highest treatments (Table 3). We observed complete mortality in all but one replicate of the two highest treatments. All fish but two died by 96 h in the 0.6-mg NH_3-N/L treatment whereas all fish died by 72 h in the 0.8-mg NH_3-N/L treatment. Mortality increased in each treatment over the course of the study. Based on the final NH_3-N concentrations, the mean MTCs were 0.53, 0.21, 0.13, and 0.14 mg NH_3-N/L for 24, 48, 72, 96 h, respectively (Table 4). The NOEL was 0.54 mg NH_3-N/L for 24 h. We could not estimate NOEL values after 24 h because all treatments caused significantly higher mortalities than were observed in the controls. The LC_{50} results are in Appendix 2.

10-d-old fish.—Mortalities increased in all treatments over the course of the study. By 96 h, mortality remained below 10% in the control but reached approximately 21-100% in the treatments, increasing with treatment concentration (Table 3). Based on the final NH_3-N

concentrations, mean MTC was at least 0.8 mg NH_3-N/L for 24 h and decreased to 0.20 mg NH_3-N/L by 96 h (Table 4). The NOEL decreased from 0.5 mg NH_3-N/L by 24 h to 0.25 mg NH_3-N/L by 72 h. No NOEL was estimated for 96 h because all treatments caused significantly more mortalities than the controls by that time.

20-d-old fish.—Mortalities increased over time in all treatments. At least half of the fish were dead within 24 h in the three highest NH_3-N concentrations, and more than 70% were dead in all treatment concentrations within 72 h (Table 3). At the highest three concentrations, we observed complete mortality in at least one replicate, and all fish died by 96 h in treatments 0.8 and 1.0 mg NH_3-N/L. The mean MTC was 0.2 mg NH_3-N/L at 24 h and 0.1 mg NH_3-N/L for 48-96 h based on final ammonia concentrations (Table 4). The NOEL could not be estimated for any time-step of the study because of significant mortalities in all treatments.

28-d-old fish.—No fish died in 96 h regardless of the NH_3-N treatment concentration. Thus, the MTC and NOEL values were undetermined. However, the NOEL (or MTC) was considered to be much higher than those of the younger age groups since no fish died in this age group at the tested higher concentrations. Based on the final NH_3-N concentrations for the highest ammonia treatment level (1.2 mg NH_3-N/L), we suggest a NOEL (or MTC) of at least 0.40 for 48 h and 0.36 mg NH_3-N/L for up to 96 h (Table 2).

DISCUSSION

Ammonia had a cumulative toxicity effect on striped bass as indicated by the consistent increases in mortalities over the course of the study, and the relationship between ammonia toxicity and striped bass age was nonlinear. We found 20-d-old fish to be the least tolerant of NH_3-N, followed by 4-, and 10-d-old fish, in that order. The 28-d-old fish was the most tolerant of ammonia toxicity since none of these fish died. Our results, in some ways, are similar to those of Bergerhouse (1993) who found 14-d-old palmetto bass more tolerant of ammonia than 5- and 20-d-old fish, an indication that the relationship between ammonia toxicity and palmetto bass age is nonlinear. The measured water quality variables, temperature, DO, and pH, did not affect ammonia toxicity in this study.

This study did not test the ammonia sensitivities of 15- to 19-d-old and 25- to 27-d-old striped bass; thus, our understanding of ammonia toxicity to striped bass of ages 4 to 32 d remains incomplete. Because the 20- to 24-d-old fish were the most vulnerable to ammonia toxicity among the ages investigated in the present study, we recommend additional studies to better demarcate the age-related transition in ammonia tolerance of striped bass. Longer-term (e.g., 28-30 d) exposures of fish to ammonia would help better understand the chronic effects of ammonia on striped bass. This information would help with effective mitigation of *P. parvum* blooms without a significant adverse effect on striped bass survival.

The first question this study intended to answer was "Are the 0.14- to -0.25-mg NH_3-N/L treatments appropriate for striped bass; in other words, can 90% of 4-d-old striped bass survive 0.25 mg NH_3-N/L for 48 h." Barkoh et al. (2004) reported the 48-h MTC for sunshine bass as 0.25 mg NH_3-N/L, the same as the upper confidence limit of the 48-h MTC for striped bass in

the current study. Thus, we conclude that the current ammonia treatments are also good for controlling *P. parvum* in striped bass fingerling production ponds. However, because the mean MTC was 0.21 mg/L we suggest using this as the maximum treatment for 4- to 6-d old striped bass.

We found the treatments inappropriate for certain sizes of striped bass during phase-1 fingerling production. The mean MTC for 22- to 24-d-old fish (0.1 mg/L) was lower than the minimum treatment; thus, these fish cannot survive the treatments. Similarly, the 7- to 8-d-old fish, with mean MTCs of 0.13-0.14 mg/L, cannot survive most of the treatments. Because the relationship between striped bass age and ammonia concentration was not a monotonic trend, there seems to be no easy answer to the question concerning if the 0.25 mg NH_3-N/L treatment can be increased as the fish grow. The 48-h MTCs suggest that NH_3-N cannot be increased in a linear or monotonic fashion with age of the fish, but rather must be seesawed to suit the different age groups of striped bass (Figure 1). Ammonia treatments may be increased up to 0.37 and 0.40 mg NH_3-N/L for 10- to 12-d-old and 28- to 30-d-old striped bass, respectively, but must be lowered to 0.1 mg NH_3-N/L for 20-22-d striped bass. For fish of ages 6- to 8-d-old and 12- to 14-d-old, maximum treatments of 0.13-0.14 and 0.20-0.30 mg NH_3-N/L may be appropriate, respectively, whereas the maximum treatment must be lowered to 0.1 mg NH_3-N/L for 22-24-d old fish. Whereas reducing ammonia concentration to 0.1 mg NH_3-N/L is possible by flowing fresh water through ponds, these ammonia concentrations are ineffective in controlling *P. parvum* (Barkoh et al. 2003; 2010). Thus, an alternative treatment should be used to treat *P. parvum*, if needed, during these ages when the fish is most vulnerable to ammonia toxicity.

The ammonia treatments (0.14-0.25 mg NH_3-N/L) were developed for 4- to 6-d old sunshine bass (Barkoh et al. 2004) but have been used to control *P. parvum* in ponds with striped bass older than 6 d old with mixed phase-1 fingerling production results. To avoid ammonia-related toxicity and achieve consistent striped bass production results, we recommend the following: Treatments that are effective for *P. parvum* control (Barkoh et al. 2003) should be selected in concert with concentrations the fish can tolerate using Table 4 as a guide. For fish 28-d and older, the maximum concentration should be 0.36 mg NH_3-N/L. Future studies should address the issue of whether or not higher ammonia treatments would allow a reduction in the frequency of ammonia reapplications, while maintaining control of *P. parvum*, in striped bass fingerling production ponds. Our experience with the 0.14- to 0.25-mg NH_3-N/L treatments is that applied ammonia declines, to levels below 0.14 mg NH_3-N/L, in 5-7 d depending on the algal biomass of the pond.

The ammonia treatments discussed are conservative in that we used the ammonia concentrations measured at the end of each time-step of the study rather than the initial exposure concentrations or the mean values. Nonetheless, these ammonia treatments must be used with caution for the control of *P. parvum* because ammonia is also toxic to *Morone* spp. (Bergerhouse 1993; Oppenborn and Goudie 1993; Weirich et al. 1993; Ashe et al. 1996; Harcke and Daniels 1999). Another reason for caution is that ammonia toxicity levels or effects on fish are influenced by several physicochemical variables including temperature, DO, pH, alkalinity, salinity, and TDS (Emerson et al. 1975; Thurston et al. 1981; Meade 1985; Bergerhouse 1993; Colt 2001).

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TABLE 1.—Mean ± SD values of temperature (°C), dissolved oxygen (mg/L), pH, and un-ionized ammonia nitrogen (NH_3-N; mg/L) in jars for testing ammonia tolerance of striped bass in 96 h. Only NH_3-N was significantly ($P > 0.05$) different among treatments.

Water quality variable	Control 0	Un-ionized ammonia nitrogen treatments (mg/L)					
		0.2	0.4	0.6	0.8	1.0	1.2
Fish age: 4 d							
Temperature	20.9 ± 0.3	20.9 ± 0.3	20.9 ± 0.3	20.9 ± 0.3	20.9 ± 0.3	N/A	N/A
Dissolved oxygen	8.6 ± 0.3	8.6 ± 0.3	8.6 ± 0.4	8.6 ± 0.3	8.5 ± 0.4	N/A	N/A
pH	8.06 ± 0.06	8.02 ± 0.04	7.98 ± 0.02	7.94 ± 0.02	7.89 ± 0.02	N/A	N/A
NH_3-N	0.003 ± 0.005	0.157 ± 0.031	0.298 ± 0.054	0.478 ± 0.093	0.588 ± 0.125	N/A	N/A
Fish age: 10 d							
Temperature	20.9 ± 0.6	20.9 ± 0.6	20.9 ± 0.5	21.0 ± 0.6	20.9 ± 0.5	N/A	N/A
Dissolved oxygen	7.9 ± 0.1	7.9 ± 0.1	7.9 ± 0.2	7.9 ± 0.1	7.9 ± 0.1	N/A	N/A
pH	8.06 ± 0.01	8.04 ± 0.02	8.04 ± 0.04	8.0 ± 0.04	8.0 ± 0.04	N/A	N/A
NH_3-N	0.0	0.161 ± 0.027	0.309 ± 0.051	0.433 ± 0.083	0.528 ± 0.056	N/A	N/A
Fish age: 20 d							
Temperature	21.2 ± 0.1	N/A	21.2 ± 0.2	21.2 ± 0.1	21.1 ± 0.1	21.1 ± 0.1	N/A
Dissolved oxygen	7.0 ± 0.2	N/A	6.6 ± 0.3	6.9 ± 0.2	6.8 ± 0.2	6.5 ± 0.2	N/A
pH	8.01 ± 0.05	N/A	7.93 ± 0.07	7.90 ± 0.08	7.90 ± 0.08	7.88 ± 0.09	N/A
NH_3-N	0.007 ± 0.004	N/A	0.258 ± 0.055	0.373 ± 0.091	0.495 ± 0.091	0.601 ± 0.152	N/A
Fish age: 28 d							
Temperature	20.9 ± 0.1	N/A	N/A	20.9 ± 0.1	20.9 ± 0.1	20.9 ± 0.1	20.9 ± 0.1
Dissolved oxygen	6.4 ± 0.9	N/A	N/A	6.2 ± 1.2	6.4 ± 1.1	6.3 ± 1.0	6.2 ± 1.0
pH	7.75 ± 0.20	N/A	N/A	7.71 ± 0.22	7.70 ± 0.26	7.73 ± 0.21	7.73 ± 0.21
NH_3-N	0.005 ± 0.004	N/A	N/A	0.274 ± 0.175	0.367 ± 0.252	0.434 ± 0.276	0.536 ± 0.332

TABLE 2.—Mean daily NH_3-N concentrations and 96-h decline values for treatments exposed to striped bass for 96 h.

Exposure time	Un-ionized ammonia nitrogen treatments (mg/L)						
(h)	0.0	0.2	0.4	0.6	0.8	1.0	1.2
			Fish age: 4 d				
0	0.000	0.189	0.358	0.625	0.794	N/A	N/A
24	0.001	0.193	0.363	0.537	0.657	N/A	N/A
48	0.001	0.139	0.263	0.423	0.512	N/A	N/A
72	0.001	0.125	0.242	0.391	0.473	N/A	N/A
96	0.004	0.138	0.265	0.413	0.501	N/A	N/A
96-h % decline		26.980	25.980	33.920	36.900	N/A	N/A
			Fish age: 10 d				
0	0.000	0.211	0.384	0.586	0.734	N/A	N/A
24	0.000	0.150	0.304	0.411	0.501	N/A	N/A
48	0.000	0.153	0.309	0.404	0.511	N/A	N/A
72	0.000	0.137	0.248	0.362	0.453	N/A	N/A
96	0.000	0.156	0.298	0.401	0.492	N/A	N/A
96-h % decline	0.000	26.070	22.390	31.570	32.970	N/A	N/A
			Fish age: 20 d				
0	0.001	N/A	0.357	0.545	0.667	0.884	N/A
24	0.012	N/A	0.265	0.356	0.475	0.564	N/A
48	0.007	N/A	0.219	0.305	0.432	0.458	N/A
72	0.008	N/A	0.221	0.341	0.440	0.573	N/A
96	0.007	N/A	0.229	0.319	0.461	0.527	N/A
96-h % decline			35.850	41.470	30.880	40.380	N/A
			Fish age: 28 d				
0	0.000	N/A	N/A	0.614	0.849	0.970	1.181
24	0.000	N/A	N/A	0.178	0.263	0.286	0.361
48	0.009	N/A	N/A	0.203	0.266	0.327	0.399
72	0.009	N/A	N/A	0.195	0.214	0.306	0.378
96	0.006	N/A	N/A	0.179	0.241	0.283	0.362
96-h % decline				70.850	71.160	70.820	69.350

TABLE 3.—Mean cumulative percent mortalities of striped bass exposed to ammonia treatments for 96 h. Data for 28-d-old fish are not included because none died during the 96-h experiment. Treatment mortalities were adjusted for control mortalities using the Schneider-Orelli's (1947) formula.

Exposure time (h)	Control	Un-ionized ammonia nitrogen treatments (mg/L)				
	0	0.2	0.4	0.6	0.8	1.0
			Fish age: 4 d			
24	1.3 ± 2.5	2.5 ± 5.0	1.3 ± 2.5	5.0 ± 0.0	50.0 ± 16.8	N/A
48	1.3 ± 2.5	10.0 ± 4.1	16.3 ± 11.1	45.0 ± 7.1	88.8 ± 8.5	N/A
72	3.8 ± 4.8	13.8 ± 4.8	45.0 ± 4.1	85.0 ± 10.8	99.5 ± 0.0	N/A
96	5.0 ± 5.8	13.8 ± 4.8	55.0 ± 13.5	97.5 ± 5.0	99.5 ± 0.0	N/A
			Fish age: 10 d			
24	2.5 ± 2.9	2.5 ± 5.0	1.3 ± 2.5	0.0	1.3 ± 2.5	N/A
48	7.5 ± 2.9	12.5 ± 5.0	3.8 ± 2.5	21.3 ± 21.4	28.8 ± 9.5	N/A
72	8.8 ± 2.5	20.0 ± 5.8	17.5 ± 11.9	46.3 ± 25.0	53.8 ± 13.8	N/A
96	8.8 ± 2.5	21.3 ± 4.8	31.3 ± 8.5	66.3 ± 25.0	100.0 ± 0.0	N/A
			Fish age: 20 d			
24	1.7 ± 3.3	N/A	26.7 ± 7.7	51.7 ± 16.7	81.5 ± 12.3	98.3 ± 3.3
48	5.0 ± 3.3	N/A	46.3 ± 9.5	80.0 ± 5.4	92.1 ± 11.8	100.0 ± 0.0
72	7.9 ± 3.7	N/A	74.6 ± 14.4	91.7 ± 8.4	97.5 ± 5.0	100.0 ± 0.0
96	9.6 ± 4.4	N/A	81.3 ± 14.6	95.0 ± 6.4	100.0 ± 0.0	100.0 ± 0.0

TABLE 4.—Means and confidence intervals of maximum tolerable concentration (MTC) and no-effect-level (NOEL) of un-ionized ammonia nitrogen for different age striped bass exposed to ammonia treatments for 96 h. Data for 28-d-old fish are not reported because none died during the 96-h experiment.

	Age of fish (d)		
Variable*	4	10	20
	Exposure time: 24 h		
MTC	0.53	at least 0.8	0.20
	0.50 - 0.56		0.15 - 0.25
NOEL	0.54	0.50	–
	Exposure time: 48 h		
MTC	0.21	0.37	0.10
	0.15 - 0.25	0.30 - 0.45	0.05 - 0.15
NOEL	–	0.40	–
	Exposure time: 72 h		
MTC	0.13	0.30	0.10
	0.10 - 0.20	0.25 - 0.34	0.01 - 0.15
NOEL	–	0.25	–
	Exposure time: 96 h		
MTC	0.14	0.20	0.10
	0.13 - 0.20	0.15 - 0.25	0.01 - 0.15
NOEL	–	–	–

*Values in mg NH_3-N/L; MTC is the concentration 90% of the fish survives; NOEL is the concentration all fish survive; MTC or NOEL for 28- to 32-d-old striped bass is suggested to be at least 0.36 mg NH_3-N/L.

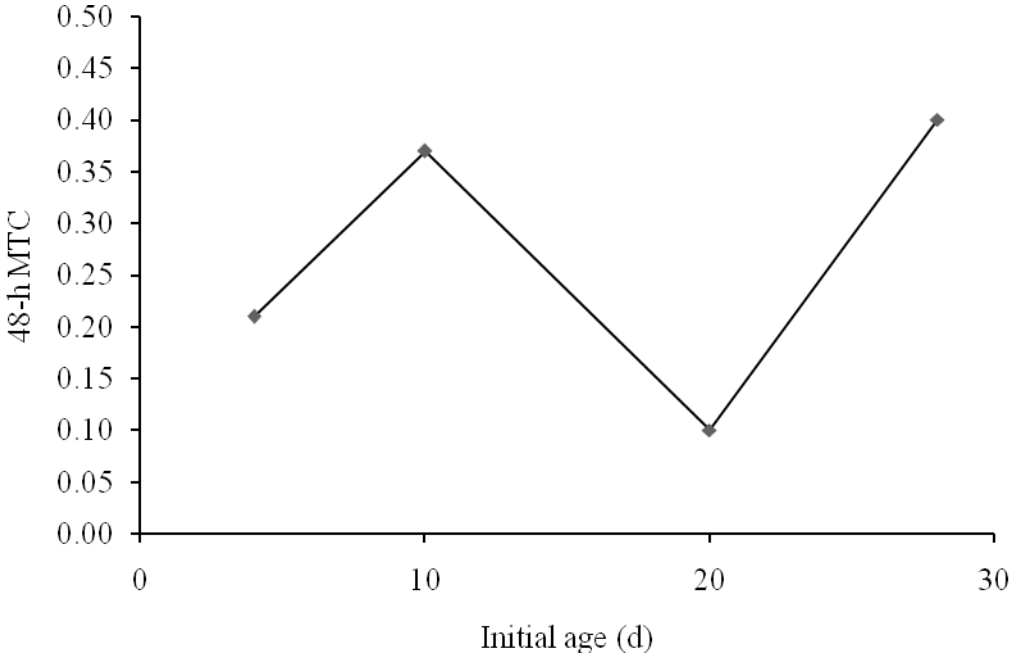
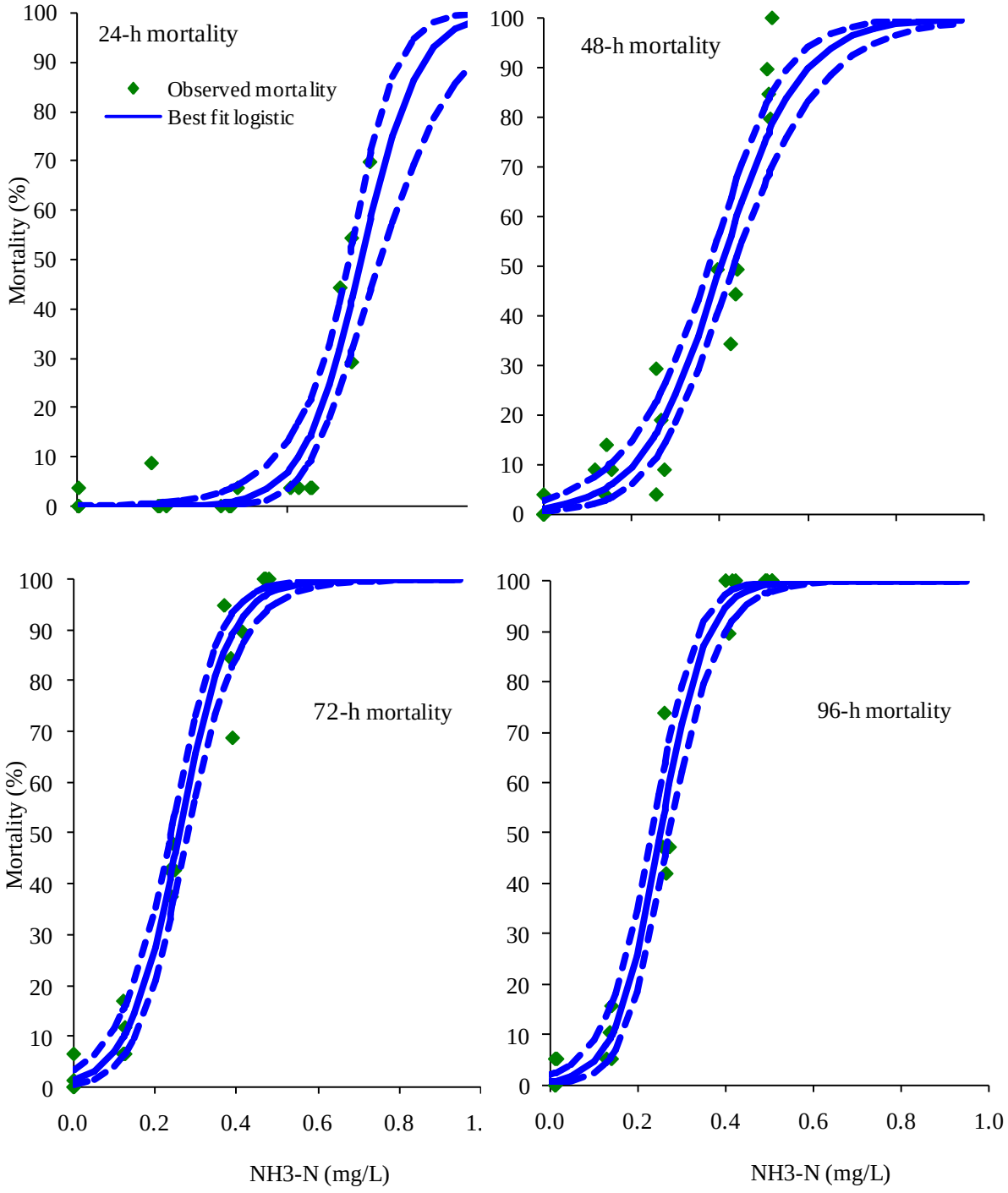


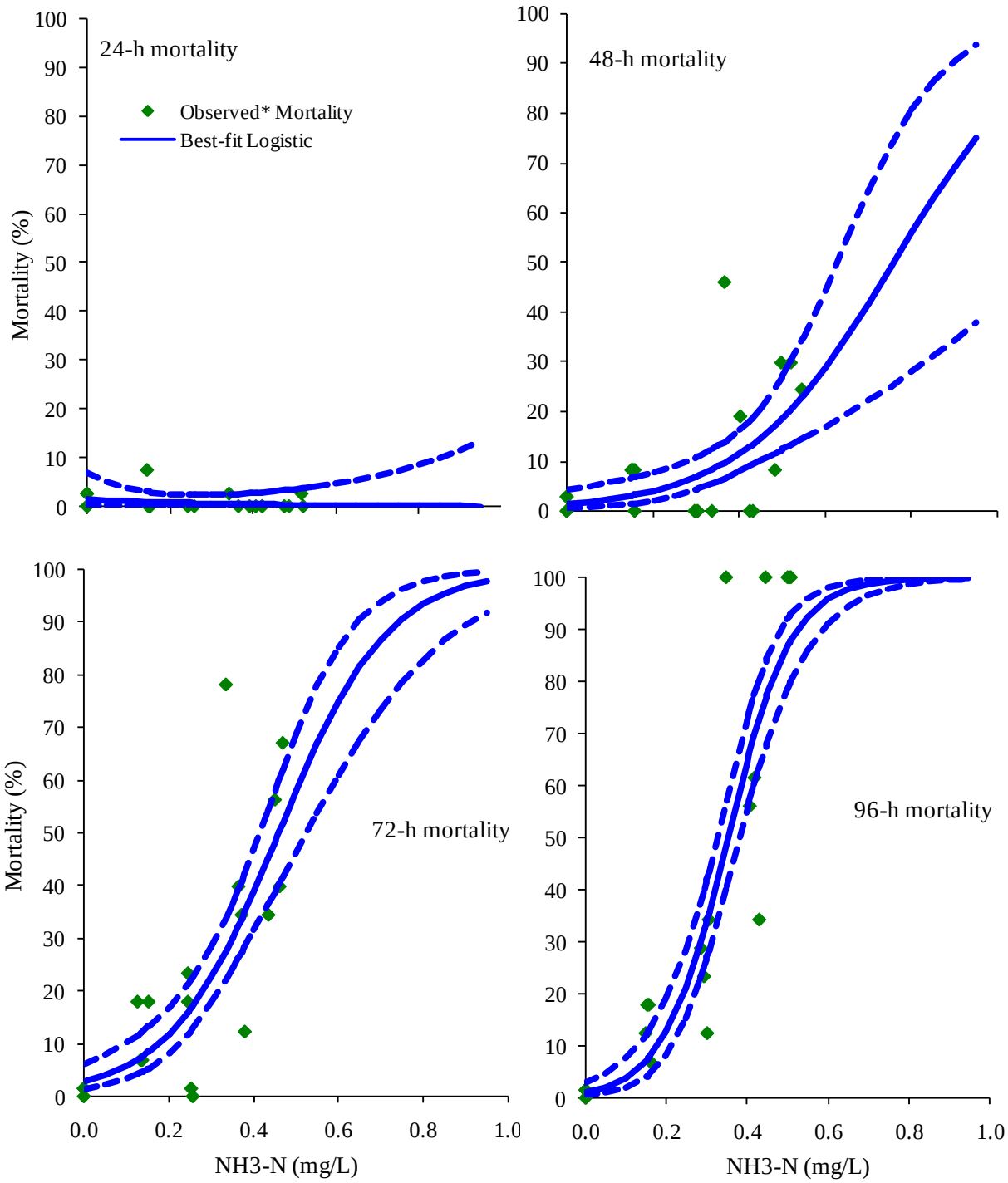
FIGURE 1.—Maximum concentrations of un-ionized ammonia nitrogen that 90% of striped bass of different ages survive in 48 h (48-h MTC).

APPENDIX 1

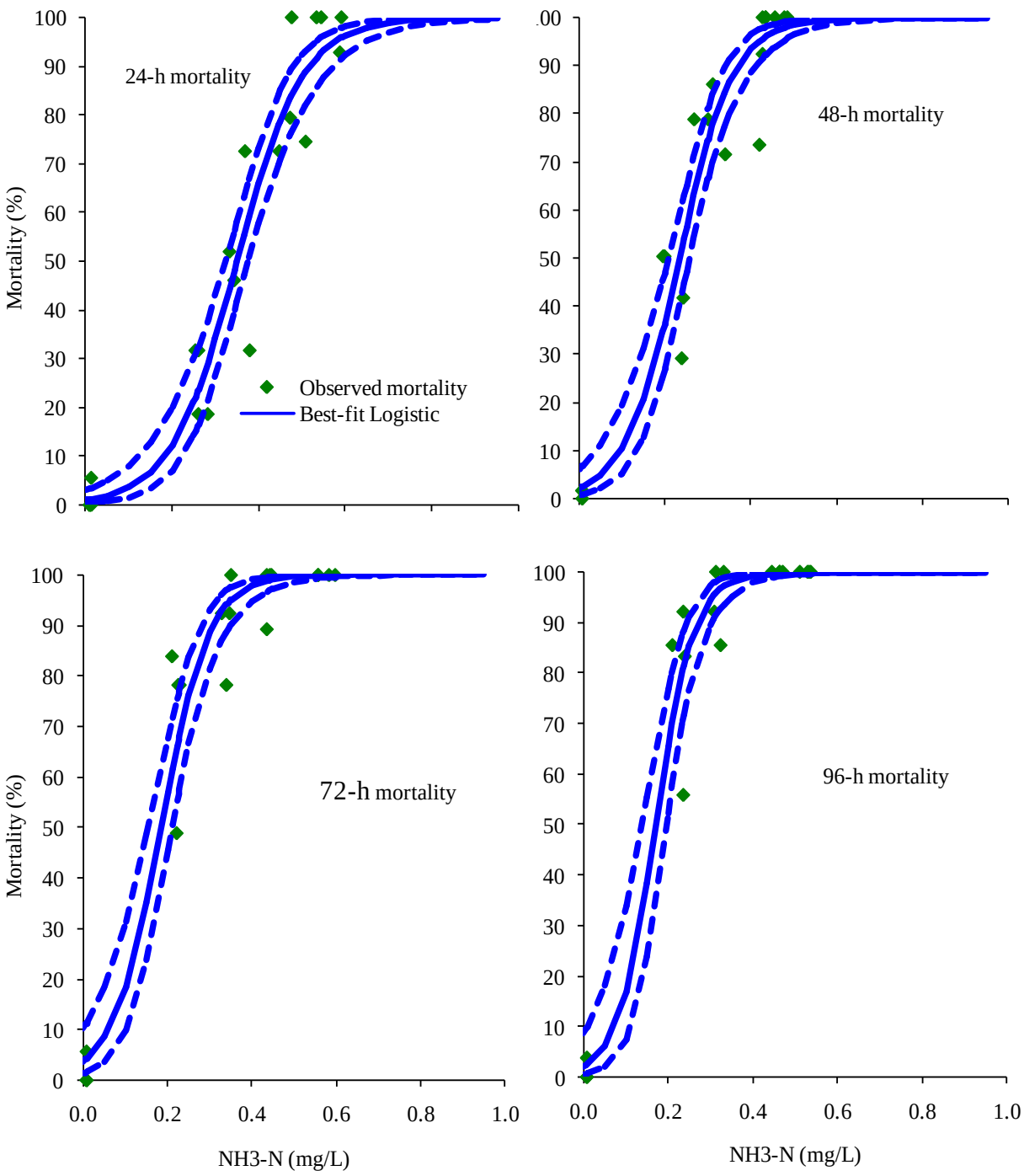
A. Mortality logistic curves for 4-d-old striped bass exposed to various concentrations of un-ionized ammonia nitrogen for 96 h. Observed mortality was mortality after correction for control mortality using the Schneider-Orelli's (1947) formula.



B. Mortality logistic curves for 10-d-old striped bass exposed to various concentrations of un-ionized ammonia nitrogen for 96 h. Observed mortality was mortality after correction for control mortality using the Schneider-Orelli's (1947) formula.



C. Mortality logistic curves for 20-d-old striped bass exposed to various concentrations of un-ionized ammonia nitrogen for 96 h. Observed mortality was mortality after correction for control mortality using the Schneider-Orelli's (1947) formula.



APPENDIX 2

Mean ± SD cumulative daily LC_{50} of un-ionized ammonia nitrogen for different age striped bass exposed to ammonia treatment for 96 h.

Age of fish (d)		
4	10	20
Exposure time: 24 h		
0.676 (0.644 - 0.734)	–	0.35 0.324 -0.375
Exposure time: 48 h		
0.402 0.375 - 0.431	0.758 0.556 - 1.19	0.234 0.207 - 0.256
Exposure time: 72 h		
0.26 0.239 - 0.28	0.459 0.413 - 0.527	0.184 0.153 - 0.21
Exposure time: 96 h		
0.253 0.234 - 0.273	0.353 0.328 - 0.381	0.171 0.137 - 0.197

LC_{50} (median lethal concentration) is the concentration that kills 50% of the fish.

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