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G. JOAN HOLT and C.R. ARNOLD
University of Texas Marine Science Institute,
Port Aransas Marine Laboratory, Port Aransas, Texas 78373

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Effects of Ammonia and Nitrite on Growth and Survival of Red Drum Eggs and Larvae[1]

G. JOAN HOLT AND C. R. ARNOLD

University of Texas, Marine Science Institute
Port Aransas Marine Laboratory, Port Aransas, Texas 78373

Abstract

The tolerances of eggs, larvae, and postlarvae of red drum *Sciaenops ocellatus* to ammonia and nitrite were determined by measuring survival and growth after continuous exposure for 1–2 weeks. Ammonia concentrations that reduced the survival rate varied with age at first exposure. Concentrations as low as 0.3 mg/liter un-ionized ammonia significantly reduced survival of newly hatched larvae in the first two weeks, whereas concentrations twice that high were tolerated by 3-week-old postlarvae. Young red drums tolerated high concentrations of nitrite, up to 100 mg/liter. Growth of survivors after 1 or 2 weeks' exposure was not significantly different from that of controls. Data indicate that un-ionized ammonia may be a potential hazard in red drum culture systems, but that, under normal circumstances, nitrite should cause no problem.

Ammonia is the principal nitrogenous end product excreted by fish. Nitrite is an intermediate in the bacterial nitrification of ammonia and can build up in biological filters of water-recirculation systems. Ammonia and nitrite concentrations can reach sublethal or lethal levels in culture systems incorporating static or recirculating water; thus it is important to identify the tolerance for these products by the organism under investigation.

Un-ionized ammonia is thought to be lipophilic and easily diffusible across respiratory membranes (Kormanik and Cameron 1981). The toxicity of an aqueous ammonia solution is directly related to the amount of un-ionized ammonia (NH_3) present; the proportion of NH_3 to NH_4^+ increases with increasing pH and temperature (Trussell 1972). Other environmental factors have been reported to increase the toxicity of ammonia, including low concentrations of dissolved O_2 and CO_2, and salinities above and below blood isotonicity (EIFAC 1973). High ammonia concentrations can occur in natural waters that receive sewage effluent, industrial wastes or runoff from agricultural lands.

Nitrite can become a problem in fish culture with water-reuse systems. Nitrite in low concentrations can be toxic to fish but increases in dissolved chloride and calcium concentrations, or in pH, reportedly decrease or inhibit its toxicity (Perrone and Meade 1977; Russo et al. 1981).

Although many studies concerning ammonia and nitrite toxicity have been reported, few deal with eggs and larvae of warmwater marine fish, and none address the tolerance of red drum *Sciaenops ocellatus*. Red drum is being investigated for its mariculture potential, because the species adapts well to, and can be spawned in, captivity (Arnold et al. 1979). The objective of this study was to determine the concentrations of ammonia and nitrite that are lethal to early life stages of red drum, and to determine the stage of development at which eggs and larvae are most sensitive to ammonia.

Methods

Eggs were obtained from laboratory spawnings induced by manipulations of photoperiod and temperature cycles (Arnold et al. 1979). Fertilized red drum eggs were exposed to controlled concentrations of ammonium sulfate or sodium nitrite in static tests that were maintained for up to 2 weeks. Culture methods followed those described by Holt, et al. (1981). Three replicates of each concentration and of the control contained an average of 65 eggs each. Eggs hatched within 24 hours and larvae began to feed on the third day posthatch. Mortality was assessed daily. Experiments with older fish included three to four replicates with 6 to 10 fish in each. Percentage hatch, percentage survival, and final standard length were variables used in evaluating the effects of toxicants.

[1] University of Texas Marine Science Institute Contribution 526.

TABLE 1.—*Temporal changes in percentage hatch and survival of red drum larvae continuously exposed to ammonia since the egg stage. The control is indicated by *. Values in the table are means (SE) of three replicates.*

Ammonia concentration (mg/liter NH_3-N)			% survival			
Total	Un-ionized	% hatch	1 day	4 days	7 days	10 days
0.2*	0.02*	94 (1.6)	95 (1.3)	95 (1.0)	52 (8.1)	49 (9.1)
1.5	0.11	93 (1.2)	95 (1.3)	94 (3.0)	52 (7.0)	42 (9.4)
3.6	0.26	93 (2.0)	95 (2.1)	85 (2.0)	60 (6.1)	0
4.5	0.31	96 (1.2)	98 (1.4)	80 (1.4)	38 (14.2)	0
7.7	0.55	97 (1.8)	99 (0.7)	18 (3.2)	0	
20.0	1.44	88 (3.1)	19 (9.9)	0		
100.0	7.20	92 (3.0)	0			
500.0	35.00	12 (2.3)	0			

Concentrations tested were 1.5, 3.5, 5, 10, 20, 100, and 500 mg/liter total ammonia, and 10, 100, and 500 mg/liter nitrite. Total ammonia-nitrogen (NH_3-N) and nitrite-nitrogen (NH_2-N) were determined by methods in Strickland and Parsons (1972), with a Gilford Spectrophotometer Model 250. Concentrations were adjusted throughout the exposure period to keep them within 10% of test doses. Un-ionized ammonia concentrations were calculated from total-ammonia measurements with formulas of Whitfield as modified by Bower and Bidwell (1978), based on pH, temperature, and salinity of the test solutions. Ammonia results are reported as mg/liter of un-ionized ammonia.

The pH of the seawater used in the tests varied from 8.0 to 8.2 and salinity varied from 28 to 32‰. Background concentrations of un-ionized NH_3 were 0.001–0.018 mg/liter; those of nitrite were 0.000–0.011 mg/liter. Temperature was maintained at either 25 or 26 C ± 0.5 C. Dissolved oxygen varied from 5.4 to 6.4 mg/liter during the experiments. The 96-hour median lethal concentration (LC50) and 95% confidence limits for ammonia were calculated by the method of Litchfield and Wilcoxon (1949). Treatment means were compared with the controls by Dunnett's test with a 5% significance level. Statistical analyses were conducted on the arc-sine, square-root transformation of percentages.

Results

Red drum eggs were relatively insensitive to un-ionized ammonia. Mean hatching success was 88–97% through concentrations as high as 7.2 mg/liter; it was substantially reduced at 35 mg/liter (Table 1). Concentrations of 0.55 mg/liter and above caused high mortality of larvae within 24 to 28 hours posthatch; survival times increased with decreasing concentrations below this threshold (Fig. 1). The 96-hour LC50 (95% confidence limits) was 0.39 (0.29–0.53) mg/liter.

Survival of larvae tested at concentrations of 0.26 and 0.31 mg/liter NH_3-N did not differ from that of controls during the first week (Table 1). These larvae, however, particularly those in 0.31 mg/liter, were notably less active than controls and remained on or near the bottom of the chamber beginning 48 hours posthatch. Larvae maintained at 0.11 mg/liter NH_3-N survived as well as controls throughout the experiment, and grew as well through 2 weeks: mean (SE) standard lengths were 4.6 (0.16) mm for controls, and 4.6 (0.19) mm for larvae exposed to 0.11 mg/liter.

Once lethal ammonia concentrations were established for eggs and first-feeding larvae, 3-week-old postlarvae were tested for 1 week to evaluate changes in acute ammonia toxicity with age and development. Postlarvae were less sensitive to ammonia than newly hatched larvae. Whereas a concentration of 0.55 mg/liter killed all newly hatched larvae within 1 week, a slightly higher exposure was tolerated by 3-week-old fish (Fig. 2). Increases in mean standard lengths (±SE) of postlarvae during 1-week exposures to 0.3 mg/liter (1.5 ± 0.21 mm) and 0.6 mg/liter (0.8 ± 0.25 mm) did not differ from those of controls (1.2 ± 0.28 mm), but the experimental fish were more darkly pigmented, which may be an indication of stress.

Nitrite concentrations up to 100 mg/liter did not significantly increase mortality or decrease growth, of newly hatched larvae during the 2-week experiment, compared with control val-

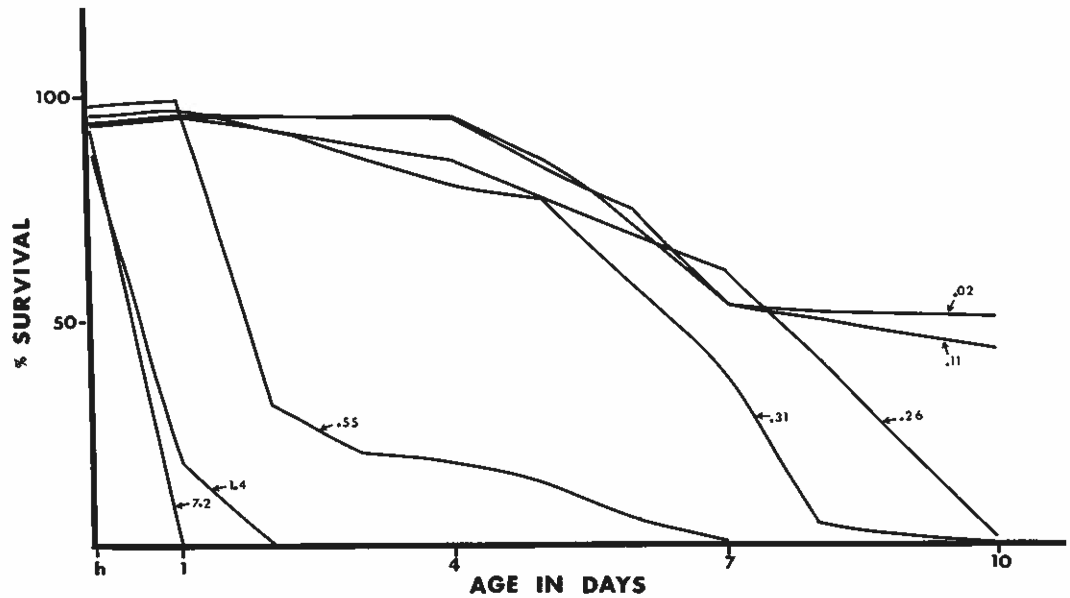

FIGURE 1.—*Percentage hatch (h) and survival over time for newly hatched red drums at concentrations of un-ionized ammonia from 0.11 to 7.2 mg/liter and for controls (0.02 mg/liter).*

ues (Table 2). The 500-mg/liter exposure was lethal to them.

Discussion

The tolerance of red drum eggs to high concentrations of ammonia parallels that reported for salmonid eggs (Rice and Stokes 1975). Rice and Stokes tested early stages of rainbow trout *Salmo gairdneri* and found that fertilized eggs and alevins were resistant to 3.58 mg/liter un-ionized ammonia, but fry (after yolk sac absorption) were very sensitive to concentrations as low as 0.07 mg/liter.

Our results also show that sensitivity to ammonia varies with stage of development and that the most critical time may be during the transition from the yolk-sac stage to actively feeding larvae. The curves in Fig. 1 indicate ammonia has different modes of action depending on concentration. Concentrations of 0.55 mg/liter or greater caused death of more than 50% of the larvae within 24 to 48 hours, whereas concentrations of 0.31 mg/liter or less did not result in death until much later. Curves for low concentrations of ammonia closely paralleled that of controls for 1 week and then dropped precipitously. Red drums begin exogenous feeding on the third day after hatch (Holt$_2$ et al. 1981) and if starved, they will not live longer than 7 days. Losses after the third or fourth day may represent larvae that did not begin feeding after exhaustion of the yolk sac. Low toxic concentrations of ammonia may act on larvae in ways that prevent or interfere with active feeding; larvae were noted near the bottom of the culture chambers one or a few days before death. We found similar results for red drum larvae exposed to low temperature (Holt$_1$ et al.

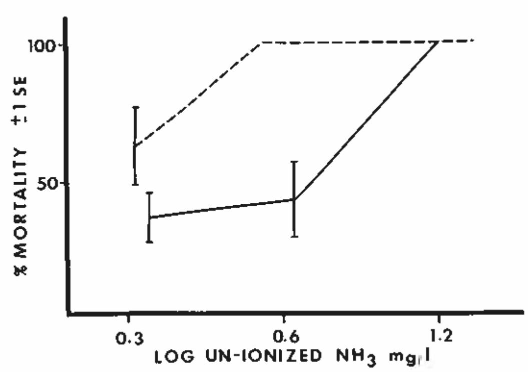

FIGURE 2.—*Ammonia toxicity as a function of ammonia concentration and age of first exposure; newly hatched (broken line) versus 3-week-old postlarvae (solid line) exposed for 1 week.*

TABLE 2.—*Percentage survival and final standard length SL of red drum larvae exposed for 2 weeks to a given concentration of sodium nitrite. Values in the table are means (SE) of three replicates.*

Nitrite concentration (mg/liter NO_2-N)	% hatch	% survival			SL, mm
		48 hours	96 hours	14 days	
0	98 (0.4)	100 (0.0)	100 (0.0)	32 (5.5)	5.6 (0.05)
10	94 (0.4)	100 (0.0)	100 (0.0)	25 (5.2)	6.0 (0.36)
100	98 (0.6)	100 (0.0)	89 (0.0)	16 (3.1)	6.0 (0.35)
500	98 (0.6)	91 (0.0)	14 (0.0)	0 (0.0)	

1981); larvae were inactive, did not feed, and eventually starved to death. Brownell (1980), investigating effects of ammonia on five species of marine fish larvae, found concentrations as low as 0.11 to 0.22 mg/liter reduced first-feeding incidence by 50% after 24 hours' exposure. The 96-hour LC50 for red drum larvae (0.4 mg/liter) is comparable to values for fry of cutthroat trout *Salmo clarki* (0.5–0.8 mg/liter) reported by Thurston et al. (1978) and for five species of British fish (Ball 1967). The short-term lethal concentrations of ammonia for a variety of fish species lie between 0.2 mg/liter for rainbow trout fry and 2.0 mg/liter for common carp *Cyprinus carpio* (EIFAC 1973).

Identification of maximum ammonia concentrations that have no effect on long-term survival and growth is of particular importance in fish culture. Maximum concentrations that permitted normal growth of red drum larvae (0.11 mg/liter) were the same as reported for young turbot *Scophthalmus maximus* (Alderson 1979), for elvers of the European eel *Anguilla anguilla* (Sadler 1981), and near the no-effects concentrations for five species of marine larvae reported by Brownell (1980). Older red drums tolerated higher ammonia concentrations without ill effects, but longer-term studies are needed to confirm this tolerance.

Nitrite toxicity to fish is indirectly correlated with salinity or the amount of dissolved solids in the test water. This is possibly because chloride competes with nitrite for transport across the gills or into tissues (Perrone and Meade 1977), or because the increased pH greatly decreases the formation of the more toxic nitrous acid (Wedemeyer and Yasutake 1978). Brownell (1980) reported high nitrite tolerance for several marine larvae during 24-hour exposures.

Nitrite concentrations in our rearing tanks never exceed 1.0 mg/liter and are generally less than 0.1 mg/liter; nitrite toxicity should not be a problem in red drum mariculture. Nitrite could be of concern if juvenile red drums are raised in fresh water (Crocker et al. 1981) because nitrite toxicity is enhanced by a reduction in chloride and calcium ions.

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