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Effects of Environmental Sodium Chloride on Percent Hatch, Yolk Utilization, and Survival of Channel Catfish Fry

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Abstract.—Only limited research has addressed the effect of salinity on hatching of channel catfish *Ictalurus punctatus* eggs, and no studies have evaluated the effect of salinity on fry development and survival. This study was undertaken to determine the effect of environmental sodium chloride (0, 1, 2, and 4 g/L NaCl) on percent hatch, yolk utilization, and survival of channel catfish fry. Experiments were conducted in recirculating systems using seven egg masses (1–2 d old). Each egg mass was divided into smaller portions which remained undissociated or were dissociated with sodium sulfite ($NaSO_3$). Eggs were incubated until hatching. Wet and dry weights were obtained for sac-fry at 1 and 5 d post-hatch to determine wet weight gain and dry weight loss, and fry were sampled 7 d after initiation of exogenous feeding to determine survival. Percent hatch, yolk utilization, and survival of fry hatched from undissociated eggs were greatest at 1 g/L NaCl. In addition, treatment of eggs with $NaSO_3$ significantly reduced percent hatch at all NaCl levels. Although our results indicate that addition of NaCl to hatchery water supplies can increase production of channel catfish fry, additional research is needed before this practice can be recommended on a commercial basis.

Although the effect of salinity on larval development of euryhaline and stenohaline marine teleosts has been extensively studied, relatively little work has addressed freshwater fishes. However, studies which have been conducted suggest that low salinity may enhance fry production of freshwater fish. For example, low salinity has been shown to increase percent hatch of koi carp *Cyprinus carpio* eggs (Froelich and Engelhardt 1996). Low salinity has also been shown to improve survival of larval silver perch *Bidyanus bidyanus* (Guo et al. 1993), European perch *Perca fluviatilis* (Bein and Ribi 1994), and walleye *Stizostedion vitreum* (Zhmurova and Somkina 1976; Krise et al. 1986). In channel catfish *Ictalurus punctatus* Phelps and Walser (1993) observed that egg masses incubated at salinities (achieved through addition of sea salt) ranging from 0.5 to 2.5 g/L had a greater hatching rate than those incubated in freshwater. However, the effect of salinity on fry development and survival was not investigated.

The addition of calcium chloride is commonly practiced by producers in northwest Mississippi whose hatcheries are supplied by calcium-deficient groundwater. This procedure was largely implemented after Tucker and Steeby (1993) determined that survival and growth of channel catfish fry were improved in water containing at least 4 mg/L calcium. A similar procedure could be utilized by hatchery producers with access to saline groundwater or an inexpensive source of salt such as sodium chloride (NaCl). This study was undertaken to evaluate percent hatch and yolk utilization of channel catfish sac-fry (eleutheroembryo stage; Balon 1975) from egg masses incubated at four levels of salinity achieved through NaCl addition. The effect of environmental NaCl on survival of fry held for 7 d after initiation of exogenous feeding was also determined.

Treatment of channel catfish egg masses with sodium sulfite ($NaSO_3$) has been shown to increase percent hatch of channel catfish eggs, presumably by reducing the incidence of fungal infestations associated with the glycoprotein matrix which binds egg masses (Ringle et al. 1992). Although we used $NaSO_3$ primarily to chemically dissociate portions of egg masses as a means to estimate the number of eggs in undissociated masses, its effect on percent hatch at varying NaCl levels was also determined.

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Materials and Methods

Experiments were conducted using four recirculating systems (one per NaCl level: 0, 1, 2, and 4 g/L NaCl). Each system was equipped with a 0.08-m^3 upwelling bead filter and 24 polyethylene tanks (38-L) supplied with blown air. Tanks had a flow rate of 4.5 L/min. Systems were filled 6 wk prior to the study and reagent grade NaCl and calcium sulfate (Sargent-Welch Co., Buffalo Grove, Illinois, USA) were added to achieve desired levels of salinity and a constant level of calcium (50 mg/L). On 14 June 1995 seven egg masses (1–2 d old) collected from a brood pond (calcium hardness ~100 mg/L as $CaCO_3$) were obtained at a commercial hatchery (26 C, calcium hardness ~50 mg/L). Egg masses were disinfected by immersion in a 100 mg/L povidone iodine solution (Aquadine®, Aquacenter Inc., Leland, Mississippi, USA) for 10 min before placement into hatchery troughs. Egg masses were transported (1.5 h) to the Louisiana State University Aquaculture Research Facility (ARF) in a 300-L hauling container filled with hatchery water and supplied with compressed oxygen. At the ARF, egg masses were placed in a 20-L ice chest containing water from the hauling container. The ice chest was supplied with blown air through an airstone. Egg masses were each divided by scissors into two halves: one to be dissociated with $NaSO_3$, the other to remain undissociated. These portions were further divided into four egg masses, each assigned to one NaCl level. Egg masses were weighed to the nearest 0.1 g and undissociated masses were placed into tanks. Remaining egg masses were immersed in a 0.75% $NaSO_3$ solution (pH 8.8) for approximately 30 sec and each group of dissociated eggs was rinsed three times in fresh water and transferred quickly to form a monolayer for image capture (Bates and Tiersch, in press) using a Microimage Video Systems Model CA2063 CCD automatic video camera (World Video Sales Co., Inc., Boyertown, Pennsylvania, USA). Dissociated eggs were placed into tanks. Eggs were incubated by the forced-air method described in Carmichael et al. (1993). The date and time at which eggs in each tank began to hatch was recorded. At 6 h after hatching, sac fry were siphoned from each tank for image capture and returned within 5 min.

Dissociated eggs and sac-fry were counted manually from digitized images produced by a Seikosha Model VP-1500 II thermal printer (Vital Image Technology, Oakwood Village, Ohio, USA). The number of eggs contained in undissociated masses from each of the seven initial masses was estimated by calculating the number of eggs/g of dissociated egg masses. An average value was obtained which was multiplied by the weight of undissociated masses to determine the number of eggs. Percent hatch for each tank was calculated by dividing the number of sac-fry by the number of eggs stocked initially, and multiplying by 100.

Wet and dry weights were obtained to determine yolk utilization according to the methods described by Tucker and Steeby (1993) with selected modifications. A total of 25 sac-fry were netted from each tank on the day of hatching (~6 h post-hatch) and again at 5 d post-hatch. Fry were held in a small amount of water from the tank. Fry were removed individually by forceps, rinsed with distilled water, blotted dry with absorbent paper, and placed in a preweighed polyethylene weighing dish. The wet weight for each group of 25 fry was recorded to the nearest 0.1 mg using an analytical balance. After weighing, fry were rinsed with distilled water into a dried and weighed aluminum weighing dish. To determine dry weights, samples were dried 72 h at 104 C, cooled in a dessicator, and weighed to the nearest 0.1 mg. Wet weight gain and dry weight loss of fry was determined by calculating the difference in each parameter between sampling dates.

The date and time when fry began swimming at the surface seeking feed was re-

corded. Fry were held in experimental systems for 7 d after initiation of exogenous feeding. During this period fry were fed a 50% protein, commercial fry feed every 3 h using automatic feeders (Sweeney Enterprises, Inc., Boerne, Texas, USA) suspended above each tank. At the termination of the study, fry from each tank were removed and sampled volumetrically according to the method described by Tucker and Robinson (1992). Percent post-hatch survival was determined by dividing the number of fry recovered 7 d after initiation of exogenous feeding by the number of sac-fry recovered 6 h after hatching, and multiplying by 100.

Water quality parameters (salinity, calcium, temperature, dissolved oxygen, pH, total ammonia-nitrogen, nitrite-nitrogen, alkalinity) were measured daily throughout the experimental period. Salinity and temperature were measured with a YSI Model 30 meter (Yellow Springs Instrument Company, Yellow Springs, Ohio, USA). Dissolved oxygen was measured with a YSI Model 51B meter. The pH was measured with a pH pen (Hach Company, Loveland, Colorado, USA). Calcium, total ammonia-nitrogen (TA-N), nitrite-nitrogen, and alkalinity were measured with Hach water quality test kits. Concentrations of un-ionized ammonia-nitrogen (UIA-N) were determined using the tables in Emmerson et al. (1975) and Bower and Bidwell (1978). Salinity and calcium were maintained within 10% of nominal concentrations. Ranges for remaining water quality parameters were: temperature, 27–29 C; pH, 8.1–8.7; dissolved oxygen, 7.7–8.6 mg/L; and alkalinity, 205–222 mg/L as $CaCO_3$. Total ammonia and nitrite-nitrogen levels remained below 0.5 and 0.1 mg/L, respectively. UIA-N levels were below 0.10 mg/L. Parameters (with the exception of salinity) did not differ among recirculating systems throughout the experimental period ($P < 0.05$, 1-factor ANOVA, df = 55).

Data were analyzed using analysis of variance in a randomized complete block design with the source of variation associated with the seven egg masses serving as the blocking factor. Percentage data were normalized prior to analysis by arc-sin-square-root transformation. Percent hatch and initial wet and dry weight data were analyzed in a 2 × 4 factorial arrangement (Factor 1 = NaCl; Factor 2 = $NaSO_3$). Main treatment effects (NaCl level and exposure or non-exposure to $NaSO_3$) were tested for statistical significance as well as the NaCl × $NaSO_3$ interaction, where appropriate, at $P < 0.05$. When significant differences in the main effects were shown to exist, Fisher's LSD was used to compare treatment means. All statistical analyses were performed using the statistical software Data Desk 4.2 (Velleman 1992).

Results

Considerable variation in percent hatch existed among eggs obtained from each egg mass. For example, percent hatch of undissociated eggs incubated at 0 g/L NaCl ranged from 10 to 65%; percent hatch at 1 g/L NaCl ranged from 39 to 86%. Although the NaCl × $NaSO_3$ interaction was not significant ($P = 0.1780$), percent hatch of channel catfish sac-fry was significantly affected ($P < 0.0001$) by NaCl level and $NaSO_3$ treatment (Fig. 1). Mean percent hatch of undissociated eggs ranged from 21 to 57% and was greatest at 1 g/L NaCl. No differences regarding percent hatch of undissociated eggs existed between 0 and 2 g/L NaCl. Percent hatch of undissociated eggs incubated at 4 g/L NaCl was significantly reduced ($P = 0.0014$) with respect to 0 g/L NaCl.

Treatment of egg masses with $NaSO_3$ significantly reduced ($P < 0.0001$) percent hatch relative to that of undissociated eggs at all NaCl levels. In addition, dissociated eggs began hatching ~24 h prior to undissociated eggs from the same egg mass at all NaCl levels ($P < 0.0001$). Mean percent hatch of dissociated eggs ranged from 2 to 27% and was lowest at 2 and 4 g/L NaCl. Percent hatch of dissociated eggs incubated

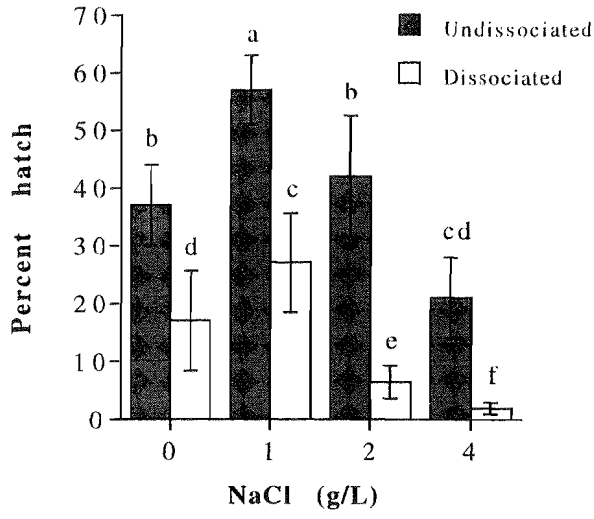

FIGURE 1. *Percent hatch of channel catfish sac-fry from undissociated and dissociated (sodium sulfite-treated) egg masses incubated at four sodium chloride (NaCl) levels. Values sharing the same letter were not significantly different (P < 0.05). Vertical bars represent mean ± SE.*

at 1 g/L NaCl was significantly greater than that at 0 ($P = 0.0056$), 2 ($P < 0.0001$), and 4 g/L NaCl ($P < 0.0001$). At 2 d post-hatch, survival of sac-fry hatched from dissociated eggs was too low to permit further collection of data.

Initial wet and dry weights of sac-fry from undissociated eggs did not differ due to NaCl level, although wet weight gain ($P < 0.0001$) and dry weight loss ($P = 0.0463$) were significantly affected by NaCl (Table 1). Wet weight gain was greatest for fry held at 1 and 2 g/L NaCl. Dry weight loss was greatest at 1 g/L NaCl. Sac-fry held at 4 g/L NaCl began exogenous feeding at the surface ~24 h later than fry held in other NaCl levels ($P < 0.0001$).

Only initial wet and dry weights were obtained for sac-fry hatched from dissociated eggs and no differences existed regarding these parameters due to environmental NaCl. Although the NaCl × $NaSO_3$ interaction was not significant ($P = 0.2242$), initial wet weights of sac-fry hatched from dissociated eggs were significantly lower ($P < 0.0001$) than those of sac-fry hatched from undissociated eggs at each NaCl level.

Survival of fry hatched from undissociated eggs 7 d after initiation of exogenous feeding was significantly affected ($P = 0.0067$) by environmental NaCl (Fig. 2). Mean survival of fry held at 1 g/L NaCl was 99%, which was at least 10% greater than that of fry held at 0 or 2 g/L NaCl. Survival of fry held at 4 g/L was estimated to be < 20%. Fry held at this NaCl level exhibited clinical signs of an infectious disease beginning 3 d after initiation of exogenous feeding. At this time 10 fry were obtained from each system for disease diagnosis at the Fish Diagnostic Laboratory, School of Veterinary Medicine, Louisiana

TABLE 1. *Initial wet and dry weights (mg; mean ± SE) for 25 channel catfish sac-fry hatched from undissociated or dissociated (sodium sulfite-treated) egg masses (N = 7) at four sodium chloride (NaCl) levels and wet weight gain and dry weight loss of sac-fry hatched from undissociated egg masses and held for 5 d at four NaCl levels. Values across columns sharing the same letter were not significantly different (P < 0.05).*

	NaCl (g/L)			
Variable	0	1	2	4
Undissociated				
Initial wet weight	336.4 ± 10.4 a	351.6 ± 13.7 a	345.1 ± 10.6 a	333.8 ± 12.1 a
Wet weight gain	244.8 ± 20.2 a	276.0 ± 15.9 b	276.2 ± 23.1 b	197.0 ± 16.8 c
Initial dry weight	112.9 ± 4.4 a	122.0 ± 5.3 a	114.2 ± 4.4 a	118.1 ± 6.1 a
Dry weight loss	11.3 ± 1.8 a	22.0 ± 3.6 b	17.0 ± 2.5 ab	11.9 ± 2.8 a
Dissociated				
Initial wet weight	330.6 ± 13.5 a	326.5 ± 14.9 a	322.7 ± 12.0 a	304.0 ± 21.6 a[1]
Initial dry weight	119.9 ± 7.7 a	120.3 ± 6.7 a	123.3 ± 6.8 a	110.7 ± 8.9 a[1]

[1] Mean values from four dissociated egg masses.

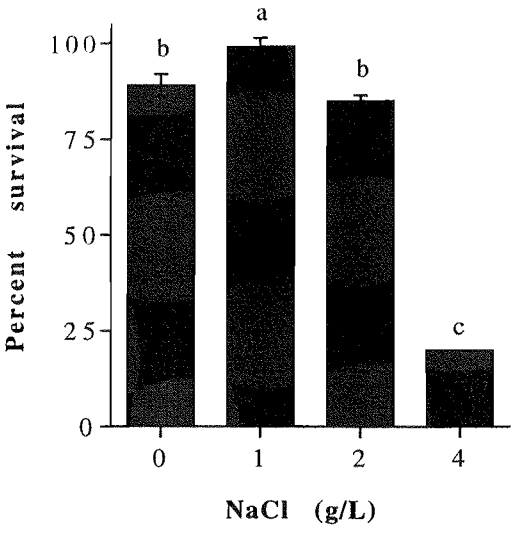

FIGURE 2. *Percent survival of channel catfish fry hatched from undissociated eggs and held for 7 d after initiation of exogenous feeding at four sodium chloride (NaCl) levels. Survival of fry held at 4 g/L NaCl was not calculated but was <20%. Values sharing the same letter were not significantly different (P < 0.05). Vertical bars represent mean ± SE.*

State University. Results indicated that only fry held at 4 g/L NaCl were infected with *Vibrio cholerae*.

Discussion

The high degree of variability regarding percent hatch of channel catfish egg masses observed in this study is consistent with findings of previous workers (Broussard and Stickney 1981; Ringle et al. 1992). However, these differences did not obscure effects due to environmental NaCl and $NaSO_3$ treatment.

The enhanced percent hatch of channel catfish eggs due to low levels of environmental NaCl observed in this study is similar to the findings of Phelps and Walser (1993). In their study, incubation of eggs at salinities ranging from 0.5 to 2.5 g/L (obtained through addition of sea salt) increased percent hatch relative to eggs incubated in freshwater. In addition, there was no decrease in percent hatch of eggs incubated at a salinity of 5 g/L. However, results presented here show that while percent hatch of undissociated eggs was increased at 1 g/L NaCl relative to 0 g/L NaCl, no difference was observed at 2 g/L NaCl, and percent hatch was reduced at 4 g/L NaCl. Similar findings were reported for koi carp eggs exposed to 0, 1, 2.5, or 5 g/L NaCl for 60 min (Froelich and Engelhardt 1996). Although treatments of 1 and 2.5 g/L NaCl improved percent hatch, treatment of eggs with 5 g/L NaCl reduced hatch relative to untreated eggs.

The difference in results between those reported by Phelps and Walser (1993) and our study may be due to differences in water quality. For example, calcium concentrations were maintained at 50 mg/L at all NaCl levels in the present study but were not reported by Phelps and Walser (1993). Although calcium is not thought to be a significant factor affecting egg hatching, it does affect survival of recently-hatched sac-fry (Tucker and Steeby 1993). Since seawater has a calcium concentration of 400 mg/L (Spotte 1979), a salinity of 0.5 g/L achieved by addition of sea salt would have a calcium concentration of ~6 mg/L. This level is above the minimal calcium concentration recommended by Tucker and Steeby (1993) to ensure optimal survival. Therefore, if freshwater used in their study was deficient in calcium, comparison of salinity treatments versus freshwater may have been confounded. In addition, the concentration of other ions present would have varied among salinities achieved through sea salt addition.

Previous studies have suggested that improved hatching of fish fry at increased salinities is due to inhibition of fungal infections of eggs such as those caused by *Saprolegnia* spp. (Taylor and Bailey 1979; Edgell et al. 1993; Phelps and Walser 1993; Froelich and Engelhardt 1996). Fungal growth was not evident in the present study at any NaCl level, suggesting that another factor associated with salinity contributed to increased hatching of channel catfish eggs observed in this study. Freshwater teleosts and their eggs are hyperosmotic to their environment and therefore must compensate for water gain and salt loss to main-

tain homeostasis (Alderdice 1988). While mechanisms controlling osmoregulation of teleost embryos are under investigation, it is currently believed that homeostasis is maintained by the low permeability of the plasma membrane to water and ions following fertilization coupled with the protective effect of the perivitelline fluid surrounding the plasma membrane (Alderdice 1988). The chorion of fertilized teleost eggs, unlike the plasma membrane, is highly permeable to water and ions (Holliday 1969) and changes in the ionic composition of the incubation medium are reflected by changes in the perivitelline fluid (Shanklin 1954; Weisbart 1968; Rudy and Potts 1969). Rudy and Potts (1969) observed that eggs of Atlantic salmon *Salmo salar* concentrated sodium ions in the perivitelline fluid when placed in media containing dilute concentrations of NaCl. This would tend to reduce the concentration gradient across the plasma membrane, and consequently the potential rate of loss of ions from the yolk (Alderdice 1988). Therefore, an environment containing low levels of NaCl may be osmotically beneficial to the developing eggs of freshwater teleosts such as channel catfish. This may explain the increased percent hatch observed for eggs incubated in a medium of 1 g/L NaCl.

In addition to percent hatch, yolk utilization by sac-fry and post-hatch survival of fry was also enhanced due to increased environmental NaCl. As sac-fry develop, low water content (high density) yolk is metabolized and converted to high water content (low density) tissue resulting in wet weight gain and dry weight loss over time (Heming and Buddington 1988; Tucker and Steeby 1993). Each of these parameters was greatest for sac-fry held at 1 g/L NaCl indicating that development of sac-fry is improved in low salinity environments. However, as with percent hatch, increased yolk utilization occurred only at low NaCl levels and was not enhanced at 4 g/L NaCl relative to freshwater. Because yolk absorption by larval marine fish has been shown to be affected by salinity (May 1974; Santerre 1976), it is reasonable to assume that variations in salinity would also affect development of larval freshwater fish. While the reason for the increased yolk utilization of sac-fry held at 1 g/L NaCl observed in this study is not known, it may have been due to reduced metabolic costs associated with osmoregulation.

A similar explanation could be offered to describe the increased survival of fry held at 1 g/L NaCl. Krise et al. (1986) observed that survival of larval walleye reared at a salinity of 1 g/L was greater than that of larvae reared in freshwater. Similarly, Bein and Ribi (1994) reported that survival of larval European perch was improved at salinities of 0.6 and 1.2 g/L. The authors of these studies suggested that the osmoregulatory capacity of larval freshwater fish may be enhanced at low salinities. Although Guo et al. (1993) reported that post-hatch survival of silver perch larvae held at a salinity of 6 g/L was greater than that of larvae held in freshwater, our results and those of Krise et al. (1986) and Bein and Ribi (1994) suggest that the beneficial effect of salinity on fry survival occurs only at low levels of this parameter.

Our observations regarding the effect of $NaSO_3$ treatment on percent hatch contrast with the findings of Ringle et al. (1992). In their study percent hatch of dissociated eggs incubated in upwelling hatching jars under freshwater conditions was as much as 20% greater than that of undissociated eggs. It is possible that the reduced percent hatch of dissociated eggs relative to undissociated eggs observed in our study may have been due to experimental procedures utilized to enumerate eggs. For example, in the process of monolayer formation, dissociated eggs were subjected to more handling than undissociated eggs. In addition, to obtain accurate estimates of the number of eggs contained in undissociated egg masses, we did not account for the weight of the egg matrix. However, based on previous experience using $NaSO_3$ in our laboratory, we

minimized exposure time to $NaSO_3$, eggs were rinsed thoroughly after treatment, and egg masses were treated with only half the $NaSO_3$ concentration commonly employed (Isaac and Fries 1991; Ringle et al. 1992). Furthermore, the matrix contributes to only approximately 17.5% of the weight of 1–2 d old egg masses (Walser and Phelps 1993). At any rate, while $NaSO_3$ treatment may be beneficial in hatcheries with a history of fungal problems, our results showed that $NaSO_3$ treatment significantly reduced percent hatch, and its effects were excaberated by increasing environmental NaCl. In addition, sac-fry from eggs treated with $NaSO_3$ hatched ~24 h earlier, were less developed, and (as evidenced by decreased initial wet weight), contained a larger percentage of yolk at hatching than did undissociated eggs. The detrimental effects of $NaSO_3$ treatment may have been caused by removal of the protective glycoprotein matrix from eggs allowing premature rupture of the chorion (Ringle et al. 1992), premature hatching of eggs, and poor survival of sac-fry. Damage to the chorion may also have reduced the osmoregulatory capacity of eggs, especially those incubated at higher NaCl levels. Indeed, dechorionated eggs of *Oncorhynchus* spp. have been shown to be less tolerant to increased salinities than eggs with intact chorions (Weisbart 1968).

Our results suggest that production of channel catfish fry can be enhanced by adjusting the salinity of hatchery water supplies. However, further research is needed before this procedure can be recommended on a commercial basis. For example, NaCl concentrations lower than 1 g/L may be sufficient to improve fry production and could be evaluated as a means to reduce costs and environmental concerns associated with salt addition.

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