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Responses of cobia *Rachycentron canadum* larvae to abrupt or gradual changes in salinity

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Abstract

Cobia *Rachycentron canadum* has recently been recognized as a potential candidate for aquaculture because this species exhibits high growth rates during the larval and juvenile stages. A series of salinity tolerance tests were performed on larval cobia in order to identify the salinity requirements of this species during culture. The effect of spawning salinity on larval tolerance is also discussed. The 18-h survival of cobia larvae 3, 5, 7 and 9 days post-hatch (dph) following abrupt transfer to salinities ranging from 4 to 48 ppt was evaluated using logistic regression. The salinity range within which 90% of the larvae would be expected to survive appeared to be age-dependent and was narrowest at 3 dph (20.1–35.6 ppt) and wider at 7 and 9 dph (7.5–32.8 ppt). The 18-h tolerance of larvae to abrupt changes in salinity was unaltered by spawning salinities of 28.0 and 36.5 ppt. In the second part of the study, rearing salinities were dropped by 5 ppt day^{-1} from 32–34 ppt (control) to 5, 10, 15 and/or 20 ppt beginning on 1, 4, 7, 10 or 13 dph. Larval survival from hatching through 10 days following the initial drop in salinity was significantly ($P<0.05$) lower (<2%) in the low salinity treatments than the control (12–15%) when the salinity drop was initiated 1 and 4 dph. No significant differences in larval survival were detected between the control (12.5%) and 20 ppt treatment (8.9%) when the salinity drop began on 7 dph but survival in the 10 ppt treatment (3.2%) was significantly lower than the control. When the salinity drop was initiated on 10 dph, no significant differences in survival (10.7–14.7%) were detected among treatments. Finally, no significant differences in survival (9.6–15.4%) were found when the salinity drop was initiated 13 dph and terminated 22 dph. However, when a similar study was extended to 28 dph survival from 13 to 28 dph was significantly lower in the 5 (49.4%) and 10 (72.5%) ppt treatments than the control (96.5%) due to disease. No significant differences in standard length were observed for larvae within each experiment irrespective of rearing salinity. The results of this study indicate that rearing cobia larvae in salinities as low as 15 ppt may be possible beginning 13 dph.

Keywords: Cobia; Salinity tolerance

1. Introduction

Cobia are distributed worldwide, with the exception of the eastern Pacific Ocean, in tropical, subtropical and temperate waters (Briggs, 1960) and are a highly prized recreational species in the Gulf of Mexico and the western Atlantic ocean (Shaffer and Nakamura, 1989). There has been considerable interest in developing reliable methods for spawning and rearing cobia in captivity because they are hardy, fast growing fish reaching weights of 6–10 kg in approximately 1 year (Liao et al., 2004). In Taiwan, roughly 80% of the open

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ocean cages are dedicated to cobia aquaculture (Liao et al., 2004). In the United States, captive broodstock have been successfully spawned via hormonal injection (Franks et al., 2001) and photothermal manipulation (Arnold et al., 2002) in recirculating aquaculture systems and the development of techniques for rearing larvae in recirculating systems is ongoing (Faulk and Holt, 2003, 2005; Hitzfelder, 2004). There is additional interest in the grow-out of cobia in commercial ponds where ambient salinities are naturally low including inland areas and those adjacent to low salinity bays and estuaries such as those located along the northern Texas coast (Longley, 1994). Recent studies conducted on juvenile cobia suggest that they are able to grow well in salinities as low as 5 ppt although they may be more susceptible to diseases and have additional nutritional requirements (Denson et al., 2003; Resley et al., in press). Hassler and Rainville (1975) reported similar hatch rates for cobia eggs collected from the wild and subsequently set in salinities ranging from 19 to 35 ppt. The authors also state that the growth and survival of larvae in salinities of 24 ppt was similar to that of larvae reared at 33 or 35 ppt but no information is provided regarding larval age. A more comprehensive study is needed to examine the tolerance of cobia larvae to a broad range of salinities throughout the larval period as such information is important for the development of successful pond culture of this species in brackish water.

Marine fish larvae are especially sensitive to short- and long-term changes in salinity. Exposure of larvae to changes in salinity may result in morphological deformities, reduced growth and decreased larval survival (Swanson, 1996; Lein et al., 1997; Kucera et al., 2002). Larvae are relatively undeveloped at hatching and do not possess the osmoregulatory abilities of juvenile and adult fishes such as gills, gut, kidneys and urinary bladder. Instead, newly hatched larvae rely on their relatively impermeable skin and cutaneous chloride cells to maintain osmotic balance during periods of fluctuating salinity (Alderdice, 1988; Rombough, 2004). The tolerance of marine fish larvae to low salinities varies throughout the larval stage with the differentiation of structures important in osmoregulation (Alderdice, 1988). Spawning salinity has also been shown to influence the tolerance of larvae to sudden changes in salinity (Kucera et al., 2002). The purpose of this study was to examine the effects of sudden and gradual changes in salinity on the growth and survival of cobia at different periods during the larval stage. The effect of spawning salinity on larval tolerance is also discussed.

2. Materials and methods

2.1. Source of eggs and larvae

Cobia eggs and larvae used in this study were acquired from two independent sources. The majority of the eggs were obtained from 2 tanks of broodstock spawned in recirculating systems (Arnold et al., 2002) at the Fisheries and Mariculture Laboratory of the University of Texas Marine Science Institute in Port Aransas, Texas (UTMSI). Following removal from the broodstock tank, cobia eggs were set in a solution of 1% formalin for 20 min (Douillet and Holt, 1994) and subsequently transferred to independent 150-l conical rearing tanks (800 eggs $tank^{-1}$). Each tank was equipped with its own internal biological filter to maintain water quality as described by Holt (1993). Hatch rates for each spawn were determined by placing 20 eggs in each of 5, 1-l beakers and recording the number of larvae hatched in each beaker the following morning. In addition, a group of larvae was obtained from the Aquaculture Center of the Florida Keys, Inc. (FL; Marathon, Florida, USA). Larvae were shipped overnight from Florida to UTMSI and were 2 days post-hatch (dph) upon arrival. Larvae were carefully moved into 150-l conical rearing tanks (described above) with the use of a 250-ml beaker (200 larvae $tank^{-1}$).

2.2. Larval rearing

For each trial, rearing tank salinities were initially adjusted to match that of spawning, temperature was maintained at 26.5±0.3 °C, mean dissolved oxygen was 6.4±0.2 mg l^{-1} and photoperiod was set to a 14-h light/10-h dark cycle. Salinity, temperature and dissolved oxygen were measured with the use of a YSI model 30 meter and a YSI model 55 meter, respectively (Yellow Springs Instruments Inc., Yellow Springs, Ohio, USA). Ammonia–nitrogen (Solorzano, 1969), nitrite–nitrogen (Strickland and Parsons, 1972) and pH (Accumet AR15 pH meter; Fisher Scientific, Pittsburgh, Pennsylvania, USA) were measured at least once a week with mean values of <0.3 mg l^{-1}, <0.1 mg l^{-1} and 7.9±0.1, respectively. Although the effects of ammonia and nitrite concentrations on the growth and survival of cobia larvae are unknown, the levels measured in this study are within acceptable ranges reported for other species (Holt and Arnold, 1983).

Standard length measurements (n=20) were taken 3 dph using a Wild Heerbrugg stereomicroscope, Summa Sketch III digitizing tablet (GTCO CalComp, Inc., Columbia, Maryland, USA) and Sigma Scan software

(Jandel Corporation, San Rafael, California, USA). Prior to measurement, larvae were anesthetized with 0.1% tricaine methanesulfonate. This procedure was used for all standard length measurements taken over the course of this study except for fish greater than 16 mm which were measured using a vernier caliper (Fowler, Chicago, Illinois, USA).

Exogenous feeding commenced on the third day after hatching at which time larvae averaged 4.6±0.1 mm standard length. Cobia larvae were fed according to the protocol described by Faulk and Holt (2005). Larvae were fed rotifers *Brachionus plicatilis* at concentrations of 4–5 ml^{-1} on 3–7 dph, newly hatched *Artemia* nauplii (<0.5 ml^{-1}) on 7–8 dph and 24 h enriched *Artemia* nauplii (0.5–1 ml^{-1}) 8 dph through the end of the experiment. Rotifers were harvested the day before feeding, placed in a 20-l plastic cylinder (400 rotifers ml^{-1}) and enriched over a 12-h period with Algamac 2000 (Aquafauna Bio-Marine; Hawthorne, California, USA) at a dose of 0.3 g per 1×10^6 rotifers. *Artemia* cysts were incubated for 24 h at which time nauplii were separated from empty cysts and either fed directly to the larvae or placed in a 10-l plastic cylinder (50–100 nauplii ml^{-1}) for enrichment over a 24-h period (Algamac 2000; 0.2 g per ~100,000 *Artemia* nauplii).

2.3. Tolerance to abrupt salinity change

Acute salinity tolerance experiments generally followed the protocol detailed in Banks et al. (1991). This test was performed on larvae from 1 spawn at UTMSI and 1 spawn from FL 3, 5, 7 and 9 dph. Larvae obtained from UTMSI and FL were spawned and reared in 28.0 and 36.5 ppt, respectively. Standard length measurements were taken on a subset of larvae ($n=20$) removed from the rearing tanks on each test day prior to setting up the experiments. Larvae sampled for standard length measurements were not subsequently used in the salinity tolerance experiments. Experiments were performed in 1-l glass beakers with three replicates per test salinity. Test salinities ranged from 4 to 48 ppt with intervals of 4 and 8 ppt and were prepared by adding dechlorinated tap water or synthetic sea salt to natural seawater. Tap water was dechlorinated with the use of sodium thiosulfate (10–15 mg l^{-1}). Larvae were fed in the morning prior to commencement of the experiment but they were not fed during the 18-h acute salinity tolerance test. Eighteen hours was chosen to avoid non-salinity effects (i.e. starvation) on larval survival. Larvae were collected from the conical rearing tanks in a 250-ml beaker and transferred to test beakers using a plastic pipette (10 larvae $beaker^{-1}$). This procedure transferred only a minimum amount of water and did not significantly alter the salinity of each test beaker. After 18 h, the number of larvae surviving in each beaker was recorded.

2.4. Tolerance to gradual salinity change

Acclimated salinity tolerance experiments were initiated on larvae obtained from UTMSI on 1, 4, 7, 10 and 13 dph. A separate spawn was used for each experiment. Larvae were reared in 150-l conical tanks (described above) with 3 replicate tanks per treatment and the control tanks were maintained at the spawning salinity (32.0–34.0 ppt). On the first day of the salinity drop, salinities in each of the treatment tanks were decreased from that of the control to 30 ppt with the addition of dechlorinated tap water. Subsequently, salinities were dropped by 5 ppt day^{-1} until the target salinities were reached for each treatment. Salinity drops were performed 2–3 h after the larvae were fed in the morning and were accomplished by draining an appropriate volume of water from each tank and slowly adding dechlorinated tap water over a period of 5–6 h. A similar method was used to exchange water in the control treatments except that the water added back to the tanks was the same salinity as that taken out. Throughout the study, salinity was monitored daily and dechlorinated tap water added as needed to maintain the target salinities (±0.2 ppt). The experiments were terminated 10 days following the initial drop in salinities at which time a subset of larvae ($n=20$) was removed from each tank and measured for standard length. Survival was estimated by taking into consideration the number of eggs set in each tank, hatch rate and the number of larvae remaining at the end of the experiment. Finally, water samples (100 ml $tank^{-1}$) were collected for determination of total and calcium hardness via EDTA titration with the use of Hach hardness reagent sets (Hach Company, Loveland, Colorado, USA).

One final experiment was conducted on larvae obtained from UTMSI to further examine the effects of salinities below 15 ppt on cobia following an initial drop in salinity on 13 dph. This study followed the procedure outlined above except that the salinity drop was performed at a rate of 10 ppt day^{-1} and the study was terminated on 28 dph. Percent survival for this experiment was calculated differently than in the previous trials. The exact number of fish in each tank on the first day of the salinity drop (13 dph) was divided by the number remaining in each tank at the end of the experiment (28 dph) and multiplied by 100.

2.5. Statistical analysis

A two-way ANOVA was used to test for differences in standard length among larvae in the acute salinity tolerance tests with spawning salinity (28 vs. 36.5 ppt) and age (3, 5, 7 and 9 dph) as the main effects and standard length as the dependent variable. The response of cobia larvae to acute changes in salinity was analyzed independently for salinities below (hyposaline) and above (hypersaline) the spawning salinity. For each variable, the LC_{10} and LC_{50} values were calculated using logistic regression with salinity as the dose and number surviving as the response. In this study, LC_{10} and LC_{50} values represent the salinity at which 10% or 50% mortality of the larvae would be expected 18 h after acute changes in salinity. Data obtained from the logistic regression were subsequently analyzed by one-way ANOVA followed by a Tukey's test for multiple comparisons of means to test for significant differences ($P<0.05$) in salinity tolerance among ages. A one-way ANOVA followed by a Tukey's test for multiple comparisons of means was used to test for significant differences ($P<0.05$) among treatments in larval growth and survival for each experiment examining the tolerance of larvae to gradual changes in salinity. Data were transformed as necessary to meet the normality assumptions of ANOVA. Linear regression was performed to examine the relationship between calcium or total hardness and salinity. Data are expressed as mean ± standard deviation. Statistical analyses were performed using SYSTAT 10.0 (SPSS Inc., 2000, Chicago, IL, USA).

3. Results

3.1. Tolerance to abrupt salinity change

The mean standard length of cobia larvae increased with age but was not significantly different ($P>0.05$) between trials. Mean standard lengths across both trials were 4.5±0.1, 4.9±0.1, 6.1±<0.1 and 6.9±0.1 mm for cobia larvae 3, 5, 7 and 9 dph, respectively. Within all ages tested, the 95% fiducial limits associated with the LC_{10} and LC_{50} values of each trial overlapped, indicating that there were no significant differences in the acute salinity tolerance of larvae spawned at 28.0 ppt and those spawned at 36.5 ppt. Therefore, data from each spawn were pooled to test for significant difference in salinity tolerance among ages. A significant difference ($P<0.05$) was detected in the hyposaline LC_{10} values between larvae 3 (20.1 ppt) and 7 (7.5 ppt) dph whereas no differences were found among the remaining ages (Fig. 1A). No significant differences in hyposaline LC_{50} values were found (Fig. 1A) with values ranging from 5.4 to 9.9 ppt. Hypersaline LC_{10} and LC_{50} values ranged from 30.2 to 35.6 ppt and 36.3 to 41.9 ppt, respectively, and were not significantly different among ages (Fig. 1B).

3.2. Tolerance to gradual salinity change

Daily observations and counts of prey items in the rearing tanks indicated that neither prey concentration nor position in the tank was different among treatments across all studies. In addition, larvae were observed actively feeding on live prey before, during and after the salinity drops for all trials.

When the salinity drop was initiated 1 dph, the percent survival of cobia larvae 10 dph was significantly lower for all low salinity treatments compared to the control (Fig. 2). Mean standard lengths of larvae in the 10 and 15 ppt treatments could not be calculated due to complete mortality of larvae in all (10 ppt) or 2 out of the 3 replicate tanks (15 ppt). However, no significant difference in standard length was detected between the 20 ppt and control treatments (Table 1). Similar results were obtained when the salinity drop commenced 4 dph where percent survival at 13 dph was significantly higher in the control treatment compared to the 10, 15 and 20 ppt treatments (Fig. 2) and no significant differences in standard length were detected among treatments (Table 1).

The percent survival of cobia larvae at 16 dph was significantly lower in the 10 ppt treatment than the control when the salinity drop was initiated 7 dph (Fig. 2). Percent survival in the 20 ppt treatment fell within this range and was not significantly different than survival in the 10 ppt or control treatments. No significant differences in standard length were found among treatments (Table 1).

When the salinity drop was initiated on 10 dph, no significant differences in growth (Table 1) or survival (Fig. 2) were detected among treatments on 19 dph. However, fish (2–3 $tank^{-1}$) in the 10 ppt treatment were discolored (white splotches covering the fish) and/or presented signs of fungal infections beginning 4–5 days after the rearing tanks reached 10 ppt. On the day after abnormal fish were observed in a tank, they were found dead in the morning and additional fish had developed similar signs of disease. In order to further assess the effects of this mortality on long-term survival, the fish were held in the treatment salinities for an additional 9 days (28 dph). The number of fish

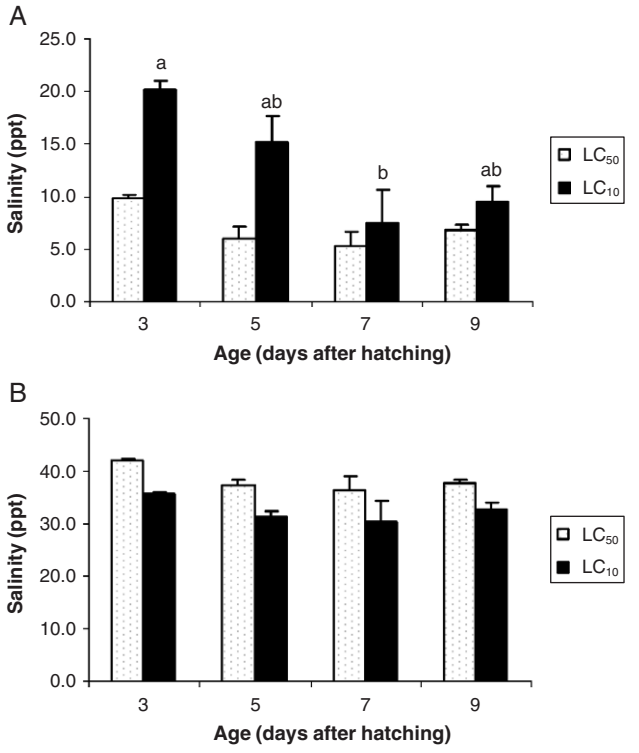

Fig. 1. Mean LC_{10} and LC_{50} salinity values (+standard deviation; $n=2$) for the acute salinity tolerance of cobia larvae 3, 5, 7 and 9 dph. Values were obtained after 18-h exposure to sudden changes in salinity below (A) and above (B) that of spawning. Letters denote significant differences ($P<0.05$) among LC_{10} hyposalinity values.

surviving from 19 to 28 dph was calculated and was significantly higher in the 15, 20 and control treatments (74.0–81.9%) than the 10 ppt treatment (35.5%; data not shown).

No significant differences in growth (Table 1) or survival (Fig. 2) were found when the salinity drop was initiated 13 dph and terminated 22 dph. However, when the study was extended beyond 22 dph the survival of fish from 13 to 28 dph (Fig. 3) was significantly lower in the 5 and 10 ppt treatments than the control while no significant differences in standard length were observed (Table 1). In both experiments, fish in the 5 and 10 ppt treatments developed similar signs of disease as seen in the

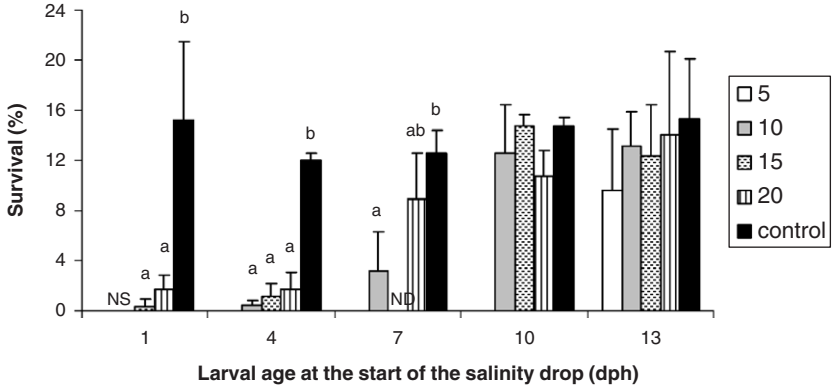

Fig. 2. Percent survival (+standard deviation; $n=3$) of cobia reared in different salinities for 10 days following an initial salinity drop (5 ppt day^{-1}) on 1, 4, 7, 10 or 13 dph. Letters denote significant differences ($P<0.05$) within age groups. NS=no survival at 10 ppt. ND=no data at 15 ppt.

Table 1
Mean standard length (±standard deviation; $n=3$) of cobia larvae reared in different salinities following an initial salinity drop on 1, 4, 7, 10 or 13 dph

Larval age (dph)		Rate of drop (ppt day^{-1})	Treatment	Standard length (mm)
Initial salinity drop	Termination			
1	10	5	20	6.4±0.2
			Control	7.1±0.4
4	13	5	10	7.6±0.3
			15	7.3±0.7
			20	7.5±0.3
			Control	7.8±0.1
7	16	5	10	11.3±0.1
			20	11.0±0.4
			Control	11.4±0.4
10	19	5	10	20.6±1.0
			15	19.3±0.7
			20	19.8±1.6
			Control	19.7±0.3
13	22	5	5	23.0±2.2
			10	21.3±1.3
			15	22.1±2.6
			20	21.7±4.0
			Control	21.0±2.1
13	28	10	5	36.0±3.5
			10	34.0±2.1
			Control	33.6±1.8

No significant differences were found ($P>0.05$) within age groups.

previous experiment beginning 3 days after the rearing tanks reached 10 ppt.

Both total and calcium hardness were positively correlated with salinity (Fig. 4). Total hardness ranged from 151.5±18.0 mg l^{-1} as $CaCO_3$ in dechlorinated tap water to 982.5±33.6 mg l^{-1} as $CaCO_3$ in full strength seawater. Calcium hardness was also lowest in dechlorinated tap water (180.3±16.8 mg l^{-1} as $CaCO_3$) and highest in full strength seawater (6181.3±294.2 mg l^{-1} as $CaCO_3$).

4. Discussion

The tolerance of cobia larvae 3, 5, 7 and 9 dph to abrupt changes in salinity was not affected by spawning salinities of 28.0 and 36.5 ppt. It is important to note that each set of eggs used in this portion of the study were obtained from broodstock maintained at different facilities which may have influenced the results. However, similarities in larval growth suggest that larvae obtained from UTMSI and FL were similar in quality. Very little information is available regarding the influence of spawning salinity on the tolerance of marine fish larvae to variations in salinity. However, there is evidence which suggests that spawning salinity plays an important role in the salinity tolerance of some marine species. Kucera et al. (2002) found that the ability of spotted seatrout *Cynoscion nebulosus* larvae to withstand sudden drops in salinity was greater when larvae were spawned at 20 or 30 ppt compared to 40 ppt. Spotted seatrout spawn in bays and estuaries over a wide range of salinities (Lassuy, 1983), the interaction between spawning salinity and larval tolerance in this species may be an adaptation to spawning in environments where substantial salinity fluctuations are common. Spawning sites of cobia throughout their distribution are relatively unknown but it is thought that this species spawns offshore and in areas where the salinity is similar to that of oceanic water (Shaffer and Nakamura, 1989). For example, cobia eggs have been collected off the coast of North Carolina along the edge of the Gulf Stream (Hassler and Rainville, 1975) and in the Crystal River Estuary, Florida in salinities of 30.5–34.1 ppt (Ditty and Shaw, 1992). In the natural environment, cobia eggs and larvae may be less likely to encounter large variations in salinity compared to species spawning in low salinity areas which may help explain the differences observed in the relationship between spawning salinity and larval tolerance in the two species.

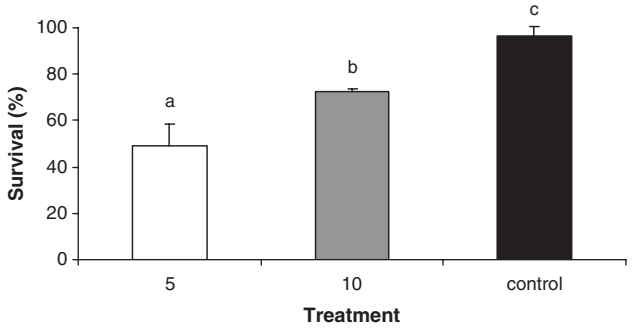

Fig. 3. Percent survival (+standard deviation; $n=3$) of cobia reared in different salinities through 28 dph following an initial drop in salinity (10 ppt day^{-1}) on 13 dph. Letters denote significant differences ($P<0.05$) among treatments.

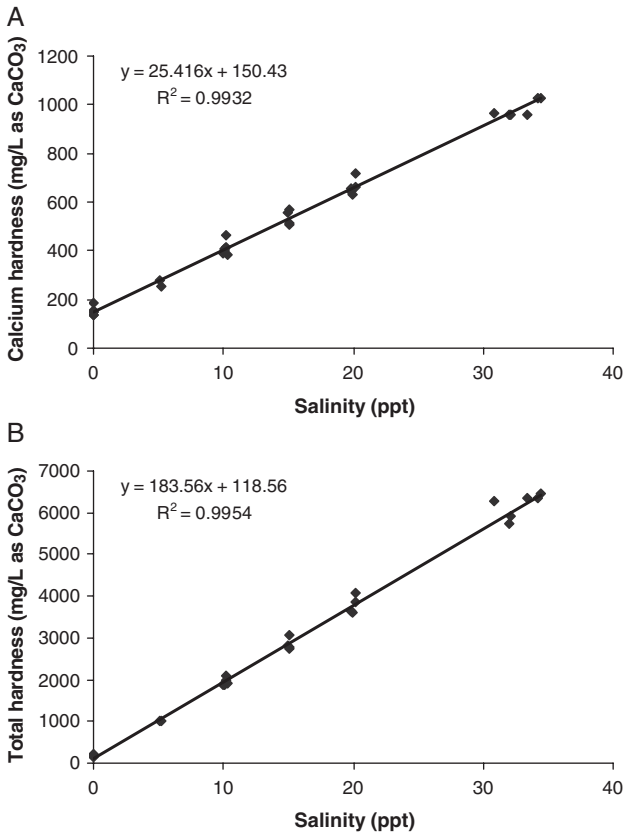

Fig. 4. (A) Calcium and (B) total hardness versus salinity for all experiments. Lines are linear regressions.

Generally, the tolerance of cobia larvae to sudden changes in salinity was similar across all ages tested (3–9 dph) although there was a pattern of increasing tolerance to low salinity with age in the LC_{10} values with older larvae exhibiting a higher tolerance to sudden drops in salinity than larvae 3 dph. These experiments suggested that 90% of the larvae would be expected to survive for at least 18 h in salinities of 20 ppt 3 dph, 15 ppt 5 dph and ~10 ppt 7 and 9 dph. The pattern of increasing salinity tolerance with age was more pronounced when cobia larvae were reared in low salinities for a longer period of time following a period of gradual decreases in salinity. Throughout the study, larval survival in the control salinities was within the normal range for this species reared under similar conditions (Hitzfelder, 2004; Faulk and Holt, 2005). The survival of cobia larvae reared in salinities of 10, 15 and 20 ppt was significantly lower than that of the controls when the salinity drop was initiated 1 and 4 dph. However, no significant difference in larval survival was detected between the control and 20 ppt treatment when the salinity drop began on 7 dph although test salinities below 20 ppt still resulted in significantly higher mortalities than observed in the controls. Only when the salinity drop was initiated on or following 10 dph was survival in the low salinity treatments similar to that of the control treatments. These results indicate that the acute salinity tolerance experiments employed in this study were a poor indicator of the ability of cobia larvae to withstand long-term changes in salinity.

Age-linked changes in the pattern of salinity tolerance have been previously reported for a number of species including Atlantic halibut *Hippoglossus hippoglossus* (Lein et al., 1997), mangrove red snapper *Lutjanus argentimaculatus* (Estudillo et al., 2000), European sea bass *Dicentrarchus labrax* (Varsamos et al., 2001) and spotted seatrout (Kucera et al., 2002). Typically, the tolerance of marine fish larvae to changes in salinity is higher for newly hatched larvae compared to first feeding larvae. Following the onset of exogenous feeding, larval tolerance increases with age as a result of the differentiation of structures important in osmoregulation. For instance, Lein et al. (1997) suggested that the increased tolerance of older, more developed halibut larvae was attributed to the presence of a

functional kidney and Estudillo et al. (2000) found that the increase in salinity tolerance of mangrove red snapper on 28 dph coincided with gill development. The relationship between ontogenic changes in the osmoregulatory ability of cobia larvae and increasing salinity tolerance with age and/or size remains to be examined.

No significant differences in growth, measured as standard length, were observed for larvae within each experiment irrespective of rearing salinity. The effects of rearing marine fish larvae in brackish or low salinity water varies among species. For example, no significant differences in larval growth were found for Atlantic halibut (Lein et al., 1997) and brown-spotted grouper *Epinephelus tauvina* (Akatsu et al., 1983) reared through 49 and 21 dph, respectively, in salinities below that of full strength seawater. On the other hand, studies conducted on gilthead seabream *Sparus aurata* (Tandler et al., 1995) and older brown-spotted grouper (Akatsu et al., 1983) larvae (21–40 dph) have shown that long-term growth is greater in lower salinities. Finally, Moustakas et al. (2004) found that the growth of southern flounder *Paralichthys lethostigma* larvae through 15 dph was greater at 32 ppt than 24 ppt. The authors of these studies have variously suggested that the positive and negative effects of low salinity on larval growth are a result of behavioral and/or physiological changes in the larvae such as swimming activity, feeding rate and increased or decreased metabolic demands due to changes in internal osmolality or buoyancy.

In this study, cobia reared in 5 and/or 10 ppt following an initial drop in salinity on 10 or 13 dph were discolored or had external lesions associated with apparent fungal infections 3–5 days after tank salinities reached 10 ppt. Although no significant differences in survival were found among treatments for the initial studies terminated on 19 or 22 dph, survival was significantly lower when the fish were held to 28 dph. There are several possible explanations for the increased incidence of disease seen in the 5 and 10 ppt treatments including increased virulence of pathogens and poor condition of fish held in low salinities (e.g. immune system suppression, insufficient nutrition, osmoregulatory stress). It is widely understood that the survival and virulence of pathogens affecting both marine and freshwater fishes is salinity dependent (Knudsen and Sundnes, 1998; Kirk et al., 2000; Yanong, 2003). Although there is very little information available regarding the effects of chronic exposure to low salinities on the immune system of marine fishes, Cuesta et al. (2005) found that humoral immune parameters were negatively affected by low salinity in gilthead seabream. In a review of fungal diseases of fish, Yanong (2003) stated that both internal and external fungal infections are frequently a secondary infection due to poor condition, environmental stress, bacterial diseases or parasite infestations. Previous studies examining the effects of salinity on juvenile cobia have suggested that this species may experience ion and/or vitamin deficiencies when reared in low salinity. Resley et al. (in press) stated that juvenile cobia reared in low salinities exhibited signs of osteopenia, inter-muscular lesions and discoloration analogous to that reported by Denson et al. (2003) when fed a commercial diet but appeared normal when fed a specially prepared diet supplemented with chelated minerals (e.g. calcium) and vitamins.

In summary, this study provides valuable information regarding the salinity requirement of cobia during the larval period and suggests that larvae may be successfully reared in salinities as low as 15 ppt once they are 13 dph or older. Although cobia exposed to decreasing salinities on 10 and 13 dph initially tolerated salinities below 15 ppt, increased incidence of disease eventually led to increased mortality when the studies were extended beyond the initial termination ages of 19 and 22 dph. In order to further assess the potential grow-out of this species in brackish water, additional research is needed to examine the effects of low salinity through larval rearing and into the juvenile stage with particular emphasis placed on possible nutritional requirements.

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