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Salinity tolerance of larvae of the mangrove red snapper (*Lutjanus argentimaculatus*) during ontogeny

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Abstract

Salinity tolerance and the effects of salinity on growth, condition factor and chloride cell (CC) densities were evaluated for *Lutjanus argentimaculatus* larvae during ontogeny. Tolerance of *L. argentimaculatus* larvae to abrupt changes of salinity from 32 ppt varied with age. Periods to 50% mortality (LT_{50}) were significantly ($P < 0.05$) longer for 0-day-old larvae than for 7-, 14- and 21-day-old larvae. Tolerance of abrupt salinity change increased remarkably, starting on day 28. Although abrupt transfer to test salinities caused substantial mortalities, *L. argentimaculatus* larvae, regardless of age (0-, 7-, 14-day-old), showed significantly longer LT_{50} when abruptly transferred to 8 and 16 ppt than for transfers to 24 and 40 ppt ($P < 0.05$). Growth of *L. argentimaculatus* larvae at 16, 24, 32 (control) and 40 ppt was not significantly different either at the end of the first rearing phase (days 0–21) or second phase of rearing (days 22–50). Survival was significantly lowest at 40 ppt (4.3%) at the end of first phase of rearing ($P < 0.05$). There were no significant differences in survival rates at the end of the second phase of rearing; however, the condition factor (K) of larvae reared at lower salinities was significantly higher than that of fish at 40 ppt ($P < 0.05$). Gill epithelia of 42- and 50-day-old larvae showed increasing density of CC with increasing salinity.

Keywords: Salinity; Chloride cell; *Lutjanus argentimaculatus*; Larvae; Condition factor

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1. Introduction

The responses of teleost fishes to osmotic gradients between the fish and their environments are well documented (see review by McCormick, 1995). Teleost larvae, like adults, osmoregulate to hold their body-fluid ion concentrations at levels between 11 and 14 ppt (350–440 mosM; Bone et al., 1995; Alderdice, 1988). Exactly how and with what organs they osmoregulate probably changes with development (Banks et al., 1991; Bone et al., 1995). Before the gills are developed, regulation is thought to be passive and largely attributable to the impermeability of the skin (Alderdice, 1988). Chloride cells (CC) are found in the early development and a likely site of osmoregulation (Ayson et al., 1994; Bone et al., 1995). As the larvae grow and the gills are fully developed, osmoregulation is thought to largely occur in the CC present in the gills (Hwang, 1987; Jürss and Bastrop, 1995).

The mangrove red snapper (*Lutjanus argentimaculatus*), an important market species in Southeast Asia, spawns in the open sea and the early larvae are planktonic (Polovina and Ralston, 1987); while the juveniles are found in mangrove estuaries and lower reaches of freshwater streams (Allen, 1985). Induced and natural spawnings have been successful in cage-reared *L. argentimaculatus* broodstock (Doi and Singhagraiwan, 1993; Lim and Chao, 1993; Emata et al., 1994); however, attempts to rear larvae in the hatchery have not been equally successful (Doi and Singhagraiwan, 1993; Lim and Chao, 1993; Singhagraiwan and Doi, 1993; Emata et al., 1994; Duray et al., 1996). Most of the rearing trials were terminated a few days after hatching and, although Duray et al. (1996) have successfully reared *L. argentimaculatus* larvae to metamorphosis at ambient salinity (32 ± 2.0 ppt), survival was low.

In their natural environment, *L. argentimaculatus* larvae are confronted with drastic changes in the external medium due to their seasonal movements from marine to fresh or brackish waters. Yet, despite the widely-recognized euryhalinity of the species, no information is available on the salinity tolerance of its early life stages. The ability to withstand salinity changes during ontogeny depends on the capacity to regulate internal ionic concentration within narrow ranges. Knowing which salinity is less stressful may enhance larval survival and growth by channeling energy saved by less osmotic work, to tissue production (Moyle and Cech, 1996). We determined the salinity tolerance limits of hatchery-reared *L. argentimaculatus* larvae during ontogeny and identified the optimum salinity for growth and survival of the larvae.

2. Materials and method

2.1. Egg source

L. argentimaculatus eggs were obtained from induced spawnings of cage-reared broodfish at SEAFDEC Igang Marine Substation, Guimaras, central Philippines. A mature male and female (average body weight (ABW): male = 4.55 kg, female = 5.0 kg) were induced to spawn by a single injection of 1000 IU/kg BW of human chorionic gonadotropin (hCG) hormone at the base of the pectoral fin. The pair was then placed in a $6 \times 6 \times 6$ m cage provided with hapa net. Fish spawned approximately 36 h after injection.

2.2. Tolerance to abrupt salinity change

The salinity tolerance limits defined as lethal time LT_{50} (time to 50% mortality) following abrupt transfer (from 32 ppt to the test salinities) of *L. argentimaculatus* larvae were determined on 0-, 7-, 14-, 21-, 35-, 42- and 50-day-old larvae. Larvae previously hatched and reared at ambient salinity (32 ppt) were transferred directly to higher salinity (40 ppt) or lower salinities (24, 16 and 8 ppt) and monitored for 48 or 72 h in 4-l plastic jars, each with 3.5-l of test salinities. Stocking density started at 50 larvae/jar on day 0 (total length, TL: 2.27 ± 0.14 mm) and was lowered to 25 larvae/jar on day 7 (TL: 3.11 ± 0.24 mm) and 14 (TL: 4.33 ± 0.89 mm), 15 larvae/jar on day 21 (TL: 6.90 ± 0.27 mm) and 28 (TL: 10.32 ± 0.43 mm), 10 larvae/jar on day 35 (TL: 17.64 ± 0.20 mm), 42 (TL: 20.35 ± 0.41 mm) and 50 (TL: 31.59 ± 3.18 mm) to avoid crowding stress effects. Larvae were starved throughout the tolerance tests. Tolerance tests were done at 27 ± 1.0°C with dissolved oxygen (D_1O_1) ranging from 5.2 to 6.8 ppm. There were three replicates in all treatments. Mortalities of the larvae in each test salinity were recorded at time intervals (1, 3, 6, 9, 12, 15, 18, 24, 36 and 48 h); 48 h was chosen and verified following Dueñas and Young (1983) as the maximum time to test for tolerance without non-salinity related complications such as starvation and poor water quality. Total ammonia (TAN) was examined before and after the experiments and it ranged from 0.00 to 0.05 ppm. In cases where mortality did not reach 50% after 48-h exposure to test salinities, survival rates at the end of the 48-h tolerance test were compared and considered as the final response of the fish to the test salinity. Alternatively, exposure to test salinities was extended to 72 h in the case of 0-day-old larvae. Exposure to test salinities in older larvae ($>$ 28-day-old) could not be extended due to cannibalism caused by starvation.

2.3. Effects of salinity on growth and survival

Fertilized eggs from cage-reared broodfish at SEAFDEC AQD Igang Marine Substation were transported to the Finfish Hatchery at Tigbauan Main Station and incubated at a stocking rate of approximately 500 eggs/l in 400-l circular fiberglass tanks supplied with filtered seawater and adequate aeration. Water temperature was 28.0 ± 1.0°C. After hatching (17 ± 2.0 h time after fertilization (TAF)), newly-hatched larvae were quantified volumetrically, acclimatized to test salinities, and stocked at a density of 30 larvae/l in 500-l circular fiberglass tanks each containing a specific treatment salinity of 16, 24, 32 (control) and 40 ppt. After 21 days of rearing (first phase of rearing), survival was determined and larval density was reduced to 0.5 larva/l in all treatments. The larvae were reared for 4 more weeks (second phase of rearing) and the survival rates were determined (day 50). Treatments were assigned at random and replicated thrice.

2.4. Condition factor

Condition factor (K), an indicator of the health or fitness of the fish, was determined on 21- and 50-day-old larvae using the formula $K = W(100)/L^3$, where W is the wet weight of the fish in g and L is the length in cm (Moyle and Cech, 1996).

2.5. Feeding and water management

The feeding and water management scheme for rearing *L. argentimaculatus* larvae used in this study was modified from Duray et al. (1996). Adult copepods and copepodids, mostly consisting of *Acartia* and *Pseudodiaptomus* species, were introduced at approximately 50–75 ind/l on the same day as newly-hatched larvae were stocked, following Toledo et al. (1997). Screened rotifers (*Brachionus plicatilis*) with an average lorica length of 0.08–0.10 mm were given once daily at 09:00 h from day 2 to day 7 and replaced with unscreened rotifers at a density of 10–15 ind/ml/day from day 8 to day 21. Nosan R_1 (Nihon, Kogyo, Japan) was used as a supplement from day 2 to day 21 at a feeding rate of 1 g/ton/day.

At the second phase of rearing (days 22–50), newly-hatched *Artemia nauplii* were provided at 1–3 ind/ml/day for about 2 weeks. As the larvae grew, *Artemia* of increasing sizes were given at 3–7 ind/ml/day until day 50. Rotifers at a density of 7–10 ind/ml were still given daily from day 21 to day 25 for smaller larvae that could not yet utilize bigger prey (*Artemia*). Larval rearing was done in "green water" (*Chlorella* sp.) until day 25. Tank bottoms were cleaned and the water was changed every other day starting day 7. Water samples were taken every other day for chemical analysis of TAN (phenate method, American Public Health Association, 1989), nitrite (colorimetric method, American Public Health Association, 1989) and pH (Cole Palmer

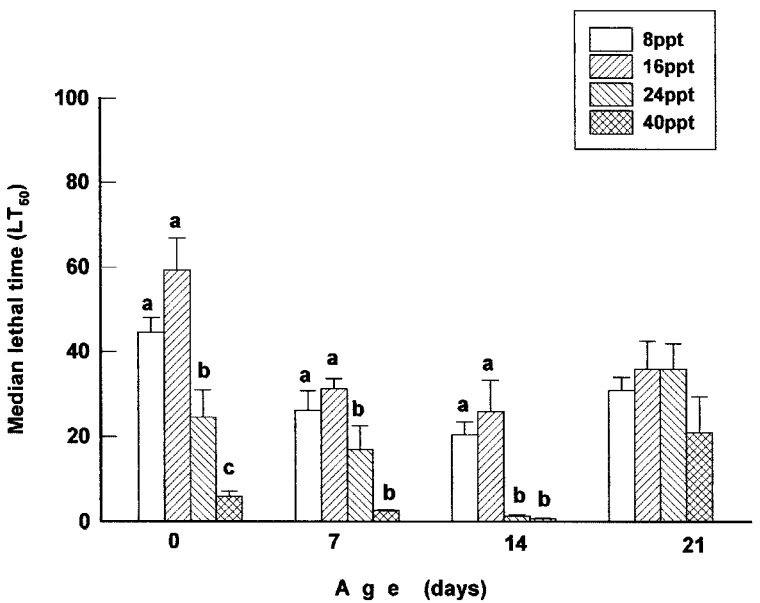

Fig. 1. Survival of *L. argentimaculatus* larvae after 72 h (0-day-old larvae) and 48 h (7-, 14-, 21-day-old larvae) of exposure to four salinities during the first phase of rearing. Vertical lines represent ±S.E. Bars with different superscripts denote significant difference at $P < 0.05$ (ANOVA). Median lethal time (LT_{50}), is the time to 50% mortality.

pH meter, model 5996-50). Dissolved oxygen and temperature were monitored daily with YSI model 51B D_1O m (Yellow Spring Instrument, Yellow Spring, OH, USA). Temperature in all test salinities ranged from 25°C to 27°C throughout the first and second phases of rearing period. There were slight variations in dissolved oxygen (range 5.8–7.0 ppm), TAN (range 0.0–0.54 ppm), nitrite (NO_2) (range 0.0–0.54 ppm) and pH (7.3–8.9), but the values obtained were below critical levels based on the review of Mohapatra and Rengaparajan (1995) and there were no significant differences observed among test salinities ($P > 0.05$).

2.6. Morphology of CC

CC in the gills were examined in 42- and 50-day-old larvae. Gill arches were excised from three live fish sampled from each tank and fixed in Champy and Maillet's solution for 18–24 h, then rinsed with distilled water and transferred to 70% ethanol for storage at −4°C (Avella et al., 1993). After fixing, gills were processed using standard histological procedures (Bell and Lightner, 1988). The number and size of CC were determined from 6-μm sections of randomly selected areas of the efferent regions of 10 primary lamellae. Estimates of CC size and density were determined by averaging the two measurements of the surface area of its maximum section profile (Cioni et al.,

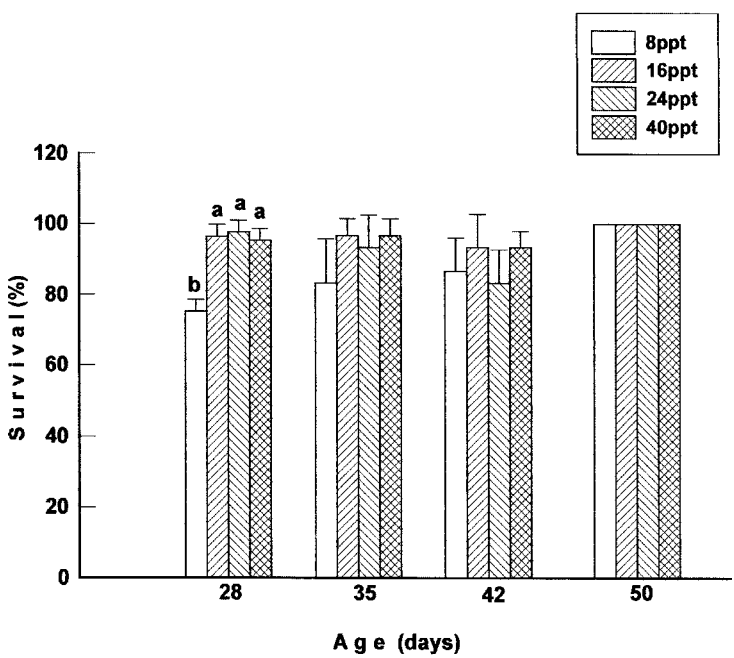

Fig. 2. Mean survival rates of *L. argentimaculatus* larvae of various ages, after 48 h of exposure to four salinities. Vertical lines represents ±S.E. Bars with different superscripts denotes significant difference at $P < 0.05$ (ANOVA).

Table 1
Survival rates (SR) and condition factor (K) (see text) of *L. argentimaculatus* during the first (days 0–21) and second (days 22–50) phases of rearing at four salinities. Values are means ± S.E.

Salinity (ppt)	First phase		Second phase	
	Condition factor (K)	SR (%)	Condition factor (K)	SR (%)
16	2.67 ± 0.29^{b}	7.50 ± 0.86^{a}	13.38 ± 9.88^{a}	16.00 ± 4.00^{a}
24	4.14 ± 0.80^{a}	6.23 ± 1.05^{a}	11.78 ± 3.02^{a}	14.50 ± 11.00^{a}
32	2.91 ± 0.10^{b}	6.44 ± 0.96^{a}	12.59 ± 2.51^{a}	22.50 ± 12.80^{a}
40	2.44 ± 0.08^{b}	4.30 ± 1.51^{b}	10.03 ± 6.20^{b}	25.00 ± 8.50^{a}

Means with different superscripts are significantly different at $P < 0.05$ (ANOVA).

1991). Measurements and determination of CC density were done under a light microscope (Nikon) equipped with a micrometer eyepiece.

2.7. Sampling and preservation of larvae

Larvae ($n = 15$) were sampled from each tank at the end of the first rearing phase (day 21) and second phase (day 50) of rearing. Larval samples were fixed in 2.5% glutaraldehyde solution (Oozeki et al., 1991) and stored for later measurement. Total lengths were measured to the nearest 0.01 mm using a profile projector (Nikon, model 6C). Body dry weights were measured to the nearest 0.01 mg with a Mettler balance (model AE 160) after drying at 60°C for 48 h.

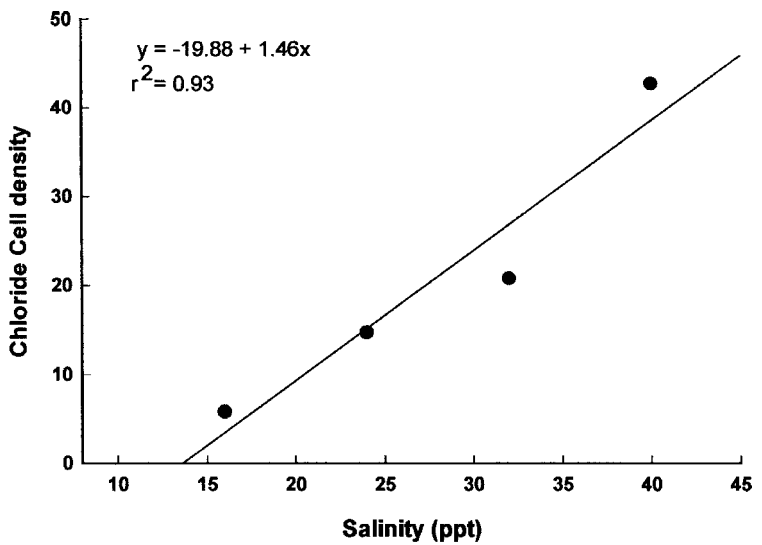

Fig. 3. Regression analysis of the density of CC in branchial epithelium of 42-day-old *L. argentimaculatus* larvae. The density of CC is linearly correlated with salinity at $P < 0.05$.

2.8. Statistical design and analyses

A completely randomized design was used in Experiments 1 and 2. LT_{50} was obtained by plotting the cumulative mortality on a probit scale against the time to death (Finney, 1962). Values, in hours (h), were then analyzed with two-way analysis of variance (ANOVA), followed by Duncan multiple range test (DMRT). The effects of salinity on growth survival and water quality were compared using one-way ANOVA followed by DMRT. CC size and density at varying salinities were compared using two-way ANOVA followed by DMRT. Regression analysis was done to determine the relationship between salinity and CC in the gills of older larvae. All percentage data were normalized by arcsin square transformation prior to statistical analyses. Statistical analyses were performed using the statistical software SAS (1998).

3. Results

3.1. Tolerance to abrupt salinity change

Newly-hatched larvae (0-day-old) showed higher tolerance of abrupt salinity changes from 32 ppt than 7-, 14- and 21-day-old larvae (Fig. 1). At the end of 72 h, LT_{50} of newly-hatched larvae was significantly longer at lower salinities (44.7 h at 8 ppt, 59.31 h at 16 ppt, and 24.7 h at 24 ppt) and shortest at 40 ppt (6 h). Two-way ANOVA showed that 7- and 14-day-old larvae were significantly less tolerant of abrupt transfer to test salinities ($P < 0.01$). LT_{50} after 48-h exposure ranged from 0.67 to 31.3 h with LT_{50}

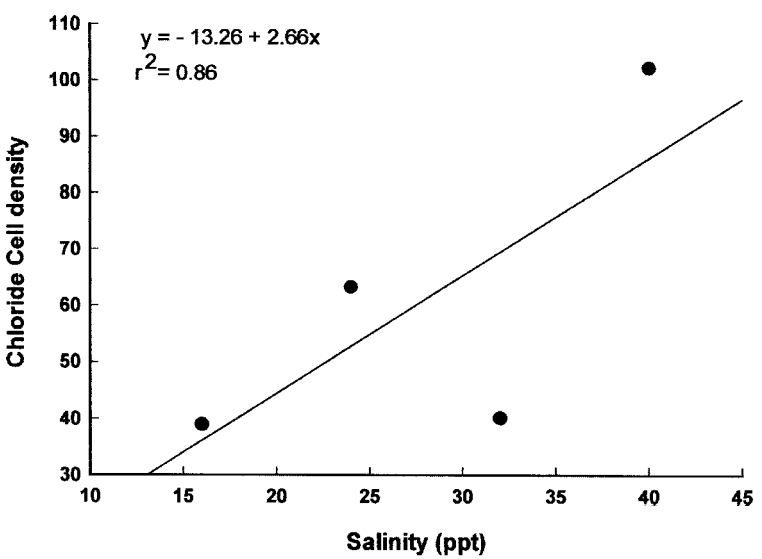

Fig. 4. Regression analysis of the density of CC in the branchial epithelium of 50-day-old *L. argentimaculatus* larvae. The density of CC is linearly correlated with salinity at $P < 0.05$.

longest at 16 ppt ($P < 0.05$) for 7- and 14-day-old larvae. Twenty-one-day-old larvae showed improved LT_{50} ranging from 21 to 36 h with slightly longer LT_{50} at intermediate salinities (24 and 16 ppt). Early larvae, regardless of age (0-, 7-, 14-day-old), showed significantly longer LT_{50} when transferred abruptly to 16 ppt, as opposed to other salinities.

Salinity tolerance increased significantly with age ($P < 0.01$) for size classes other than newly-hatched larvae. No LT_{50} values were obtained in 28-day-old larvae at the end of 48-h exposure to the test salinities. In this case, the final survival rates at the end of 48 h were considered as the final response of larvae to the test salinity. Survival of 28-day-old larvae ranged from 75% to 96% with lowest values at 8 ppt ($P < 0.05$). For 35- and 42-day-old larvae, survival did not vary significantly with salinity (Fig. 2). Apparently, 50-day-old larvae were able to adapt effectively to all salinities, because they all survived the experiment.

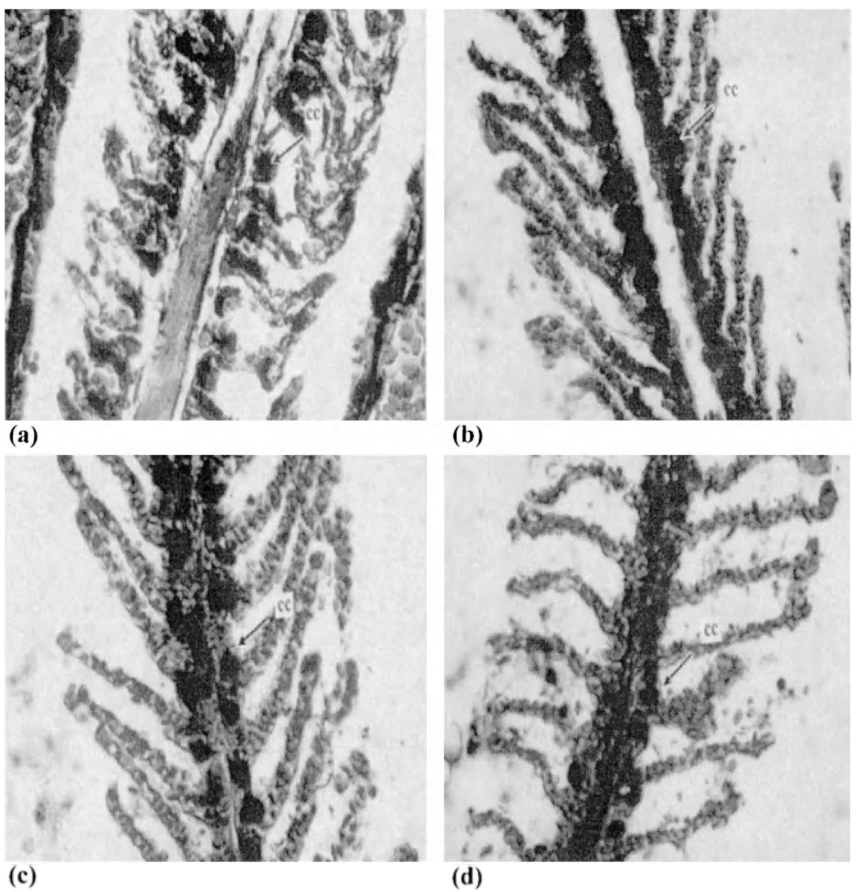

Fig. 5. Light microscopy (×100) of brancial epithelium of 50-day-old *L. argentimaculatus* larvae reared at four salinities. (a) 32 ppt control, (b) 40 ppt, (c) 24 ppt, (d) 16 ppt. CC = chloride cell.

3.2. Effect of salinity on growth and survival

The summary of salinity effects on survival and growth of *L. argentimacultaus* during the first (0–21 days) and second (22–50 days) phases of rearing is presented in Table 1. Survival was significantly lowest at 40 ppt (4.3%) during the first phase of rearing. There were no significant differences in survival during the second phase of rearing. Growth was not significantly different either at the end of the first or second phase of rearing.

3.3. Condition factor

Condition factor was significantly greatest at 24 ppt (4.14) during the first phase of rearing, while at the end of the second phase of rearing, K value was significantly lowest at 40 ppt (10.03) ($P < 0.05$) (Table 1).

3.4. Morphology of CC

There were significantly higher numbers of CC in the gills of 42- and 50-day-old larvae reared at 32 and 40 ppt than those reared at 24 and 16 ppt ($P < 0.01$). The size of the CC did not vary significantly with salinity. The density of CC in the gills of 42- and 50-day-old larvae (Figs. 3 and 4) was linearly correlated with salinity ($r^2 = 0.93$, $P < 0.05$; $r^2 = 0.86$, $P < 0.05$, respectively), while no significant correlation was found with respect to size of CC ($r^2 = 0.79$, $P > 0.05$; $r^2 = 0.83$, $P > 0.05$, respectively). Light microscopy ($\times 100$) of branchial epithelium of 50-day-old *L. argentimaculatus* larvae showing CC is shown in Fig. 5.

4. Discussion

4.1. Tolerance to abrupt salinity change

The tolerance of *L. argentimacultus* larvae to abrupt salinity change varies with age. Newly-hatched *L. argentimaculatus* larvae showed an effective ability to tolerate abrupt salinity change. The ability of newly-hatched larvae to tolerate a wider range of salinity compared to adults is true to many teleost species (see reviews by Alderdice, 1988; Bone et al., 1995). In rabbitfish (*Siganus guttatus*), Young and Dueñas (1993) reported that the larvae could tolerate salinity ranges of 10–45 ppt at 12 h after hatching and 14–37 ppt at 24 h after hatching. Newly-hatched larvae of milkfish (*Chanos chanos*) can tolerate salinities ranging from 8 to 37 ppt (Dueñas and Young, 1983) and 15–55 ppt (Swanson, 1996). Banks et al. (1991) reported that 1-day-old larvae of spotted sea trout, *Cynoscion nebulosus*, can tolerate salinity ranges of 4–40 ppt; 3-day-old larvae 8–32 ppt and 9-day-old larvae 8–48 ppt. These authors suggest that osmoregulation at very early stages (eggs and yolk sac larvae) occurs through the skin and probably with the aid of ion pumps, such as CC (Ayson et al., 1994). The tolerance of *Tilapia mosambicus* embryos and larvae was influenced by the presence of CC in the yolk-sac

membrane. These CC increase in size and density at higher salinities (Ayson et al., 1994). In the present study, LT_{50} was longer (33.7 ± 23.4 h) for newly-hatched larvae, shorter for day 7-(19.2 ± 12.6 h), 14-(13.0 ± 13.9 h) and 21-(31.0 ± 7.1 h) day-old larvae and improved again starting from 28-day-old larvae.

Low tolerance of 7-, 14- and 21-day-old *L. argentimacustus* larvae is difficult to explain. Other factors, such as critical time to first feeding, and swim bladder inflation, may have influenced survival, as well as salinity. Development associated with first feeding caused lower tolerance of *C. nebulosus* to abrupt salinity change (Banks et al., 1991). The increase in salinity tolerance limits of *L. argentimaculatus* larvae, starting on day 28, could be attributed to the development of the gills, which have been reported by various authors to be the site for ionic regulation (see reviews by Perry and Laurent, 1993; McCormick, 1995). *L. argentimaculatus* larvae under the stereomicroscope showed the formation of gill-like structures starting on day 21. The gills became distinct in 28-day-old larvae. Furthermore, although 7-, 14- and 21-day-old *L. argentimaculatus* showed lower tolerance to salinity change, larvae survived better at 16 ppt than at the other test salinities used. Similar observations were reported by Tyler and Blaxter (1988) in herring (*Clupea harengus*), plaice (*Pleuronectes platessa*) and cod (*Gadus morhua*), and they also observed that the drinking rates in 32 ppt doubled those in 16 ppt. Marine teleosts have body fluids of 11.5–15 ppt (Wootton, 1995), thus 16 ppt is nearly isosmotic to their body fluid. At 16 ppt, *L. argentimaculatus* larvae probably survive better because there is a reduction of metabolic cost for osmoregulation.

4.2. Effects of salinities on growth and survival

Substantial information is available on the short-term effects of salinity changes on growth and respiration (Tyler and Blaxter, 1988; Banks et al., 1991; Cioni et al., 1991; Avella et al., 1993; Moser and Miller, 1994), but only a few deal with the effects of long-term exposure to different salinities. This approach is essential for a more realistic assessment of optimal salinity for larval rearing (Johnson and Katavic, 1986; Lee and Menu, 1986). The responses of teleost fishes to different rearing salinities affect growth and, possibly, survival (Johnson and Katavic, 1986; Lee and Menu, 1986; Alderdice, 1988; Banks et al., 1991; Murashige et al., 1991; Bone et al., 1995). The extra costs for ionic regulation may reduce energy available for growth unless the fish can compensate by increasing its feeding rate (Wootton, 1995). The results of the present study show that growth of *L. argentimaculatus* is not affected by the rearing salinities. In contrast, larvae of grey mullet (*Mugil cephalus*) obtained maximum growth at 22–23 ppt (Lee and Menu, 1986) and at 16 ppt (Murashige et al., 1991) after 15 days of rearing. For seabass (*Dicentrarchus labrax*), Johnson and Katavic (1986) reported maximum growth at 10–20 ppt after 18 days of rearing. Although growth of *L. argentimacultus* larvae was not affected by the rearing salinities, survival rates were significantly higher at lower salinities (16, 24 and 32 ppt).

In the second phase of rearing (days 21–50), final larval growth and survival rates were similar in all rearing salinities. These results indicate that older (> 21-day-old) *L. argentimaculatus* larvae can adapt relatively well within salinities of 16–40 ppt. The same observation was reported by Murashige et al. (1991) for striped mullet (*M.*

cephalus) reared at 32–35 and 22–25 ppt for 50 days. Similarly, Ogasawara et al. (1978) reported that striped fish (*Monodactylus sebae*) larvae tolerated salinities of 8.5–34 ppt shortly after it reached the juvenile stage at about 10 days after hatching.

The condition factors of 21- and 50-day-old larvae were lowest at 40 ppt indicating that, although *L. argentimaculatus* larvae could tolerate and survive at high salinity, they were leaner compared to those at lower salinities. These results may indicate that larvae reared at lower salinities are less stressed and therefore are healthier compared to those at 40 ppt. Moreover, although growth of *L. argentimaculatus* did not differ significantly between test salinities, larvae at 16 and 24 ppt grew a bit faster than those at 32 and 40 ppt. The rate of metamorphosis was not monitored in this study. However, concomitant observation showed that metamorphosis was size-related rather than age-related. *L. argentimaculatus* larvae metamorphosed earlier at 16 and 24 ppt.

4.3. Morphology of CC

The majority of studies we reviewed indicated that the density and size of CC in the gill epithelia of marine teleosts is greater in isotonic (ca. 16 ppt) and hypertonic (> 25 ppt) environments (see review by McCormick, 1995). The increasing density of CC in the gills of *L. argentimculatus* larvae with increasing salinity indicates the osmoregulatory function of CC at higher salinities. Our results suggest that salinity fluctuations have relatively little effect on survival of older *L. argentimaculatus* larvae (> 28-day-old), which may already have functional osmoregulatory organs such as gills. However, in cases where fluctuations of salinity cannot be avoided, it is suggested that feeding frequency/rate should be increased in extreme salinities to compensate for energy loss due to ionic imbalance (Moyle and Cech, 1996).

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