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Original Article

Effects of habitat structure and salinity on growth and survival of juvenile mangrove red snapper *Lutjanus argentimaculatus* (Forsskal, 1775)

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Abstract

Mangrove Red Snapper (*Lutjanus argentimaculatus*) is a large, palatable fish that has become increasingly valuable for aquaculture in South East Asia. Unfortunately, the bulk of current mariculture of *L. argentimaculatus* depends almost entirely on fingerlings collected from the wild, since hatchery-raised fry are regarded as inferior by fishermen. Wild Mangrove Red Snapper spawn on offshore reefs, but their larvae occupy coastal lagoons and estuaries. In this study, we examined some of the factors (habitat structure and salinity) associated with the natural recruitment habitat of *L. argentimaculatus* to determine whether they might play a role in the perceived superiority of wild-caught fry. We found statistically significant relationships between survival and salinity, and between somatic growth and habitat structure, but no interaction between the factors. Juvenile *L. argentimaculatus* survived best at moderate (17ppt) salinities, with fresher water (10ppt) providing better outcomes than more saline (25ppt) conditions. We found also that juveniles grew best when provided with hard, complex structures (rock piles and mangrove roots), rather than in tanks without such structures. Here, we have demonstrated two simple environmental factors that improve survival and growth of juvenile *L. argentimaculatus* and may provide a substantial boost to the performance of hatchery-reared fish.

Keywords: *Lutjanus argentimaculatus*, reef fish, habitat structure, salinity, stock viability

1. Introduction

Mangrove Red Snapper (*Lutjanus argentimaculatus*) is an important commercial and recreational fish throughout its range (Allen, 2002; Norris-Piddocke *et al.*, 2015; Norris-Zagars *et al.*, 2012; Russell, McDougall, Fletcher, Ovenden, & Street, 2003). They are not an abundant resource, however; this species has never been found in large quantities (Anderson & Allen, 2001). The growing demand for this large, palatable fish has led to increased interest in the development of its aquaculture (Chou & Lee, 1997; Emata & Borlongan, 2003; Liao, Su, & Chang, 1995; Wong, 1995). Unfortunately, the bulk of current mariculture of *L. argentimaculatus* depends almost entirely on fingerlings collected from the wild. Although *L. argentimaculatus* have spawned both spontaneously and under aquarium conditions in concrete tanks and floating net cages (Emata, Damaso, & Eullaran, 1999), there are variations in egg and larval quality, and larval

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survival is generally poor (Doi, Ohno, Kohno, Taki, & Singhagraiwan, 1997; Emata, Ogata, Garibay, & Furuita, 2003). The supply of wild fingerlings is seasonal, variable, and probably unsustainable: such harvesting of juvenile fish can deplete natural recruitment, and consequently reduces the natural resource (Gjertsen, Hall, & Squires, 2010). Moreover, the few coastal ecosystems where the fingerlings can still be harvested in commercial quantities are under pressure. The coastal lagoons that support high juvenile densities, and contribute juveniles to adult populations provide habitats that – so far – do not seem to be emulated by current aquaculture hatcheries, but which seem to make a vital difference to the viability of juvenile fish.

L. argentimaculatus is threatened by both over-fishing and habitat loss, but little attention has been directed towards understanding those aspects of its larval and juvenile ecology that ensure successful recruitment and juvenile survival. In Vietnam and elsewhere, the increasing pace of mangrove forest destruction (Rajarshi & Rajib, 2013; Richards & Friess, 2016), or coastal wetlands and seagrass losses (Airoldi & Beck, 2007) means that critical juvenile habitats of Mangrove Red Snapper (as well as some other fish species) are under threat.

L. argentimaculatus, like many lutjanids, spawns at deep, offshore reefs (Day, Blaber, & Wallace, 1981; Doi & Singhagraiwan, 1993). It is not known whether they form spawning aggregations, but such reefs are often known to local fishermen and are subject to intensive fishing. After hatching, *L. argentimaculatus* larvae spend several weeks in the plankton before settling in brackish coastal waterways (Norris-Zagars *et al.*, 2012; Russell *et al.*, 2003). Doi, Kohno and Singhagraiwan (1994) reported that juveniles >16mm in length (about 30 days old) acquire sufficient swimming or cruising ability to migrate to coastal and estuarine waters and thus to seek settlement habitat.

Larvae and juveniles of Mangrove Red Snapper are found in estuaries and coastal areas, and also move into freshwater areas (Doi & Singhagraiwan, 1993; Ebner & Morgan, 2013; Lake, 1971; Russell & McDougall, 2005). The extent of their movement into freshwater is generally limited. Mangrove Red Snapper is an euryhaline species, but their tolerance for hyper- and hyposalinity varies by ontogenetic stage (Estudillo, Duray, Marasigan, & Emata, 2000). It is known, however, that juveniles occasionally venture into quite fresh water: in northern Australia juveniles and sub-adults were found 130 km up the Burdekin River and well upstream in the Tully River near its headwaters (Merrick & Schmida, 1984).

In aquaculture hatchery production, lutjanid juveniles mostly are reared in highly saline (close to oceanic) seawater. In various studies, we found that larvae were stocked at 30ppt until day 50 (Leu, Chen, & Fang, 2003), at 35ppt until day 55 (Duray, Alpasan, & Estudillo, 1996), and at 29 – 35ppt from day 30 to 80 after hatching (Thanh, 2012). The majority of small juveniles (<3cm in length) cultured in Vietnam are captured from brackish coastal lagoons and estuaries where salinity seldom rises to 25ppt. Although the general assumption is that nursery areas offer an abundance of food and protection from predators, a preference for low-salinity waters might also imply that escape from the profusion of stenohaline marine predators into relatively depauperate brackish water habitats is the dominant factor. If this is so, then it makes sense that juvenile fishes will demonstrate preferences for differing salinities commensurate with documented ontogenetic habitat shifts, independent of habitat structure.

In nature, adult Mangrove Red Snappers are often associated with snags (Grant, 1997), or rocky areas (Day *et al.*, 1981). A more detailed description by Russell *et al.* (2003) suggests that Mangrove Red Snapper mostly used rocks or snags as their refuges, however there appear to be habitat shifts between size classes. In that study, most fish under 10 cm were caught amongst rocks, while those of larger size chose snags as hiding places (Russell *et al.*, 2003). These authors also found that very few fish were caught in open water where there was no structure. In aquaculture, and for aquaculture research, however, juveniles are typically raised in featureless tanks.

There is a general belief that most fishes have at least some connection with solid structures as foraging, sheltering or spawning habitats at some life stage (Nikolsky, 1963), especially as juveniles. Mostafa, Malcolm, and Graham (1998) found that there was a positive correlation between growth rate of *Clarias gariepinus* with the extent of shelters, while Dou, Seikai, and Tsukamoto (2000) observed that the availability of refuges significantly reduced mortality due to cannibalism in *Paralichthys olivaceus*. Lutjanids tend to be generalist mesopredators, and cannibalism likely limits stocking rates in featureless habitats. As the juveniles become more competent predators, it might be expected that their reliance on structure as ambush sites eclipses its utility as refuge. The nature of the structure is thus potentially as important as having access to some such structure; while a planktonic larva may gravitate towards floating leaves or sticks, newly-settled larvae are more likely to seek out crevices as refuge. As predatory competence increases, and the range of potential prey expands, it is likely that snags and mangrove prop roots offer increasingly better foraging opportunities for juveniles, and hence become more desirable. Here, we explore the interplay between the two dominant habitat characteristics of the coastal lagoons favored by juvenile mangrove jacks in Vietnam: salinity and structure. Effects on juvenile survival rate associated with either of these factors offers an easy remedy for the poor viability of hatchery-raised juveniles, which can be used to take pressure off the fragile natural supply of these fish.

2. Materials and Methods

Field surveys were undertaken to examine the natural recruitment habitats in two provinces (Thua Thien Hue and Binh Dinh) (Figure 1) in the northern and middle parts of central Vietnam, where juvenile Mangrove Red Snapper are harvested in large numbers for the aquaculture industry. The local fishermen have been harvesting juvenile Lutjanidae from these sites for decades and are very familiar with their target species' habits and how and where to find them at different life stages. Under the guidance of local fishers, observers made notes of the ecological characteristics of the localities where juveniles were most common. In all, twelve localities were examined. Perhaps surprisingly, the most common habitat of newly-settled *L. argentimaculatus* is dominated by rocky berms interspersed with mangrove roots, broken branches and thickets of seaweed, and the occasional large

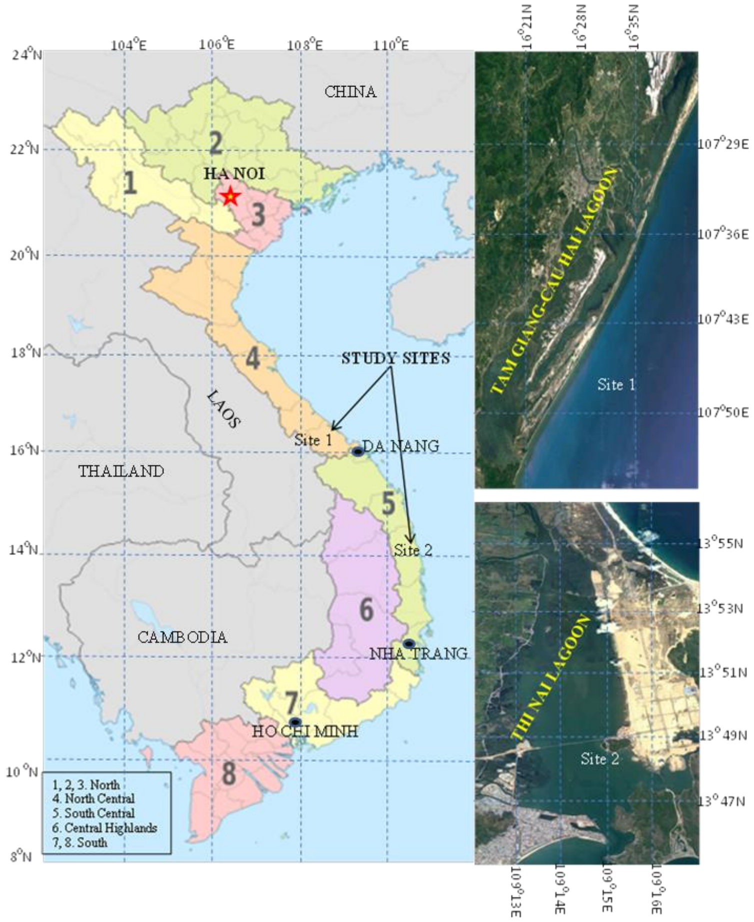


Figure 1. Study sites (Site 1: “Tam Giang-Cau Hai” in Thua Thien Hue province, located from 16^{0}14’ to 16^{0}42’ N and 107^{0}22’ to 107^{0}57’ E; Site 2: “Thi Nai” lagoon in Binh Dinh province, located from 13^{0}45’ to 13^{0}57’N and 109^{0}12’ to 109^{0}17’ E)

bivalve shell. Juveniles were found mostly at the freshwater end of the estuarine salt wedge in a salinity range from 10ppt to 25ppt. The results of these initial surveys (Figure 2) informed the experimental design and parameters.

We conducted a factorial experiment in controlled conditions to investigate effects of shelter and salinity on growth and survival of juveniles. Sufficient wild juveniles (initial TL 24-27mm, mean 25.7mm) were obtained from local fishermen to conduct an orthogonal experiment to test different types of habitat shelter under varying salinities. After one week of acclimation, we selected 36 random groups of 30 healthy juveniles and exposed each group to one of four kinds of habitat structure: piles of fist-sized rocks, mangrove root snags, bundles of plastic string emulating seagrass (anecdotally associated with juvenile mangrove jacks), and no structure (control). Test structures were large enough to occupy 50% of the floor area of the tanks. Seawater at three salinity levels (10, 17 and 25 ppt) was provided for each habitat type by dilution with clean fresh water, with 3 replicate tanks for each combination of salinity and habitat (i.e. N=36). We assigned 30 healthy fish to each replicate 20L tank. Water was cycled through a basic aquarium treatment process to maintain quality and was subjected to continuous aeration. Photoperiod was held at 12:12h light:dark cycle.

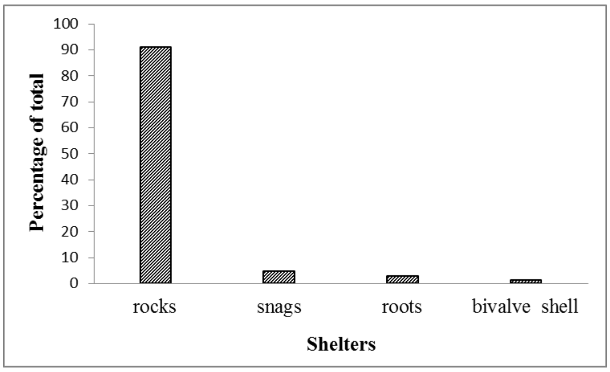


Figure 2. Natural habitat features in estuarine areas where juvenile Mangrove Red Snapper are most commonly caught by fishermen.

During the experiment, 30% by volume of water was exchanged daily. Water temperature during the experimental period ranged within 26.5-29^{0}C; dissolved oxygen was maintained at 5.3-5.7 mg/L; pH was kept within 7.8-8.3; and ammonia was consistently less than 0.1 mg/L for the duration of the experiment.

Fish were fed minced fresh fish at 7:00 and 17:00, and fed *Artemia* nauplii at 12:00 pm (midday), except on the days of measuring and weighing. After each meal, any uneaten food was manually siphoned out of the culture tanks. Tanks and shelters were cleaned by hand at regular intervals to minimize algal buildup. The experiment ran for 30 days. Total length (from the point of the nose to the end of the caudal fin), and weight of a subset of 10 randomly-selected fish from each tank were examined at the start of the experiment, and subsequently every ten days. A pilot study had indicated that repeated handling can cause stress to juvenile *L. argentimaculatus*; by measuring a random sub-sample of the fish in the tanks, potential differences in survival between treatments also could be measured without excessive handling stress, and each measurement thus represented a random sample of the tank population. Mean values from these subsamples provided replication at the tank level, without confounding the effects of different conditions.

We used two-way repeated measures analysis of variance (ANOVA) to distinguish the individual and inter-action effects of salinity and habitat conditions on weight and length of juveniles over the study period. All statistical ana-lyses were done using SPSS version 15.0 (SPSS Inc. 2006).

3. Results

3.1 Survival

Habitats had no statistically significant effect on survival rates of juveniles. Salinity was the major determinant of fish survival during the course of this experiment. We found that juveniles survived better in brackish water than in near-oceanic salinity ($F(2, 4)=90.89$, $p<0.001$). There was no difference detectable in any salinity regime that could be attributed to type of habitat structure. When analyzed using the same repeated measures model as for the growth increments, we found that only the survival rate at the highest salinity was significantly different (lower) than in other cases. However, it was apparent that habitat has no discernable effect on survival rates ($F(1.2, 2.39)=0.18$, $p=0.75$). Nor was there an interaction effect of habitat and salinity ($F(1.8, 3.6)=0.1$, $p=0.89$). Therefore, when the habitat groups are pooled it is evident that the lowest (10ppt) and highest (25ppt) salinities are significantly less beneficial to juvenile survival than moderately brackish (17ppt) water (Figure 3, 4; Table 1).

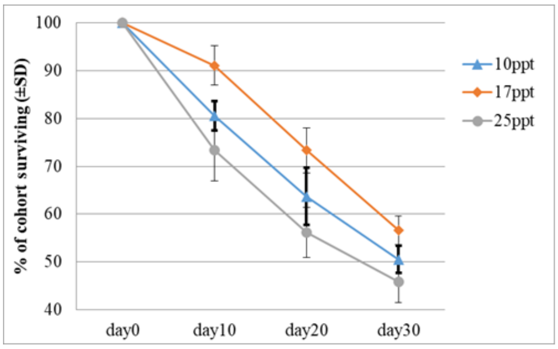
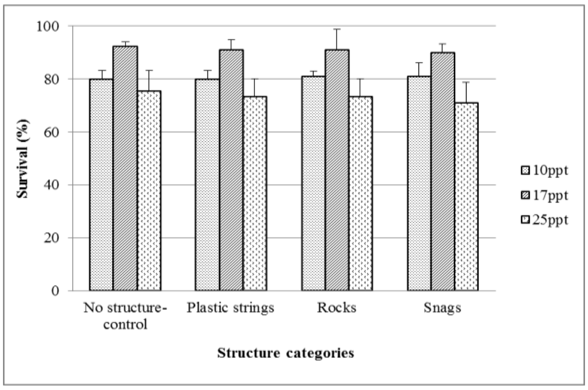


Figure 4. Survival of fish by salinity as time progressed



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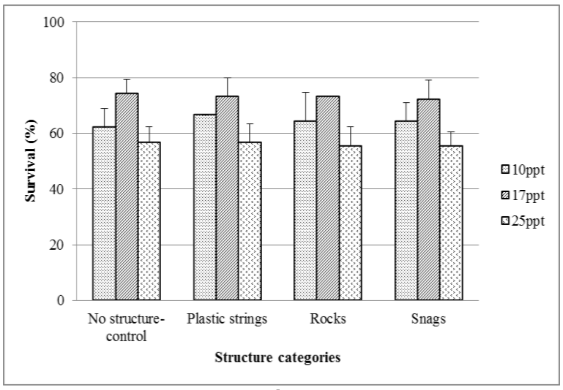

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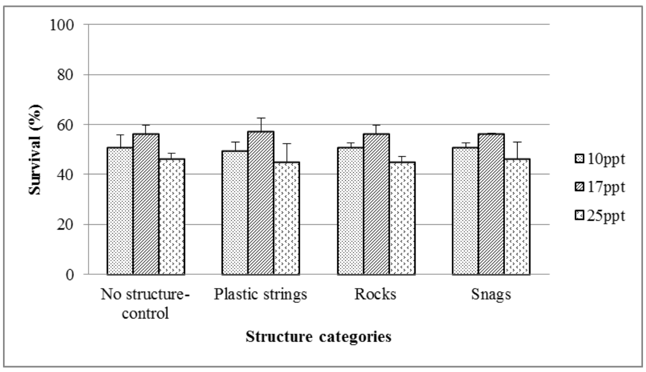


c

Figure 3. Survival of fish with different shelters and salinities (a, b, c: at day 10, day 20 and day 30, respectively)

Table 1. The differences of fish survival between treatments

(I) salinity	(J) salinity	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower bound	Upper bound
10ppt	17ppt	-7.592	1.306	0.085	-17.579	2.396
	25ppt	7.778*	0.735	0.026	2.158	13.399
17ppt	10ppt	7.592	1.306	0.085	-2.396	17.579
	25ppt	15.370*	1.286	0.021	5.534	25.207
25ppt	10ppt	-7.778*	0.735	0.026	-13.399	-2.158
	17ppt	-15.370*	1.286	0.021	-25.207	-5.534

* The mean difference is significant at the 0.05 level.

3.2 Growth rate

Total length of juveniles varied significantly between habitat structures (F(3, 87)=42.98, p<0.001), but not between salinities (F(2, 58)=0.23, p=0.79). The effect of sea-grass-emulating plastic structure was the same as having no structure, whereas both rock and snag structures provided a considerable boost to growth (>4%, on average) over 30 days. Likewise, mean weight of juveniles varied significantly between habitat structures (F(1.98, 3.97)=12.58, p=0.02), but not between salinities (F(2, 4)=0.33, p=0.74). Complex structures (rocks, snags) appear to provide the best habitats for juvenile growth, and weight of the fish in these habitats was consistently higher than in tanks with no structures or with plastic strings (~9% greater at 30 days) (Figure 5).

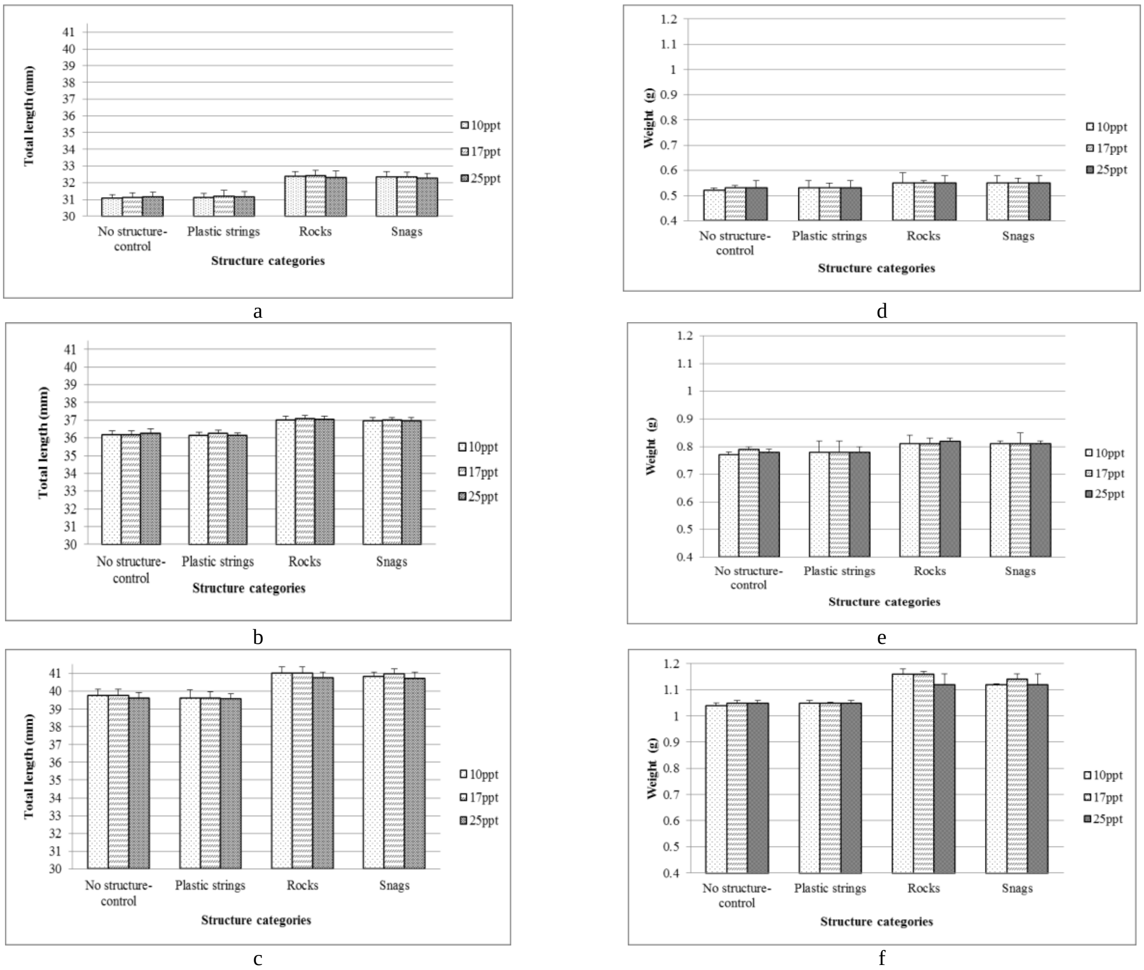
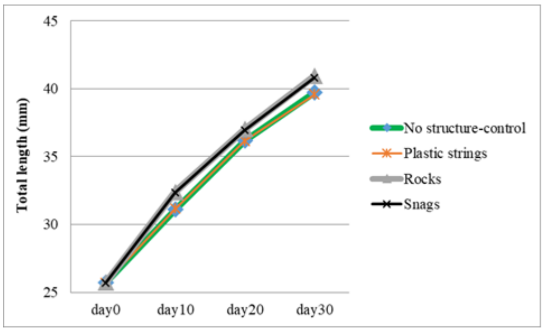


Figure 5. Growth of fish with different shelters and salinities (a, b, c: Total length of fish at day 10, 20 and 30, respecitvely; d, e, f: weight of fish at day 10, 20 and 30, respectively)

Interestingly, salinity had no effect at all on linear extension or weight gain of the juveniles in any of the structured environments. Juvenile fish grew as well in quite fresh (10ppt) and quite saline (25ppt) waters (Figure 6).



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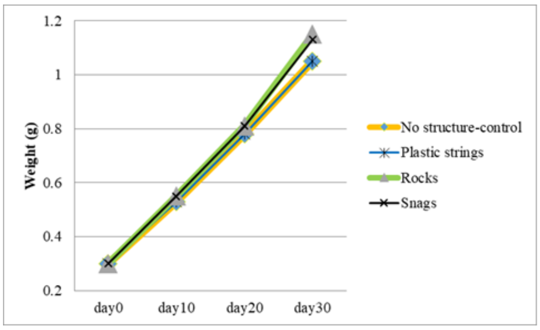

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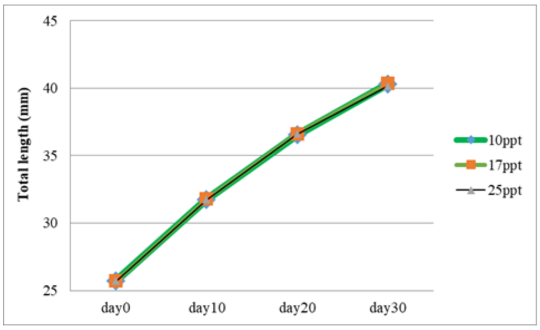

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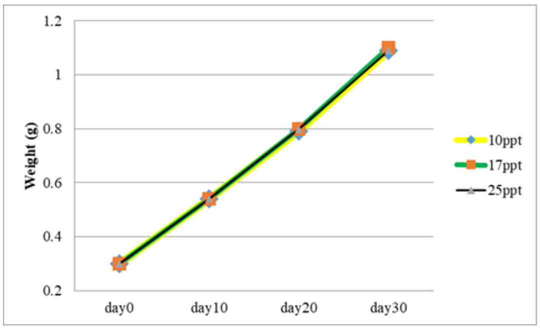

d

Figure 6. Growth of fish as time progressed (a, b: length and weight growth of fish by structure; c, d: length and weight growth of fish by salinity)

4. Discussion

L. argentimaculatus culture relies almost exclusively on wild-caught fry because Vietnamese mariculturists believe that hatchery-raised juveniles are unthrifty and weak. Although induced and natural spawning has been demonstrated in cage-reared *L. argentimaculatus* (Doi & Singhagraiwan, 1993; Emata *et al.*, 1994; Emata, 2003), hatchery culture of larvae has not been nearly as successful (Doi & Singhagraiwan, 1993; Duray *et al.*, 1996; Emata, 2003; Emata, Eullaran, & Bagarinao, 1994; Estudillo *et al.*, 2000; Lim & Chao, 1993). Our results suggest that this may be at least partly because hatchery-reared juveniles are raised under inappropriate conditions. Typically, Mangrove Red Snapper juveniles are cultured in featureless tanks at salinities approaching oceanic. The main effects of the experiment described here (the presence or absence of complex, hard structures, and moderate salinity) appear to operate independently on different aspects of juvenile growth and survival. That is, certain types of habitat correspond to increased growth rates amongst juveniles, but have no effect on survival, whereas the survival of juveniles was greatest at intermediate salinities that had no effect on growth rate of the survivors.

Key among our findings is that the salinity of water strongly influences rate of juvenile survival, without appearing to have any impact on the growth rate of the juveniles that survive (Figures 3 and 4). After 30 days of culture, the moderate (17ppt) salinity treatment exhibited a 20% increase in survivorship over the more oceanic (25ppt) treatment, and 11% increase over low salinity (10ppt), regardless of habitat structure (Figure 3). It is noteworthy that even this highest salinity treatment (reflecting the dry season salinity of the coastal lagoon where the fishermen collect juveniles for culture) is substantially less saline than the cases in most published accounts (e.g. Abbas, Jamil, Akhtar, & Hong, 2005; Abbas & Siddiqui, 2013; Estudillo *et al.*, 2000), although the strong preference for moderate salinities by juveniles has been known for a long time (Estudillo *et al.*, 2000), and it is not uncommon for similarly estuary-located juveniles of other species to attain maximal growth at these intermediate salinities (e.g. *Mugil cephalus* at 22-23ppt (Norris-Murashige *et al.*, 1991) and *Dicentrarchus labrax* at 10-20ppt (Johnson & Katavic, 1986)). It is clear that the practice of culturing fish whose juveniles settle in estuarine waters at oceanic salinities is at odds with current evidence, and should be modified to maximize survival of the cultured juveniles.

What is surprising about this result is that the juveniles kept at the higher and lower salinities grew as fast as those at the intermediate salinity preferable for survival (Figure 5). This suggests that the osmotic differences between treatments do not have severe metabolic costs (cf. Boeuf & Payan, 2001; Estudillo *et al.*, 2000) on the juveniles (hence compromising growth), yet in some way both low and high salinities affect viability. Further investigation of ontogenic shifts in salinity preferences (and their possible epigenetic consequences) may reveal ways to refine culture to further improve survival and maximize aquaculture returns on these fish.

Mangrove Red Snapper juveniles are known to recruit to mangrove-lined estuaries and coastal lagoons (Russell & McDougall, 2005). Adult Mangrove Red Snappers are often associated with snags (Grant, 1997), or rocky areas (Day *et al.*, 1981). In the coastal lagoons of Thua Thien Hue and Binh Dinh provinces, the recently-settled juvenile *L. argentimaculatus* are most commonly harvested from rock-lined areas of moderate salinity; in the winter, larger juveniles are primarily associated with shallow seaweed beds where they hunt sergestid shrimps (Vo & True, n.d.). Despite the similarity of the experimental plastic string habitat to this winter seaweed habitat, our experiment demonstrated that it offered no more benefit to the juveniles in terms of growth than did bare tank substrate. In contrast, both rocks and snags improved juvenile growth substantially. Both length and weight of fish in these complex structure habitats were consistently higher than those in tanks with no structures or with plastic strings (~4% greater length, ~9% greater weight after 30 days). This pattern held true regardless of salinity regime. It is not possible to ascertain whether this preference for hard, complex shelters is "instinctive", or a consequence of recruitment of the study animals to a natural habitat leading to risk-averse behavior or stress in the absence of shelter (despite the absence of potential predators in the aquaria). Closer observation reveals that the winter seaweed habitat grows on top of boulders as substrate, suggesting that the juveniles are actually using the underlying substrate for shelter and foraging in the seaweed above. It would be useful to repeat this experiment using both wild-caught and hatchery-raised juveniles to determine whether this result is from innate shelter-seeking behavior, or is learned by wild-settled juveniles. If the response is innate, then, regardless of its origin, it is clear that a simple way to improve weight gain and conditioning in juvenile *L. argentimaculatus* culture is for the aquaculturist simply to add hard structures to the culture tanks.

Several factors could potentially cause mortality in fish when rearing early juveniles, such as (inappropriate) food, rearing environment, or cannibalism. It is difficult to identify exactly which factors are the main causes of fish mortality in our experiment. Because of our observations (reported in another paper, Vo & True, n.d.), we believe that the "hatchery standard" food used in this trial might not be optimal for juveniles of this stage. Indeed, in the second experiment we reared juvenile fish using live *Acetes* and mysid shrimps that were found as the dominant prey in fish stomachs, to feed the juveniles, resulting in faster growth and higher survival than in the current study. It is clear that there are many interacting factors affecting the growth and survival of juvenile fishes under culture conditions, some of which are quite simple to address, and may provide large benefits with little effort on the part of the mariculturist.

Mangrove Red Snapper are a popular food fish, and a lucrative aquaculture product, and probably face local extinctions as both adults and juveniles are overharvested to supply for the demand. The supply of wild-caught fry is seasonal, unpredictable, and quite limited; on undertaking this research, we found that fishermen in several locations in Thailand and Vietnam have effectively ceased juvenile harvesting operations because the numbers have dropped catastrophically. Since *L. argentimaculatus* spawns readily in captivity, it seems obvious that hatchery-reared juveniles would provide a more consistent and ecologically sustainable resource for aquaculture, if the fishermen could be convinced that they perform as well as wild-caught fry. Here, we have demonstrated two simple environmental factors that improve juvenile survival and growth. By growing juveniles in moderate (15-20ppt) salinity water, in the presence of hard, complex structures such as rock piles or mangrove roots, juvenile survival rate can be improved by 20% and growth by almost 10%, without changing diet or stocking rates.

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