Effects of habitat structure and salinity on growth and survival of juvenile Mangrove Red Snapper *Lutjanus argentimaculatus* (Forsskal, 1775)

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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 recruit to 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 survivorship and salinity, and between somatic growth and habitat structure, but no interaction between the factors. Juvenile *L. argentimaculatus* survive 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 survivorship and growth of juvenile *L. argentimaculatus* and may provide a substantial boost to the performance of hatchery-reared fish.

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

Introduction

Mangrove Red Snapper (*Lutjanus argentimaculatus*) is an important commercial and recreational fish throughout its range (Allen, 2002; Norris-Zagars et al., 2012;
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 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 fishes.

*L. argentimaculatus* is threatened by both overfishing 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 survivorship. In Vietnam and elsewhere, the increasing pace of mangrove forest destruction (Rajarshi & Rajib, 2013; Richards & Friess, 2016), or coastal wetlands and seagrasses loss (Airoldi
& Beck, 2007) means that critical juvenile habitats of Mangrove Red Snapper as well as 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 subject to intensive fishing. After hatching, *L. argentimaculatus* larvae spend several weeks in the plankton before settling in brackish coastal waterways (Russell et al., 2003, Norris-Zagars et al., 2012). 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 differs between ontogenetic stages (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 of fish under 10cm were caught in amongst rocks while larger classes chose snags
as hiding places (Russell et al., 2003). These authors also found that very few fish were
catched 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 for foraging, sheltering or spawning habitats at some life stage (Nikolsky,
1963), and especially for juveniles. Mostafa, Malcolm, and Graham (1998) found that
there was a positive correlation between growth rates of *Clarias gariepinus* with
increase in 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
is likely to limit 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 as potentially important as that it exists; while a planktonic larva may gravitate towards floating leaves or sticks, newly-settled larvae are more likely to seek out crevices as refugia. As predatory competence increases, and the range of potential prey expands, it is likely that snags and mangrove prop roots offer increasingly greater foraging opportunities for juveniles, and hence become more desirable. Here, we explore the interplay between the two dominant habitat characteristics of the coastal lagoons favoured by juvenile mangrove jacks in Vietnam: salinity and structure. Changed juvenile survivorship associated with either of these factors offers an easy remedy for unviability in hatchery-raised juveniles, which can be used to take pressure off the fragile natural supply of these fish.

Materials and methods

Field surveys were undertaken to examine natural recruitment habitats in two provinces (Thua Thien Hue and Binh Dinh) (figure 1) in the northern and middle part 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 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.

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

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

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 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 subjected to continuous aeration. Photoperiod was held to a constant 12:12h light: dark cycle. During the experiment, 30% by volume of water was exchanged daily.
temperature during the experimental period ranged between 26.5-29°C; dissolved oxygen was maintained at 5.3-5.7 mg/L; pH was kept between 7.8-8.3; ammonia was consistently less than 0.1 mg/L for the duration of the experiment.

Fish were fed minced fresh fish at 7:00am and 17:00, and fed *Artemia* nauplii at 12:00pm, 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 subsample of the fish in the tanks, potential differences in survivorship 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 separate and synergistic effects of salinity and habitat conditions on weight and length of juveniles over the study period. All statistical analyses were done using SPSS version 15.0 (SPSS Inc. 2006).

**Results**

**Survivorship**

Habitats had no statistically significant effect on survivorship rates of juveniles. Salinity was the major determinant of fish survivorship 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 analysed using the same repeated measures model as for the growth increments, we found that only survivorship at the highest salinity was significantly different (i.e. lower). However, it was apparent that habitat has no discernable effect on survivorship rates (F(1.2, 2.39)=0.18, p=0.75). Nor was there an interactive effect on habitat and salinity (F(1.8, 3.6)=0.1, p=0.89). Therefore, when the habitat groups are pooled, the apparent effect size increases across salinities, and it is evident that the lowest (10ppt) and highest (25ppt) are significantly less beneficial to juvenile survivorship than moderately brackish (17ppt) waters (figure 3, 4; table 1).

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

**Figure 4.** Survival of fish according to salinities through time

**Table 1.** The difference of fish survival between treatments

**Growth rates**

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 the seagrass-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 fishes in these habitats was consistently higher than those in
tanks with no structures or with plastic strings (~9% greater at 30 days).

**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 regime had no effect at all on linear extension or weight
gain of the juveniles in any of the structure treatments. Juvenile fishes grew as well in
quite fresh (10ppt) and quite saline (25ppt) waters.

**Figure 6.** Growth of fish throught time (a, b: length and weight growth of fish
according to structures; c, d: length and weight growth of fish according to salinities)

**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, Eullaran, & Bagarinao, 1994; Emata, 2003; 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 survivorship. That is, certain
types of habitat correspond to increased growth rates amongst juveniles, but have no
effect on survivorship, whereas survival of juveniles was greatest at intermediate salinities (which had no effect on growth rates).

Key among our findings is that the salinity of the water strongly influences rates of juvenile survival, without appearing to have any impact on the growth rates of the juveniles that survive (figure 3, 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 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 survivorship of 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 (figure 5). This suggests that the osmotic differences between treatments are not having 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 survivorship 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, in review). 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 substrate. Contrastingly, both rocks and snags improved juvenile growth substantially. Both length and weight of fishes 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 a boulder 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.

There may be many factors thought as to be causes of mortality of fish when rearing early juveniles, such as (inappropriate) food, rearing environment, or cannibalism. It is difficult to identify exactly which factors are the main cause for fish mortality in our experiment. Because of our observations (reported in another paper: Vo & True (in review), we believe that the “hatchery standard” food used in this trial might not be completely optimal for juvenile 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 juveniles, resulting in faster growth and higher survival than in the current study. It is clear that there are many interacting factors in the growth and survival of juvenile fishes under culture conditions, some of which are quite simple to address, and provide large benefits for little effort on the part of the mariculturist.

Mangrove Red Snapper are a popular food fish, and a lucrative aquaculture product, and is probably facing local extinction as both adults and juveniles are overharvested to supply demand. The supply of wild-caught fry is seasonal, unpredictable, and quite limited; in undertaking this research, we found that fishermen in several locations in Thailand and Vietnam have effectively ceased juvenile harvesting operations because 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 survivorship 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 survivorship can be improved by 20% and growth by almost 10%, without changing diet or stocking rates.

Acknowledgement

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Reference


Western Central Pacific - Bony fishes part 3 (Menidae to Pomacentridae) (pp. 2791-3380). Rome: FAO.


The figure caption list

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Figure 2. Natural habitat features in estuarine areas where juvenile Mangrove Red Snapper are most commonly caught by fishermen.
Figure 3. Survival of fish with different shelters and salinities (a, b, c: at day 10, day 20 and day 30, respectively)

Survivorship was largely determined by salinity; survivorship at 25ppt salinity was significantly less than at lower salinities. Although not significant under a 2-factor repeated measures model, survivorship rates under these salinity treatments were significantly different when habitats were pooled.

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* The mean difference is significant at the 0.05 level.