Habitat Specificity and Feeding Ecology of Juvenile Mangrove Red Snapper (*Lutjanus argentimaculatus* Forsskal, 1775)

Vo Van Chi

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biology
Prince of Songkla University
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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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Abstract

Mangrove Red Snapper (*Lutjanus argentimaculatus*) is an important commercial and recreational fish throughout its range and considered to be an excellent food fish. In Vietnam, Mangrove Red Snapper has been a favourite fish species for mariculture, however, the bulk of Vietnam's mariculture of Mangrove Red Snapper relies on the depends on fingerlings recruitment in the two remaining locations where juveniles recruit in sufficient numbers to support harvesting.

In this study, I investigated habitat specificity and feeding ecology of juvenile Mangrove Red Snapper in central Vietnam by combining field surveys with confirmatory experimental studies. The results showed that there was only one major recruitment season of juvenile in central Vietnam, from July to August. During the recruitment period, juvenile fish mostly occupied complex rocky habitats within a salinity range of 10-25ppt, and consumed a wide range of planktonic and benthic prey. Fish revealed seasonal and ontogenetic changes in feeding habit, and the intensity of their feeding activity was also governed by tidal rhythm, being maximal on the rising tide. Consumption of prey varied with maturity of the juveniles, with mysid shrimps providing the bulk of prey for the smallest size class. Larger juveniles foraged in different strata of the water column and consumed both benthic and planktonic prey, but relied entirely on sergestid shrimps during the winter months. It was found that hard-complex habitat structure had benefits in term of growth while moderate salinity water improved survival of fish in experiments. My experimental findings also demonstrated that using natural food prey species (*Acetes* and mysid shrimps) as the diet of cultured juveniles strongly promoted both growth and survival of juvenile fish. Therefore, the findings of this study reveal that a simple way to enhance commercial production is to culture juveniles in moderately saline water (15-20ppt), in the presence of hard, complex structures such as rocks or snags, and using live shrimp prey as *Acetes* and mysids to feed fish. It is clear that the presence of complex rocky habitats within mangrove-lined estuaries and the associated food resources play an important role for growth and/or survival of juvenile Mangrove Red Snapper, and represent critical habitat for juvenile recruitment.

ACKNOWLEDGEMENT

Firstly, I would like to give many thanks to my supervisors, Asst. Prof. Dr. James Dominic True, for his support and guidance throughout my project. He helped me become better in conducting scientific researches and scientific writing.

I would like to thank the President of Prince of Songkla University for offering me the fellowship. I am also gratefully acknowledge the “PSU.GS. Financial Support for Thesis, Fiscal Year 2015” from Graduate School, Prince of Songkla University. I am also thankful to Department of Biology, Faculty of Science, Prince of Songkla University for all supports during my study.

I am thankful to my committees of both proposal and thesis examination, who gave me valuable suggestions to accomplish my study.

I am also thankful to my teacher, Mr. Vo Dieu, and his students from Hue University of Agriculture and Forestry who supported me to do interviews and field surveys, and helped me identify some natural food organisms.

I am also grateful Dr. Koraon Wongkamhaeng from Marine and Coastal Resource Institute (MACORIN), Prince of Songkla University for helping me identify zooplankton.

I am thankful to the vice Director of Center for Aquatic Breeding of Binh Dinh province, Mr. Phan Thanh Viet, and his staffs for supporting me to do my experiment.

I also thank fishermen and my undergraduate students who helped me complete the field surveys and experiments.

I would like to thank my friends in Thailand who helped and encouraged me to overcome difficulties during my study.

Finally, I would like to thank my parents, my brothers and sisters who supported and encouraged me during my study. I would like to give special thanks to my wife and my son who always encouraged me and tried to overcome all difficulties during my study.

Vo Van Chi

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CHAPTER 1

INTRODUCTION

1.1. BACKGROUND AND RATIONALE

Mangrove Red Snapper (*Lutjanus argentimaculatus*) is an important commercial and recreational fish throughout its range and considered to be an excellent food fish (Allen, 2002; Russell et al., 2003; Zagars et al., 2012; Piddocke et al., 2015). This fish species is distributed widely in the Indo-West Pacific from Samoa and the Line Islands to East Africa and from Australia northwards to Ryukyu Island, Japan (Doi & Singhagraiwan, 1993).

This large, palatable fish has become a sought-after aquaculture species in Southeast Asia because its size and eating qualities command consistently high market price (Liao et al., 1995; Wong, 1995; Chou & Lee, 1997; Emata & Borlongan, 2003). The development of this industry through SEA has brought important income to coastal farmers.

In Vietnam, Mangrove Red Snapper is also a valuable economic species, and is exported to nearby countries such as China, Taiwan, and Japan. It is also a favoured item in the domestic market (Thanh, 2012). Mariculture of this species has become common throughout Vietnam's coastal areas. However, its aquaculture mostly depends on a wild fingerling supply that is limited, seasonal and unpredictable although this fish species has been produced in several research projects (Thanh, 2012). Interviews with fishermen and mariculturists throughout Vietnam have indicated that this once-common wild resource is rapidly depleting, to the extent that many areas can no longer support the harvesting of wild juveniles (Vo, unpubl.). The bulk of Vietnam's mariculture of Mangrove Red Snapper relies on the production of the two remaining locations where juveniles recruit in sufficient numbers to support harvesting. It is clear that such widespread and intense gathering of natural juveniles has caused noticeable pressure on the wild fish resource. Mangrove Red Snapper is a desirable table fish at all stages of its life cycle, and fishery pressure on subadults and sexually mature adults is intense. *L. argentimaculatus* is a long-lived fish (up to 31 years), and is vulnerable to recruitment overfishing of the deep water adults and the subadults that inhabit coastal areas (Fry et al., 2006). Moreover, this species is never found in large quantities anywhere in its range (Anderson & Allen, 2001), while the demand for aquaculture stock is increasing. Given the lack of fishery-restricted marine protected areas in Vietnam and elsewhere in SEA, long term prospects for the persistence of this species in the wild are unpromising, and depend greatly on a continuing supply of juveniles recruiting into the adult population.

In nature, juvenile *L. argentimaculatus* tend to be associated with mangrove-lined habitats (Sheaves, 1995; Zagars et al., 2012). The ecosystem quality of the mangrove habitat apparently exerts a strong influence on the abundance and size of *L. argentimaculatus* in the mangroves (Nanjo et al., 2014). Unfortunately, Vietnam like many other countries in Southeast Asia have a high rate of mangrove deforestation (Rajarshi & Rajib, 2013; Richards & Friess, 2016). Therefore, if the associated habitats, and ecological goods and services of mangroves are essential for Mangrove Red Snapper recruitment, this loss of critical habitat is likely to depress both recruitment and survivorship of juveniles, further depleting the pool of fish available to local fishers, and for natural replenishment.

Although the survival of early juvenile stages of marine fish species is crucial bottleneck leading to the success or failure of subsequent year fish classes (Bailey & Spring, 1992), studies on the ecological determinants of juveniles of Mangrove Red Snapper remain scarce. This lack of knowledge hinders attempts to conserve a key fishery, since several large projects or programs are being carried out to recover destroyed coastal habitats in Vietnam; if certain types of coastal aquatic habitats are necessary for enhancing the recruitment of livelihood-related fishes, then it is important to identify the critical factors associated with high levels of recruitment and survivorship in fish settlement magnets. Moreover, the accelerating push of intensive aquaculture into coastal areas to generate foreign income threatens areas perceived to be “beneficial” to juvenile growth, since these are desirable. The threats posed by intensive pond aquaculture include mangrove conversion into ponds, use of antibiotics and chemicals leading to drug resistance, and the dumping of pond effluents which pollute neighboring ecosystems.

Therefore, in this thesis, I will attempt to describe ecosystem factors associated with the successful recruitment and growth of juvenile *L. argentimaculatus* by examining habitat specificity and feeding ecology of this species in coastal areas in central Vietnam. This study will provide important knowledge for the conservation and management of marine resources in general, and Mangrove Red Snapper in particular. It is also hoped that this study will provide important baseline information to support large projects for coastal ecosystem conservation and fisheries resource management being undertaken in Vietnam, and as a case study for the wider SEA area.

1.2. REVIEW OF LITERATURE

1.2.1. General information concerning Mangrove Red Snapper

In Asia, *L. argentimaculatus* (Forsskal, 1775) is generally called Mangrove Red Snapper. In Australia, however, it is most commonly known as Mangrove Jack but is also known as Creek Red Bream, Dog Bream, Purple Sea Perch, Red Bream and Red Perch (Grant, 2002). There are 103 species in the family Lutjanidae, of which there are 65 species of the genus *Lutjanus*. Of these 39 species occur in the Indo-Pacific, 9 in the eastern Pacific, 12 in the western Atlantic, and 5 in the eastern Atlantic (Doi and Singhagraiwan, 1993).

Mangrove Red Snapper is a carnivorous species. They consume a wide range of prey, but adults feed mainly on fishes, crustaceans, gastropods and cephalopod molluscs. As ambush predators, they often dwell around mangrove roots, fallen trees, rock walls, and any other snag areas where smaller prey reside for protection. (Martin F. Gomon & Dianne J. Bray, *Lutjanus argentimaculatus* in Fishes of Australia, accessed 06 August 2017, http://fishesofaustralia.net.au/home/species/548)

Mangrove Red Snapper can be identified morphologically using the following characteristics (Allen, 1985):

http://www.fishbase.org/Summary/SpeciesSummary.php?ID=1407&AT=mangrove+red+snapper

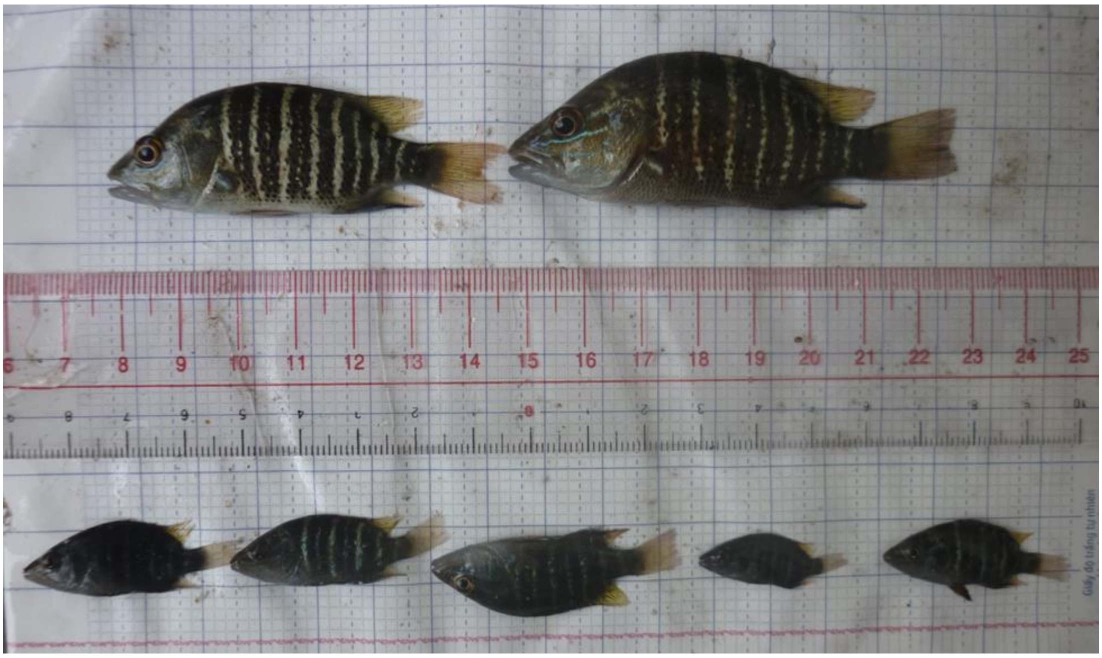

Figure 1.1. Juvenile Mangrove Red Snapper at different life stages caught in central Vietnam.

Figure 1.2. Adult of Mangrove Red Snapper caught in central Vietnam.

Body moderately deep (greatest depth 2.5 to 3.1 times standard length: D_{max}= (2.5→3.1)xTL). Snout somewhat pointed; preorbital bone relatively broad, wider than eye diameter; preopercular notchand knob poorly developed; vomerine tooth patch crescentic, without a medial posterior extension; tongue with a patch of granular teeth; gill rakers on lower limb of first arch (including rudiments) 9 to 12, total gill rakers on first arch 16 to 20. Dorsal fin with 10 spines and 13 or 14 soft rays; anal fin with 3 spines and 8 soft rays; posterior profile of dorsal and anal fins rounded; pectoral fins with 16 or 17 rays; caudal fin emarginate to nearly truncate. Scale rows on back more or less parallel to lateral line, or parallel below spinous part of dorsal fin and sometimes rising obliquely posteriorly, or rarely with entirely oblique rows, belly silvery or whitish; specimens from deep water frequently overall reddish; juveniles with a series of about eight whitish bars crossing sides, and 1 or 2 blue lines across cheek.

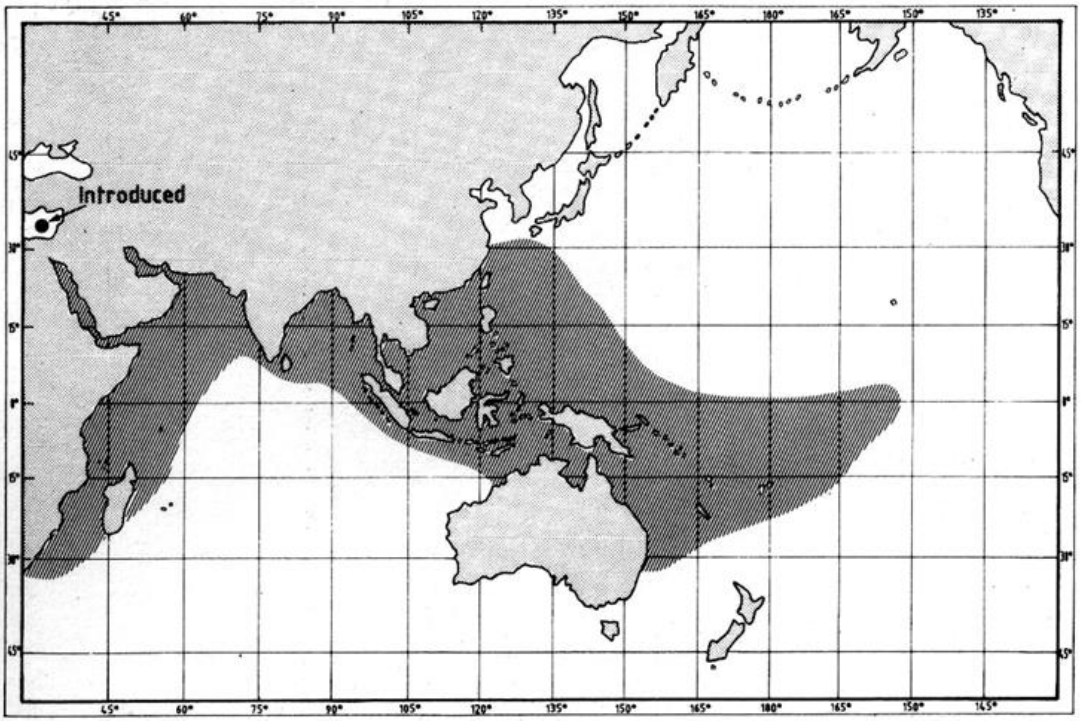

Figure 1.3. Global distribution of Mangrove Red Snapper (Allen, 1985).

Mangrove Red Snapper is distributed widely in the Indo-west Pacific from Samoa and the Line Islands to East Africa and from Australia northwards to Ryukyu Island, Japan (Doi & Singhagraiwan, 1993) (Figure 1.3). It is also believed to migrate between the Red Sea via the Suez Canal to the Mediterranean coasts of Israel and Lebanon but is not believed to have become well established in the wild in these areas (Anderson & Allen, 2001). In east Africa, this species is common in mangrove areas, estuaries and sheltered coastal and reef areas (Talbot, 1960). It is commonly found in Mozambique and extends in decreasing numbers to the Transkei (Day et al., 1981). In Australia, it is distributed from northern New South Wales, around the northern coast to Shark Bay, Western Australia; there are records of Mangrove Red Snapper being caught as far south as Sydney (Allen et al., 2002). In Vietnam, Mangrove Red Snapper are caught in most of coastal areas from north to south, especially in southern central areas, where many river-fed lagoons are associated with mangroves, and Truong Sa archipelago (Nguyen et al., 1995; Thanh, 2012; Figure 1.4).

Figure 1.4. Distribution areas (marked as stars) of Mangrove Red Snapper in Vietnam (Nguyen et al., 1995; Thanh, 2012).

L. argentimaculatus, like many lutjanids, spawns near deep, offshore reefs (Day et al., 1981; Doi & Singhagraiwan, 1993). After hatching, larvae spend several weeks in the plankton before settling in brackish coastal waterways (Russell et al., 2003, Zagars et al., 2012). Juveniles and subadults are often found in brackish estuaries and in the lower reaches of freshwater streams (Russel & McDougall, 2005). As they mature, fish are commonly found in mangrove-lined estuarine systems, before they migrate to offshore reefs, sometimes hundreds of kilometers from the coast, to spawn when reaching reproductive maturity at 5-7 years of age (Allen & Erdmann, 2012). *L. argentimaculatus* is a long-lived fish (up to 31 years: Fry et al., 2006).

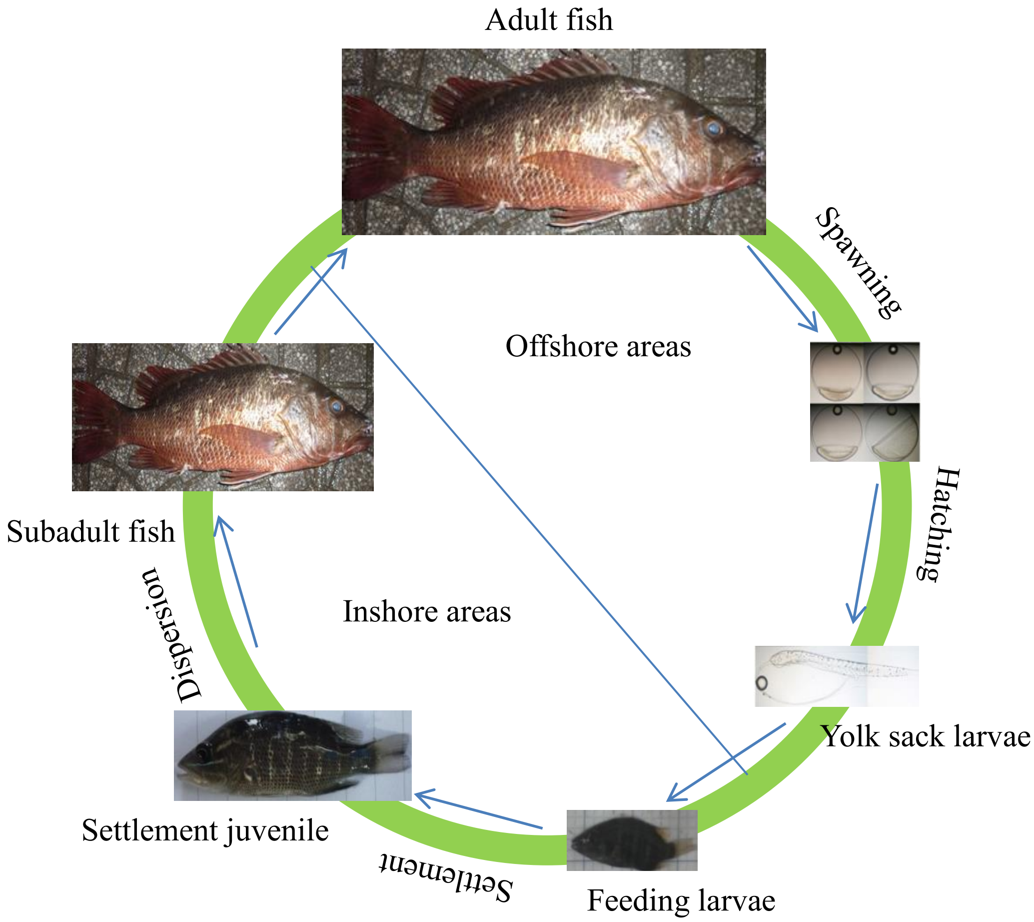

Figure 1.5. Life cycle of Mangrove Red Snapper.

1.2.2. Habitat

Like other animals, fish need healthy habitats to survive, grow, and reproduce, and the quality and quantity of habitats, therefore, can directly or indirectly affect fish populations. Coastal areas are thought to be vital habitats for fish in general and Mangrove Red Snapper in particular, especially during its juvenile stages. However, habitat degradation, such as mangrove forest destruction (Rajarshi & Rajib, 2013; Richards & Friess, 2016), or coastal wetlands and seagrasses loss (Airoldi & Beck, 2007), loses critical habitats of this fish species, and thus seriously threatens natural fish resource through direct removal or by controlling recruitment. Therefore, examining factors associated with critical habitats such as estuaries and mangroves is essential to ensure continued resources of this species.

Mangrove Red Snapper is an euryhaline species. Wild fish can be found in a salinity range from 8-40ppt (Estudillo et al., 2000). The larvae and juveniles of this fish are found in estuaries and coastal areas, and but also are reported to move into freshwater areas (Lake, 1971; Doi & Singhagraiwan, 1993; Russell & McDougall, 2005; Ebner & Morgan, 2013). The extent of their movement into freshwater is generally limited, although, 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). Similarly, also in northern Australia, Russell and McDougall (2005) found that fish less than 50mm TL appeared to recruit into freshwater riverine habitats. In eastern Thailand, juveniles (mostly 18-25mm total length) occur seasonally in rivers and canal estuaries (Doi & Singhagraiwan, 1993). Doi et al. (1998) suggested that juveniles larger than 16mm total length would acquire swimming or cruising ability strong enough to migrate from offshore spawning grounds to coastal waters and estuaries. These authors also observed that juveniles appeared to move into the estuary after the wet season (from late October to January, on the South China Sea coast) and suggested that the upstream movement of *L. argentimaculatus* was governed by freshwater run off resulting from high seasonal rainfall. In Vietnam, fishers comment that juveniles Mangrove Red Snapper are mostly caught at coastal lagoons where are associated with estuaries and mangroves, however, there is no specific study undertaken on this aspect. Generally, it is likely that above studies only focus on generally investigating sites of occurrence and movement of juveniles, while understanding of their habitat specificity is still limited. However, information on details of habitats is an important key for management and conservation of fish resource, thus studies on this aspect are needed.

Some studies shows that estuaries are important habitats for Mangrove Red Snapper. In eastern Australia, Sheaves (1995) suggested that estuaries were important development grounds for *L. argentimaculatus* and that estuarine populations appeared to consist entirely of immature fish. In the Emberly River in the north-eastern Gulf of Carpentaria (Australia), *L. argentimaculatus* was more abundant in the middle reaches of the estuary than either upstream or in the lower reaches (Blaber et al., 1989). These authors also found that mangrove red snapper was one of 14 species whose juveniles were found exclusively in estuarine waters, although adults were found both inshore and offshore.

Apart from a few efforts at general assessment, there were few studies looking in detail at the juvenile habitat of this fish. In estuaries, freshwater and inshore areas Mangrove Red Snappers were often associated with snags (Grant, 2002) or coastal reefs (Allen, 1997). In coastal areas of southern Africa, juveniles from Morumbene estuary to the Mngazana were mainly found in rocky areas (Day et al., 1981). A more detailed description by Russell et al. (2003) for *L. argentimaculatus* in rivers and creeks of north east Queensland showed that Mangrove Red Snapper mostly utilized rocks, snags and roots as their shelters, however, there was habitat shift between size classes. These workers indicated that most of the fish less than 10cm in length were caught in amongst rocks, while larger classes chose snags to hide. These authors also found that very few fish were caught in open water where there was no cover. In general, the above studies imply that rocks, snags and roots are important shelters associated with habitats of Mangrove Red Snapper in the wild. The question generated is whether these shelters are always chosen by *L. argentimaculatus* in different geographical areas, or why mangrove red snapper choose such shelters.

On the other hand, both tropical and subtropical mangrove habitats are recognised worldwide as important nursery habitats for juvenile fish (Weinstein and Brooks, 1983; Wright, 1986; Robertson & Duke, 1987; Little et al., 1988; Chong et al., 1990). It has been proved that high number of fishes and marine invertebrates depends to a large extent on mangrove habitats during the juvenile phase of their life cycles (Bennett, 1989). The availability of mangrove nursery habitats has been demonstrated to have a striking impact on the community structure and biomass of reef fish. The biomass of several species is more than doubled when the reefs are connected to rich mangrove resources (Mumby, 2004). The larvae of *L. argentimaculatus* usually recruit to mangrove-lined estuaries (Russell et al, 1999; Zagars et al., 2012), and therefore mangrove is thought to play an important role for this species. However, the true reliance of juvenile Mangrove Red Snapper on mangroves is somewhat unknown, given the apparently strong influence of estuarine habitats on recruitment, however this apparent conflict may simply represent different requirements at different development stages of juvenile: the published data from the few studies worldwide are not sufficiently comparable to resolve this. It has been shown that the quality of the mangrove habitat apparently exerts a strong influence on the abundance and size of *L. argentimaculatus* in the mangroves (Nanjo et al., 2014), so if mangroves are destroyed, they can affect seriously on both recruitment and survivorship of juvenile fish. This may mean that the extremely high rate of mangrove deforestation which has occurred in Southeast Asia as well as in Vietnam (Rajarshi & Rajib, 2013; Richards & Friess, 2016) may have had profound effects on the pool of juveniles recruiting into the fishery. Despite the apparent importance of mangroves as nursery areas for life history of *L. argentimaculatus*, there is no research on the early stages of this fish species and their habitat ecology undertaken in Vietnam.

L. argentimaculatus inhabit quite a range of coastal habitats apart from estuaries or mangrove forest. For instance, in the tidal Leanyer swamp of northern Australia, transient juveniles were reported to be using the swamp as a nursery (Davis, 1988). This author found that the numbers of juvenile *L. argentimaculatus* entering the swamp were correlated with the environmental parameters and tidal height. Higher tides provide greater assistance for the upstream movement of juvenile fish and also enable them to penetrate further into upstream areas.

It is thought that characteristics of the diverse habitats of Mangrove Red Snapper are different in various geographical regions, and this varied ecology can impact on recruitment, survivorship and abundance of juveniles. The roles of key elements of habitats structure and ecology may have profound importance to the rate of replenishment of this heavily fished species. Although there have been some few studies of habitat associations, little is yet known about the functions of those habitats or how they are used by the juveniles at different ontogenic stages.

1.2.3. Trophic ecology

Studies of feeding habits and diet are the key to understand many aspects of the biology, ecology, physiology, and behaviour of fish (Goncalves & Erzini, 1998; Rita et al, 2006). Therefore, accurate description of fish diets and feeding habits would provide the basis information to understand trophic interactions in the food web as well as to examine important ecological aspects such as behavior, habitat use, and energy intake of fish.

Diet may be a major factor initiating the shift toward adult habitat, although other factors may be involved (Cocheret et al., 2003). However, the feeding habits of juveniles at each stage may be different, due to changes in buccal development, body shape or behaviour and may change during transition between ontogenetic stages. While many studies have examined trophic shifts in marine species, few have looked in detail into changes in snapper trophism. Szedlmayer and Lee (2003) believed that the habitat shift would increase food resources and protection from predators. This implies that there are certain relations between fish diets and their habitats. Thus, Szedlmayer and Lee (2003) suggested that the diet shifts of red snapper *Lutjanus campechanus* were attributed to opportunity for feeding more on reef-associated prey than on open-water prey, and diets were separated by habitat type rather than fish size for the size ranges whose habitats overlapped. This viewpoint is supported by Nakamura et al. (2008) who found that *Lutjanus fulvus* (42–100mm SL) caught in mangroves fed predominantly on estuary-associated crabs and shrimps while individuals collected from the coral reef fed mainly coral reef-associated crabs. The similar results were also observed for two species *Lutjanus fulviflamma* and *Lutjanus ehrenbergii* (Berkström et al, 2013). These studies imply that diet of fish mostly depends on natural food availability at the habitats where they are settling but give no insight into why fish are migrating between habitats at different growth stages. However, studies on feeding habits of fish in linking to natural food resources as well as habitats are still limited, and seems that this work has been examined for small numbers of lutjanids. And, it is worth to note that although Mangrove Red Snapper is an economically and recreationally important species, very little information on their trophic ecology is documented.

Feeding habits of fish not only shift between their habitats but also change between size classes. Berkström et al. (2013) found that individuals of *L. ehrenbergii* from 3.2-10cm in mangrove habitats fed upon crabs and fish, while the larger size class of 16.4-19.4cm mostly consumed crabs. Similar results were also reported by Nakamura et al. (2008); in the same mangrove habitat, small *L. argentimaculatus* (6.6-9.2cm SL) mostly fed on estuary-associated grapsid crabs while the larger size class (12-18.9cm SL) fed on fishes and shrimp in addition to crabs. In another lutjanid, *L. gibbus*, small individuals (5.4-7.3cm SL) consumed shrimps and isopods, while larger fish (14.2-27.5cm SL) consumed predominantly coral reef-associated crabs (Nakamura et al., 2008). Ontogenetic diet shifts were also recorded on other fish species (Sedberry & Cuellar, 1993; Burke, 1995; Rooker, 1995; Lowe et al., 1996; Szedlmayer & Lee, 2003). Carnivorous fish as snappers often tend to catch bigger prey with increasing their size (Johnson et al., 2012). However, some authors observed that when food is abundant, then fish predominantly selects prey of the largest size class available, but large prey become scarce, this fish tend to eat more prey from smaller size classes (e.g. for *Lepomis macrochirus* O'Brien et al. (1976)). That means that fish can shift their dietary to adapt food resource availability in water environment. Therefore, information on the feeding habits of fishes in linking to habitats, in particular preferred-prey abundance or availability, is useful in order to assess the role of fishes in the ecosystem as well as to make plans for fish resource management.

The time of year of an ecological survey can have a profound effect on the findings of a survey of fish diets, especially if – like *L. argentimaculatus* – recruitment is seasonal, and the climate of the locality changes between seasons. Monteiro et al. (2009) found that dog snapper *Lutjanus jocu* between 8.4 to 31cm TL consumed mainly penaeid shrimps in the dry months, while in the wet months they displayed a widening of the trophic spectrum consuming Grapsidae, Porcellanidae, Portunidae, Penaeidae and Xanthidae. These authors did not offer an explanation for the diet shift in this study, except to support the idea that they could be related to the distribution, abundance and availability of prey in each season (Rooker, 1995). Snyder (1984) and Lucena et al. (2000) suggested that seasonal changes in a trophic guild can often be attributed to the changes in life history patterns of their food organisms, which would make sense in this context if cohorts of larval recruitment coincide with cycles of prey replenishment.

A common theme amongst trophic studies of lutjanids appears to be the flexibility of their diets related to habitat characteristics and seasonal fluctuations and the strong ontogenetic dietary shifts that are associated with changes in habitat with age. In particular, it would appear that the prey composition of the estuarine habitats and coastal habitats of the early juvenile stages of most snappers is a key component of their trophic ecology.

Moreover, although there were a number of studies carried out for lutjanids, very few studies have been done on Mangrove Red Snapper, so information on trophic ecology of this species is still scarce. The question is raised as to whether *L. argentimaculatus* is similar to other snappers in their trophic ecology; in particular, whether they change feeding habits between size classes, habitats, and seasons.

1.3. STUDY SITES

This study was carried out at sites in two provinces (Thua Thien Hue and Binh Dinh) in the north and south central Vietnam (Figure 1.6) where juvenile Mangrove Red Snapper are harvested annually to supply aquaculture. Both provinces have river-fed coastal lagoons connected to the sea, such as “Tam Giang-Cau Hai” in Thua Thien Hue province and “Thi Nai” in Binh Dinh province.

The Tam Giang - Cau Hai lagoon is located along the coastal area of Thua Thien Hue province, north central Vietnam. This lagoon prolongs 70km, is connected to the sea by two inlets named Thuan An in the north and Tu Hien in the south. It has 21,600ha in area, 1 - 10 km in width, 1.5 - 2m in depth on average and over 10m maximum at the Thuan An inlet (Thanh & Nam, 2002). This lagoon is the largest in Southeast Asia and typical for monsoon tropics. It plays a very important role for the coastal ecology and socioeconomic development of Thua Thien Hue. It has taken an important part in maintaining the stability of hydrological, biological and ecological features related to the livelihood of about one million people in surroundings. It serves as a climate regulation lake able to restrict storm surge, flood-inundation, saltintrusion and to stabilize ground water level as well as to conserve biodiversity. Tam Giang - Cau Hai is also favorable nursery for many fish species and others due to its rich food supply and good environmental conditions. Moreover, it is also an ideal fishing ground for local residents. There is an increasing perception that this is a good place for ecotourism development. Thus, Tam Giang - Cau Hai serves an especially great role in the socioeconomic development in the region (Thanh et al., 1996; Thanh & Nam, 2002). However, under the pressure of economic and population development, the lagoon ecosystem resources and environment are facing severe problems, including the pollution from industrial oil and domestic wastes, over exploitation and habitat degradation (Thanh & Nam, 2002).

Thi Nai lagoon covers an area of 5,060 ha and represents a major wetland ecosystem in Binh Dinh province. There is about 36tonnes of finfish, 75tonnes of crustacean and 600tonnes of molluscs captured annually. Besides, the mangrove forest associated with the lagoon is also the habitat of 10 resident bird species and 37 species of migratory water-birds. However, although mangrove and seagrass habitats in this lagoon are known as vital living environment of many species, unregulated resource exploitation has destroyed or reduced their ecosystem functions (Fisheries Department, Binh Dinh province, 2008). Recently, the Thi Nai lagoon has acquired particular economic importance because it is one of the key areas addressed to drive the socioeconomic development in central Vietnam that is scheduled for 2020 (Stefania et al., 2013).

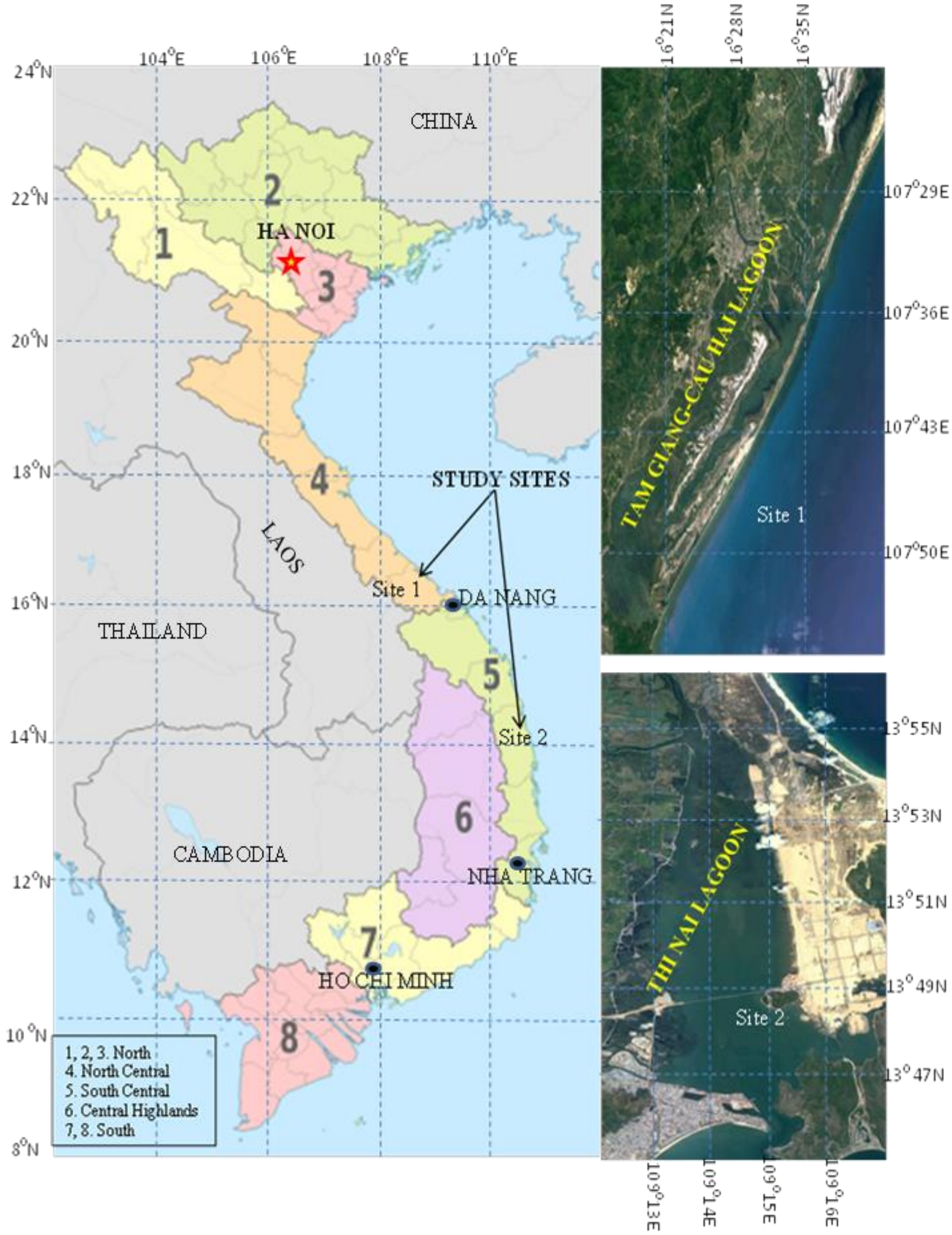

Figure 1.6. Study sites (Site 1: "Tam Giang-Cau Hai" in Thua Thien Hue province; Site 2: "Thi Nai" lagoon in Binh Dinh province.

1.4. RESEARCH OBJECTIVES

This study was undertaken with intent to investigate factors associated with the recruitment of juvenile *L. argentimaculatus* by examining habitat specificity and feeding ecology of this fish species in coastal areas in central Vietnam. The information obtained from this study will be important knowledge for the conservation and management of marine resources in general, and Mangrove Red Snapper in particular.

1.5. RESEARCH QUESTIONS

In overall, this study aims to answer the question “What are critical habitats during recruitment of juvenile Mangrove Red Snapper in central Vietnam?”. To clarify this big question, we will go to interpret specific considerations followed:

1. When does the recruitment of juveniles occur? What are prefered habitats of juveniles in central Vietnam?

2. Do habitat structure and salinity influence growth and survival of fish?

3. What are the natural diets of fish? Are there ontogenetic and seasonal shifts in feeding of fish?

4. Do natural food prey found in the stomachs affect growth and survival of fish?

1.6. STRUCTURE OF THE THESIS

This thesis is composed in 5 chapters, of which manuscript versions of Chapter 2, Chapter 3 and Chapter 4 have been submitted as manuscripts for publication. The chapters are arranged as follows:

In Chapter 1, I present definitions of the key terms, review published information related to the biology and ecology of *L. argentimaculatus*, and outline the contents of each chapter.

In Chapter 2, I investigate ecological characteristic of the environments from which juvenile fishes are harvested to supply the aquaculture industry, to find out what sort of habitats are preferred at different juvenile size classes in the wild, as well as conditions which might enhance recruitment of juvenile Mangrove Red Snapper in the coastal lagoons of central Vietnam.

For Chapter 3, I examine habitat condition changes such as habitat structure and salinity on growth and survival of juvenile fish. Based on the results collected we can infer relationships between recruitment and habitat use of fish. As a corollary of the findings in this chapter, several simple recommendations may be made to increase efficiency of aquaculture practice for grow-out of this fish.

In Chapter 4, I present information on the feeding habits of fish. In particular, I pay attention to the ontogenetic and seasonal changes in the natural diet of fish. In addition, I examine the manner in which tidal shifts influence the feeding habits of fish. Moreover, the stomach contents of wild-caught fishes are described, and the prey found in stomachs identified and their relative trophic importance analysed. The role of different natural food organisms for different ontogenic stages, as well as recommendations to enhance aquaculture production of fish are discussed.

In Chapter 5, the results of the data chapters are combined into a coherent exploration of key habitat characteristics associated with recruitment, growth and survivorship of juvenile *L. argentimaculatus.* This chapter synthesizes the findings of previous chapters to provide insights into habitat specificity and feeding ecology linked to the recruitment of fish, and proposes reasons why certain habitats are recruitment foci. Recommendations to enhance the usability of hatchery-raised juvenile fish, and thereby reduce pressure on the vulnerable wild resource are presented here.

CHAPTER 2

HABITAT ECOLOGY

2.1. INTRODUCTION

Mangrove Red Snapper 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. The successful recruitment to juvenile habitats, and adequate growth and survival within juvenile habitats are important requirements to reach adulthood to replenish the spawning population (Minello et al., 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 et al., 2010). Moreover, the few coastal ecosystems where the fingerlings can still be harvested in commercial quantities are under intense 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. The practice of fishing for juveniles to supply the aquaculture industry, coupled with the apparent loss of suitable nursery grounds in many coastal areas have affected the sustainability of Asian populations of this fish (Yamada, 2010). Knowledge of habitat characteristics critical to the recruitment process is urgently needed, both to focus management of coastal areas, and to enhance artificial culture of this species to reduce harvest pressure on stocks of wild juveniles.

L. argentimaculatus, like many lutjanids, spawns at deep, offshore reefs (Day et al., 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, Zagars et al., 2012). Doi et al. (1994a) 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 (Lake, 1971; Doi & Singhagraiwan, 1993; Russell & McDougall, 2005; Ebner & Morgan, 2013). 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 et al., 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). Grant (1997) stated that Mangrove Red Snapper individuals were often associated with snags, although Day et al. (1981) and Russell et al. (2003) reported that this fish was mainly found in rocky areas.

In Vietnam, Mangrove Red Snapper aquaculture mostly depends on fingerlings collected from the wild, even though induced spawning of *L. argentimaculatus* is a relatively well-known and straightforward procedure (Doi & Singhagraiwan, 1993;

Cowden, 1995; Emata, 1996). This means that increased juvenile fishing is causing serious pressure on the wild fish resource.

The majority of wild juveniles *L. argentimaculatus* for mariculture in coastal Vietnam are captured from two central provinces, where coastal lagoons fed by rivers are associated with mangrove forest. Interviews with older fishermen and mariculturists indicate that previous generations of fishermen were able to harvest these fish throughout coastal Vietnam. It is likely that degradation of coastal ecosystems, including the widespread use of defoliants for mangrove deforestation during the Vietnam War, and the ubiquitous conversion of mangroves for shrimp aquaculture throughout Southeast Asia (Richards & Friess, 2016), and losses of critical nursery habitats have contributed to the depauperisation of the Mangrove Red Snapper populations.

Unfortunately, the key attributes of these critical habitats are largely unknown. Local fishermen are extremely familiar with patterns of recruitment and habitat use by these fish, but, until now there has been no ecological study on recruitment or distribution of juvenile Mangrove Red Snapper in Vietnam. Although this is a valuable aquaculture fish, and although the techniques for spawning and hatching of larvae are well-known, little is known of the manner in which critical habitats influence the development and survivorship of the settled juveniles. Nor is it known how these key habitat characteristics influence the perceived viability advantage of wild-caught juveniles over hatchery-raised fry.

In aquaculture hatchery production, lutjanid juveniles mostly are reared in highly saline (close to oceanic) seawater. In various studies, i found that larvae were stocked at 30ppt until day 50 (Leu et al., 2003), at 35ppt until day 55 (Duray et al., 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). 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, and most of fish under 10cm in that study were caught in amongst rocks while larger classes chose snags as hiding places. 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), and especially for juveniles. Mostafa et al. (1998) found that there was a positive correlation between growth rates of *Clarias gariepinus* with increase in the extent of shelters, while Dou et al. (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. In this chapter, I 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.

2.2. MATERIALS AND METHODS

2.2.1. Study sites

This study was carried out in two provinces (Thua Thien Hue and Binh Dinh) in the north and south central Vietnam (Figure 2.1). Field surveys were undertaken to examine natural recruitment habitats in the two provinces, where juvenile Mangrove Red Snapper and other fish such as groupers or rabbitfish are harvested in large numbers for the aquaculture industry. Both provinces have river-fed coastal lagoons connected to the sea; “Tam Giang-Cau Hai” in Thua Thien Hue province is the largest lagoon system in Vietnam, with area of 21,600ha. Binh Dinh province also has several lagoons, especially “Thi Nai” lagoon, with an area of over 5,000ha.

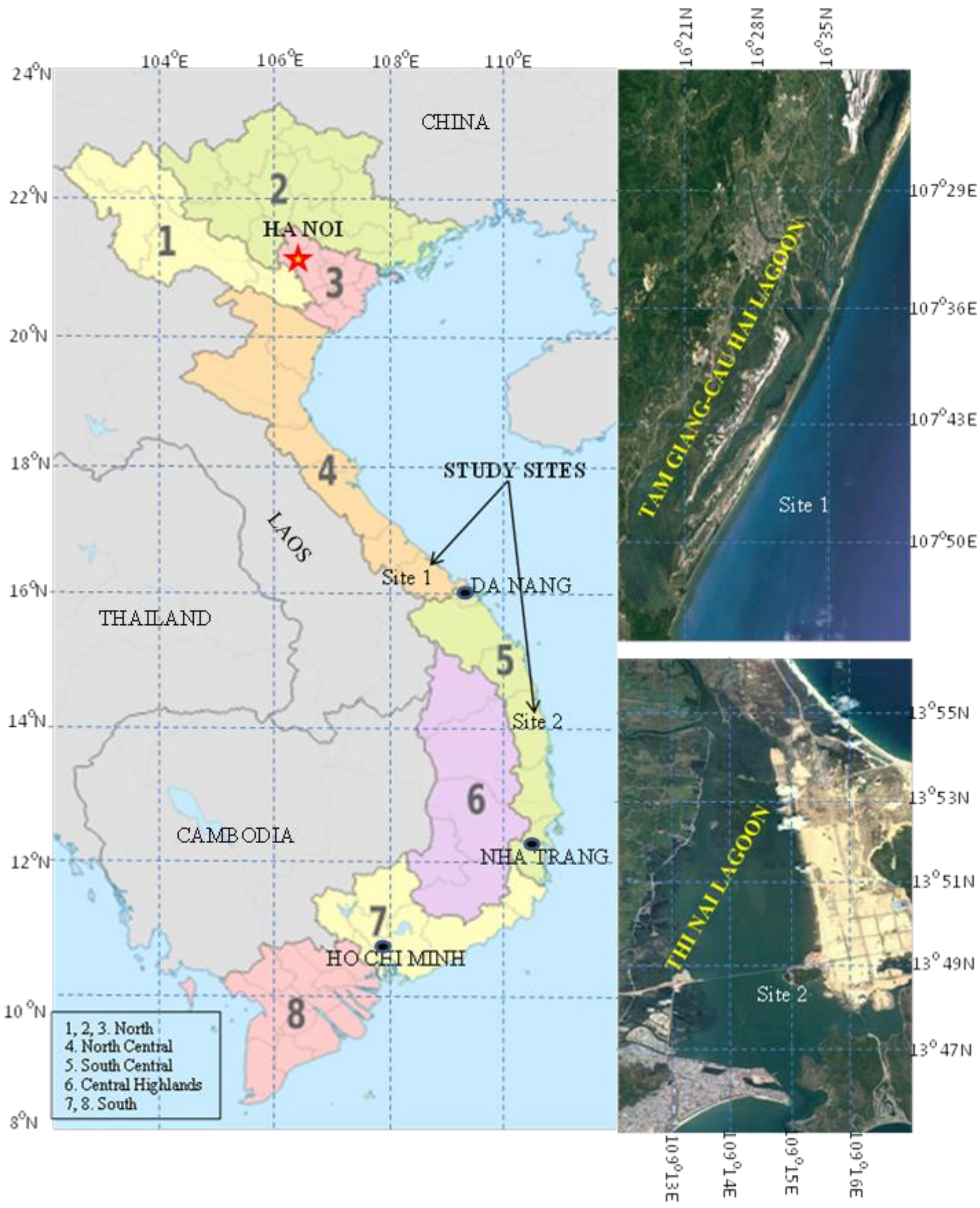

Figure 2.1. Study sites (Site 1: "Tam Giang-Cau Hai" in Thua Thien Hue province; Site 2: "Thi Nai" lagoon in Binh Dinh province).

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 2.2) informed the experimental design and parameters.

2.2.2. The data collection and analysis

Interviews with local fishermen were undertaken at study locations using a standardized questionnaire to collect information related to recruitment, habitat and fishing of juveniles Mangrove Red Snapper. A total of 73 fishermen (of whom 48 were in Thua Thien Hue and 25 in Binh Dinh) experienced in catching, rearing, or trading juveniles Mangrove Red Snapper were interviewed during study period.

Field surveys were made to record details of habitat as well as directly observe fishing methods. By applying transects, details of habitat structure were recorded and expressed as percentage of total area of transects investigated. Salinity was measured using salinity meter (LH-Y100) during every collection event.

During the field surveys, we also collected natural food organisms at habitats investigated and preserved them in 10% Formalin. In the laboratory, these prey items were separated, counted, measured and identified to the lowest possible taxon using a stereomicroscope (Meiji EMTR-3) and binocular microscope (Olympus CX22). Data of food analysis were expressed as percentage by number (%N) and percentage by volume (%V).

2.3. RESULTS

2.3.1. Recruitment

Results of interviews (Table 2.1) showed that the recruitment of juveniles is different between the two study provinces. However, fish smaller than 3cm were caught mostly in the short period between July and August in both provinces. Larger juveniles, in contrast, were collected in different months (e.g. during May-June and October-November in Thua Thien Hue, and during February and September to December in Binh Dinh). The fishermen reported that about 1,700,000 juvenile fish (in Thua Thien Hue) and more than 58,000 fish (in Binh Dinh) were caught per year. Of these, about 85% were fish less than 3cm TL, which were collected mostly during July to August. This period is therefore considered as the major recruitment season of juveniles in central Vietnam.

2.3.2. Habitats of fish

Juveniles Mangrove Red Snapper were caught at sites in lagoons connected to rivers and mangrove forest where the salinity ranges from 10-25ppt. The information collected from fishermen (Table 2.1) showed that fish were mainly caught in rocky habitats. The exception being the smallest size class (less than 3 cm), which usually was captured in sandy habitats in Thua Thien Hue and in seagrass habitats in Binh Dinh. Similarly, results from our field surveys (Figure 2.2) also showed that rocky areas were preferred habitat of all size classes of juvenile Mangrove Red Snapper.

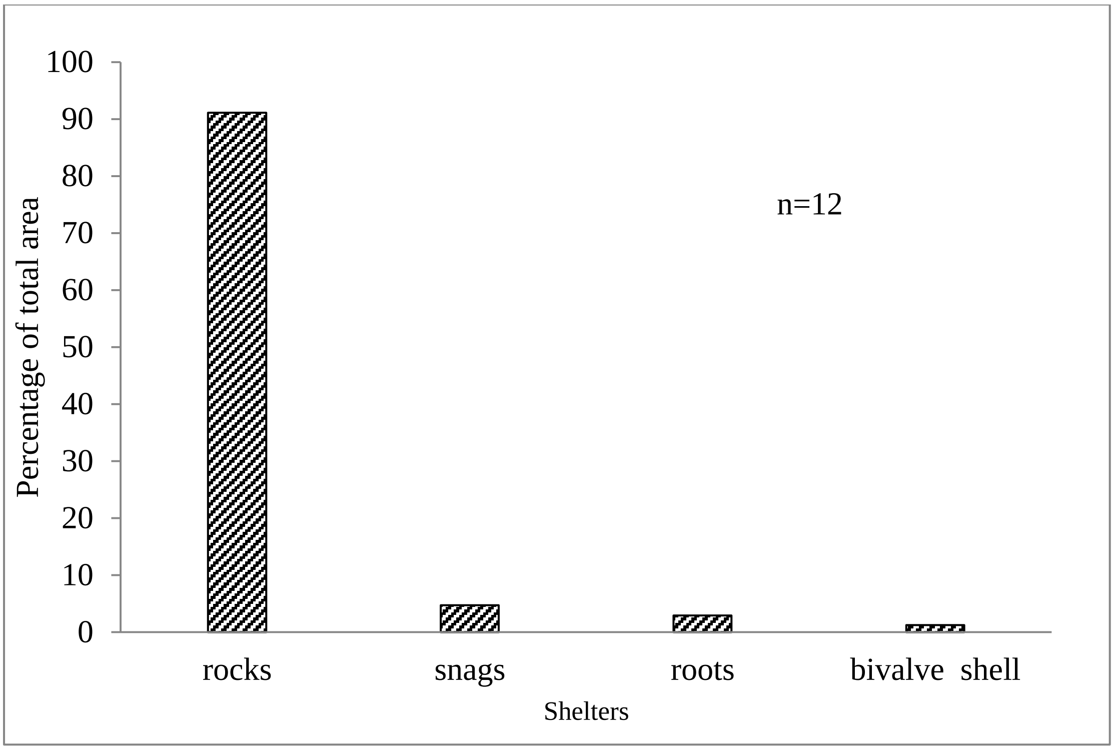

Figure 2.2. Natural habitat features in estuarine areas where juvenile Mangrove Red Snapper are most commonly caught by fishermen.

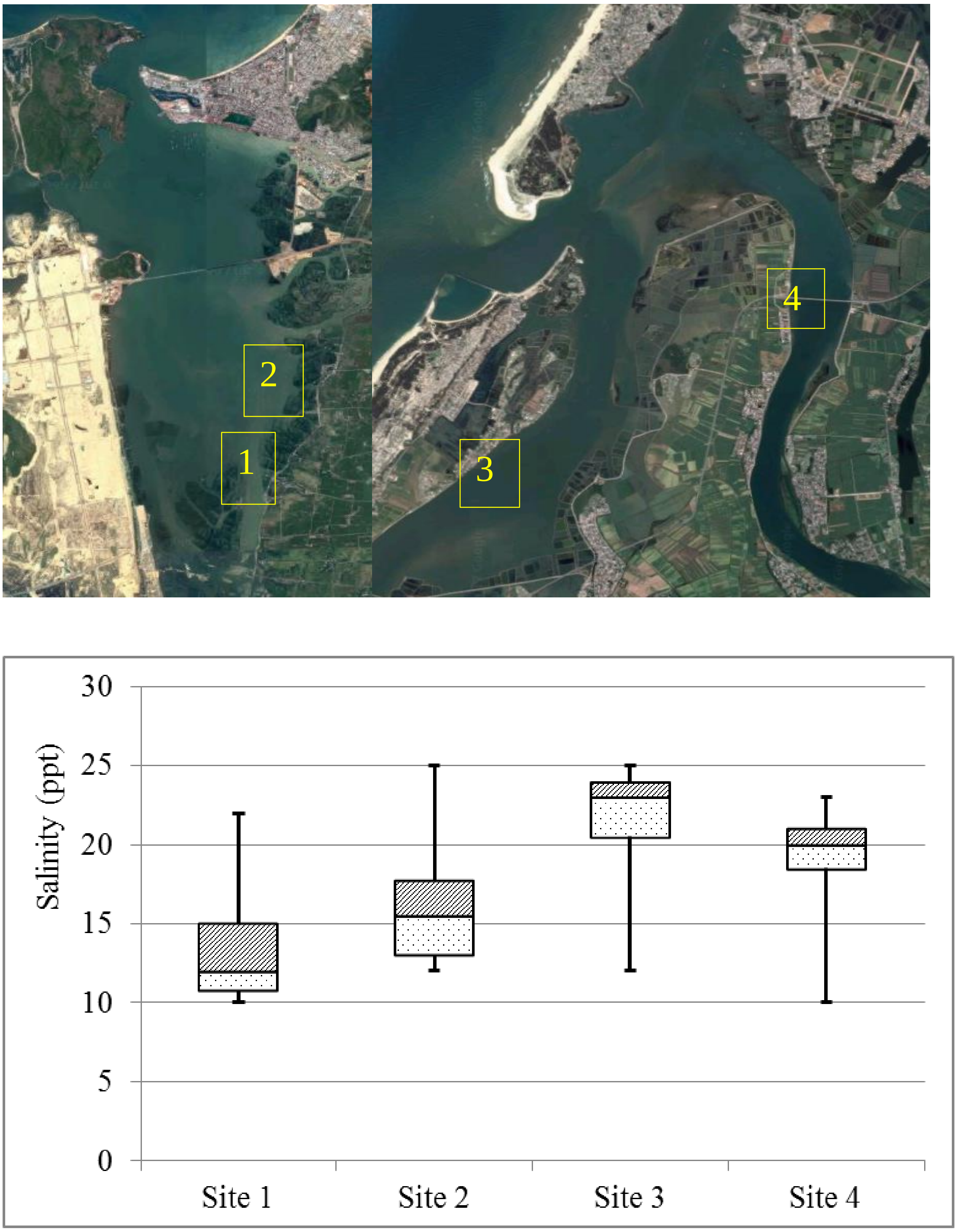

Figure 2.3. Salinity variation (in September-November, 2015 and February-April, 2016) at study sites (site 1 and site 2 in Thi Nai lagoon, Binh Dinh; site 3 and site 4 in Tam Giang-Cau Hai lagoon, Thua Thien Hue).

Figure 2.4. Overview of site having seagrass bed linked to mangrove and estuary in Binh Dinh.

Figure 2.5. Overview of site having rocky habitat associated with mangrove and estuary in Binh Dinh.

Firue 2.6. Rocky habiat in Thua Thien Hue.

Figure 2.7. Seagrass habitat in Binh Dinh.

Table 2.1. The recruitment and habitats of juvenile Mangrove Red Snapper.

Study sites	Fishing period (recruitment seasons)	Number of interviewed fishermen per total	Predominant size class	Main habitat	Total number of juveniles caught per year
Thua Thien Hue	May	3/48	3-10cm	Rock	1,691,800
	June	4/48	3-10cm	Rock	
	July - August	35/48	<3cm	Sand	
	October	3/48	5-7cm	Rock	
	November	3/48	5-10cm	Rock	
Binh Dinh	February	1/25	7-10cm	Rock	58,200
	July - August	20/25	<3cm	Seagrass	
	September - November	2/25	5-10cm	Rock	
	December	2/25	5-10cm	Rock	

2.3.3. Natural food

The results of natural food analysis (Figure 2.8) showed the wide range of prey species associated with fish habitats, including shrimps, fish, crab, zooplankton and zoobenthos. Of which, shrimps were the dominant prey in both number and volume (e.g. *Acetes indicus* took 30.61% by number and 40.57% by volume; another species of *Acetes* held 10.2% by number and 20.17% by volume; or Mysidae comprised 17.86% by number and 13.6% by volume). Several zooplankton species (e.g. Calanoida or *Melita longidactyla*) were also abundant in number. These might be important food resource of juvenile Mangrove Red Snapper in the wild.

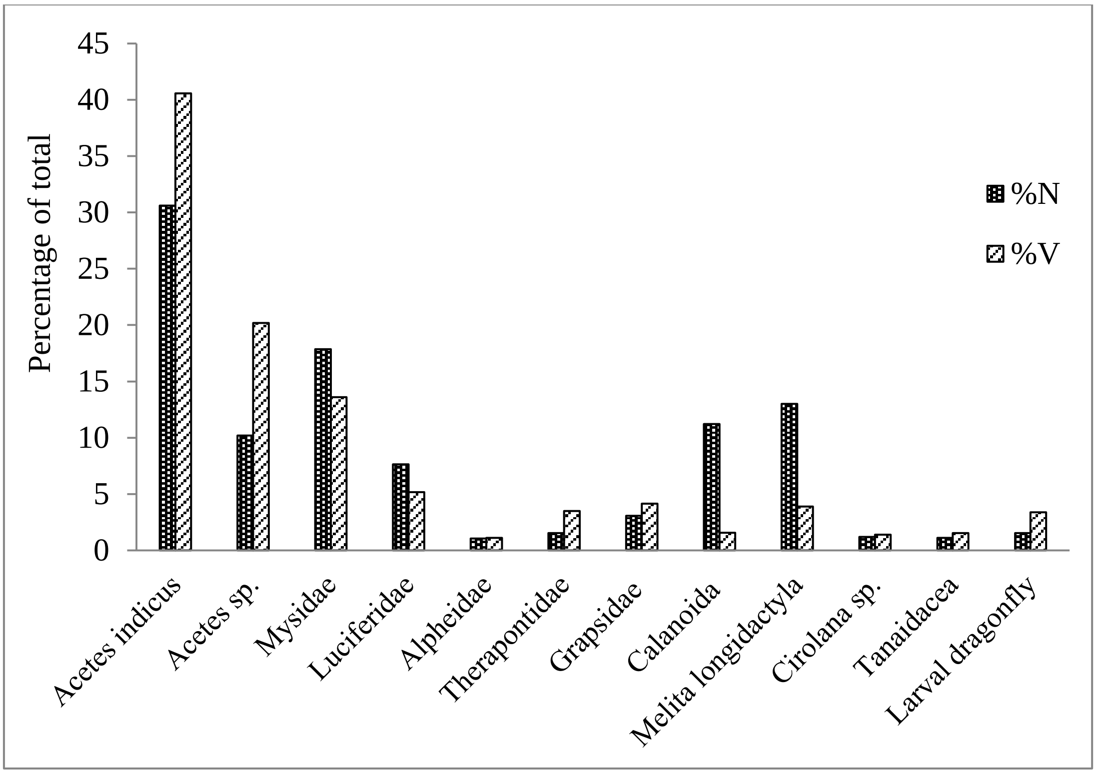

Figure 2.8. Natural food collected from lagoon environment in the first collection period of September-November, 2015 (expressed as percentage by number and volume).

2.3.4. Juvenile fishing

I found that the range of fishing gears used by fishermen was quite diverse, and varied between locations (Table 2.2). Stow nets and fish corrals were considered as the main fishing gears in Thua Thien Hue, which caught 51.66% and 33.31% of total fish per year, respectively, while seine nets were the preferred gear in Binh Dinh; this gear captured 85.91% of total fish per year. Most of the smallest juveniles (less than 3cm) were caught by these gears, whereas larger fish were captured by other techniques.

Table 2.2. Diversity of gears and practice of juvenile fishing by local fishermen at study locations.

Study sites	Fishing gears	Catching time	Proportion of fish caught per total (%)	Predominant size class of fish caught
Thua Thien Hue	Stow net	Night	51.66	<3cm
	Fish corral	Day and night	33.31	<3cm
	Scoop net	Day	0,65	3-10cm
	Lift net	Day and night	3,28	3-10cm
	Long trap cage	Night	11.10	5-10cm
Binh Dinh	Seine net	Day	85.91	<3cm
	Scoop net	Night	5,50	5-10cm
	Lift net	Day and night	3,44	3-10cm
	Long trap cage	Night	5,15	3-10cm

The time of deployment of fishing gear differed according to the type of gear used. Some fishing gears such as stow nets, long trap cages were operated at night, while seine nets were generally deployed during daytime. Passive gears such as fish corrals, lift nets were fished both day and night.

Most of fishers focused on catching juveniles <3cm, because they were considered as the main supply for aquaculture both within province and for export to other provinces. The larger juveniles comprised only a small proportion of the total number of juveniles captured per year, and these tended to supply only small local culture operations.

Figure 2.9. Site where stow nets were operated in Thua Thien Hue.

2.4. DISCUSSION

Data collected from fishermen showed that in both locations the main recruitment of juvenile Mangrove Red Snapper often occurs in July to August, with a large number of <3cm fish caught. Whereas, there is small number of fish captured in other seasons, and most of them are >3cm fish. However, it is noteworthy that aquaculturists also comment that the recruitment season of fish can vary between different years due to weather variation resulted from climate change. This is supported by my field surveys; that in 2015, principal settlement of fish changed to only occur in period of September-November, especially peak in September-October with a large number of small juveniles captured. In general, there might be only one major period of recruitment in year in central Vietnam despite its change due to annual different weather. Therefore, further studies are recommended to investigate how the weather affects the recruitment of this fish species.

It can be seen that although juvenile Mangrove Red Snapper can be caught in several periods of year, most fish are often captured in July and August. However, in eastern Thailand, large numbers of small juveniles (2-3cm total length) recruit into estuaries after wet season, during late October to January (Doi et al., 1992; Doi et al., 1994b), while in northern Australia, less than 5cm fish appear to recruit into freshwater riverine habitats in autumn and winter, between February and July (Russell & McDougall, 2005). It is clear that the recruitment of juvenile Mangrove Red Snapper occurs differently among geographical regions. It is believed that the different climate may be an important factor contributing to this difference.

During the field surveys, I found that juvenile Mangrove Red Snapper were mostly collected from waters with salinity range from 10ppt to 25ppt. Interestingly, although Australian researchers found juvenile Mangrove Red Snapper far upstream in rivers (Russell & McDougall, 2005), there is no information indicating appearance of fish in freshwater in central Vietnam. This may reflect differences in the river systems and the way they are utilized by juveniles between the countries, or the result of intensive juvenile fishing in Vietnam removing surplus juveniles, which does not occur in Australia.

I found that larger juveniles (>3 cm total length) were mostly caught at rocky habitats. Similar results were also observed by Day et al. (1981) and Russell et al. (2003). In contrast, <3cm fish were collected at seagrass bed in Binh Dinh and sandy bed in Thua Thien Hue. However, during the field surveys, I observed that in Binh Dinh, small juveniles only appeared at seagrass habitats several days before they were captured or moved to other habitats. Similarly, in Thua Thien Hue, most small fish were caught as soon as they recruited into the lagoon from the sea. Moreover, fish smaller than 3cm were also found at rocky habitats in both provinces; therefore, it is thought that seagrass and sand bed might not be preferred habitats of fish. In general, rocky areas could be the favourite habitats of juvenile Mangrove Red Snapper (≤10cm total length) in central Vietnam.

I also observed that fishing gears were diverse and different between locations. This might depend on traditional experiences of fishermen at each location. However, it is worth noting that use of these gears seem more or less to be related to movement of fish during the recruitment. The preferred fishing gears, such as stow nets and fish corrals in Thua Thien Hue or seine nets in Binh Dinh, are usually operated at sites in lagoons which are the nearest to the lagoon access to the sea, and result in a large number of fish <3cm caught. This indicates that most of the small juveniles are captured as soon as they recruit into coastal lagoons from offshore waters. Moreover, the chief harvesting period is the main recruitment season of juveniles (often in July-August: Table 2.1). However, when fish move to estuary or mangrove associated-rocky habitats, the preferred mass-harvest fishing gears as stow nets or seine nets appear not to be as effective; fishers then switch to use other gears such as scoops or long trap cages to collect fish. As a result, subsequent to the initial recruitment event, fishermen catch only small numbers of juveniles (mostly the bigger juveniles). Capture of juveniles becomes more haphazard and fishermen capture fish in different months (e.g. May, June, September, October, November).

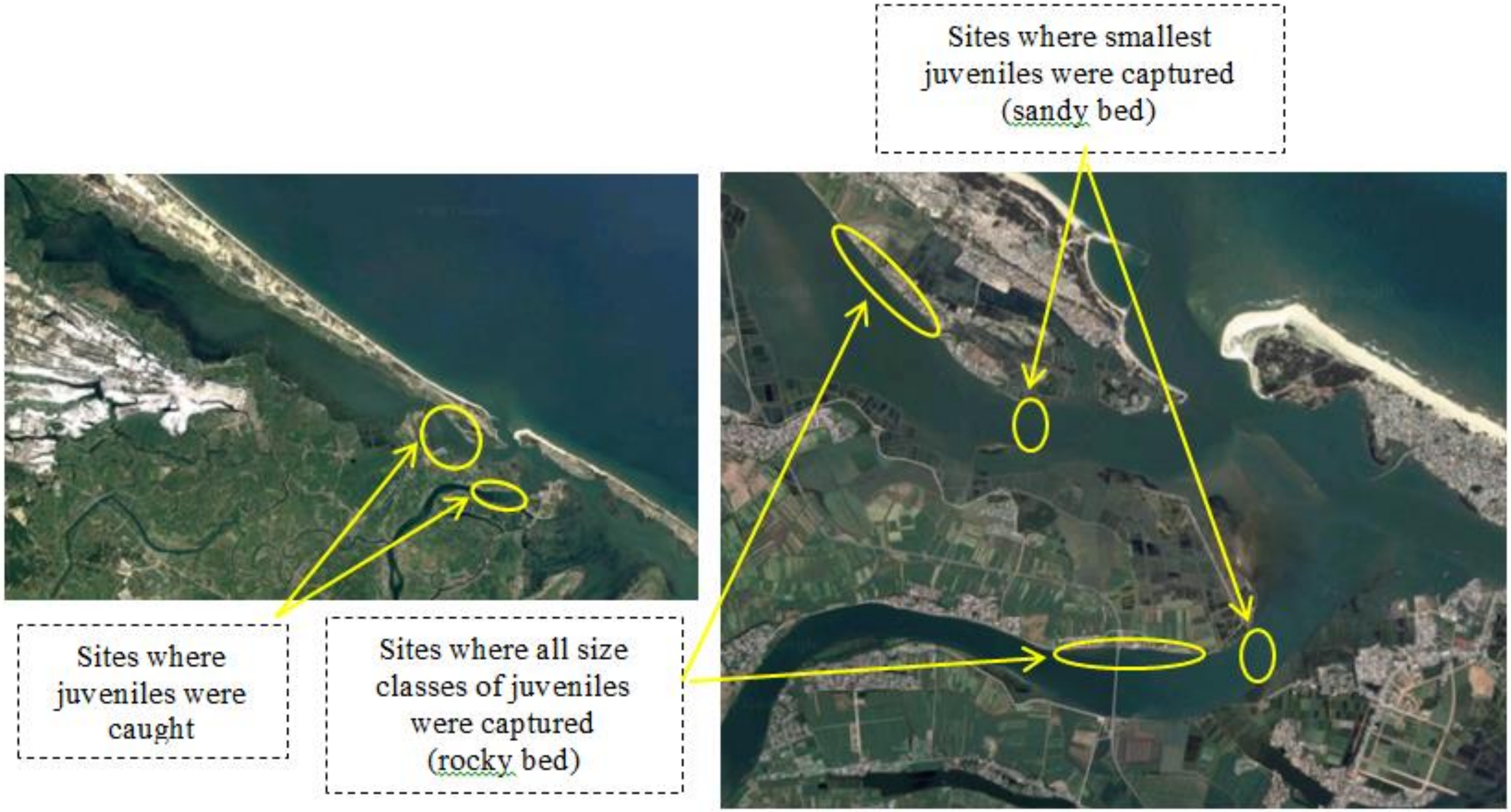

Figure 2.10. Sites where juveniles were caught in Tam Giang-Cau Hai lagoon in Thua Thien Hue in September-November, 2015.

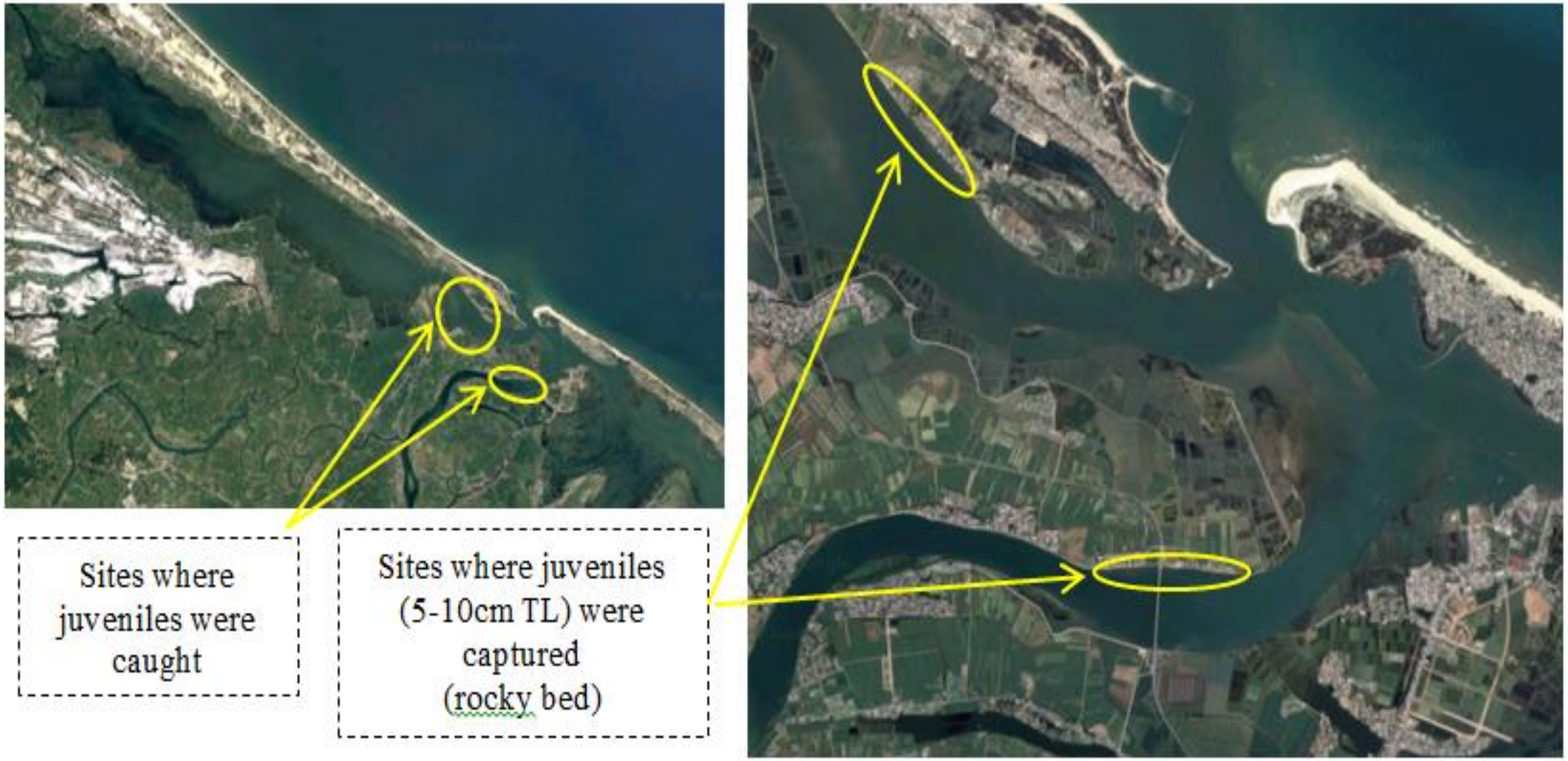

Figure 2.11. Sites where juveniles were caught in Tam Giang-Cau Hai lagoon in Thua Thien Hue in February-April, 2016.
Note: Only the largest fish (5-10cm TL) were captured in February-April. No small juvenile was found in this period.

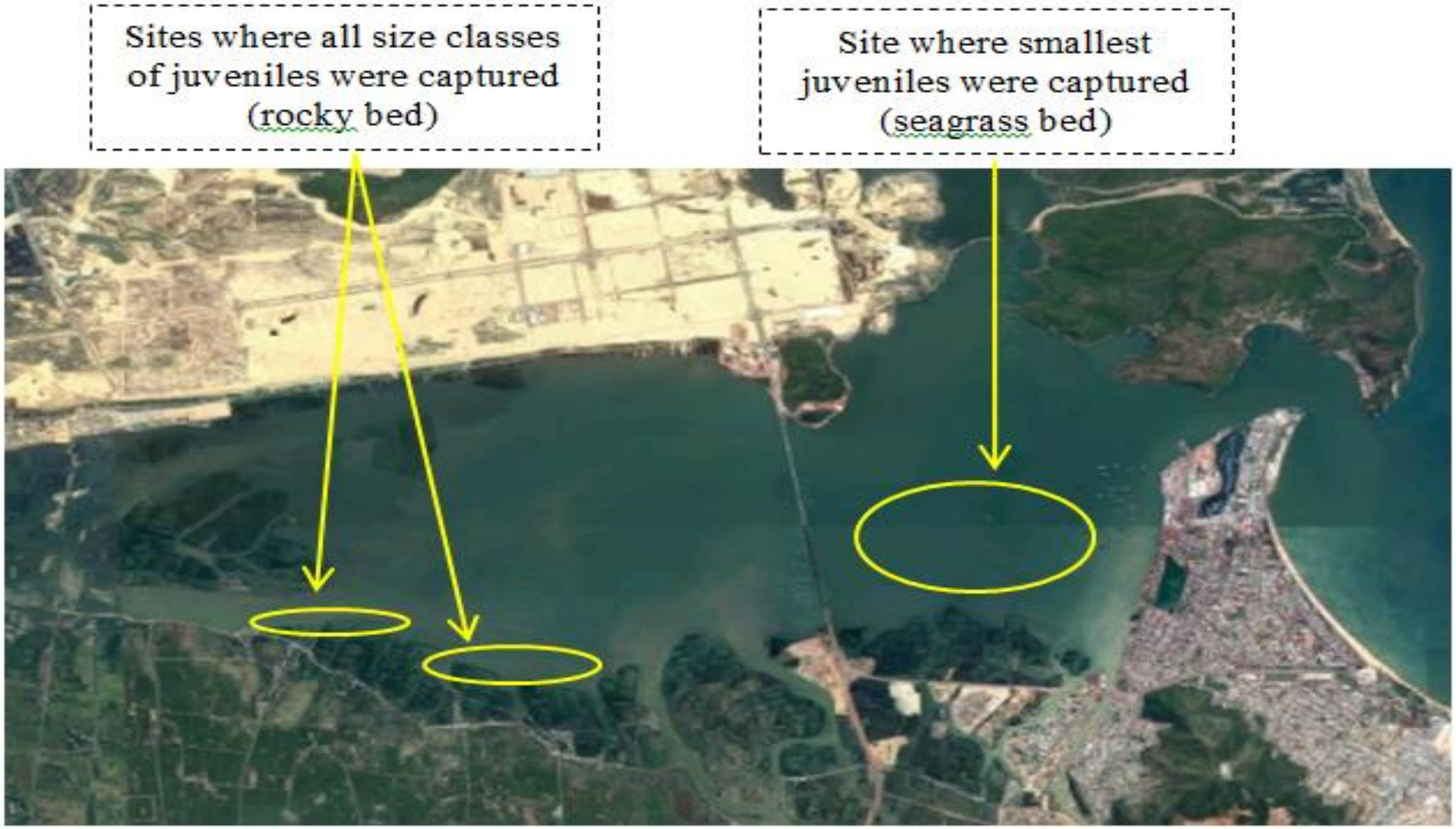

Figure 2.12. Sites where juveniles were caught in Thi Nai lagoon in Binh Dinh in September-November, 2015 (*No fish was collected in February-April, 2016 in Binh Dinh).*

This study collected basic information related to the recruitment and habitat of juvenile Mangrove Red Snapper that might be useful for fish resource conservation. Understanding the habitat characteristics that attract planktonic juveniles and cause them to settle and the factors that enhance recruitment are vital to the continued existence of the resource. The primary cue for settlement appears to be the presence of the low-salinity coastal lagoon environment, rather than any particular structural cue, since the smallest juveniles initially settle in shallow sandy meadows within the lagoon, but quickly move upstream to structurally complex rocky habitats. Juveniles were found in low salinity water, but not in fresh water, suggesting that either the foraging opportunities, osmotic gradient or habitat characteristics of the brackish lagoons provided the best environment for juveniles.

Some ecological questions remain, however, such as why fish select rocky habitats to reside or whether habitat selection is related to salinity, shelters and natural food availability. Moreover, the reliance of aquaculturists on wild-caught fingerlings has meant that the few places in Vietnam where juvenile *L. argentimaculatus* still recruit are under increasing heavy pressure from collectors, which is likely impacting a much wider fishery resource. In particular, although a large number of juveniles are caught every year, interviewed fishermen commented that this fish resource varied every year and in recent years has tended to be less than previously.

CHAPTER 3

THE IMPORTANCE OF HABITAT CHARACTERISTICS

3.1. INTRODUCTION

Mangrove Red Snapper (*Lutjanus argentimaculatus*) is an important commercial and recreational fish throughout its range (Allen, 2002; Russell et al., 2003; Zagars et al., 2012; Piddocke et al., 2015). 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 (Liao et al., 1995; Wong, 1995; Chou & Lee, 1997; Emata & Borlongan, 2003). 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 et al., 1999), there are variations in egg and larval quality, and larval survival is generally poor (Doi et al., 1997; Emata et al., 2003). The supply of wild fingerlings is seasonal, variable, and since such harvesting of juvenile fish can deplete natural recruitment, probably unsustainable (Gjertsen et al., 2010). Moreover, the few coastal lagoons that support high juvenile densities where the fingerlings can still be harvested in commercial quantities are under pressure. The coastal ecosystems contribute juveniles to adult populations and 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.

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 et al., 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, Zagars et al., 2012). Doi et al. (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.

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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 appears 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 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), and especially for juveniles. Mostafa et al. (1998) found that there was a positive correlation between growth rates of *Clarias gariepinus* with increase in the extent of shelters, while Dou et al. (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. *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. 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.

3.2. MATERIALS AND METHODS

Field surveys were undertaken to examine natural recruitment habitats in two provinces (Thua Thien Hue and Binh Dinh) at the northern end and southern middle 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* appears to be dominated by rocky berms intersperse 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 (Chapter 2, Figure 3.2) informed the experimental design and parameters.

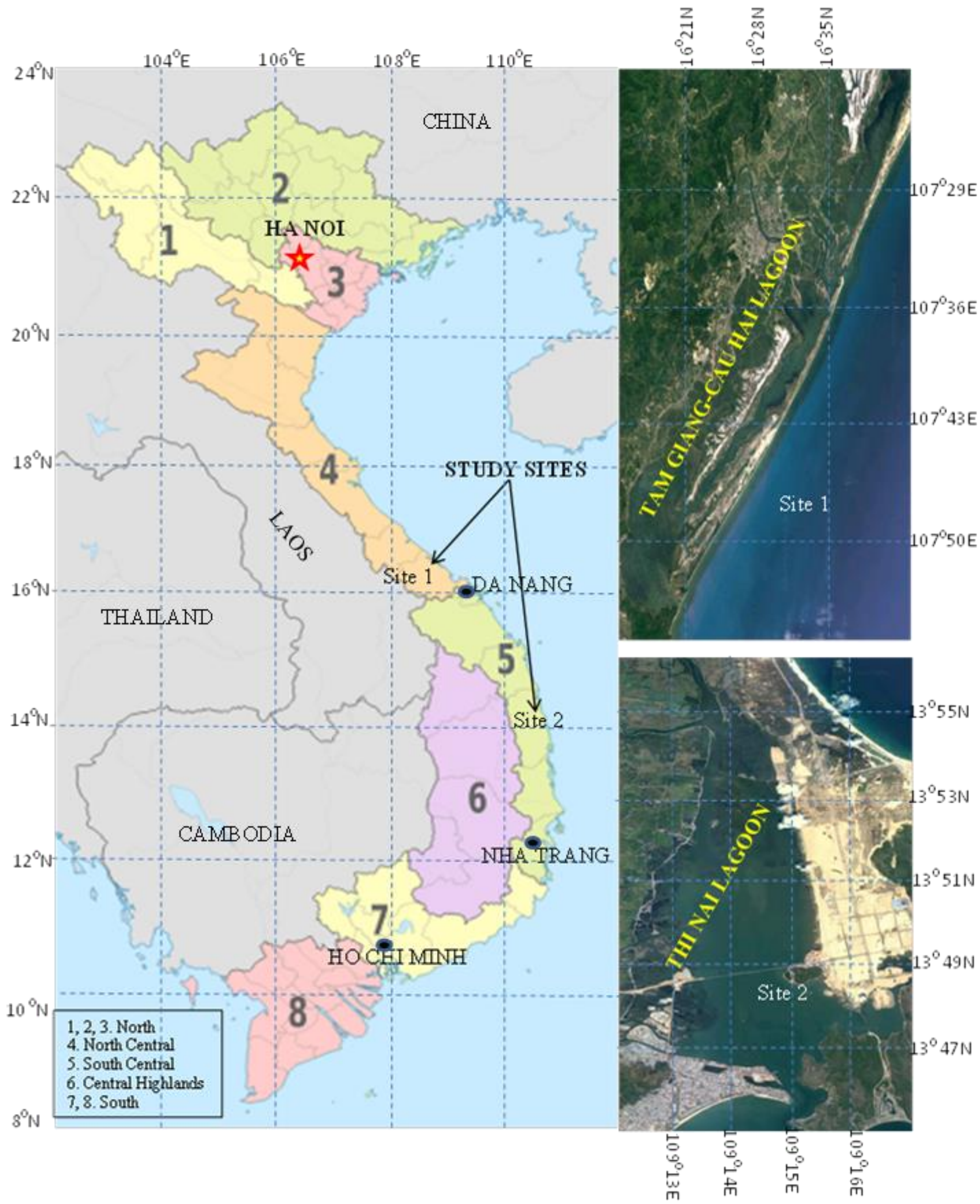


Figure 3.1. Study sites (Site 1: "Tam Giang-Cau Hai" in Thua Thien Hue province; Site 2: "Thi Nai" lagoon in Binh Dinh province). The habitats are described in more detail in Chapter 2.

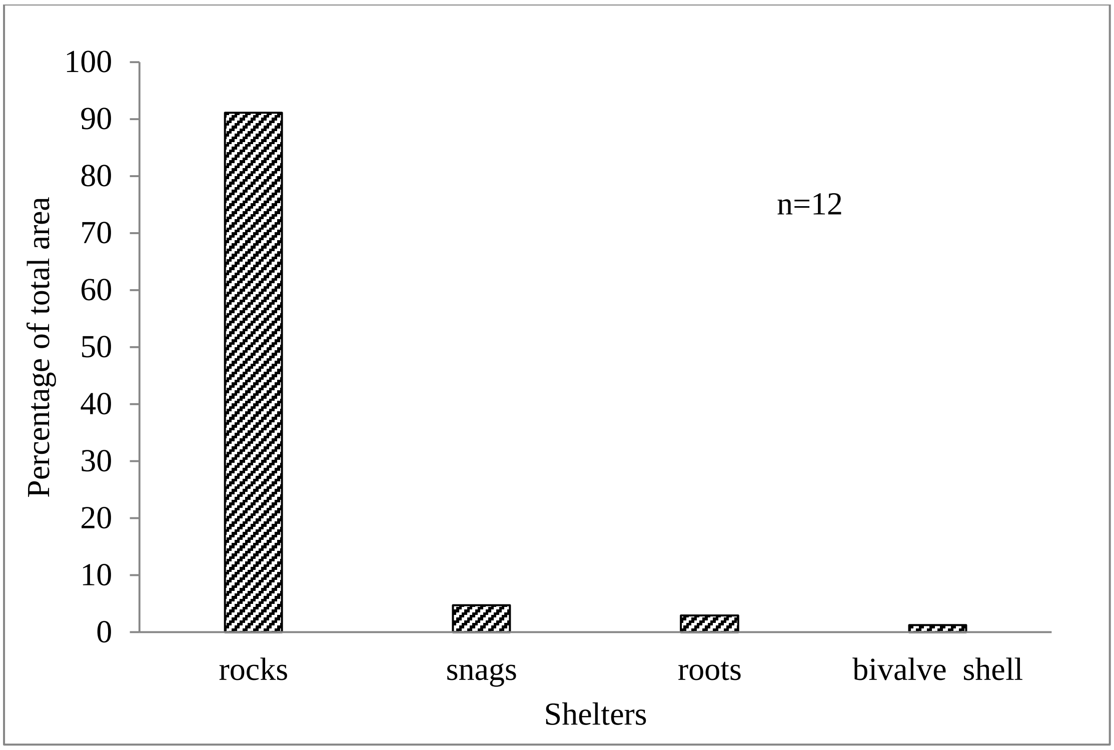


Figure 3.2. Natural habitat features in estuarine areas where juvenile Mangrove Red Snapper are most commonly caught by fishermen (Chapter 2).

I conducted a habitat-effect experiment in controlled conditions to investigate effects of hard and soft shelter and level of 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, I selected random groups of 30 healthy juveniles and exposed each group to one of four kinds of habitat structure: piles of fist-sized rocks (similar to the wild habitats where juvenile fish can be observed), 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 25ppt) 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). I 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. Water temperature during the experimental period ranged between 26.5-29^{0}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 7:00 and 17:00, and fed *Artemia* nauplii at 12:00, 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 fish from each tank fish were examined at the start of the experiment, and subsequently every ten days. A pilot study indicated that repeated handling can cause stress to juvenile *L. argentimaculatus*; 10 randomly collected fish were measured at each data point, so that survivorship could also be measured without confounding. Mean values from these subsamples provided replication at the tank level, without confounding the effects of different conditions.

I used repeated measures analysis of variance (ANOVA) to examine growth of fish according to time, and identify effects of salinity and habitat structure as well as interactive effects of these two factors on weight, length and survivorship of juveniles over the study period. Repeated measures uses time as a blocking factor, which is appropriate in this case, because it is likely that fish within the same tank are likely to respond in the same way; thus there is no interaction with the treatment factors. All statistical analyses were done using SPSS version 15.0 (SPSS Inc. 2006).

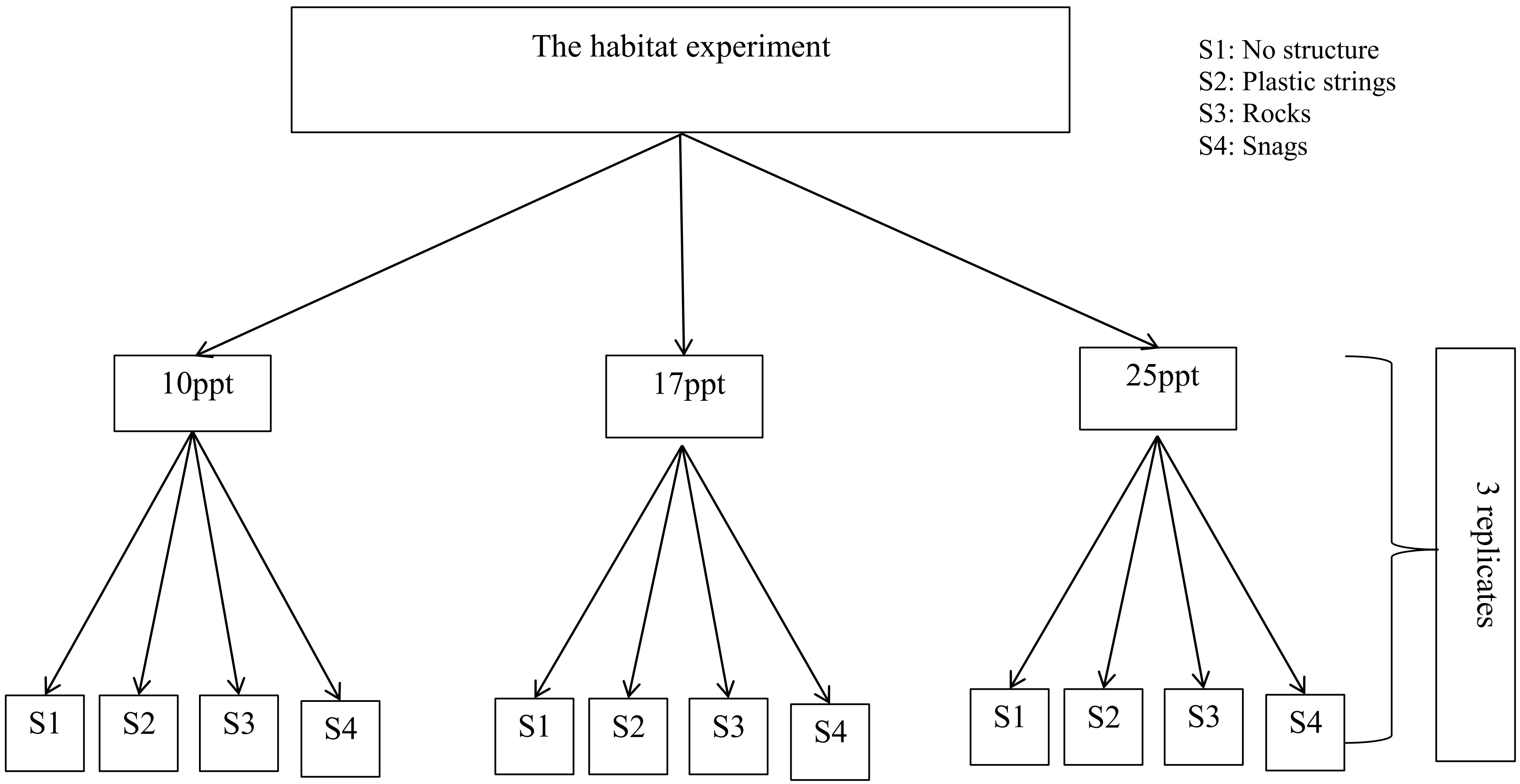


Figure 3.3. The structure of the experiment examining the effectes of salinity and habitat structure.

3.3. RESULTS

3.3.1. Growth rates

There was no interaction between salinity and structure on length of fish ($F(6, 174)=0.05$, $p=0.999$). 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, there was no interaction between salinity and structure on weight of fish ($F(6, 12)=0.32$, $p=0.914$). 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).

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.

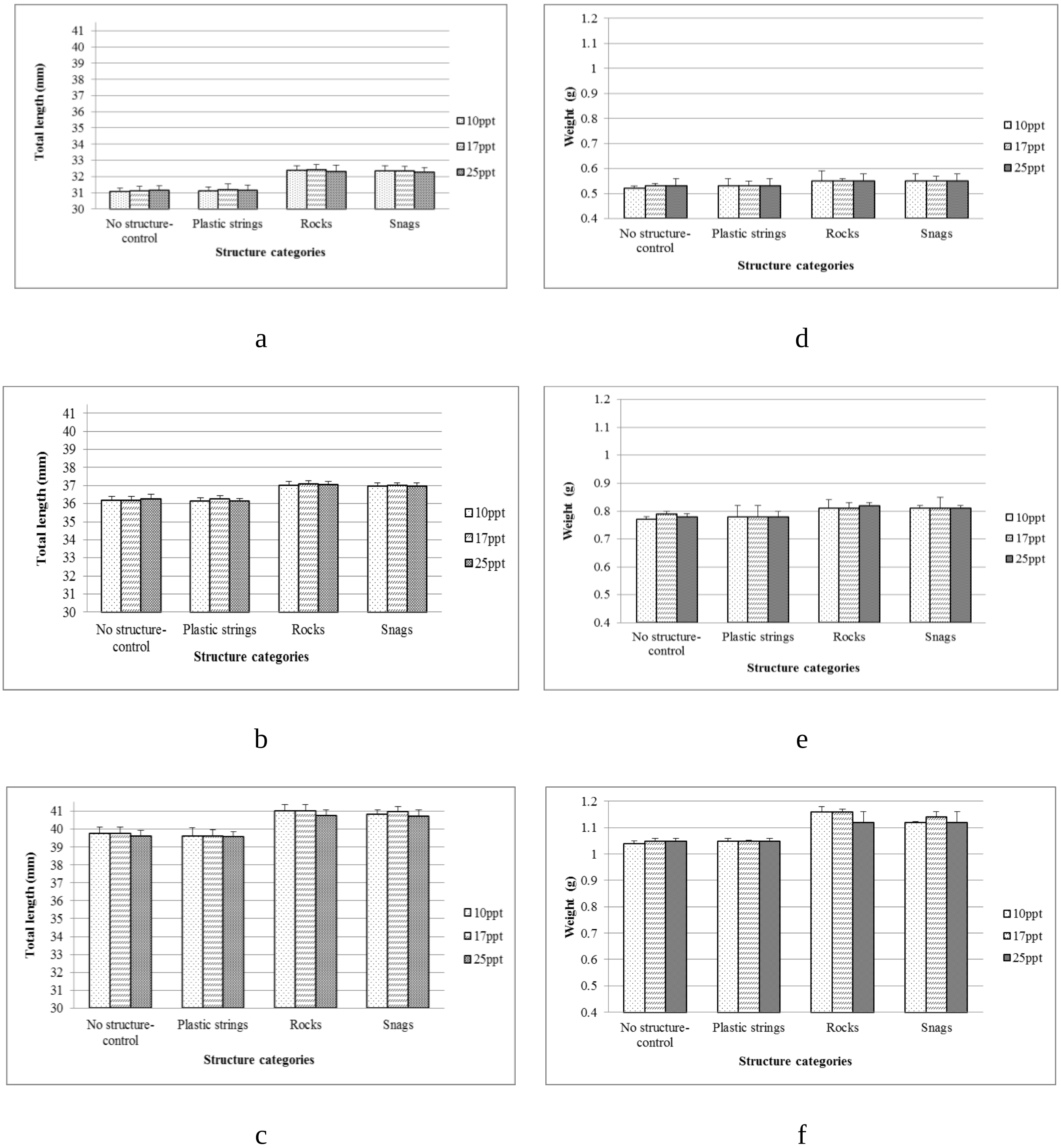
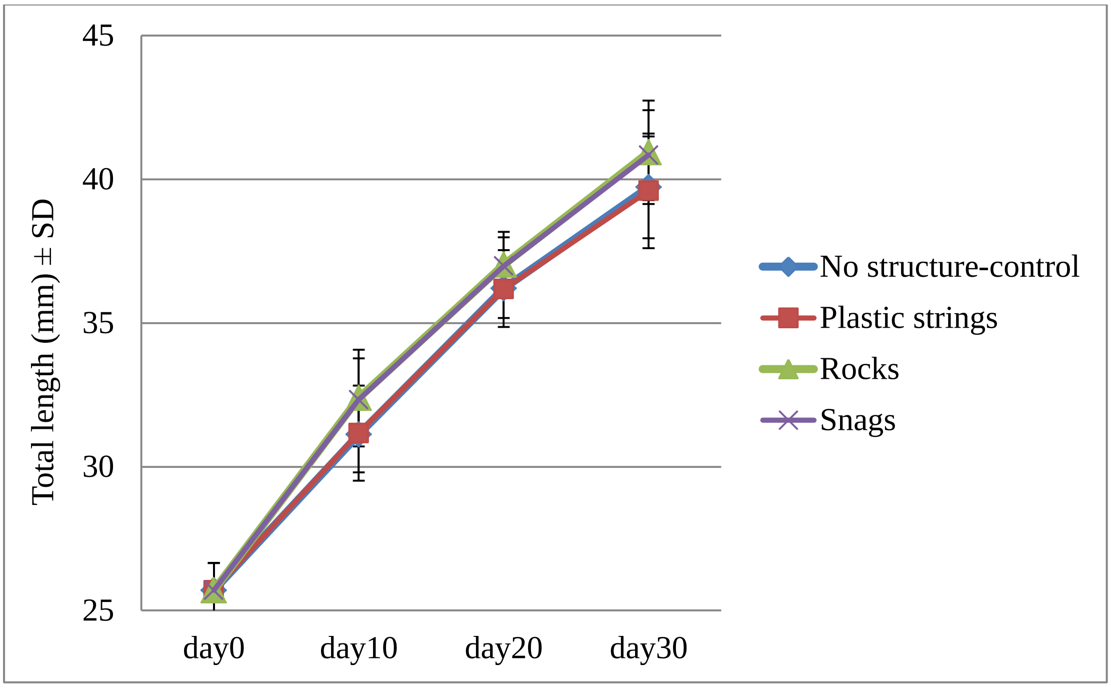
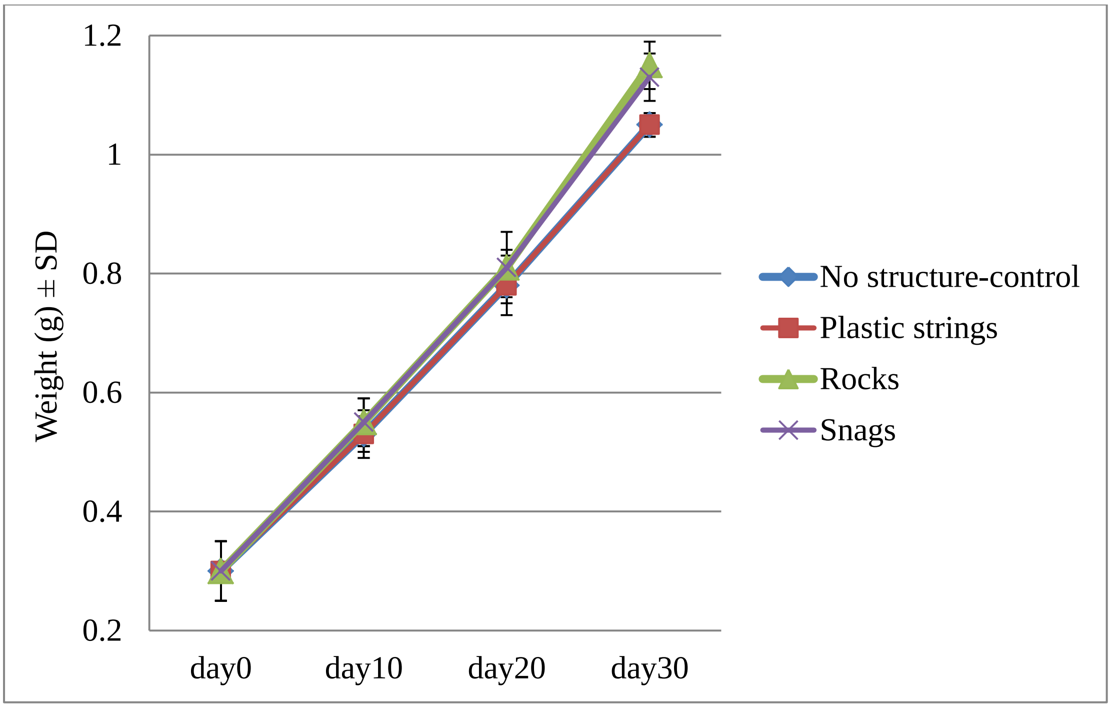


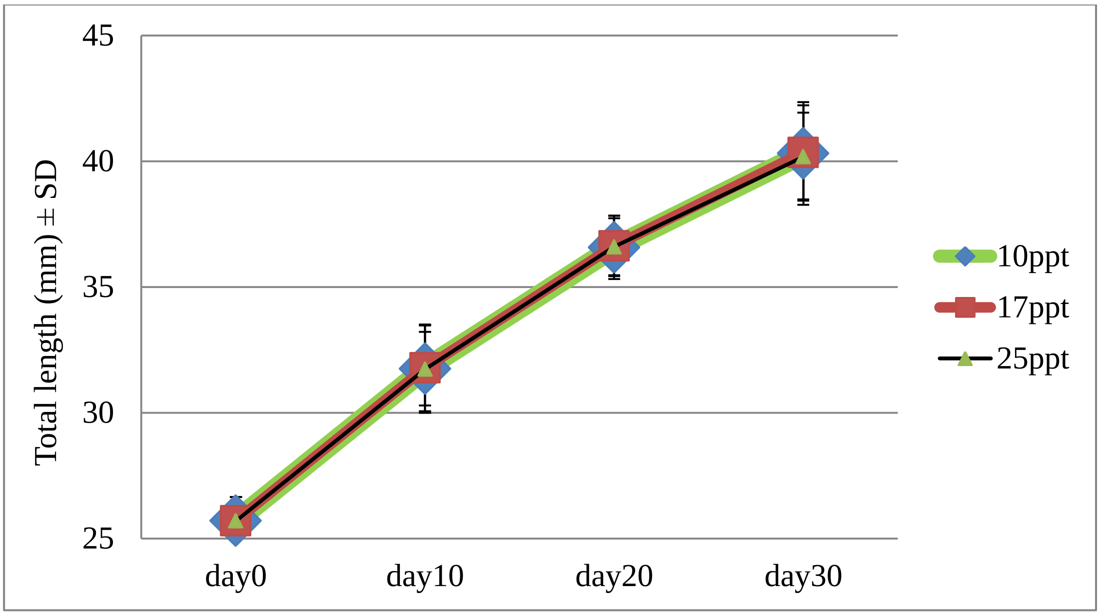
Figure 3.4. Growth of fish with different shelters and salinities (a, b, c: Total length of fish at day 10, 20 and 30 respectively; d, e, f: weight of fish at day 10, 20 and 30 respectively).


45
40
35
30
25
Total length (mm) ± SD
day0
day10
day20
day30
No structure-control
Plastic strings
Rocks
Snags

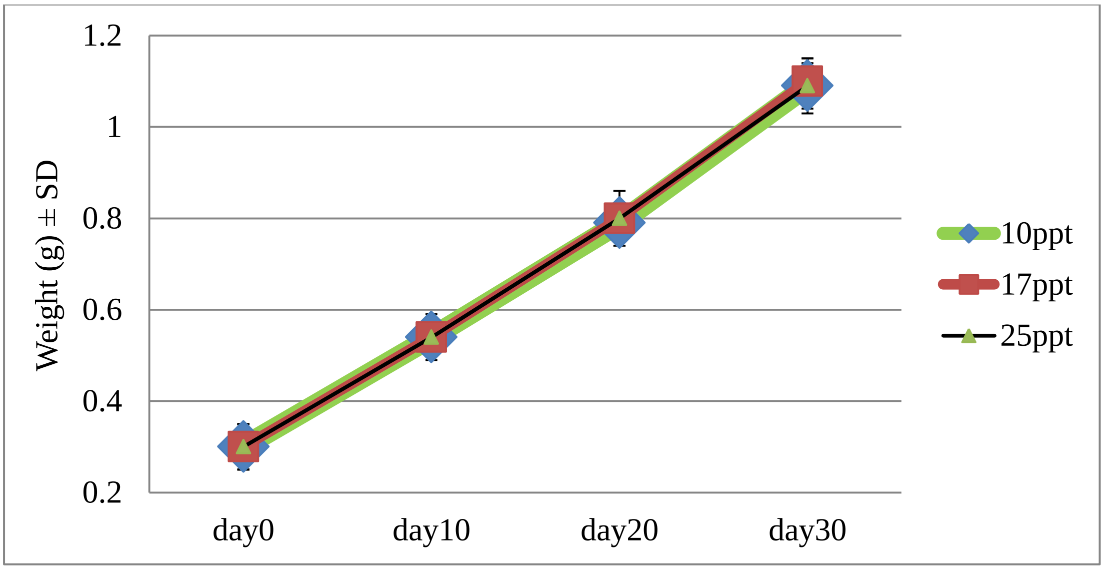
a


1.2
1
0.8
0.6
0.4
0.2
Weight (g) ± SD
day0
day10
day20
day30
No structure-control
Plastic strings
Rocks
Snags

b



c



d

Figure 3.5. Length and weight gain of fish through time (a, b: according to structures; c, d: according to salinities). Because there was no interaction between factors, salinity levels were pooled for the habitat graphs (a, b) and habitat structures are pooled for salinity (c, d)

Table 3.1. Difference of total length between structure treatments.

(I) structure	(J) structure	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference (a)	
					Upper Bound	Lower Bound
Rocks	Plastic strings	0.856*	0.120	0.000	0.516	1.195
	No structure	0.828*	0.108	0.000	0.522	1.134
	Snags	0.053	0.098	1.000	-0.226	0.331
Plastic strings	Rocks	-0.856*	0.120	0.000	-1.195	-0.516
	No structure	-0.028	0.106	1.000	-0.328	0.272
	Snags	-0.803*	0.099	0.000	-1.084	-0.521
No structure	Rocks	-0.828*	0.108	0.000	-1.134	-0.522
	Plastic strings	0.028	0.106	1.000	-0.272	0.328
	Snags	-0.775*	0.072	0.000	-0.979	-0.571
Snags	Rocks	-0.053	0.098	1.000	-0.331	0.226
	Plastic strings	0.803*	0.099	0.000	0.521	1.084
	No structure	0.775*	0.072	0.000	0.571	0.979

* The mean difference is significant at the 0.05 level.

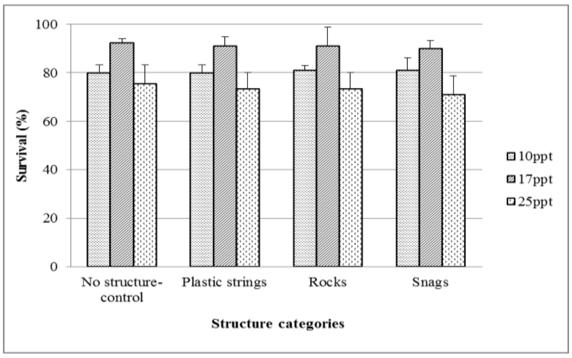
Table 3.2. Difference of weight between structure treatments.

(I) structure	(J) structure	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference (a)	
					Upper Bound	Lower Bound
Rocks	Plastic strings	0.037*	0.008	0.003	0.010	0.064
	No structure	0.039*	0.010	0.002	0.012	0.066
	Snags	0.005	0.010	1.000	-0.022	0.032
Plastic strings	Rocks	-0.037*	0.008	0.003	-0.064	-0.010
	No structure	0.002	0.002	1.000	-0.025	0.029
	Snags	-0.032*	0.008	0.014	-0.059	-0.005
No structure	Rocks	-0.039*	0.010	0.002	-0.066	-0.012
	Plastic strings	-0.002	0.002	1.000	-0.029	0.025
	Snags	-0.034*	0.009	0.009	-0.061	-0.007
Snags	Rocks	-0.005	0.010	1.000	-0.032	0.022
	Plastic strings	0.032*	0.008	0.014	0.005	0.059
	No structure	0.034*	0.009	0.009	0.007	0.061

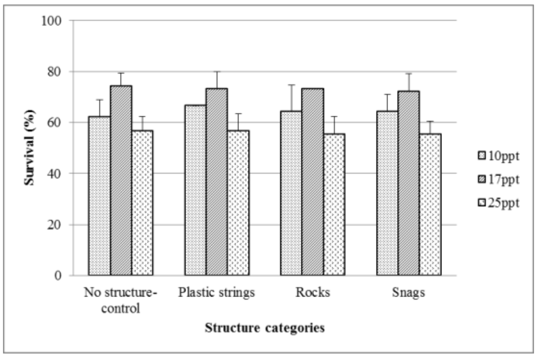
* The mean difference is significant at the 0.05 level.

3.3.2. Survivorship

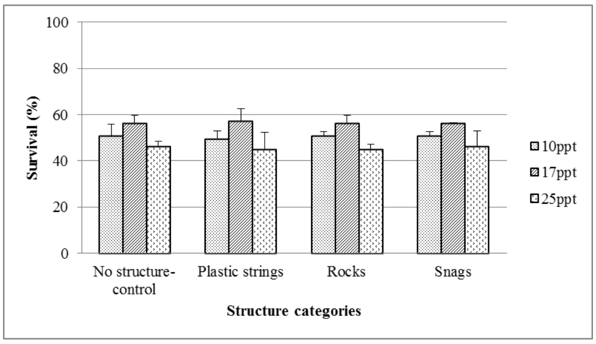
There was no interactive effect of habitat and salinity on survival of fish ($F(1.8, 3.6)=0.1$, $p=0.89$). Habitats had no statistically significant effect on survivorship rates of juveniles. Salinity was the major determinant of fish survivorship during the course of this experiment. The study found 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, I 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). 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 5, 6; Table 1).



a



b



c

Figure 3.6. Survival of fish with different shelters and salinities (a, b, c: at day 10, day 20 and day 30, respectively).

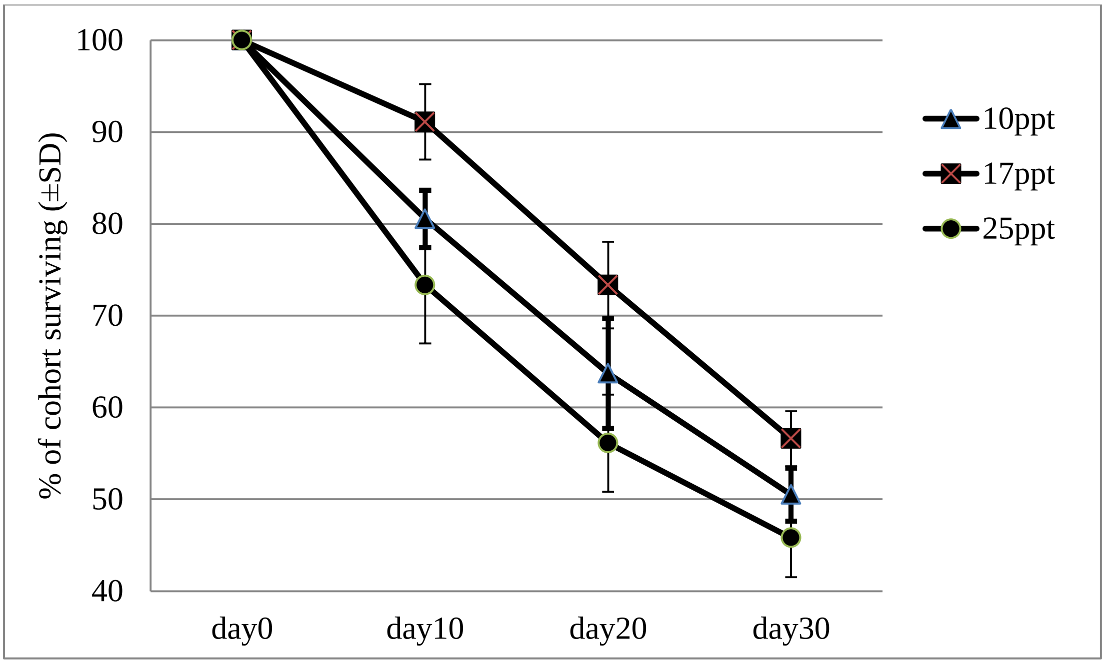


Figure 3.7. Survival of fish according to salinities through time.

Note: 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.

Table 3.3. The difference 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.

3.4. DISCUSSION

Lutjanus 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 (Lim & Chao, 1993; Doi & Singhagraiwan, 1993; Emata et al., 1994; Duray et al., 1996; Estudillo et al., 2000; Emata, 2003). My 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.

Key among the findings in this chapter 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 3.6, 3.7). 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 3.6). 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. Estudillo et al., 2000; Abbas et al., 2005; Abbas & Siddiqui, 2013), 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 16ppt (Murashige et al., 1991), *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 that were cultured at the higher and lower salinities grew as fast as those at the intermediate salinity (Figure 3.4). This suggests that the osmotic differences between treatments are not having severe metabolic costs (cf. Estudillo et al., 2000; Boeuf & Payan, 2001) on the juveniles (hence compromising growth), yet in some way 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 (Chapter 4, Vo & True, in review). Despite the similarity of the experimental plastic string habitat to this winter seaweed habitat, my 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). 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. 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.

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.

CHAPTER 4

FEEDING ECOLOGY

4.1. FEEDING ECOLOGY

4.1.1. Introduction

Mangrove Red Snapper (*Lutjanus argentimaculatus*) is an important commercial and recreational fish throughout its range, and considered to be an excellent food fish (Allen, 2002). This species has been considered as a profitable candidate for aquaculture in many countries, especially in Southeast Asia (Liao et al., 1995; Wong, 1995; Chou & Lee, 1997; Emata & Borlongan, 2003). *L. argentimaculatus* is distributed widely in the Indo-West Pacific from Samoa and the Line Islands to East Africa and from Australia northwards to Ryukyu Island, Japan (Doi & Singhagraiwan, 1993). It is commonly found in mangrove-lined estuarine systems, especially as juveniles and sub-adults, although it is known that spawning occurs on deep offshore reefs (Allen & Erdmann, 2012). Larval fish recruit into brackish coastal waterways, and move into progressively more saline areas as they mature. Although they are most commonly associated with mangrove-lined estuaries (Allen & Erdmann, 2012), local fishermen harvest huge numbers for the aquaculture industry from shallow coastal lagoons with substrates ranging from gravel to sand and seagrass. Despite their importance for artisanal fisheries and aquaculture, little is known about the feeding ecology and habitat requirements of juveniles.

The role of diet as a major factor initiating the shift toward adult habitat (Cocheret et al., 2003) is unclear, particularly since the feeding habits of many lutjanid fish can change between life stages and between size classes. For example, Szedlmayer and Lee (2003) found that juvenile *Lutjanus campechanus* from 1.8 to 28cm (SL) in the northeast Gulf of Mexico changed diets with increasing size. Nakamura et al. (2008) also showed that small *L. argentimaculatus* (6.6 – 9.2cm SL) exclusively fed on estuary-associated grapsid crabs, while larger size class (12 – 18.9cm SL) within the same mangrove habitat mostly fed on crabs but in addition to fishes and shrimp. In a coral reef habitat, small *L. gibbus* individuals (5.4–7.3cm SL) similarly consumed shrimps and isopods, whereas larger fish (14.2–27.5cm SL) consumed predominantly coral reef-associated crabs (Nakamura et al, 2008). Berkström et al. (2013) reported change in feeding habit between size classes for *L. ehrenbergii*; in mangrove habitat, individuals with size of 3.2-10cm fed crabs and fish while size class of 16.4-19.4cm mostly consumed crabs. Additionally, diet shifts with increasing size have been reported for other fish species (Sedberry & Cuellar, 1993; Burke, 1995; Rooker, 1995; Lowe et al., 1996), although this may be a characteristic mainly of carnivorous fishes (Johnson et al., 2012), and O'Brien et al. (1976) believed that fish might shift their dietary preferences to adapt to food resource availability in the environment.

This is somewhat supported by Monteiro et al. (2009), who found that feeding habits of *Lutjanus jocu* changed seasonally, consuming mainly penaeid shrimps in the dry months while exhibiting a wide trophic spectrum in the wet months, consuming Grapsidae, Penaeidae and Porcellanidae. These authors did not offer an explanation for the diet shift in this study, except to support the idea that they could be related to the distribution, abundance and availability of prey in each season (Rooker, 1995). Snyder (1984) and Lucena et al. (2000) suggested that seasonal changes in a trophic guild can often be attributed to the changes in life history patterns of their food organisms, which would make sense in this context if cohorts of larval recruitment coincide with cycles of prey replenishment.

Although several studies have examined juvenile diets of lutjanids, almost nothing is known of the diet of Mangrove Red Snapper, especially in Vietnam where there has been no research undertaken on the wild population. Importantly, few studies have examined the role of a succession of critical habitats in larval and juvenile recruitment. Anecdotal evidence from Vietnamese fishermen indicates that the number of sites where juvenile *L. argentimaculatus* can still be caught is diminishing, which means that it is increasingly important to identify factors that may contribute to recruitment success or failure.

This study is the first to examine shifts in the diet of very young Mangrove Red Snapper, and seeks to create links between dietary preferences and seasonal patterns in juvenile habitat use.

4.1.2. Materials and methods

4.1.2.1. Study sites

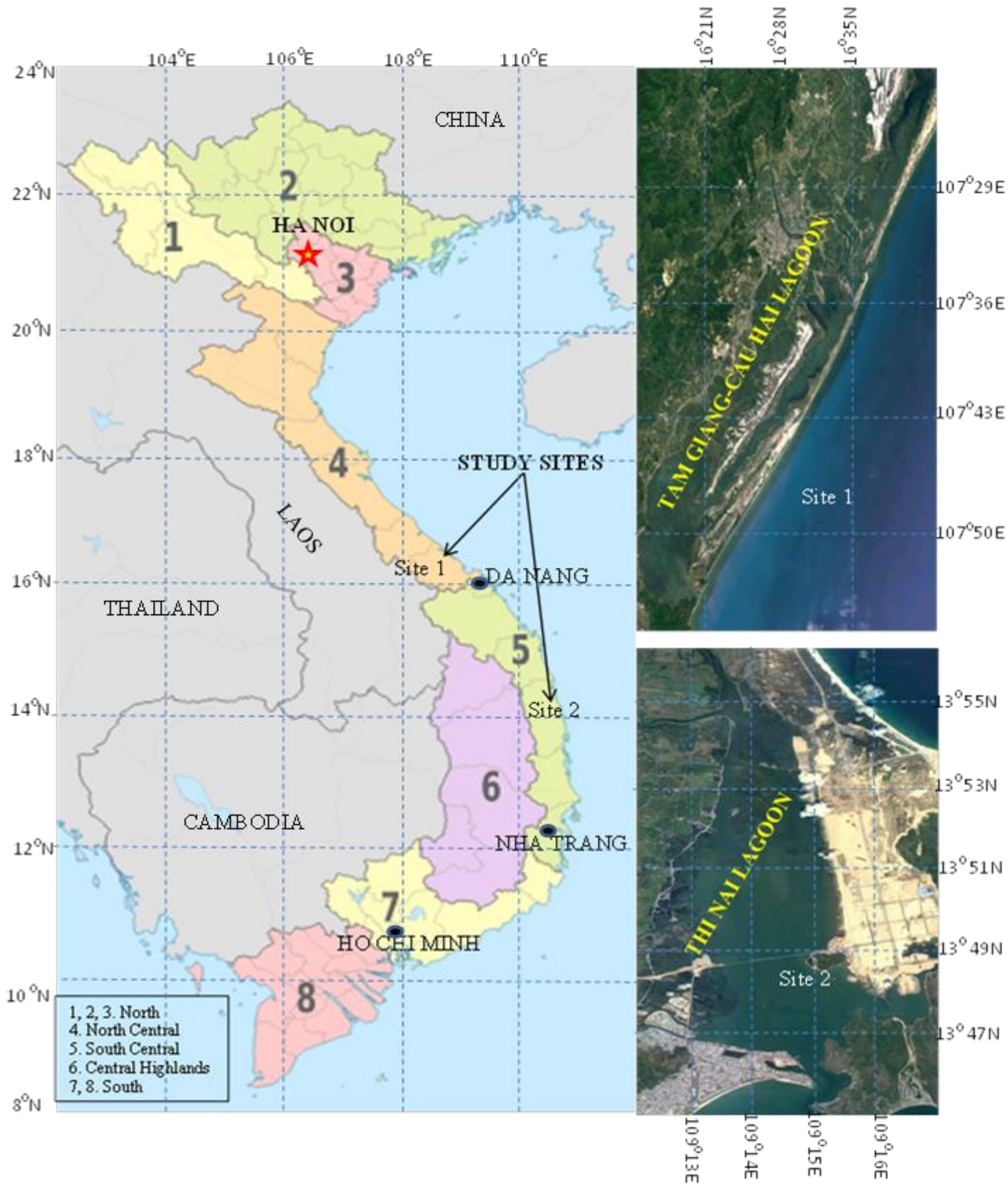


Figure 4.1. Study sites (Site 1: “Tam Giang-Cau Hai” in Thua Thien Hue province; Site 2: “Thi Nai” lagoon in Binh Dinh province.

This study incorporated sites in two provinces (Thua Thien Hue and Binh Dinh) in the north and south central Vietnam (Figure 4.1) where juvenile Mangrove Red Snapper are harvested annually to supply aquaculture. Both provinces have river-fed coastal lagoons connected to the sea; “Tam Giang-Cau Hai” in Thua Thien Hue province is the largest lagoon system in Vietnam, with area of 21,600ha. Binh Dinh province also has several lagoons, especially “Thi Nai” lagoon, with an area of over 5,000ha.

4.1.2.2. Sample collection and prey composition analyses

Sampling was undertaken during two periods: from September to November 2015 and from February to April 2016, which coincided with the annual peak in larval settlement. During each period, fish were trapped 3 times per day, in accordance with common practice of local fishermen: after sunrise (7-11am), after sunset (7-10pm), and midnight (12pm-2am). Trapping was concentrated in locations where the experience of local fishermen indicated that juvenile fish were aggregating. The total of 121 fish caught were divided into 3 size classes; <3cm TL (33 fish), 3-5cm TL (35 fish), 5-10cm TL (43 fish), and preserved in 10% formalin. Potential natural food organisms (for comparison to gut contents) were also collected at the same time and preserved in 10% formalin.

In the laboratory, all fish stomachs were dissected and contents were placed onto petri dishes. Using a stereomicroscope (Meiji EMTR-3) and binocular microscope (Olympus CX22), the prey items were separated, counted, measured and identified to the lowest possible taxon. The same procedure was applied to the potential prey organisms sampled from the water column. Stomachs of fish caught during the rising tide mostly contained relatively intact prey, making these prey items relatively simple to identify. Identification of partially digested prey was carried out by comparison with prey identified from stomachs of fish and collected from the estuarine environment. Some prey that could not be identified due to the advanced state of digestion was labelled as unidentified food. Data were expressed as percentage by number (%N), percentage by frequency of occurrence (%O), and percentage by volume (%V), as proposed by Hyslop (1980).

$$\%\mathrm{N} = \left(\frac{\text{Number of particular prey item}}{\text{Total number of prey items in stomach}}\right) \times 100$$

$$\%\mathrm{O} = \left(\frac{\text{Number of stomach containing particular prey item}}{\text{Total number of examined stomach}}\right) \times 100$$

$$\%\mathrm{V} = \left(\frac{\text{Volume of particular prey item}}{\text{Total volume of prey items in stomach}}\right) \times 100$$

A volumetric measure was chosen to provide an estimation of biomass, since gravimetric methods can produce large errors in small volumes because of water content and blotting may damage samples in some cases (Cocheret et al., 2003). In very small stomachs (such as those from newly-recruited juvenile fishes), individual prey items were difficult to weigh and therefore the volume of the food items found was visually estimated, as percentage of total volume of all stomach contents (Hynes, 1950; Hyslop, 1980).

No dietary index is without bias, since foraging behaviour and encounter frequency exert stochastic influences on stomach contents. A way around this is to employ a frequently-used quantitative description of fish diet and the relationship of diet to other characteristics of fish populations that estimates importance of individual prey taxa in their diet. Contribution of each prey item to fish diets can be estimated according to the Index of Relative Importance (IRI), a compound index (Pinkas et al., 1971; Cortés, 1997).

$$\text{IRI} = (\%\text{N} + \%\text{V})\ \%\text{O}$$

To facilitate comparisons among prey categories, IRI was standardized to %IRI (Cortés, 1997).

$$\%\text{IRI} = \left(\frac{IRI}{\sum IRI}\right) \times 100$$

The feeding intensity (stomach fullness) was estimated according to a 5 level scale (empty, 25% full, 50% full, 75% full, completely full) as described by Pillay (1952) and Kock et al. (1994).

Empty 25% full 50% full 75% full 100% full

Figure 4.2. The different stomach fullness of juvenile fish

5 levels of the stomach fullness of juvenile Mangrove Red Snapper can be described as follows:

- Empty: Stomach looks a straight pipe, has no prey inside
- 25% full: Stomach is slightly bloated with few amount of prey
- 50% full: Stomach is bloated with considerable amount of prey
- 75% full: Stomach is sufficiently bloated with large amount of prey
- 100% full: Stomach is immensely bloated with large amount of prey

4.1.3. Results

4.1.3.1. Feeding variations with increasing fish size

In this study, I examined the stomachs of 121 wild-caught Mangrove Red Snapper juveniles (24 empty and 97 with contents). In general, identification of the components of their stomach contents revealed that the diets of all juvenile size classes were dominated by various types of shrimps, with lesser quantities of crabs and other motile zooplankton (Table 4.1 to Table 4.3). However, there were strong variations in stomach contents between size classes of fish. In the smallest size class, the bulk of the diet (84.9% by occurrence frequency, 30.8% by number, 37.3% by volume) was composed of mysid shrimps (59.5%IRI). Calanoida occurred in stomachs in equal numbers to Mysidae (30.8%), but were found in fewer stomachs (42.4% by occurrence frequency) and 16.8% by volume, and thus ranked second in importance at 20.8%IRI. Luciferidae was also important prey for fish in this stage (14.3%IRI).

For size class of 3-5cm, *Acetes indicus* predominated in all occurrence frequency (65.7%), number (49.0%) and volume (40.6%), and formed the most important prey (59.3% IRI). Grapsidae, were the second most common prey item in stomachs, comprising 62.9% by occurrence frequency, 15.7% by number, 31.8% by volume and 30.1%IRI.

Table 4.1. Stomach contents of <3cm juvenile collected in September - November (no juvenile fish < 3cm were caught in February - April)

Contents in stomach	%O	%N	%V	%IRI
Shrimp				
Acetes indicus	15.2	4.4	11.4	2.5
Mysidae	84.9	30.8	37.3	59.5
Luciferidae	27.3	23.9	27.0	14.3
Zooplankton				
Calanoida	42.4	30.8	16.8	20.8
Melita longidactyla	21.2	7.6	4.9	2.5
Tanaidacea	6.1	1.3	1.1	0.2
Unidentified	6.1	1.2	1.5	0.2

Number of juveniles examined: 33

Table 4.2. Stomach contents of 3-5cm juvenile collected in September - November (no 3-5cm juvenile fish were caught in February-April)

Contents in stomach	%O	%N	%V	%IRI
Shrimp				
Acetes indicus	65.7	49.0	40.6	59.3
Acetes sp.	14.3	3.3	10.5	2.0
Mysidae	22.9	17.6	7.9	5.9
Alpheidae	5.7	1.3	1.0	0.1
Crab				
Grapsidae	62.9	15.7	31.8	30.1
Zooplankton				
Calanoida	8.6	2.0	0.3	0.2
Melita longidactyla	8.6	4.6	1.0	0.5
Zoobenthod				
Larval dragonfly	14.3	3.2	2.5	0.8
Unidentified	14.3	3.3	4.4	1.1

Number of juveniles examined: 35

Table 4.3. Stomach contents of 5-10cm juvenile collected in September - November and February - April.

Sampling periods	Stomach contents	%O	%N	%V	%IRI
September to November	**Shrimp**				
	Acetes indicus	75.8	26.1	22.8	47.2
	Acetes sp	15.2	7.2	5.9	2.5
	Crab				
	Grapsidae	36.4	33.3	29.4	29.1
	Fish				
	Therapontidae	24.2	10.8	32.6	13.4
	Zooplankton				
	Melita longidactyla	24.2	15.3	3.1	5.7
	Cirolana sp.	12.1	3.6	3.1	1.0
	Unidentified	12.1	3.7	3.1	1.1
February to April	**Shrimp**				
	Acetes sp.	100	100	100	100

Number of juveniles examined: 43

Remark: percentage by number (%N), percentage by frequency of occurrence (%O), and percentage by volume (%V) and proportion of relative importance (%IRI)

At the largest size class (5-10cm), Mangrove Red Snapper juveniles consumed predominantly *Acetes indicus* (75.8% by occurrence frequency, 26.1% by number, 22.8% by volume), and this prey became most important for them (47.2%IRI). Grapsidae ranked second in importance (29.1%IRI), with 36.4% by occurrence frequency, 33.3% by number and 29.4% by volume. The largest fish marked a dietary and behavioral change. Therapontid fish (13.4%IRI) appeared in the stomach contents for the first time, although juveniles of this size class still consumed grapsid crabs and relied predominantly on sergestid shrimps.

4.1.3.2. Seasonal diet shift

Only the largest size class of juvenile fish (5-10cm) were caught during the February to April period. In the September to November sampling period, the larger juveniles consumed a diverse range of prey items (Table 4.3), of which *Acetes indicus* was the most important prey (47.2%IRI), followed by Grapsidae (29.1% IRI) and Therapontidae (13.4% IRI). However, in the February-April season, *Acetes indicus* were absent from the coastal lagoon (Figure 4.3 and Figure 4.4). A second species of *Acetes*, somewhat larger than *A. indicus*, was found hiding amongst filamentous seaweeds or emerged rocks near the bottom; this species exclusively was consumed by the juvenile snapper during the February to April period.

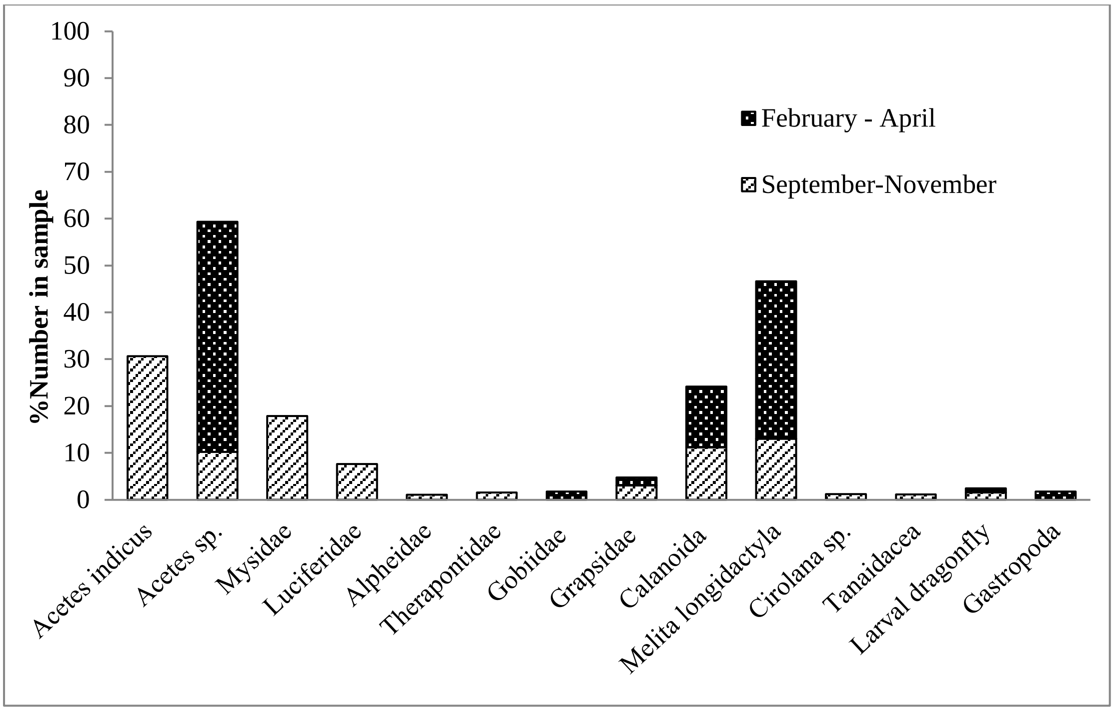


Figure 4.3. Percentage by number of natural food organisms collected from water environment.

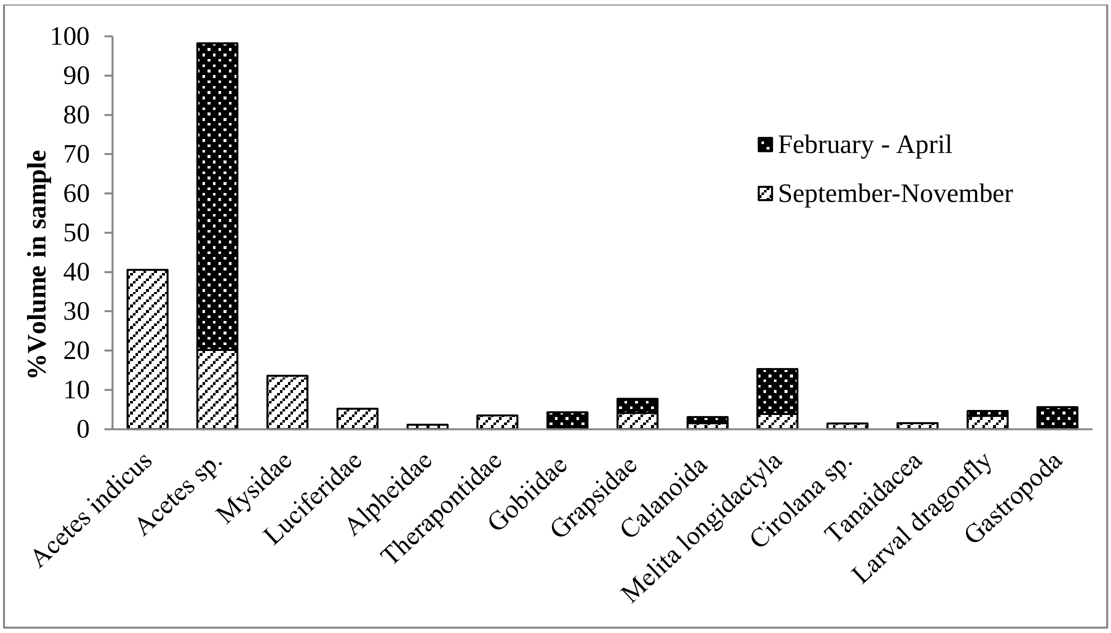


Figure 4.4. Percentage by volume of natural food organisms collected from water environment.

4.1.3.3. Feeding behavior of fish

Feeding intensity of fish was affected by tidal cycle (Table 4.4), with the majority of feeding apparently occurring during the incoming tide. More than 75% of all captured fish had full stomachs when caught during the incoming tide (100% of 3-5cm size class captured in Thua Thien Hue province). In contrast, more than 40% of all fish collected during the outgoing tides presented empty stomachs (except <3cm size class collected in Binh Dinh). No captured fish of any size class had full stomachs during the outflowing tidal cycle, suggesting that partial fullness may indicate the remnants of feeding on the previous inflow cycle.

Table 4.4. Stomach fullness of fish (%) according to tidal cycle

Tide	Stomach fullness	Binh Dinh			Thua Thien Hue		
		<3cm	3-5cm	5-10cm	<3cm	3-5cm	5-10cm
Incoming	Empty	0.0	0.0	0.0	0.0	0.0	0.0
	25% full	0.0	0.0	0.0	0.0	0.0	0.0
	50% full	0.0	0.0	0.0	0.0	0.0	0.0
	75% full	14.3	12.5	25.0	15.4	0.0	10.0
	Full	85.7	87.5	75.0	84.6	100.0	90.0
Outflowing	Empty	33.3	57.1	62.5	40.0	50.0	40.0
	25% full	50.0	28.6	25.0	40.0	30.0	40.0
	50% full	16.7	14.3	12.5	20.0	20.0	20.0
	75% full	0.0	0.0	0.0	0.0	0.0	0.0
	Full	0.0	0.0	0.0	0.0	0.0	0.0

As well as tidal cycles, I found that within the same main habitats, fish used different microhabitats; the smallest fish usually travelling around surface water preferred to catch small prey moving in surface water or suspending in water column as Mysidae and Calanoida. Larger fish size classes, which preferred to move around lower water layers, gradually changed to eat larger prey living in these water layers, such as grapsid crabs or therapontid fish. It can be seen that prey selection of Mangrove Red Snapper not only depends on food availability associated with preferred microhabitats of fish but also depends on prey size that may be suitable for mouth size of each size class of juvenile.

4.1.4. Discussion

Aquaculture of Mangrove Red Snapper in Vietnam mostly depends on capture of juvenile fish from coastal lagoons, but the availability of the stock depends strongly on the role of a succession of critical habitats in larval and juvenile recruitment. The sustainability of the industry requires understanding of factors which influence the survivorship and growth of early life stages of fish. My study found important links between the diet of juvenile *L. argentimaculatus* and natural prey associated with particular estuarine habitats.

Key among the findings in this chapter is that juveniles *L. argentimaculatus* reveal ontogenetic feeding changes. Although juvenile fish in general fed on shrimp prey, the smallest size classes (<3cm total length) consumed predominantly mysid shrimps, in addition to copepods and luciferid shrimps. Larger juveniles (3-5cm) fed mostly on *Acetes* and grapsid crabs. These prey were also the preferred food of the largest juveniles (5-10cm), although the larger fishes also consumed small fishes.

Similar dietary changes have been also reported for *L. argentimaculatus* in other locations (e.g. Nakamura et al., 2008), although the continued importance of *Acetes* has not been previously recognised. Availability and ease in capture could be a key as to why shrimp prey are important for Mangrove Red Snapper in all studied size classes. In the wild, *Acetes* and mysid shrimps were the most prominent prey items (in both number and volume: Figure 4.2, 4.3) associated with rocky habitats where juveniles fish were caught..

I also found seasonal variations in diet of fish. *L. argentimaculatus* consumed a wide food spectrum in period of September-November, including shrimps, crabs, fish and zooplankton. Whereas, they uniquely fed *Acetes* in period of February-April. Similar diet shifts were also recorded on *Sciades herzbergii* (Tommaso & Ulrich, 2008), *Lutjanus jocu* (Monteiro et al., 2009), *Mustelus schmitti* (Juan & Andrea, 2011). Only 5-10cm fish were caught in period of February-April, therefore, seasonal diet changes of smaller fish classes have been still unknown. It is believed that a possible cause for seasonal difference in diet could be related to the distribution, abundance and availability of prey in each season (Monteiro et al., 2009; Rooker, 1995), or changes in life history patterns of food organisms, but also feeding activities of the fishes themselves (Snyder, 1984; Lucena et al., 2000).

Food availability in water environment clearly affects feeding habit of fish, although abundance of certain prey items was not a good indicator of juvenile diet. My findings also indicate selective feeding by juvenile Mangrove Red Snapper throughout ontogeny. This seems to be related to habitat use of fish and prey size. Specifically, I found that despite occupying the same main habitats, fish of different size classes used different microhabitats; the smallest fish usually travelling around surface water preferred to catch small prey moving in surface water or suspended in the water column (like mysids or calanoid copepods). Larger fish classes, which preferred to move around the lower water layers, gradually changed to eat bigger prey living in the bottom water layers, such as grapsid crabs or small therapontid fishes. Dietary shifts related to habitat use were also found by Bowen and Allanson (1982), but little attention has been paid to where either prey or predator are located in the water column. Although not examined in the current study, other authors have reported that anatomical and morphological variation may be also important aspects associated ontogenetic changes in diet of reef fishes (Schmitt & Holbrook, 1984; Lukoschek & Mccormick, 2001). A follow-up survey after this study (in February, the coldest time in the study periods) found that only the largest size class of *L. argentimaculatus* were present, sheltering amongst small rocks supporting the shallow seaweed comunity in which they hunted *Acetes* to the exclusion of all other potential prey. The juveniles appeared to have adjusted their foraging strategy to minimize energy expenditure in the cold conditions. That might be the reason as to why juvenile consecutively consumed *Acetes* in period of February to April.

Also interesting is that feeding intensity of Mangrove Red Snapper is affected by tidal cycle. Mostly fish caught on rising tide had full stomachs, whereas a fish captured during time of falling tide generally had empty or nearly empty stomachs. Sheaves (2005) and Zagars et al. (2012) made similar observations. The finding is consistent with studies conducted on *Pleuranectesp fatessa* by Kuipers (1975) and *Platichthys flesus* by Summers (1980).

The time of day of the tidal cycle had negligible influence on feeding intensity of juvenile Mangrove Red Snapper. Ebeling and Bray (1976) stated that microcarnivores are often active only during the day and seek refuges at night for protection against predators. In contrast, it is supposed that mesocarnivores maintain peak activity during crepuscular or nocturnal periods in addition to opportunistic feeding during the day (Collette & Talbot, 1972; Ebeling & Bray, 1976). My findings seem not to support these general feeding strategies. Feeding activity of juvenile Mangrove Red Snapper is governed by tidal cycle rather than time of day. This confirms suggestions by Zagars et al. (2012), who also found that movement of juveniles of this species mostly occurred at high tide regardless day or night, which implied that feeding occurred during high tide.

4.2. THE IMPORTANCE OF LIVE FOOD

4.2.1. Introduction

Mangrove Red Snapper (*Lutjanus argentimaculatus*) has been a profitable species for aquaculture in many countries, especially in Southeast Asia (Liao et al., 1995; Wong, 1995; Chou & Lee, 1997; Emata & Borlongan, 2003). Mariculturists in Vietnam prefer wild-caught juveniles to hatchery-raised fish. Larval Mangrove Red Snapper that survive settlement have been exposed to strong environmental filters, which eliminated unthrifty, weaker juveniles. They are therefore thought to have some advantage over cultured fish. Part of this advantage may be the preference of juvenile of this fish species for intermediate salinities, since cultured fry tend to be grown in oceanic conditions (Chapter 3), but some of the advantage may lie with their diet (Chapter 4-Section 4.1).

Studying feeding habits and natural diet of fish is the key to understanding many aspects of their biology, ecology, physiology, and behaviour (Goncalves &

Erzini, 1998; Rita et al, 2006). Moreover, knowledge of the natural diet of a given species is generally essential for studies of its nutritional requirements, its interactions with other organisms and its potential for culture (William, 1981). However, despite certain success in breeding (Leu et al., 2003, Thanh, 2012), it is likely that limited understanding of optimal juvenile environment and food leads to low and variable survival rates for cultured Mangrove Red Snapper. Thus, it is needed to examine natural diet and relative aspects of this fish species to maintain a supply of fingerlings for its aquaculture to supplement or (eventually) replace the wild-caught resource, which is better left to replenish the wild spawning population.

The idea of using live food organisms in aquaculture is not particularly new. Lian et al. (2003) stated that the success in the fingerlings production of hatcheries was largely dependent on the availability of suitable live food organisms for feeding larvae, fry and juveniles. Even more than 20 years ago, productions of at least 60 marine finfish species and 18 species of crustaceans were relying on live food organisms (Dhert, 1996). In addition, provision of appropriate live food at the proper time plays a major role in achieving maximum growth and survival of finfish and shellfish in early stages of the life (Pronob et al., 2012). This implies that seeking suitable food for stages of larvae and fingerlings would be indispensable contribution for successful production. However, what juvenile Mangrove Red Snapper consume at different life history stages is virtually unstudied.

Typical aquaculture diets for juvenile Mangrove Red Snapper comprise compounded pellets with varying proportions of fat, protein and bulk designed to optimize trophic efficiency. This the addition of live food to juvenile diets has been advocated for more than a decade (Surtida, 2003). A number of studies have examined the role of live food for fish species (Awais et al., 1992; Sorgeloos et al., 2001; Lian et at., 2003; Evjemo et al., 2003; Jan et al., 2003; Wang et al., 2005), most studies have carried out on larval stages, while only a few have been done on juvenile stages (Ethiraj et al., 1994; Domingues et al., 2003; Eduardo et al., 2006). Moreover, although natural diets of many fish species have been examined (Szedlmayer & Lee, 2003; Nakamura et al., 2008; Monteiro et al., 2009; Sedberry & Cuellar, 1993; Rooker, 1995; Lowe et al., 1996), there remains a knowledge gap regarding Mangrove Red Snapper.

Juvenile Mangrove Red Snapper have shown feeding patterns similar to many other marine fishes. After settlement, from approximately 2cm to 10cm total length, they showed a diverse range of prey that included shrimps, crabs, fish, zooplankton and zoobenthos. Of which, it is worth to note that shrimp prey contributed more than two thirds of the diet of the examined fish (Chapter 4-Section 4.1). It is likely that shrimps often appear in diet of lutjanids (e.g. these prey were found in natural diet of *L. jocu* (Monteiro et al., 2009); *L. campechanus* (Stephen and Jason, 2003); *L. fulvus* and *L. gibbus* (Nakamura et al., 2008); or *L. ehrenbergii* and *L. fulviflamma* (Berkström et al., 2013)). In general, shrimps play an important role in the life history of many lutjanids, however, their real role for these fish or Mangrove Red Snapper in particular is somewhat unknown. This study investigated the natural diet of very young Mangrove Red Snapper and also examined effects of live food organisms on survival and growth of cultured juveniles.

4.2.2. Materials and methods

4.2.2.1. Experimental design and management

The results of the stomach contents analysis (Chapter 4-Section 4.1, Table 4.1, 4.2 and 4.3) showed that *Acetes* and Mysidae were important components of the natural diet of juvenile Mangrove Red Snapper.

Sufficient wild-caught juveniles of the smallest (<3cm in total length, ~0.3g in weight) size class were obtained from local fishermen to conduct the experiment. After one week of acclimation, 150 healthy fish were assigned to each of nine fine-mesh net cages (50x40x50cm) placed in a well-flushed culture pond. The cages were assigned to 3 dietary treatments:

1) a mixture of live cultured *Acetes*, and freshly-collected mysid shrimps from the nearby mangrove area;

2) minced fresh anchovy obtained daily from fishermen;

3) commercial pelleted feed (INVE-NRD 2/3),

Each group contained 3 randomly-assigned replicates for each treatment (i.e. N=9).

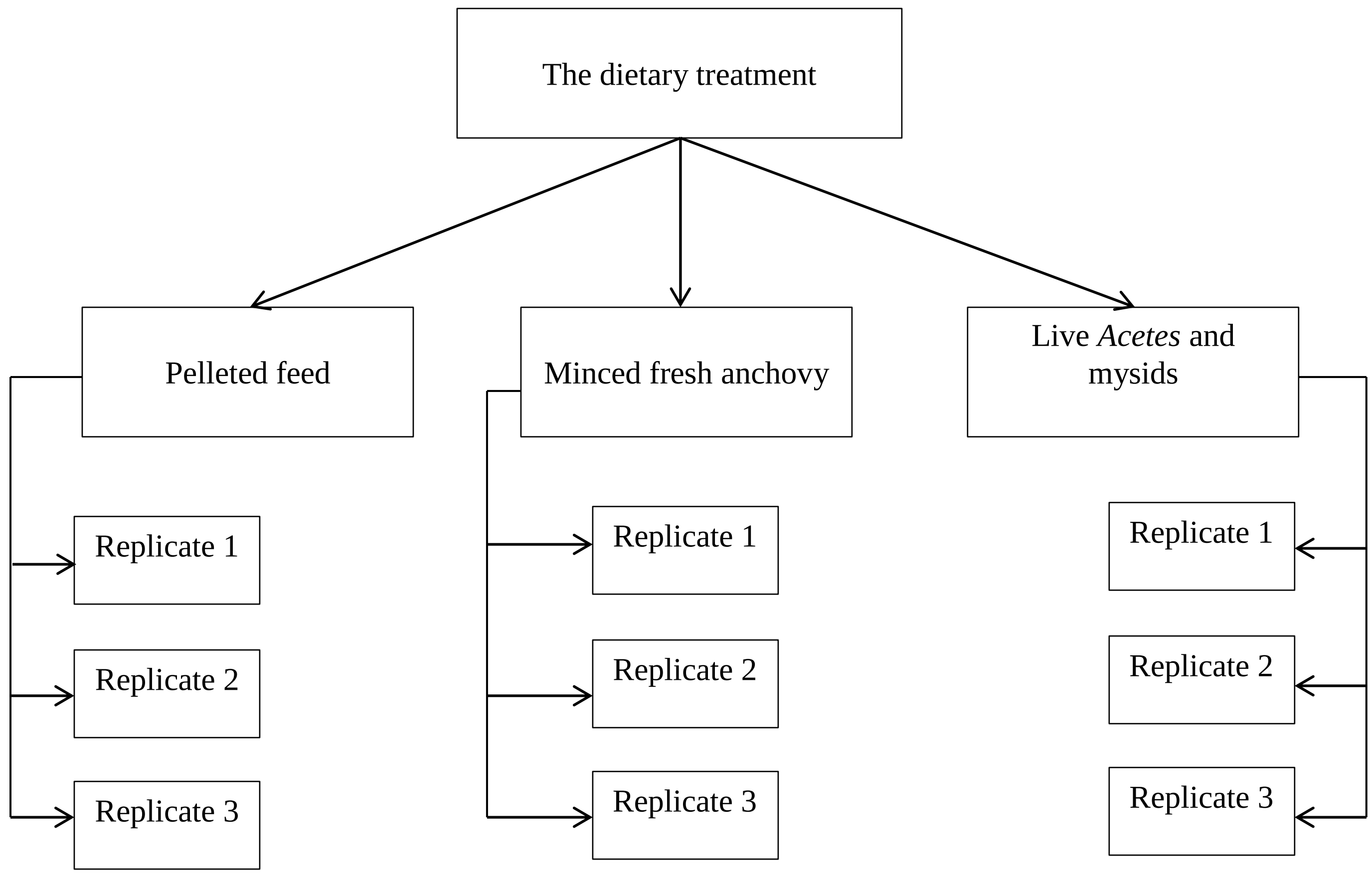


Figure 4.5. The experimental plan for effects of different diets on growth and survival of fish

The net cages (50x40x50cm) were made of 80µm plankton net to prevent other organisms in the pond environment from entering the cages. Therefore, fish only consumed the supplied food. The cages were suspended in nursery pond that was a closed system, surrounded by mangroves (Figure 4.6), and allowed water from the pond to exchange easily. Water temperature during the experimental period ranged between 28-30^{0}C; dissolved oxygen was from 5.5-6.0 mg/L; ambient pH was from 8-8.5; ammonia was consistently less than 0.01 mg/L for the duration of the experiment. Comparative nutritional yield of the different feeding regimes is listed in Table 4.5.

Figure 4.6. Nursery pond where the dietary experiment was conducted.

Fish were fed 4 times a day, at 7am, 10am, 2pm and 5pm, except on the days of measuring and weighing. After each meal, any uneaten food was manually removed out of the culture cages. Net cages were cleaned every week by hand. The experiment ran for 28 days. I measured total length (from the point of the nose to the end of the caudal fin) and weight of a subset of 10 fish from each cage every week. Number of surviving juveniles in each net was counted at similar weekly time points.

Table 4.5. Approximate composition of food used to feed juvenile Mangrove Red Snapper (percentage by dried weight).

Composition	Pelleted diet	Minced fresh anchovy	Mysidae + *Acetes*
Protein	55.00	76.00	69.90
Lipid	9.00	8.13	6.33
Ash	14.50	13.50	15.50
Fiber	1.90	0.26	2.19

4.2.2.2. Statistical analysis

I used repeated measures analysis of variance (ANOVA) to identify growth and survival of juveniles fish according to different foods over the study period. All statistical analyses were done using SPSS version 15.0 (SPSS Inc. 2006).

4.2.3. Results

4.2.3.1. Effects of live food on growth of fish

Total length of juveniles varied significantly between treatments ($F(2,58)=220.85$, $p<0.001$). The use of *Acetes* and Mysidae provided a considerable boost to growth over 28 days compared to juveniles fed on minced fresh anchovy (~22%, on average) and pelleted diet (37%, on average).

Likewise, the kinds of experimental food provided to the juveniles significantly affected mean weight at the end of the experiments ($F(2,58)=274.7$, $p<0.001$). Mean weight of fishes fed sergestid and mysid shrimps was much higher than those in treatments with anchovy (29% greater) and with pelleted feed (~67% greater) over a period of 28 days.

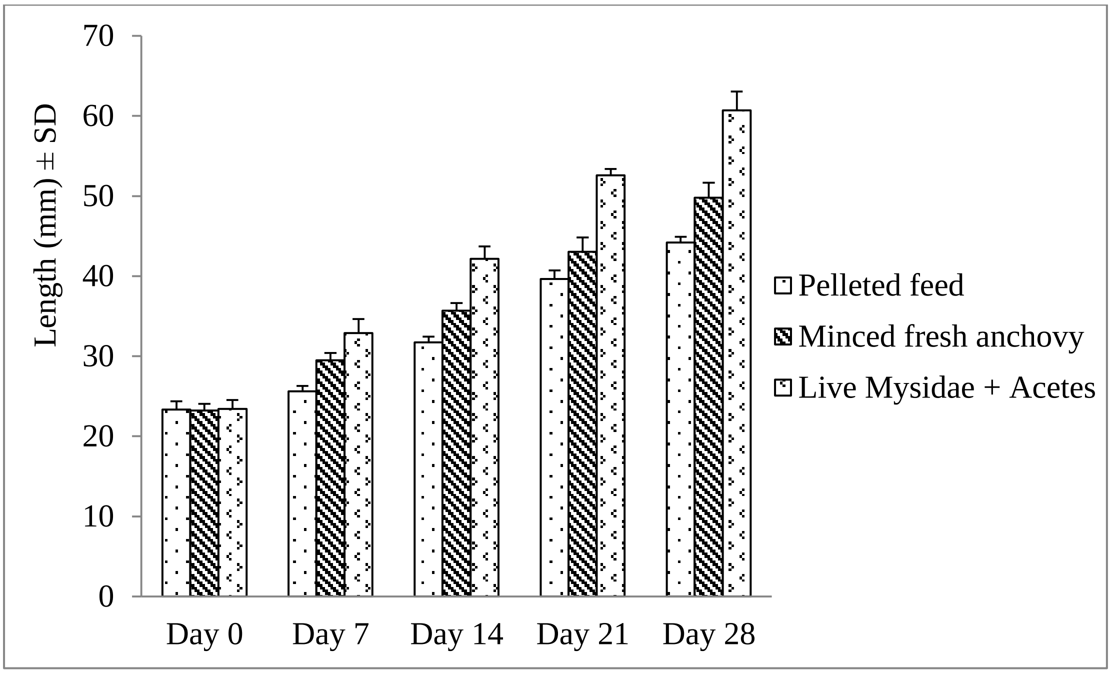


Figure 4.7. Length of fish fed different food.

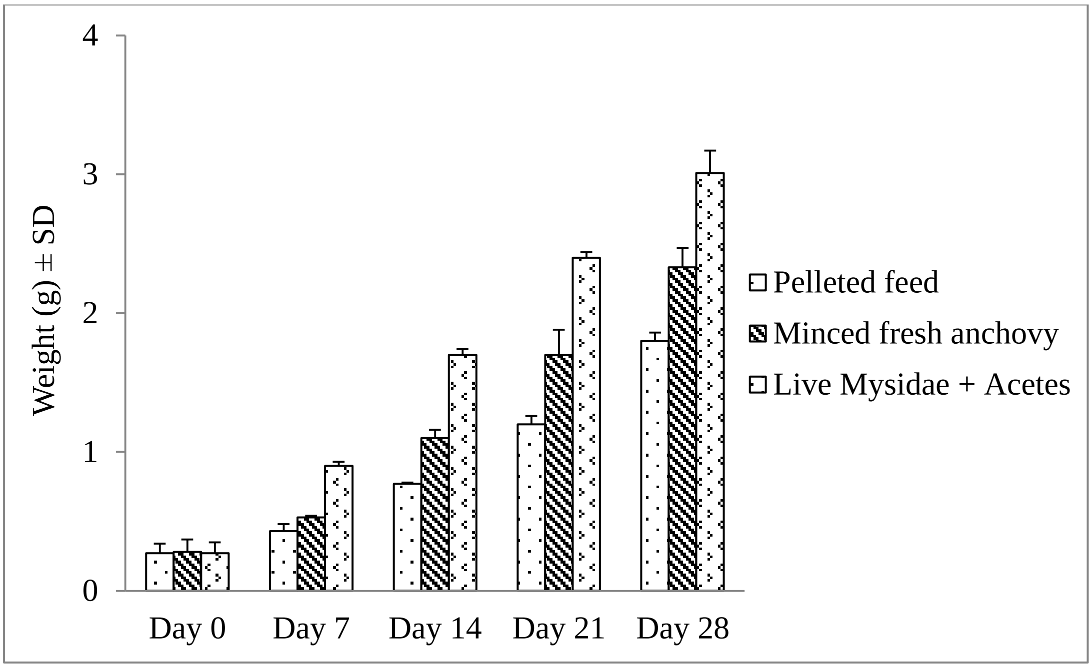


Figure 4.8. Weight of fish fed different food.

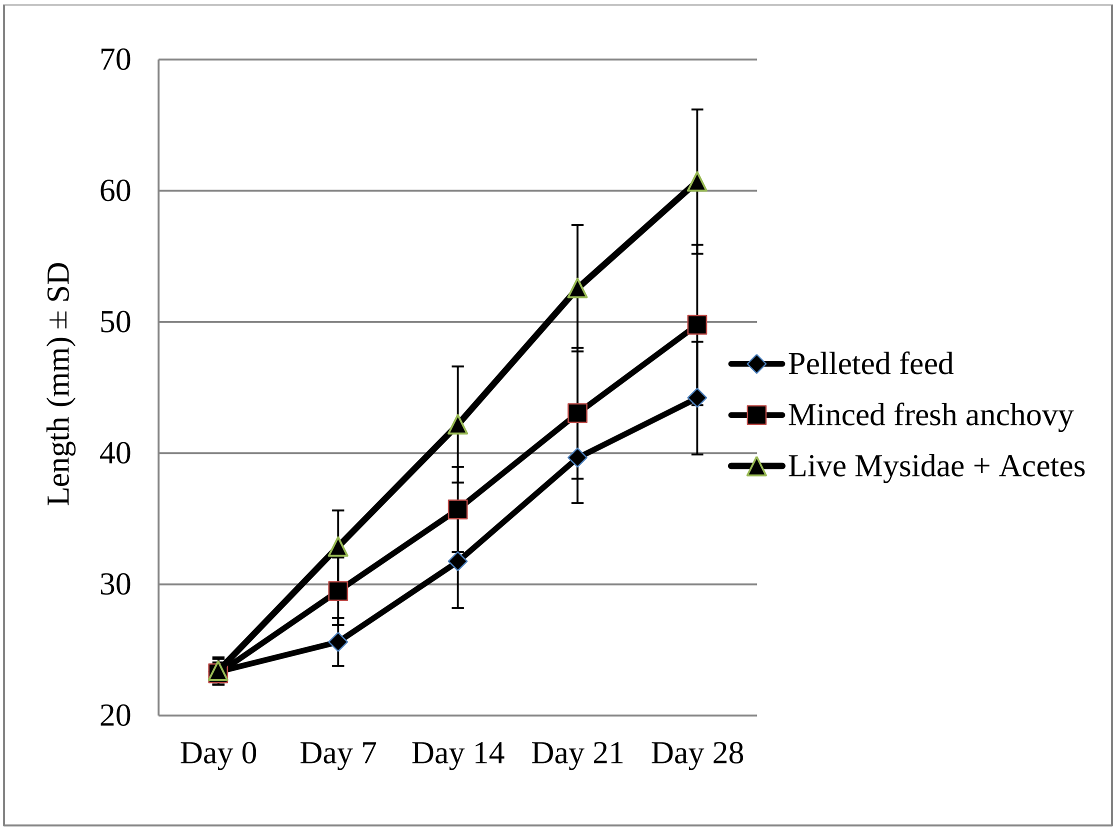


Figure 4.9. Length trajectories of fish through time according to food regime.

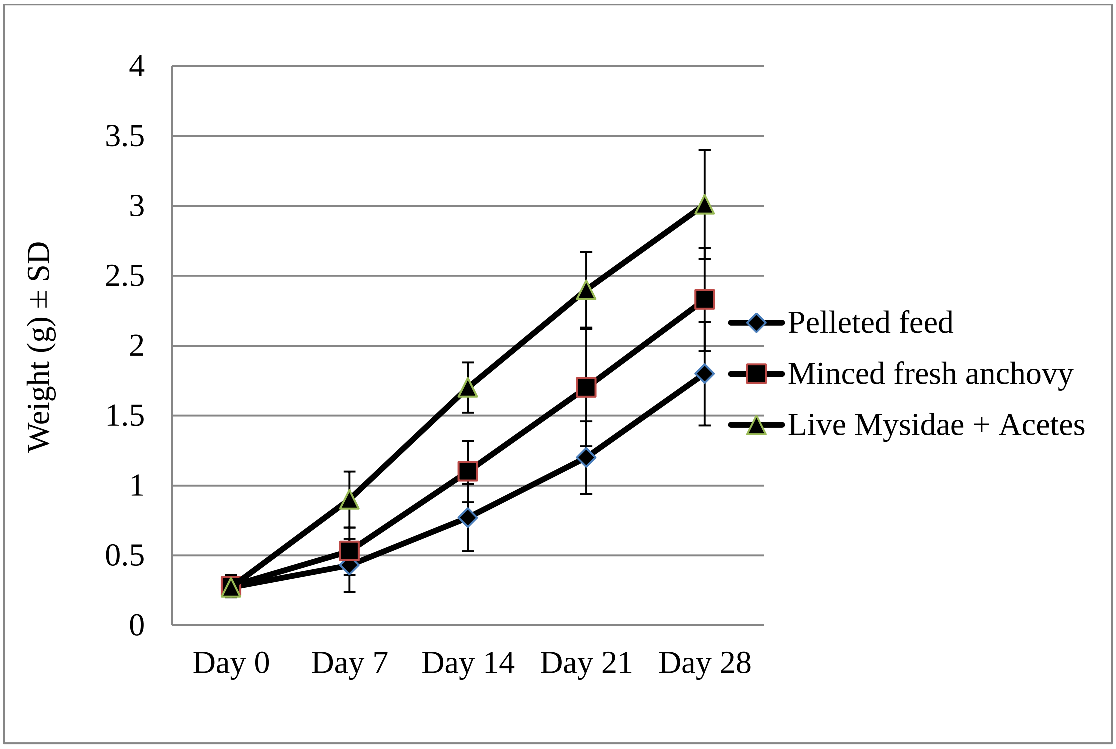


Figure 4.10. Weight trajectories of fish through time according to food regime.

4.2.3.2. Effects of live food on survival of fish

Survival of fish differed significantly between experimental treatments ($F(2,4)=106.75$, $p<0.001$). I found that diet of *Acetes* and Mysidae was significantly more beneficial to juvenile survivorship than the others ($p<0.05$); there was no significant difference in rates of survival of fish between anchovy and pelleted feed treatment ($p>0.05$). At the end of experiment (day 28), survival of fish on the shrimp diet of *Acetes* and Mysids was 93.78%, which was ~4% greater than that of fish fed anchovy and ~6% greater than this of fish consuming pelleted feed.

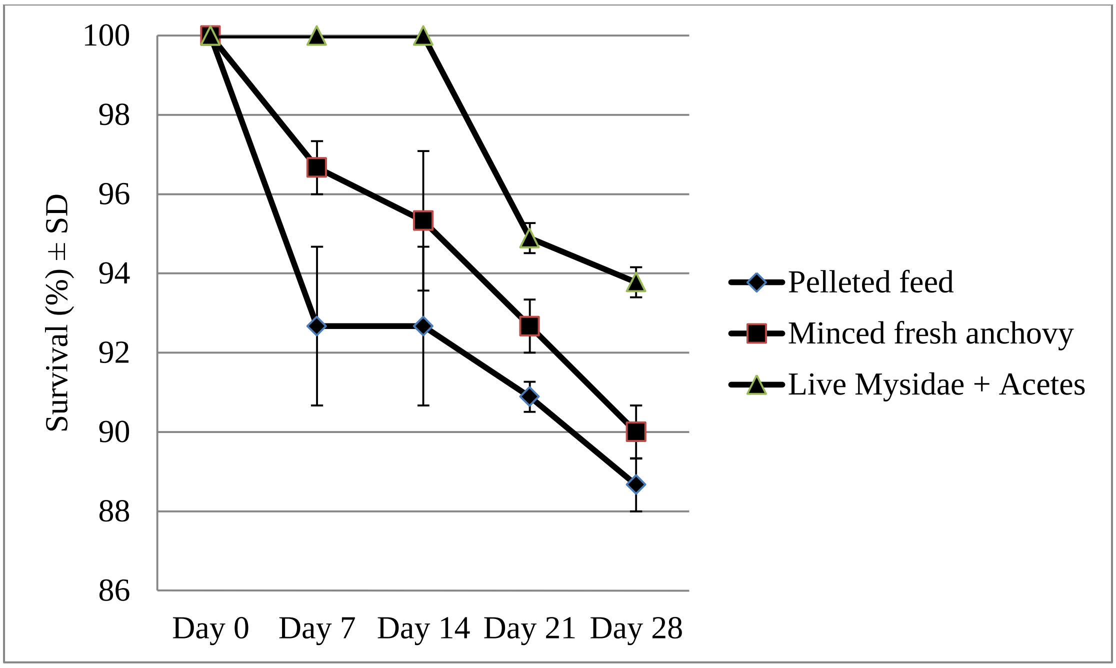


Figure 4.11. Survival trajectory of fish on the experimental diets through time.

4.2.4. Discussion

Studying feeding ecology of fish and the selection of a suitable feed are crucial factors contributing to operation of a successful aquaculture industry. However, despite certain successes in captive breeding, understanding of the role of natural food of Mangrove Red Snapper in linking to its growth or survival is still limited.

The interesting findings here are that *Acetes* and Mysidae strongly influence growth of captive juvenile fish (i.e. greater by ~22% to 37% in terms of average length, and 29% to ~67% in terms of average weight than other food), and also improve survival of fish (i.e. ~4 to ~6% increase over other food used). Eusebio et al. (2010) reported that that mysids are superior live food organisms to *Artemia* biomass for grouper larvae, acting in increase the activity of several biomarkers associated with food assimilation.

Similar growth improvement of fish reared on a diet of shrimp prey was also found for *Oreochromis mossambicus* (Ethiraj et al., 1994); the benefits of such foods are not restricted to teleosts: Domingues et al. (2003) and Eduardo et al. (2006) found similar results with cuttlefish *Sepia officinalis*. These authors, however, did not mention whether shrimps were preferred prey of fish in the wild. Whereas, it is clear in this study that shrimps, particularly sergestids and mysids, are the most important natural prey in the diet of juvenile Mangrove Red Snapper at the early post-recruitment phase. Although there may be subtle differences between the wild and cultured mysids in terms of nutritional components (Herrera et al., 2011), harmonising the diet of captive juveniles with their wild kin appears to have strong benefits for their culture, and demonstrates clear gains in culture efficiency.

The ratios of protein and lipid are important factors affecting growth or even survival of many fish species (Joly et al., 2011; Kyoung et al., 2012; Arredondo et al., 2012; Stavros et al., 2012; Fatime et al., 2012; Minerva et al., 2012). Generally, an increase of protein and lipid levels in diet can improve fish production, especially for carnivorous fish (Arredondo et al., 2012). The results of present study seem not to support this, however. In particular, although the crude protein content in anchovy was higher than that of mysids and *Acetes*, juveniles fed anchovy returned lower growth rates than those consuming mysids and *Acetes*. Similarly, fish fed pelleted feed and anchovy that contain high lipid levels (9% and 8.13%, respectively) exhibited lower growth and survival compared to fish fed mysids and *Acetes* which contain only 6.33% lipid. It should be noted that these groups of shrimps often have high polar lipid content, something that may improve growth of fish during early life history stages (Domingues et al., 2003). Moreover, live food contribute digestive enzymes assisting in the digestive process (Dabrowski and Glogowski, 1977) that can improve feeding efficiency or help fish effectively absorb nutrient elements, leading to higher growth and/or survival. On the other hand, there may be no degradation of nutrition when feeding live organisms to fish. It can be noted, moreover, that minced fish lose a certain amount of material upon first introduction to water (which dissipates in a fine cloud around larger chunks), perhaps diminishing the nutritional value or accessible fraction of the minced fish.

Although there were several studies estimating optimal levels of protein for juvenile *L. argentimaculatus* (e.g. 40% to 42.8% (Abbas et al., 2011), or 44% (Catacutan et al., 2001)), these studies were carried out on much larger juveniles than fish used in our study. Therefore, the nutritional requirement of early juveniles has remained largely unknown. Despite a certain amount of success rearing larvae or fingerlings of Mangrove Red Snapper using artificial diets (Leu et al., 2003) or minced fish (Duray et al., 1996), these appear sub-optimal. The provision of live mysids and sergestids showed clear benefits in terms of growth and survivorship of fish. The addition of such live foods at critical stages is likely to have similarly large benefits if introduced into common aquaculture practice.

CHAPTER 5

GENERAL DISCUSSION

Lutjanus argentimaculatus is commonly called Mangrove Red Snapper in Asia, although elsewhere it is known by different names, including Mangrove Jack, Creek Red Bream, Dog Bream, Purple Sea Perch, Red Bream or Red Perch. Perhaps unsurprisingly, Mangrove Red Snapper individuals are commonly found in mangrove-lined estuarine systems, although adults are known to migrate to open waters to breed, sometimes hundreds of kilometers from the coast (Allen & Erdmann, 2012).

Mangrove Red Snapper is an important market species that is considered to be an excellent food fish, and also a major recreational species throughout its range. It has recently become a profitable candidate for aquaculture in many countries, especially nations in Southeast Asia including Vietnam. This desirability places strong pressures on the species at all its life stages, and makes the species especially vulnerable to recruitment overfishing. However, its mariculture mostly depends on a source of wild juveniles that is seasonal, variable, and probably unsustainable. In addition, fishing juveniles to supply aquaculture and coastal habitat depression reduces the pool of viable recruits to the spawning population of this fish species. However, despite certain successes in captive breeding, understanding of its habitats, trophic ecology as well as recruitment is still limited. Therefore, in this study, I investigated important factors associated with the recruitment of juveniles *L. argentimaculatus* by examining their habitat specificity and feeding ecology in coastal areas in central Vietnam. This study aims to supply important background information to facilitate its production as well as further support fish resource conservation.

In Vietnam, most of the juveniles Mangrove Red Snapper supplying its aquaculture are caught from the wild. Although the techniques for induced spawning of *L. argentimaculatus* have been well-established for several decades, aquaculturists prefer the wild fry, citing a perceived advantage in terms of viability and thrift over hatchery-raised fry. The smallest (<3cm) size class of fish is considered as the best suited to grow-out culture; the coastal lagoons explored in this study are the major source of fry for mariculture throughout Vietnam's coastal regions. Therefore, information related to the recruitment of juvenile fish as recruitment season or habitats is more and more important for managers tasked to maintain this resource. As reported in chapter 2, the main recruitment of juvenile Mangrove Red Snapper appears to occur from July to August in central Vietnam. The season of recruitment appears to be different between geographical regions in similar latitudes in SEA; in eastern Thailand, juvenile settlement occurs from late October to January (Doi et al., 1992; Doi et al., 1994). In northern Australia (the same latitude south of the equator), recruitment occurs from February to July (Russell & McDougall, 2005). The timing of the tail-off period of the "wet" monsoon in different regions may explain part of this difference; in each case, a monsoon that ends earlier or later than average may influence the timing and intensity of recruitment pulses. Even within a country recruitment season of juvenile recruitment changes due to annual weather change (e.g. in 2015, I found that major settlement of juvenile occurred from September to November, with a peak in September and October). Since *L. argentimaculatus* spawns on deep offshore reefs, and the cues for natural spawning unknown (even whether the species forms spawning aggregations), it is difficult to speculate as to whether the monsoon causes shifts in spawning behaviour, or determines the duration and movement of the planktonic larval phase. The mechanism by which the monsoon or seasonal weather patterns might affect the recruitment of fish is still unknown, so further studies are encouraged to clarify this issue.

Understanding key factors influencing natural recruitment is especially vital because, although mariculture of Mangrove Red Snapper has been undertaken throughout coastal Vietnam, the majority of wild juveniles for mariculture are captured from two central provinces (Thua Thien Hue and Binh Dinh). In these provinces, coastal lagoons fed by rivers are associated with mangrove forest. It is not immediately clear why these lagoons have become preferred nursery grounds of many fish species including Mangrove Red Snapper; it may be that they are simply the last remaining intact examples of this type of habitat. Other areas traditionally associated with *L. argentimaculatus*, such as Ca Mau in southern Vietnam, have been seriously degraded – some from the lingering effects of widespread deployment of American defoliants during the Vietnam War, and some from conversion to intensive shrimp production or urban encroachment. The end result has been a contraction of the harvest of lutjanid juveniles to these last two locations. There is still question, however, as to why these sites have persisted and what cues promote recruitment there. Although there are different fishing gears in use, most fishers focus on catching fish less than 3cm that are trapped at sandy or seagrass bed sites in lagoons nearest to the lagoon access to the sea. It is not clear whether these sites are real habitats of fish, or are simply convenient locations where the fish congregate prior to movement into habitats further upstream.

To identify preferred habitats of fish, I carried out field surveys. My findings (Chapter 2) showed that smallest juvenile Mangrove Red Snapper usually inhabited habitats associated with hard structures. Specifically, I found that juveniles less than 10cm total length were mostly associated with rocky habitats, while only small number of fish selected other shelters such as snags. The finding seems counter-intuitive, since mangrove habitats are conventionally associated with muddy, snag-filled ecologies (e.g. Nagelkerken et al 2008), but similar results were also recorded by Day et al. (1981) and Russell et al. (2003). However, it is likely that habitat structure is not the most important factor governing the recruitment of this fish species. For reef fish species, their juveniles usually occupy coastal habitats such as estuaries, where salinity varies frequently, so salinity might be more important than other factors. During the field surveys, I observed that in both provinces juveniles it was clear that although the preferred shelter of juvenile Mangrove Red Snapper (rocks) can be found in different waters, juveniles only occupied estuarine areas, with salinity range from 10ppt to 25ppt. I did not find fish in freshwater in central Vietnam (contrasts with Russell et al. (2003), concurs with Piddocke (2015)). This implies that juvenile fish might prefer to only inhabit brackish waters as mangrove-lined estuaries, then migrate to coastal reefs or offshore when they reach certain maturity.

Although Mangrove Red Snapper is an euryhaline species, their tolerance for hyper- and hyposalinity differs between ontogenetic stages (Estudillo et al., 2000), and optimal salinity for early juvenile fish has been somewhat speculative. The results of experiment (Chapter 3) showed that both the lower salinity (10ppt) and the higher salinity (25ppt) are less beneficial to juvenile survivorship than moderately brackish waters (17ppt). Improving survivorship of fish reared at moderate salinity suggests that brackish water habitats are important loci for recruitment of juvenile Mangrove Red Snapper. It should be noted that the effects of modifying salinity did not extend to growth rates of juvenile fish: fish in both low and high salinity treatments grew as well as those in the moderate treatment. The difference is in the number of juveniles which survived. In aquaculture, hatchery production (e.g. Duray et al., 1996; Leu et al., 2003; Thanh, 2012), juveniles are mostly reared in highly saline (close to oceanic) seawater. Such practice is at odds with current evidence, and thus should be modified to maximize survivorship of hatchery-produced juveniles.

As mentioned previously, juvenile *L. argentimaculatus* are usually associated with rocks or snags in the coastal lagoons of Thua Thien Hue and Binh Dinh provinces. The effects of these types of habitat structure on fish growth and survivorship were examined in a manipulative experiment. The results of this experiment (Chapter 3) showed that both rocks and snags improved juvenile growth over bare substrate or artificial seaweed. Perhaps surprisingly, no effect of substrate was observed in terms of survivorship, suggesting that cannibalism is not an issue for culture at the moderate stocking density used for the experiment. Although 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, this may be a good cue for the aquaculture industry. Simply providing hard substrate for the juveniles increased their grow-out performance. Rather than growing juveniles in bare tanks, significant gains in grow-out efficiency may be made by incorporating shelter structures into grow-out tanks.

From the experimentation undertaken in Chapter 3, and the information garnered in the field survey of Chapter 2, it is possible to make recommendations to the mariculture industry to improve performance of early juvenile culture. By growing juveniles in moderate salinity water (15-20ppt), in the presence of hard, complex structures such as rocks or snags, mariculturists can improve juvenile survivorship and growth without changing diet or stocking rates.

Apart from examining abiological aspects of habitat such as structure or salinity, the investigation of biological factors such as the composition of natural food is also important to identify critical habitats of fish. Barry et al. (1996) stated that in a natural water environment, food availability may be a principal factor influencing on the nursery function of shallow inshore habitats. This suggestion is also supported by Rozas and Odum (1988); that availability of food is one of main factors that are invoked to explain the high density of fish in a certain habitat. The diversity of natural food organisms in brackish waters is not unpopular because there might be mixture between fresh water and seawater prey. Therefore, besides the positive effects of moderate salinity on fish survival (Chapter 3), selecting brackish water areas may be an indication that Mangrove Red Snapper juveniles might be following the preferences of important natural prey species. My findings (Chapter 4) showed that food selection of fish is at least partially related to availability and abundance of food prey associated with the habitats. Specifically, the remains observed in stomachs of juvenile *L. argentimaculatus* largely reflected the communities of potential prey species identified from water column samples. It is noteworthy, however, that the proportions of those prey species was not necessarily reflected in the gut contents.

I found ontogenetic changes in diet of juveniles *L. argentimaculatus*. Specifically, although juvenile fish of all size classes fed generally on shrimp prey, the smallest size class (<3cm total length) consumed predominantly mysid shrimps in addition to copepods and luciferid shrimps, while larger juveniles (3-5cm) fed mostly on sergestids (*Acetes spp.)* and grapsid crabs. These prey were also the preferred food of the largest juveniles (5-10cm), although the larger fishes consumed small fishes in addition. Mysid shrimps, in particular, inhabit a relatively narrow salinity range in estuaries, and provide an energy-dense meal for the smallest juveniles. It is probably no coincidence that the smallest juveniles occupy habitats where mysid shrimps are most abundant.

Although it is clear that food availability in water environment affects feeding habit of Mangrove Red Snapper, my findings also indicate selective feeding of juveniles of this species throughout ontogeny. This seems to be related to habitat use of fish and prey size. Indeed, I found that despite occupying the same main habitats, fish of different size classes used different microhabitats; the smallest fish usually travelled around surface water, and preferred to catch small prey moving close to the water surface or suspended high in the water column (like mysids or calanoid copepods). Larger fish classes, which preferred to move around the lower water layers, gradually changed to eat bigger prey living demersally or in the bottom water layers, such as grapsid crabs or small therapontid fishes. Although it is not examined in the current study, other authors have reported that anatomical and morphological variation may be also important aspects associated ontogenetic changes in diet of reef fishes (Schmitt & Holbrook, 1984; Lukoschek & Mccormick, 2001). Therefore, further studies are encouraged to find information on these aspects.

On the other hand, repeated field sampling of juvenile Mangrove Red Snapper also reveal seasonal variation in diet. As mentioned above, there is only one major recruitment season of juveniles Mangrove Red Snapper in central Vietnam, but the fishermen are able to harvest juveniles over much of the boreal winter period. The fish caught at other times of the year recruited as 3cm fry in October; thus only juveniles in the 5-10cm size class were collected in period of February-April. My findings showed that these juveniles uniquely fed on a single unidentified species of *Acetes* in this period, while they consumed a wide food spectrum in period of September-November, including shrimps of different families, crabs, fish and zooplankton. Interestingly, the species of *Acetes* consumed in the February-April sampling period was not the same species that comprised the bulk of their diet in the earlier period (*Acetes indicus*). The *Acetes* observed in February-April was associated with the thalli of seaweeds growing on top of the rocky berms inhabited by the juvenile fishes, and was present during the earlier sampling episode, but not consumed. Likewise, *Acetes indicus* was observed in the water column in February, but was not a component of the gut contents of captured juveniles. The reasons for the prey shift in this case was not obvious, but may reflect behavioral changes associated with winter temperatures on the part of either predator or prey, and may provide more insight into the nature of the critical juvenile habitats.

The seasonal feeding changes were also recorded for *Lutjanus jocu, Sciades herzbergii,* and *Mustelus schmitti* (Tommaso & Ulrich, 2008; Monteiro et al., 2009; Juan & Andrea, 2011). Some researchers believed that distribution, abundance and availability of prey in each season (Monteiro et al., 2009; Rooker, 1995), or changes in life history patterns of food organisms or feeding activities of the fishes themselves

(Snyder, 1984; Lucena et al., 2000) could be causes of such change. In the present study, it is clear that natural food resource in both seasons was diversified but Mangrove Red Snapper had selective feeding strategy for each season. It is believed that reducing water temperature in the period of February to April is one of important factors affecting feeding of fish. The juveniles appeared to have adjusted their foraging strategy to minimize energy expenditure in the cold conditions.

I also found that feeding activities of juvenile Mangrove Red Snapper is governed by tidal rhythm. Feeding activity of juveniles *L. argentimaculatus* is strongly biased, and occurs prior to high tide. Juveniles captured on the falling tide predominantly had empty stomachs. Similar findings of inflowing tide feeding behaviour are recorded in some studies (e.g. Kuipers, 1975; Summers, 1980; Zagars et al., 2012). It is believed that tidal cycle might govern prey behavior which affects feeding activity of fish (Robin and Marcha, 1986), or that high tide makes foraging habitats more accessible (Tommaso & Ulrich, 2008) that might help fish easily find their prey. Although some researchers observed day-night cycle-linked feeding strategies of fish (e.g Ebeling & Bray, 1976; Collette & Talbot, 1972; Ebeling & Bray, 1976), my findings showed that feeding activity of fish is governed by tidal cycle rather than time of day. This is supported by research of Zagars et al. (2012) on the same species. Therefore, it would be useful to do further experimental studies to examine this issue as well as find out the real reasons of such feeding habit. If tidal rhythm strongly affect food intake of fish, it would be beneficial to improve aquaculture production by exploiting the urge to feed on tidal inflow and controlling tidal feeding of cultured fish.

Although fish exhibit ontogenetic or seasonal feeding changes, it is interesting that shrimps are always the important prey items in the diet. Shrimp prey are also known as preferred food of other lutjanids, although their role for these fishes is less well known. The results from my experiment (Chapter 4) showed that provision of preferred prey such as *Acetes* and mysids to captive fishes strongly improved both growth and survival of juvenile fish. It is also interesting that crude protein and lipid content in *Acetes* and mysids are less than that of other experimental food, but juveniles fed live shrimps as food exhibited higher growth and survival compared to fish consuming other diets. It is clear that *Acetes* and mysids have certain advantages, such as no degradation of nutrition when feeding fish, compared to other experimental food. However, it is also known that these groups of shrimps often possess high polar lipid content that may improve growth of fish during early life history stages (Domingues et al., 2003). Moreover, such live food may also contribute digestive enzymes, assisting in the digestive process (Dabrowski & Glogowski, 1977) that can improve feeding efficiency, leading to higher growth and/or survival. However, my data of diet nutritional analysis are not detailed enough to clarify these aspects, so the follow-up studies should address this issue.

It is clear that habitat structure and natural food resource associated with habitat play an important role for growth and/or survival of juvenile Mangrove Red Snapper. However, my findings shows that salinity seems to be major factor controlling habitat selection of juvenile of this fish species. Further studies are encouraged to examine whether there is any interaction between these.

In general, there are a number of studies examining ecology of lutjanids but very few studies have been done on Mangrove Red Snapper. In this study, although I found important information on habitat and feeding ecology of juvenile Mangrove Red Snapper in central Vietnam, there are still gaps remaining that need to be studied. For example, discovering if habitat structure selection of fish, as mentioned in chapter 3, is from innate shelter-seeking behavior; or examining micro-nutrients of food used in chapter 4 to clarify improved growth and survivorship of fish; or investigating role of less dominant natural prey found in stomachs for fish in comparison with important prey. Moreover, until now aquaculture of Mangrove Red Snapper in Vietnam has mostly depended on wild fingerlings, while this fish resource is perilously close to the type of local extinction seen in southern Thailand. Therefore, besides continuing research on wild population, applied studies to enhance the viability of artificial spawning and culture of this species would be greatly beneficial to reduce harvest pressure on stocks of wild juveniles.

Based on results of current study, I would like to give suggestions as follows:

1) Coastal brackish water habitats with solid structure as rocks or snags are important for the recruitment of fish, therefore, to conserve wild population of this species (and provide natural recruitment loci), then it is also needed to address preservation of these habitats.

2) A simple way to enhance production is to culture juveniles in moderate salinity water (15-20ppt), in the presence of hard, complex structures such as rocks or snags.

3) Shrimp prey as *Acetes* and mysids take an important role in promoting growth and survival of fish, they, therefore, should be used to feed juvenile Mangrove Red Snapper in aquaculture.

4) Feeding activity of fish strongly occurs during high tide, therefore, it would be beneficial to improve production by controlled tidal feeding of fish.

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