CHAPTER 1

INTRODUCTION

1.1 BACKGROUND AND RATIONALE

The Gulf of Thailand (the east coast of the Isthmus of Kra) faces the South China Sea; many organisms around this coast are representative of the western Pacific Ocean/South China Sea biota. The Andaman Sea (the west coast of the Isthmus of Kra) forms the eastern-most margin of the Indian Ocean, partly separated from the Bay of Bengal by the arc of the Andaman-Nicobar Islands; west coast organisms are representative of this Ocean. The biota of these two coasts was mostly separated in historical glacial periods, when the sea level was lower than the present day (Voris, 2000; Bird et al., 2005). Superficially, however, the biota of the two coasts are not completely distinct, because of the existence of a connection via a persistent northwesterly Holocene (and warm inter-glacial) water flow through the Straits of Malacca (Kimura et al., 2009; Satapoomin, 2011). However, the water current from Malacca Straits does not flow throughout the entire Andaman Sea (Wyrtki, 1961 (in Rizal et al., 2010)); a countervailing current from the north opposes the Malacca Strait current, creating what may be a semi-permeable barrier to dispersal around the central Andaman coast of Thailand. Water currents from western Pacific/South China Sea potentially only influence organisms in the southern part of the Andaman Sea, whereas organisms in northern part may be more influenced by water currents from the northern Indian Ocean via the northern Bay of Bengal. The confluence of two opposing currents around middle part of Andaman Sea may serve as a barrier to dispersal between northern and southern stocks; some larvae of northern may not migrate to the southern part of Andaman Sea or Gulf of Thailand. Satapoomin (2007) reported sub-regional variation in reef fish populations of the central and northern Thai Andaman region, hinting at the biogeographic complexity of the system.

The target species of this study, Moses perch, *Lutjanus russelli* (Bleeker, 1849) is a common fish in Thai coastal waters and is an important fish for local food security. Since the artisanal fishers who most rely on lutjanids in their catch tend not to range far from home, depletion of local stocks represents a serious threat to the sustainability of local artisanal fisheries and consequently, to the food security of local people. Moreover, several authors (e.g. Allen, 1985; Satapoomin, 2011) have reported consistently different color morphs in Indian Ocean and Pacific Ocean populations of *L. russelli*, hinting that the species may be partitioned into discrete population and/or species.

In this study, I aimed to establish the existence of geographic variants within Lutjanus russelli by examining both external morphology and population genetic variability within the larger Thai Andaman Sea region and also between the Gulf of Thailand and the Andaman Sea. Potential differences between these regions may imply the existence of dispersal barriers between the two coasts of Thailand (despite the presence of a putative corridor through the Malacca Strait) and unsuspected geographical barriers within the larger Andaman Sea region. Knowledge about population genetics and replenishment of marine organisms is important for the conservation and management of marine resources (Ovenden and Street, 2003; Robertson, 2000; Zhang et al., 2004). Knowledge about the population structure of L. russelli may enhance understanding of the degree of larval migration between biogeographic provinces. In seeking to establish whether the populations in each biogeographic area represent distinct genetic stocks or components of a larger metapopulation, it is worth noting that there are further consequences of stock isolation due to potential dispersal barriers. The existence of hitherto unsuspected stock partitioning may have important consequences for stock management of a vital food fish. Moreover, despite the prolonged isolation between the east and west coasts, even a relatively small amount of gene flow between populations may prevent them from evolving into different species, although, it may reveal an incipient taxonomic instability

within this species and creates questions about the distribution and taxonomy of Lutjanidae.

1.2 REVIEW OF LITERATURE

1.2.1 Moses perch *Lutjanus russelli* (Bleeker, 1849)

1) General information concerning Moses perch (Perciformes: Lutjanidae: Lutjaninae)

Lutjanids are small to medium-sized perciform fishes with a robust, relatively elongate body, a single dorsal fin (usually with 10 or 12 spines and 10 to 19 soft rays), two nostrils on each side of snout, and well-developed canine teeth in jaws; they are principally marine in habit (Figure 1). The Lutjanidae includes 17 genera and around 103 species collectively known as snappers. Four lutjanid subfamilies include the Lutjaninae, which contains the type genus *Lutjanus* and refers to fishes with are slender to deep-bodies and a truncate to deeply forked caudal fin, scaly sheaths at the bases of the dorsal and anal fins, teeth present on the vomer, and without filamentous soft dorsal or anal fin rays (Allen, 1985). *Lutjanus russelli* (Moses perch, Fingermark Bream, Russell's Sea-perch, Russell's Snapper) is a relatively typical example of the type genus of this family. The specific characters of this fish are outlined in Figure 2.



by W.D. Anderson, Jr. and G.R. Allen

Diagnostic characters: Typical perch-like fishes, oblong in shape, moderately compressed (size to 160 cm). Eye usually moderate. Premaxillae usually moderately protrusible (fixed in *Aphareus* and *Randallichthys*). Two nostrils on each side of snout. Mouth terminal and fairly large. Maxilla slipping for most or all of its length under lacrimal when mouth closed. Supramaxilla absent. Jaws usually with more or less distinct canines (canines absent in *Aphareus, Parapristipomoides,* and *Pinjalo*). Vomer and palatines usually with teeth. Pterygoids usually toothless. Cheek and operculum scaly; maxilla with or without scales; snout, lacrimal, and lower jaw naked. Opercular spines 2. Branchiostegal rays 7. Dorsal fin single, spinous portion sometimes deeply incised posteriorly where it joins soft portion. Caudal fin truncate to deeply forked. Dorsal fin with X to XII spines and 10 to 19 soft rays. Anal fin with II spines and 7 to 11 soft rays. Principal caudal-fin rays 17 (9 in upper lobe, 8 in lower lobe of fin). Pectoral-fin rays 14 to 19. Pelvic fins with I spine and 5 soft rays. Scales moderate to rather small, ctenoid. Lateral line complete. Pelvic axillary process usually well developed. Vertebrae 24 (10+14). <u>Colour</u>: highly variable; mainly from red through yellow to blue; often with blotches, lines, or other patterns.



Figure 1 Diagnostic characters of Lutjanidae (Carpenter and Niem, 2001).



Figure 2 Identification character of *Lutjanus russelli* (Carpenter and Niem, 2001).



Figure 2 Identification character of *Lutjanus russelli* (Carpenter and Niem, 2001) (continued).

Generally, Moses Perch are seldom an important commercial component of the catch anywhere within their distribution range from Tahiti to East Africa, and from Australia to southern Japan (Allen, 1985), although the flesh is highly regarded. *L. russelli* is found either in small groups or as solitary individuals; fishermen rarely catch large numbers at any particular site (Allen, 1985; Newman, 2002). Quite often, however, they were commonly found for sale in local fish markets – especially in the inner Gulf of Thailand (personal observation); likewise, they are commonly grown in floating fish cage culture and contribute strongly to food security for local artisanal fishers in Thailand. Moreover, *L. russelli* is an important component in reef ecosystems and for recreational fishing in coral reefs (Newman, 2002); in this it is similar to other species of fishes in this genus, such as *L. decussatus* and *L. kasmira*.

Adult Moses Perch inhabit inshore and offshore coral reefs and other rocky areas. They only occasionally enter estuaries for feeding. Juvenile Moses Perch, on the other hand, are often seen in the lower reaches of freshwater streams and mangrove estuaries, and appear to utilize sea grass meadows as nursery habitat (Allen, 1985; Sheaves, 1995). These fishes are generally found at depths anywhere from 3 m to 80 m (although Newman, 2002, reported trawl catches down to 200m in Western Australia and Kuiter and Tonozuka (2001) indicated that adults prefer depths around 50m), and may grow to around 45cm in length (Allen, 1985) and reach more than 20 years of age (Newman, 2002). On the average, lutjanids reach first maturity at about 43 to 51% of the maximum total length, with males maturing at a slightly smaller size than females. It is therefore likely that Moses Perch juveniles recruit into the fishery around the time of sexual maturity, which has been reported to occur at around 4-5 years old (Newman, 2002). Adults and juveniles feed primarily on benthic invertebrates and fishes. *Lutjanus russelli* have a planktonic larval stage, but the duration and vagility of this phase is not well known; recruitment appears to be exclusively coastal. Seasonality and duration of reproductive activity have not been reported for *L. russelli* outside of Australia; it is likely that Thai *L. russelli* behave similarly — that is, with an annual spawning period spaced over 2 or 3 months prior to the onset of the monsoon

The reproductive pattern of *L. russelli* is that of gonochorism: following sexual differentiation, the sex remains constant throughout the life cycle. *L. russelli* exhibits no sexual dimorphism — i.e. variations of color pattern between sexes — but variation between geographical regions has been reported by several authors. Lutjanids are dioecious (separate sexes) and generally display little or no sexual dimorphism in structure or color pattern. Geographic variation between color morphs found in the Indian Ocean and Pacific Ocean has been reported. These color pattern variants are apparently not correlated to sex, but depend on age (maturity) and location.

The Pacific morph of Moses Perch has a grey body, often with a reddish tinge. There is a diffuse black spot (sometimes faint) mostly above the lateral line below the soft part of the dorsal fin. Juvenile Moses Perch look similar to adults, but have four to five brown stripes on the body, and an ocellus (distinct black spot) on the back. The Indian Ocean color morph has or 8 narrow golden-brown stripes on sides. The chromatic patterning of the juvenile of the Indian Ocean morph has not been reported. Allen (1985) described a complicated pattern from Pacific Ocean samples: adults have no lateral stripes, whereas juveniles from the Southwest Pacific have 4 black stripes with a back spot on their side, and juveniles from the Northwest Pacific present only stripes and have no black spot. Adults from the Indian Ocean display an obvious pattern of yellow color stripes (Carpenter and Niem, 2001) (Figure3). Ages of fish or length of their body at stripes disappear and turn to be an adult with only spot were not reported by any authors, although it may coincide with recruitment of the juveniles into the adult population at around yearclass 5. Unsurprisingly, the consistent identification of *L. russelli* in different regions has been problematic. FAO criteria (Allen, 1985; Carpenter and Niem, 2001) cannot partition this species into different group.



Figure 3 Color variation of *Lutjanus russelli* between Pacific Ocean and Indian Ocean; left is adult and juvenile from Pacific region and right is adult from Indian Ocean region (Allen, 1985)

2) Taxonomic problem of this species.

A few publications show some level of misunderstanding of classification within snapper because of morphological similarity of several species within the genus (Sarver *et al.*, 1996). There are at least 25 species of *Lutjanus* reported form Andaman Sea (Satapoomin, 2011) and an additional species from Gulf of Thailand, *Lutjanus carponotatus* (personal observation). Following the FAO identification guide above (Allen, 1985; Carpenter and Niem, 2001), it can be seen that *Lutjanus russelli* can be discriminated from sixteen of these species by their body shape alones. Another ten species exhibit quite different color patterns when compared with the target species; for instance *L. decussatus* exhibits dark brown bars and stripes surrounding rectangular, whitish "windows"; lower half of sides with 2 dark brown stripes, and *L. kasmira* has only yellow stripes on its body, without a black spot.

Seven species found in Thai waters, however, show similar shape and color pattern to *L. russelli*. It is quite possible to distinguish them based on the published FAO criteria, however, since while two species exhibit a black spot near the caudal peduncle similar to the Pacific Ocean color morph of *L. russelli*, viz: *L. johnii*, *L. monostigma* (Figure 4; upper row), it is evident that *L. johnii* has a series of horizontal lines on the sides of its body, and the centre of each scale often has a reddish-brown spot. This is different from *L. russelli*, which possesses obliquely positioned longitudinal scale rows above the lateral line. Likewise, *L. monostigma* possesses thirteen (rarely fourteen) soft dorsal fin rays, a crescentic vomerine tooth patch, without a medial posterior extension and all fins are always yellowish whereas *L. russelli* usually has fourteen of soft dorsal-fin rays, triangular or diamond- shaped vomerine tooth patch with a medial posterior extension, and normally a dorsal and a caudal fin are red, it is not yellowish.

Another three species are superficially similar with the Indian Ocean color morph of *L. russelli*, which possesses both black spot and stripes. These species are *L. ehrenbergii*, *L. fulviflamma*, and *L. quinquelineatus* (Figure 4; lower row).

L. ehrenbergii has scale rows on its back parallel to the lateral line and it often possesses a series of 4 or 5 narrow yellow stripes on its sides below the lateral line, whereas *L. russelli* possesses a quite different pattern of scale rows and has 7 or 8 narrow golden – brown stripes. It is also relatively simple to distinguish from *L. quinquelineatus*, which has a series of 5 bright blue stripes on its sides. *L. fulviflamma* is more apparently similar to the Indian Ocean color morph of *L. russelli*, however there are fin color differences between the species. All fins of *L. fulviflamma* are yellowish, the lower sides of its body are whitish or light brown, and belly is whitish to yellow, whereas only the pectoral, pelvic and anal fins of *L. russelli* are yellow; the lower sides of *L. russelli's* body and belly are pink to whitish with a silvery sheen. Moreover, the number of the gill rakers, and pectoral fins rays are also different between the two species.

FAO identified species of *Lutjanus* based on meristic morphometric and some standard measurements which are readily taken by an observer in field surveys. However, *L. monostigma* has occasionally been misidentified as *L. russelli* because of similarity within the "black spot: group, and also *L. fulviflamma* which is consistent with *L. russelli* from Indian Ocean, although this misunderstanding is rare these days. Moreover, misidentification can also occur within species, especially in the target species for this study, *L. russelli*, which shows strong chromatic variation between populations in the Pacific Ocean and India Ocean. Uncertain identification within Lutjaninae may contribute to both misidentification of species encountered in ecological or fisheries surveys, and also to issues of unsupported synonymy within the subfamily.

Many phylogenetic studies of Indo-Pacific snapper have been taken only from South China Sea samples (Guo *et al.*, 2007a; Miller and Cribb, 2007; Wang *et al.*, 2010) and similar snapper fish can be broadly categorized into three color pattern groups: the black spot, yellow line and blue line groups (Miller and Cribb 2007). An insufficiency of broad-scale sampling from various regions has been a persistent weakness of phylogenetic investigation of this genus, especially for species such as *L. russelli* that exhibits some degree of geographic variation. Samples from South China Sea only partly explain the phylogeny of Pacific Ocean color morph and reported close relatives within the "black spot" species group (Miller and Cribb 2007). The studies preceding the present study might not categorize the Indian Ocean color morph of *L. russelli* into the same group because Indian Ocean fish possesses different color morph with Pacific; it may group to the "yellow-lined: subgroup instead. The existence of two separate color morphs of *L. russelli* begs the question of whether purely morphological criteria should be applied to this species. Regional variation in colour morph may disguise or obscure population partitioning, cryptic species or monophyly for this species



Figure 4 Two color pattern of snapper that are often misidentified because of similar morphs; upper row is the "black spot" group and the lower row is the "both spot and stripe" group.

Several researchers have attempted to resolve the taxonomic difficulties of the genus using molecular phylogenies and DNA barcoding to confirm classification and elucidate phylogenetic relationships within lutjanid genera. Miller and Cribb (2007) categorized snapper into three groups (blue-lined, black spot and yellow-lined) and studied phylogenetic relationships between samples from the South China Sea. These workers observed that while the general morphology and external colouration of many Indo-Pacific species of lutjanids allows the recognition of several species groups, such as the "black spot" complex containing *L. ehrenbergii*, *L. fulviflamma*, *L. fuscescens*, *L. johnii*, *L. monostigma* and *L. russelli* (Miller and Cribb, 2007). They regarded the employment of molecular techniques to the classification problem as indispensible in resolving such difficult groups,

3) Population genetics in Lutjanus

Population genetic structure of the genus *Lutjanus* has been examined in many species and regions (Table 1). Mostly, the results of such studies showed no genetic difference between populations. This can imply that the life histories of these – generally long-lived – fishes are such that they are highly connected (whether through migration or juvenile dispersal). Even in studies examining large geographic regions, there was a tendency for snappers to exhibit genetic homogeneity of population. For example, one such study of *Lutjanus erythropterus* from the East China Sea reach to western Pacific Ocean around northern Malaysia (Zhang *et al.*, 2004) or studied of *L. malabaricus* and *L. erythropterus* along the central and eastern Indonesia and northern Australia around 2400 km (Salini *et al.*, 2006), etc.(Table 1).

Reference	Таха	Geographic locale	Genetic marker	Summary of study
Ovenden and Street,	Lutjanus argentimaculatus	Queensland, Australia	Microsatellite and	No genetic differentiation
2003			mtDNA	
Zhang <i>et al</i> ., 2004	Lutjanus erythropterus	East Asia	mtDNA	No genetic difference
Pruett <i>et al.</i> , 2005	Lutjanus campechanus	northern Gulf of	mtDNA	No genetic differentiation
		Mexico	sequences	
Salini <i>et al</i> ., 2006	Lutjanus erythropterus and	Central and eastern	Allozyme and	No genetic differentiation
	Lutjanus malabaricus	Indonesia and northern	mtDNA	
		Australia		

Table 1 Summary of studies on population structure of lutjanid fishes

1.2.2 The geography of Thailand: The area of interest

The coastal regions of Thailand are some of the most unique biogeographic areas in that that they are located in two oceans (Figure 5). The eastern coast of peninsular Thailand, the Gulf of Thailand, faces the western Pacific Ocean/South China Sea. The Gulf of Thailand is generally shallow, with high turbidity because of topography and coastal development (Satapoomin, 2002) and weak surface currents. During the low sea level stands of the Pleistocene glaciations, the entire Gulf region was episodically immersed and forested, with the bed of the paleo-Chaopraya river providing an early colonization path upon the next cycle of sea level rise. The present Gulf dates only to the late Holocene (around 7000 years before present). The western coast of peninsular Thailand represents the eastern-most portion of the Indian Ocean; the Andaman Sea (up to 3000m deep) abuts a narrow continental shelf and is a much more oceanic environment than the eastern side of the peninsula. Even extreme eustatic sea level changes associated with glaciations did not affect the proximity of the coast in the way it did in the east.



Figure 5 The coastal waters of Thailand face the western Pacific Ocean (South China Sea) on the eastern side (Gulf of Thailand) and Andaman Sea on the western side.

In the last glacial period, the two coasts of Thailand were completely separated for long periods (Bird *et al.*, 2005; Voris, 2000). The depth contours map of Voris (2000) at 120 m. below present-day sea level around 17,000 yr bp showed exposure of contiguous Sunda shelf landmass over the south and east of the Isthmus of Kra. Dry land connected Thailand and area of the Sunda Shelf (including Sumatra, Java and Borneo and even the Philippines) in to a large barrier which was virtually exhibit connectivity of marine organisms between Pacific Ocean and Indian Ocean. After sea level was rising to 40 m. below present-day many coastal islands in Gulf of Thailand was still connect to the mainland, whereas offshore island in the Andaman Sea such as Adung-Rawi and Surin Island split. Satapoomin (2002) argued that different durations exposed to the sea of the two coasts of Thailand may be one of the reasons of dissimilarity reef fish composition of these two coasts.



Figure 6 Surface current in Malacca Straits, Andaman Sea and Indian Ocean in February (1a) and August (1b) (Wyrtki, 1961(cited in Rizal *et al.*, 2010)). Modeled details of surface current at the layer 30-50 m around Malacca Straights in February (2a) and August (2b) (Rizal *et al.*, 2010).

Potentially, however, two coasts of Thailand have been linked via Straits of Malacca since 7000 yr bp (Sathiamurthy and Voris, 2006). Sources of fish recruitment in the Andaman Sea can potentially come from both of South China Sea and northern Bay of Bengal (Satapoomin, 2007). Importantly, the South China Sea waters mass more influence southern Andaman Sea population than northern because the current from Malacca Straights does not flow throughout the entire coast of the Andaman Sea both the northeast monsoon and southwest monsoon (Wyrtk, 1961(cited in Rizal *et al.*, 2010)) (Figure 6). The water mass from Malacca Strait flows northwards to meet with the northern water mass from Bay of Bengal and move offshore to Indian Ocean around middle part of Andaman Sea. This counter current may allow water mass form SCS to mix only with southern Andaman Sea. However the ability of this water current to serve as a barrier of marine organism to maintain population within the large Andaman Sea region or Thai water was still ambiguous.



Figure 7 Relative dissimilarity between fish composition between offshore and inshore and present of discriminated assemblages of fish between the southern part (blue line) and the northern part (red line) in each habitat within the Andaman Sea (Satapoomin, 2007).

Satapoomin (2007) reported dissociation amongst habitats in terms of fish community composition in the Andaman Sea (Figure 7). This worker reported not only differentiation between inshore and offshore location, but also dissimilarity between the southern part and the northern part of the Andaman Sea. The fish communities of offshore localities in the southern part of the Andaman Sea (Figure 7, blue line) and from the northern part (red line) have high relative dissimilarity. Additionally, there appear to trends of dissimilarity of fish in inshore locality between the southern and northern part of the Andaman Sea. It may be that community structures are different between inshore and offshore because many factors are different such as water quality, structure of coral reef and degradation of coral reefs, whereas the cause of differences between the southern and the northern part of Andaman Sea is not well known. Therefore, it is increasingly interesting to investigate within-regional variation amongst the reef fishes of Thailand. Many kinds of research, not only taxonomy but also population studies and questions regarding the replenishment of fish stocks are required.

1.3 OBJECTIVE

This thesis is an attempt to establish the existence of geographic variants within *Lutjanus russelli* (Bleeker, 1849) by examining both external morphology and population genetic between the Gulf of Thailand and the Andaman Sea and also within the larger Andaman Sea region. The information obtained from this present study will have important consequences for stock management of a vital food fish and it may reveal an early taxonomic instability of this species and creates questions about the distribution and taxonomy of Lutjanidae.

1.4 STRUCTURE OF THE THESIS

This thesis is organized into chapters that follow a logical progression to address the project goals. .

In Chapter 2, I examine morphological characters to confirm the ability of FAO identification characters to discriminate this species from morphologically similar congenerics. I also test the power of differentiation of another general character measurement of fish (that is not reported in FAO literature) between the Gulf of Thailand and the Andaman Sea. An analysis of the morphometric characteristics of populations of the two coasts of Thailand is provided. Chapter 3, I use microsatellites markers to explore differences in population genetics of *L. russelli* between the Gulf of Thailand and the Andaman Sea and also between northern and southern Andaman Sea population. The set of population genetic parameters including Analysis of molecular variance analysis (AMOVA), pair-wise F_{ST} , pair-wise R_{ST} , structure of population and isolated by distance is provided.

Finally, Chapter 4 is a general discussion chapter that combines the results of the data chapters into a coherent exploration of the relative power of morphometric versus molecular techniques to discriminate geographic variants within a species, and discusses the validity of the current criteria used to describe this species. In this chapter, I also speculate about the potential existence of cryptic species within the Lutjanidae, and contemplate future research directions.