CHAPTER 2

EXTERNAL MORPHOLOGY OF *Lutjanus russelli* FROM THE ANDAMAN SEA AND GULF OF THAILAND

2.1 INTRODUCTION

The family Lutjanidae is composed of 17 genera and 103 nominal species. The biggest genus is *Lutjanus*, comprising 65 known species (Allen, 1985). Most of the fish in this genus are not strictly commercial species but are important staples of the artisanal fishery and they provide local food security throughout their distributional ranges. Information about fisheries, taxonomical, biological and ecological patterns is rare for most lutjanids, however, because of the nature of the artisanal fisheries they support; landings of snapper are mostly undocumented (Allen, 1985).

The taxonomy of the Lujanidae is far from settled: some species are debatable synonyms and many species are morphologically similar to each other, or chromatically polymorphic within species. Several species are frequently confused during identifications, especially in their larval stage (Allen, 1985; Chow *et al*., 1993; Zhang *et al*., 2004). Juveniles and adult fishes of many species, for instance, have a black spot sited posteriorly below the soft dorsal fin (examples include species such as *Lutjanus johnii* , *L. monostigma*, *L. fulviflamma*) and have superficially similar body dimensions, making these species close enough in appearance to be frequently conflated.

Moses Perch (*Lutjanus russelli* (Bleeker, 1849)) is the one of several lutjanid species that has bewildered classification. It is one of the “black spot” group that is morphologically confusing between species; its morphology also obscures identification within species because color patterns of this fishes from Pacific Ocean and Indian Ocean are truly different. Allen (1985) reported that only adult fishes from the Indian Ocean have 7 or 8 narrow golden-brown stripes on their sides, whereas fishes from Pacific Ocean have no stripes. This color pattern difference is generally assumed to be simply geographic variation (Satapoomin, 2011), with no real implications for phylogenetic classification of this species. This has obviously been a recurrent problem for Thai fish taxonomists, who always found specific color pattern differences between two coasts of Thailand (Satapoomin, 2011) which represent both Indian Ocean and Pacific Ocean bioregions. It raises all sorts of questions about researchers' ability to classify this fish by using the standard FAO definitions (Allen, 1985) that mostly apply to previously-identified fish samples. FAO identified species based on meristic morphometric and some standard measurements (Guo *et al*., 2007a) that do not discriminate between the two forms of this fish, and so there is no information about the actual geographic distribution of the color morphs. Further studies that place more emphasis on external morphology comparison may potentially explain this question. Importantly, it begs the questions: Are regional differences in colour patterns consistent, or are they the result of historical sample biases? If so, can we still group this lutjanid fish of different stripe pattern into the same species?

Therefore, in this study I investigated external morphology to identify the range and consistency of morphological variations of *Lutjanus russelli* between the Andaman Sea and the Gulf of Thailand. The purpose of the study was to confirm the ability of FAO identification characters (Carpenter and Niem, 2001), to discriminate this species from morphologically similar congenerics and to test the capacity for differentiation of other general character measurements of fish that are not utilized in the FAO literature. Moreover, the study provides opportunity to investigate new characters whose measurement might potentially provide insight into differences of morphology between two geographic forms of this fish. This study increases the corpus of knowledge about geographic variations in external morphology and makes some progress in resolving species classification status of this fish.

2.2 OBJECTIVES

Investigate potential differentiation of *Lutjanus russelli* morphology between the Andaman Sea and the Gulf of Thailand by

1) Testing the ability of FAO identification characters to discriminate fish between Indian Ocean (Andaman Sea) form and Western Pacific Ocean/South China Sea (Gulf of Thailand) form.

2) Exploring the potential of other general morphology measurements that have not been used for identify to this species and determine the usefulness of new measurable characters that have not been studied in this species before to discriminate geographical variants of this fish.

2.3 RESEARCH QUESTION

What (if any) are the differences in external morphology between *Lutjanus russelli* from the Andaman Sea and the Gulf of Thailand?

2.4 MATERIAL AND METHOD

2.4.1. Sampling locations and collection

Peninsular Thailand is that part of the Thai-Malay peninsula that divides the South China Sea from the Andaman Sea between latitudes 6-12° North to the north of peninsular Thailand, the Andaman Sea extends into the Gulf of Martaban (Myanmar) and from thence connects with the northern Bay of Bengal. South of Thailand, the Andaman Sea is connected to the South China Sea via the Straits of Malacca, adjacent to the northern tip of Sumatra. The east coast of peninsular Thailand forms the western margin of the South China Sea north of the Straits of Malacca.

The east coast sample population was collected from middle peninsular part of the Gulf of Thailand (GT), in Surat Thani and Nakhon Si Thammarat provinces (Figure 8). The west coast sample population was collected from both the northern part of the Thai Andaman Sea coast, in Ranong and Phangnga provinces (NA), and from the southern path of the Thai Andaman coast, in Satun province (SA) (Figure 8). North Andaman and Gulf of Thailand sampling localities are at the same latitude. Samples were collected by local artisanal fisherman and also bought from local fish markets (where these same artisanal fishers habitually sell their catch) at locations on both eastern and western coasts of Thailand. All size classes vulnerable to the capture techniques of local fishermen were represented in the samples from both coasts. The smallest individual captured was 8.7cm Standard Length (SL), and corresponds to the reported size of newly-settled juveniles (Sheaves, 1995). The largest individual in the sample set was 32.9 cm SL, which lies comfortably within the range reported for reproductively mature adults of this species (Sheaves, 1995). Local fishermen targeted both inshore (mangrove, seagrass and inshore coral reef) and offshore (coral reef) fishing grounds, and appeared to be able to capture fishes of all size classes. For this study, I obtained 40 usable (see below) samples from Gulf of Thailand (GT) and 53 samples from Andaman Sea (AN).

Fish obtained from all sources were categorized into “juvenile” and “adult” groups following the habitat assumption outlined in Sheaves (1995). Young fishes or reproductively immature fishes are overwhelmingly more frequently found in mangrove estuaries and lower reaches of freshwater streams, and seagrass than reproductively mature fishes (Allen, 1985; Newman, 2002; Sheaves, 1995), whereas fishes that have been collected from offshore coral reef habitats are exclusively adult (Allen, 1985; Sheaves, 1995). Although adult fishes do occasionally venture into mangroves or estuarine habitats, they can be separated from immature fishes based on body length. Inshore coral reef fish samples were not arbitrarily grouped to any life stage; potential candidate to sub-adult were categorized to juvenile if their standard length (SL) fell in the normal range of juveniles (maximum SL about 15 cm), and categorized to adult if SL exceeded the minimum size range of adults (i.e. SL greater than 20 cm, since reproductive maturity of *L. russelli* has been reported as occurring at around 40-50% of maximum size for this species —which is around 45 cm SL (Sheaves, 1995)), although the maximum standard length of any fish captured for this study was 32.9 cm. Any fish from any habitat that fell within the SL “gap” between juvenile and adult size classes were excluded from the sample and not used in this stud. Size selective sampling was thus undertaken in a way that avoided potential size effects from different life history developmental stages.

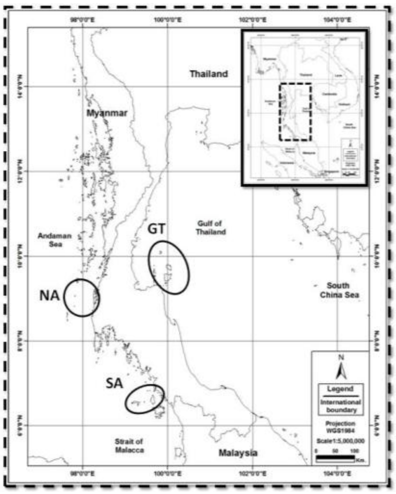

Figure 8 Sites where fish samples for this study were obtained.

2.4.2 Measurement of morphometric Characteristics

All character measurements of fish were obtained using a manual vernier caliper. Fishes were photographed after capture or immediately after being obtained from fishermen using a Canon S95 digital camera (under natural light, using automatic white balance, aperture and exposure, juxtaposed with a sheet of white underwater paper and a millimeter-scale rule for calibration); the appearance of any stripes or other color markings on their body was noted and counted at the time of photography, and confirmed later from the digital images.

General characters and new character measurement that were not suggested by Allen (1985) were explored for potential to discriminate differences in external morphology between the two populations. Eleven general morphology measurements reported from a FishBase and Fish Taxonomy training session in 2007 (Jordan, 2007) (Figure 9) were selected. New character measurements, including nineteen inter-landmark distances (ILD) from ten recognizable landmarks were adapted from Vasconcellos *et al.* (2008) (Figure 10). Measurements of all characters of each sample were standardized by the standard length of the fish, which is a traditional general method to reduce the effect of size and reduce autocorrelation within groups (Cadrin, 2000) in a non-weighted analysis. Descriptions of all characters and their abbreviation are outlined in Appendix A.

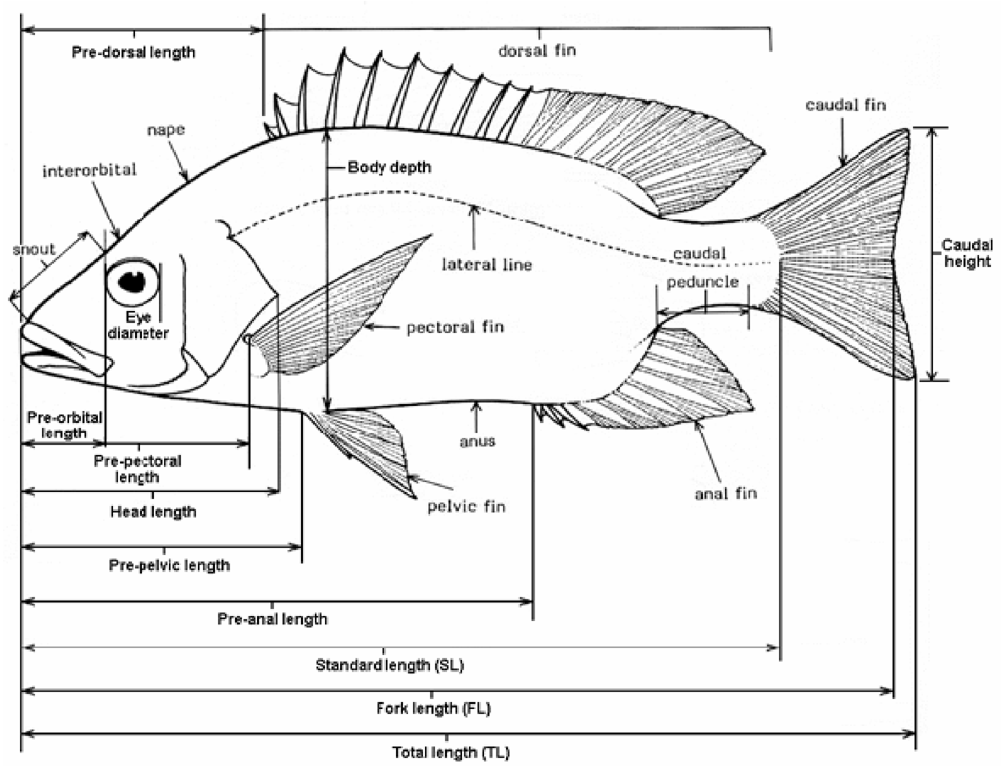

Figure 9 General morphology measurements in fish; all abbreviations was detail in Appendix A

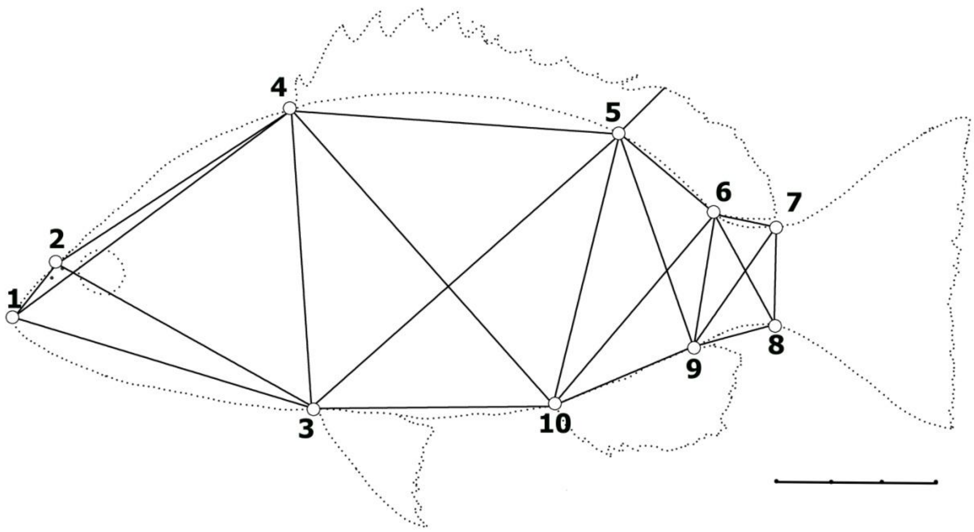

Figure 10 Outline drawing of *Lutjanus russelli*, 19 inter-distance from 10 landmarks; Note: all abbreviations was detail in Appendix A

2.4.3. Statistic analysis

Mean values of each character were compared in samples from Gulf of Thailand and Andaman Sea population by one-way analysis of variance (1-way ANOVA) for both adults and juveniles. Transformed data were used for some character data that didn't have normal distribution and/or homogeneity of variance in groups, both of which might have affected the validity of the ANOVA test. Log transformation was use to reduce effects from heteroscedasticity (Bro and Age, 2003; van den Berg *et al*., 2006). As a final option, if any characters could not be transformed to fit the assumptions of ANOVA, I used the non-parametric Kruskal-Wallis test to examine differentiation of means between populations. Characters useful for discriminant analyses should have nominal independence from other characters, so pairwise correlation between characters was tested by Spearman's rank correlation (Appendix B).

All general and new measurable characters that satisfied the selection criteria (i.e. were different between the two populations (data on descriptive statistics of all characters is tabulated in Appendix C) and independent from other characters (correlation statistics between characters is detailed in Appendix B) were selected as parameters of a principle component analysis (PCA). It was hoped that this analysis would reveal a metric for discriminating groups based on the underlying morphometric data. A scree plot (Appendix D) revealed that the first two principle components explained the majority of variation, and were used to create a biplot of the specimens mapped to this reduced dimensional space. All data analysis were analyzed by SPSS program version 11.5 (SPSS, 2002), PC-ORD version 5.10 (McCune and Mefford, 2006) and Past (Hammer *et al*., 2001).

2.5 RESULTS

It was apparent from the sampling undertaken during this study that the Andaman Sea and Gulf of Thailand populations *of L. russelli* are consistently different in color patterning (Figure 11). All of the 53 fishes observed (both juvenile and adult) from the Andaman Sea (AN) sample have golden-brown stripes on each side and a black spot located around the upper half of the lateral line below the posterior soft dorsal fins. Conversely, the 40 fishes obtained from the Gulf of Thailand (GT) that represent the western Pacific Ocean/South China Sea population have did not exhibit stripes along their sides, possessing only black spots similarly located around the upper half of the lateral line below posterior soft dorsal fins. Body colour and general overall appearance of the fishes from each side of the Thai peninsula was otherwise similar, although proportionality differed somewhat (described in the section below).

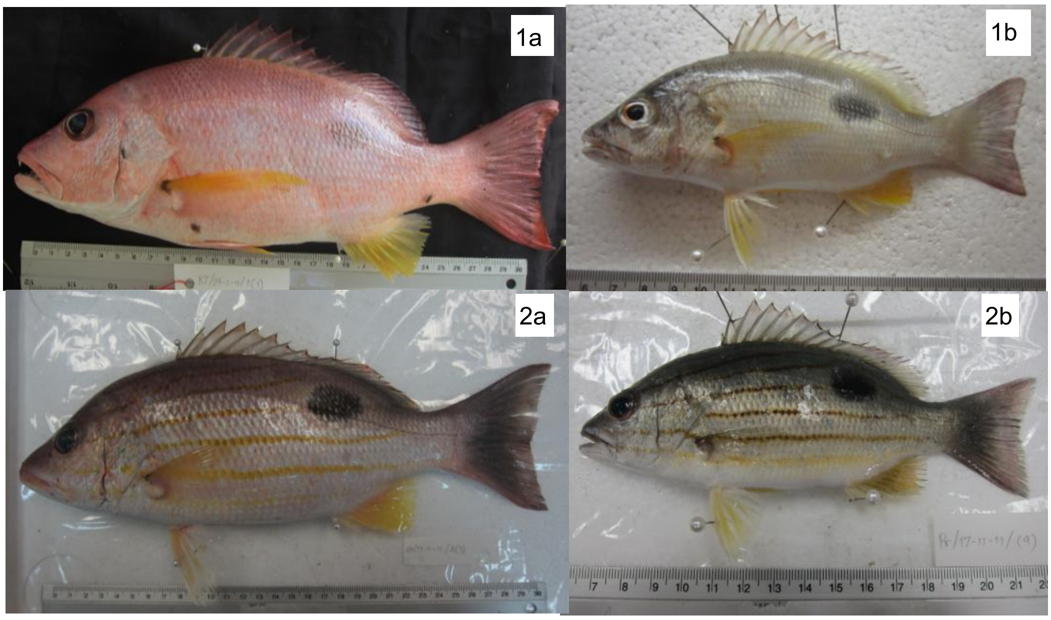

Figure 11 Configuration compare the present of stripes of both adult (a) and juvenile (b) fishes from Gulf of Thailand (1) and the Andaman Sea (2)

2.5.1 General characters measurement and new characters measurement

Of the 32 characters measured for this analysis (13 general characters and 19 new characters, as described above) four characters of the adult population were significantly different between the Andaman and Gulf sites, according to the results of ANOVA and the parallel ANOVA (Table 2). The two general characters that differed were pre-orbital length and length of pelvic fin (F= 14.243, df= 31, p= 0.001 and F=13.776, df= 31, p= 0.001 respectively). New characters that exhibited statistically significant differences were distance between the anterior tip of the snout on the upper jaw to posterior nostril (between landmarks1 and 2: Figure 10) and distance from the anterior tip of the snout on the upper jaw to origin of spinous dorsal fin (between landmarks1 and 4: Figure 10) (F= 5.801, df =31, p=0.022 and H = 5.398, p=0.020 respectively).

Table 2 Key characters differentiation of adult fish between Gulf of Thailand and Andaman Sea.

Measurable characters		AN		GT		F value	df	H-value (Kruskal-Wallis)	P-value
		Average raw data ±SD	Ratio by SL±SD	Average raw data ±SD	Ratio by SL±SD				
General characters	POL	2.6 ±0.3	0.1 ± 0.0	3.1 ±0.4	0.1±0.0	14.243	31	-	0.001**
	PVFL	4.8±0.4	0.2±0.0	5.2 ±0.9	0.2±0.0	13.776	31	-	0.001**
New characters	1-2	1.8±0.2	0.1±0.0	2.1±0.4	0.1±0.0	5.801	31	-	0.022*
	1-4	10.3±0.9	0.4±0.0	10.3±1.3	0.4±0.0	-	-	5.398	0.020*

Note: all abbreviations was detail in (Appendix A)

Note: * indicated statistical significance when p-value < 0.05

** indicated statistical significance when p-value < 0.01

Morphometric variation of the four characters between two adult populations was visualized via scatter plot of the first two principle component axis. These axes were highest eigen values, indicating that they explained the majority of the variation that could be explained by the model (Appendix D). PC1 shows more separation of two groups in the horizontal axis; PC2 separates specimens along the vertical axis (Figure 12). The plot showed an area of partial overlap; it was evident that some samples did not fall into particular discrete groups. It is apparent that the centroids of each group are quite distinct, however, with the Andaman population trending towards shorter, slightly less deep heads and noticeably shorter pelvic fins, amongst some other minor variations.

Examination of the component values of the Eigenvector revealed that pelvic fin length (PVFL), pre-orbital length (POL), and distance of anterior tip of the snout on the upper jaw to posterior nostril (inter-landmark 1-2) were consistent within the Gulf of Thailand (GT) and separated these specimens from Andaman Sea (AN) samples. These three characters explained high variation of data on PC1. Much of variation on PC2 was explained by distance between anterior tip of the snout on the upper jaw to origin of spinous dorsal fin (inter-landmark 1-4) (Figure 13).

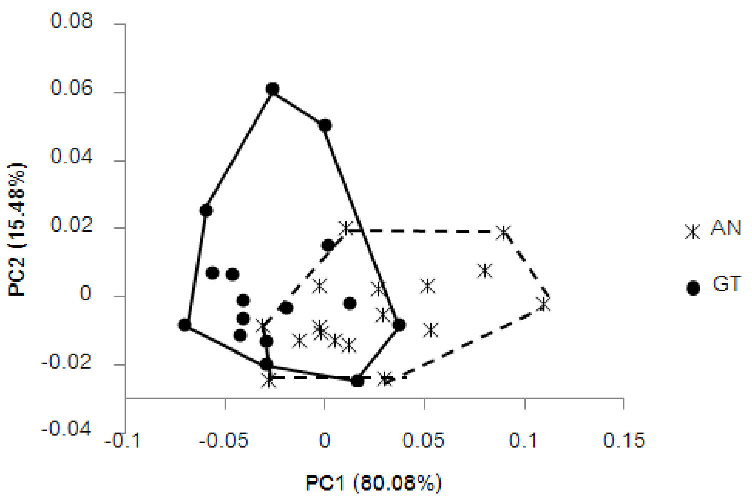

Figure 12 Scatter plot from the first two principle component and convex hulls by some general characteristic of adult fish between the Andaman Sea (AN) and the Gulf of Thailand samples.

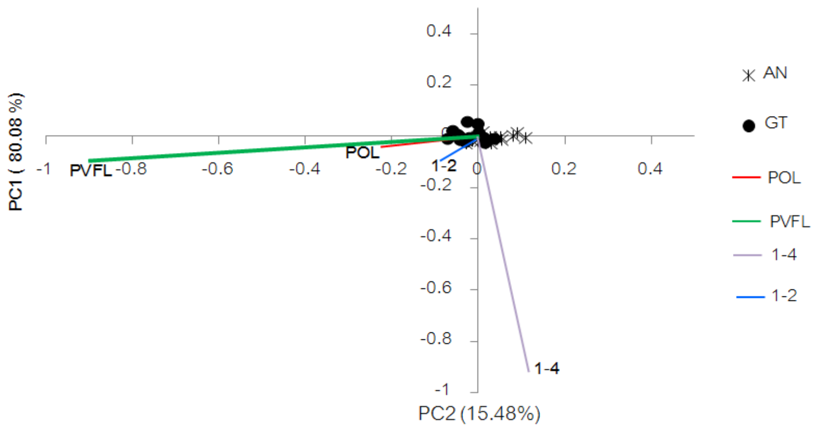

Figure 13 Eigenvector reveal effectiveness of each adult's character to distributions of data.

In the case of the juvenile populations, their characters were also significantly different between the Andaman and Gulf of Thailand population, although it was not the same characters that separated the adult groups. Fourteen characters were significantly different between the juvenile groups (Table 3). The four general characters that differed were total length, pre-pelvic length, pre-pectoral length, eye diameter (F= 4.598, df= 29, p= 0.041, F= 6.804, df= 29, p= 0.014, F= 11.840, df= 29, p= 0.002 and F= 4.622, df= 29, P= 0.004 respectively). Ten of new measurable characters were significant different, viz.: Origin of spinous dorsal fin to original of soft dorsal fin (between landmarks4 and 5) (F= 5.732, df= 29, p= 0.023), Origin of spinous dorsal fin to origin of anal fin (between landmarks 4 and 10) (F= 6.162, df= 29, p= 0.019), Origin of pelvic fin to original of soft dorsal fin (between landmarks 3 and 5) (Kruskal-Wallis = 9.129, p= 0.002), Original of soft dorsal fin to origin of anal fin (between landmarks 5 and 10) (F=10.758, df= 29, p= 0.003), Original of soft dorsal fin to insertion of anal fin (between landmarks 5 and 9) (Kruskal-Wallis = 19.69, p< 0.000), End of soft dorsal fin to insertion of anal fin (between landmarks 6 and 9) (Kruskal-Wallis = 9.002, p= 0.003), Insertion of 1st dorsal caudal fin ray to insertion of 1st ventral caudal fin ray (between landmarks 7 and 8) (F= 4.719, df= 29, p= 0.038), Insertion of anal fin to insertion of 1st ventral caudal fin ray (between landmarks 9 and 8) (Kruskal-Wallis = 8.016, p= 0.005), Posterior nostril to origin of pelvic fin to origin of pelvic fin (between landmarks 2 and 3)

(F= 8.035, df= 29, p= 0.008), End of soft dorsal fin to origin of anal fin(between landmarks 6 and 10) (F= 5.029, df= 29, p= 0.033).

Table 3 Key characters differentiation of juvenile fish between Gulf of Thailand (GT) and Andaman Sea (AN)

Measurable characters		AN		GT		F value	df	H-value (Kruskal-Wallis)	P-value
		Average raw data ±SD	Ratio by SL±SD	Average raw data ±SD	Ratio by SL±SD				
General characters	TL	15.8±1.4	1.2±0.0	15.1±1.6	1.2±0.0	4.598	29	-	0.041*
	PPVL	5.0±0.7	0.4±0.0	5.0±0.6	0.4±0.0	6.804	29	-	0.014*
	PPTL	4.6±0.5	0.3±0.0	4.6±0.4	0.4±0.0	11.84	29	-	0.002**
	ED	1.2±0.1	0.1±0.0	1.1±0.1	0.1±0.0	4.622	29	-	0.004**
New characters	4-5	4.1±0.5	0.3±0.0	3.7±0.4	0.3±0.0	5.732	29	-	0.023*
	4-10	6.1±0.6	0.5±0.0	5.6±0.7	0.4±0.0	6.162	29	-	0.019*
	3-5	0.8±0.6	0.4±0.0	5.2±0.7	0.4±0.0			9.129	0.002**
	5-10	3.8±0.5	0.3±0.0	3.4±0.5	0.3±0.0	10.758	29	-	0.003**
	5-9	4.4±0.4	0.3±0.0	4.0±0.5	0.3±0.0	-	-	19.69	0.000**
	6-9	2.5±0.3	0.2±0.0	2.2±0.3	0.2±0.0	-	-	9.002	0.003**
	7-8	3.9±0.3	0.3±0.0	3.5±0.6	0.3±0.0	4.719	29	-	0.038*
	9-8	3.8±0.4	0.3±0.0	3.4±0.4	0.3±0.0	-	-	8.016	0.005**
	2-3	4.6±0.6	0.3±0.0	4.6±0.6	0.4±0.0	8.035	29	-	0.008**
	6-10	3.8±0.4	0.3±0.0	3.4±0.4	0.3±0.0	5.029	29	-	0.033*

Note: all abbreviations was detailed in (Appendix A)

Note: * indicated statistical significance when p-value < 0.05

** indicated statistical significance when p-value < 0.01

Morphometric variation between two juvenile populations was visualized via scatter plot of the first two principle component axes (highest eigen value (Appendix D)). The plot showed slight overlap of data points (Figure 14). It shows clear separation based on PC1 and this axis explains around 32% of data variation. Pre-pelvic length (PPVL), pre-pectoral length (PPTL), total length (TL), posterior nostril to origin of pelvic fin (inter-landmark 2-3) and Insertion of anal fin to insertion of 1st ventral caudal fin ray (inter-landmark 9-8) affect the body shape of specimens in the Gulf of Thailand (GT) sample (Figure 15) and separate them from Andaman Sea samples. Other characters were consistent with Andaman Sea population (AN). This implied high potential to discriminate juvenile samples into different groups using these fourteen variable characters.

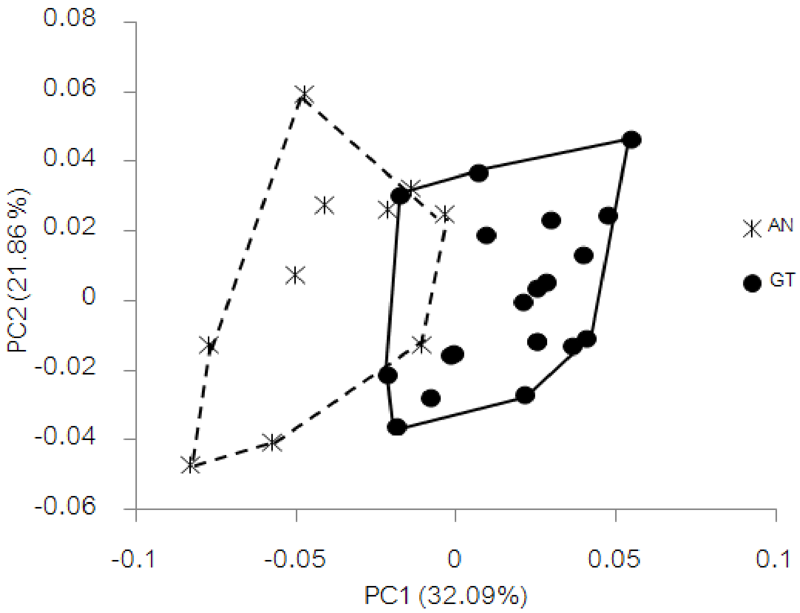

Figure 14 Scatter plot by the first two principle components from four general characters and ten new measurable characters of juvenile fish; there is considerable separation between the Andaman Sea (AN) and the Gulf of Thailand samples (GT).

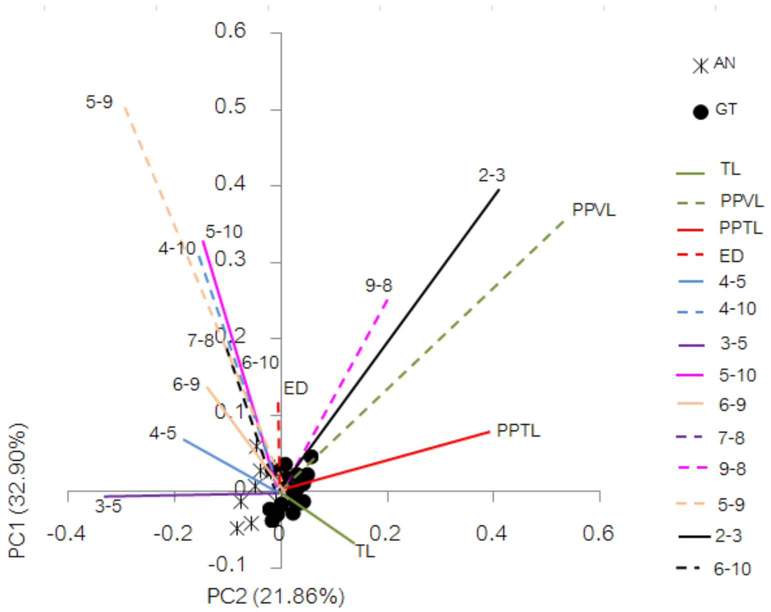

Figure 15 Eigenvector plots reveal contributions of each juvenile's character to the component axes.

2.6 PRELIMINARY DISCUSSION

The results of this study indicate that the diagnostic characters described in the FAO identification guide did not discriminate between AN and GT populations. The FAO guide book (Allen, 1985) is a very complete identification key but it is designed for use for snapper fishes which have wide distribution; especially, it has to account for all the variability within the species over a broad range, so that characters are not used to detect any difference of morphology between geographic regions.

This study reported some differences in body proportion, described by general that have not been used to identify to this species and new measurable characters, as well as chromatic differences between the two populations. Most characters of adult fishes that exposed a degree of potential population differentiation were on or around the head area; adults in GT samples had some head characters that were longer than those in AN samples. Moreover, in juveniles, many characters represent deeper body proportions in AN samples than those from GT.

Differences in stripe patterning of the SCS/WPO and Indian Ocean morphs of *L. russelli* are consistent and obvious in each population. The present or absences of stripes show obvious differences between GT and AN populations; there appears to no overlap between the eastern and western chromatic morphs.

This is the first report that shows the color pattern of juveniles from the Indian Ocean. Juveniles from this region have seven stripes as adults, the results of this study reveal that the reported difference in stripe patterning between the SCS/WPO and Indian Ocean morphs occurs not only in the adult but also in juvenile fishes. Surprisingly, the color morph within Gulf of Thailand juveniles was different from the Pacific Ocean morph reported by Allen (1985). I did not obtain any juveniles with 4 stripes or any spots during the course of this study, although a younger specimen with 4 stripes was reported from Songkhla Lake — which is a semi enclosed estuarine lake in the lower part of Gulf of Thailand (Source: Report by Department of Marine and Coastal Resources Research Center, Lower Gulf of Thailand, 2009). The absence of stripes in juveniles in this study may be the result of sampling bias (since the specimens were obtained by artisanal fishermen, only bigger/older juveniles that were vulnerable to their capture gear were obtained) or perhaps it reveals more variation of juvenile morphs within the Pacific Ocean than was previously thought. Many more samples from locations along the distribution range will be required to gain a more complete understanding of this question. Ontogenic variation can play an important role in morphological development in fishes, but it is unclear how the presence or absence of stripes might be so consistently divergent in a connected population. Phylogeography of this fish by morphology and genetic study will perhaps answer this question.

Eustatic sea level change associated with past glacial periods created a strong east-west barrier along the western margin of south-east Asia (Randall, 1998; Sathiamurthy and Voris, 2006; Satapoomin, 2011; Voris, 2000). The lack of connectivity between populations in the past created possible geminate species pairs of fishes of around 65 marine species along this region (Randall, 1998). The present patterns of surface water current via the Straits of Malacca (Wyrtki, 1961 (cited in Rizal *et al.*, 2010) eventually connected population between east and west but because of the relative weak of surface current and the shorter period of recent connection (around 7,000 year before present (Sathiamurthy and Voris, 2006)) maybe not enough to homogenize the two populations into similar color patterns. The lack of connectivity in the past geographically history may be a main cause of external morphology difference between two coasts of Thailand.

Many characters that were examined in this study showed highly significant differences in external morphology between AN and GT samples. Especially, it was apparent that differences in the presence of stripes on their body should be push to become a diagnostic character to identify this ambiguous species. The stripe did not easily disappear when the specimens were preserved in alcohol/formalin; as a color pattern, it can be a candidate to be a good diagnostic character. Differences of morphometry may reveal potential subspecies in this region, rather than the existence of geographic “regional types” on two coasts of Thailand as previous reports.

Population genetics and genetic diversity within *L. russelli* in Thailand should be a research priority to gain insight into the phylogeographic basis of the morphological differences and to confirm species classification status of this fish.