

**DISTRIBUTION OF GREENBACK MULLET *Liza subviridis*
(Valenciennes, 1836) IN RELATION TO ENVIRONMENTAL
PARAMETERS OF PINANG RIVER ESTUARY, BALIK PULAU,
PENANG.**

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PENANG.**

by

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**Thesis submitted in fulfillment of the requirements for the degree of
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TABURAN IKAN BELANAK, *Liza subviridis* (Valenciennes, 1836)
BERHUBUNG DENGAN PARAMETER PERSEKITARAN MUARA SUNGAI
PINANG, BALIK PULAU, PULAU PINANG

Ikan belanak (*Liza subviridis*) dan parameter persekitaran (iaitu pH, saliniti, konduktiviti, jumlah pepejal larut (TDS), suhu, oksigen terlarut, kekeruhan, jumlah pepejal terampai (TSS), nutrien, partikel pasir dan bahan organik) telah dikutip di empat stesen yang berbeza di sepanjang muara Sungai Pinang di Balik Pulau semasa air pasang perbani untuk tempoh satu tahun (Mac 2013 – Februari 2014). Pukat hanyut dan jala dengan keluasan kawasannya diketahui telah digunakan untuk mengutip sampel ikan. Sebanyak 2286 individu (635 ikan jantan dan 1651 ikan betina) telah disampel. *L. subviridis* dijumpai di kesemua stesen tetapi hanya ikan bersaiz besar dengan saiz panjang 13.2 cm – 19.6 cm ditemui di kawasan persisiran pantai manakala pelbagai ukuran panjang (9.3 cm – 19.6 cm) boleh didapati di kesemua tiga stesen sungai. Jumlah individu yang banyak dengan pelbagai julat panjang (9.3 cm – 19.6 cm) dapat dilihat di tengah muara dengan keadaan polihalin (6 – 31 ppt) dengan jumlah individu yang banyak dilihat semasa musim hujan. Hubungan panjang-berat dan corak pertumbuhan adalah $W = 0.0117 L^{2.9989}$ (isometrik) untuk semua individu, $W = 0.0157 L^{2.8787}$ (alometrik negatif) untuk ikan jantan dan $W = 0.0109 L^{3.0284}$ (isometrik) untuk ikan betina. Nilai b untuk ikan betina adalah lebih tinggi daripada ikan jantan. Tiada perbezaan yang signifikan dikesan untuk nilai b antara musim hujan dan kering. Nilai purata faktor keadaan relatif bagi *L. subviridis* adalah 1.01 ± 0.13 . Tiada perbezaan yang signifikan dikesan untuk nilai Kn antara musim hujan dan kering dan juga diantara ikan jantan dan ikan betina. Daripada ujian ANOVA satu hala untuk parameter in-situ (iaitu TSS, kekeruhan, TDS, konduktiviti dan saliniti) adalah terendah manakala nutrien (iaitu ortofosfat)

adalah tertinggi di hulu muara berbanding dengan kesemua tiga stesen lain. Ujian-t menunjukkan hanya ammonia, pH dan TSS adalah berbeza secara signifikan ($p < 0.05$) antara musim hujan dan kering. Hasil dari Analisis komponen prinsipal (PCA) telah mengklasifikasikan tengah dan hulu muara mengandungi kepekatan nutrien, bahan organik, dan partikel pasir yang lebih tinggi manakala hilir muara dan kawasan persisiran pantai mengandungi saliniti, konduktiviti, TDS dan kelodak dan tanah liat yang lebih tinggi. Analisis regresi pelbagai telah menunjukkan jumlah individu ikan belanak berkorelasi secara positif dengan kepekatan nutrient, bahan organik dan partikel pasir dan berkorelasi secara negatif dengan saliniti, TDS, konduktiviti dan peratusan kelodak dan tanah liat.

DISTRIBUTION OF GREENBACK MULLET *Liza subviridis* (Valenciennes, 1836) IN RELATION TO ENVIRONMENTAL PARAMETERS OF PINANG RIVER ESTUARY, BALIK PULAU, PENANG.

ABSTRACT

The mullet (*Liza subviridis*) and environmental parameters (i.e. pH, salinity, conductivity, total dissolve solid (TDS), temperature, dissolved oxygen, turbidity, total suspended solid (TSS), nutrients, sand particle and organic matter) were collected in four different stations along Pinang River estuary in Balik Pulau during spring tide for one year period (March 2013 – February 2014). Gill net and cast net of known area were used for fish sample collection. A total of 2286 individuals (635 males and 1651 females) were sampled. *L. subviridis* were found in all stations but only larger individuals with length size of 13.2 cm - 19.6 cm were found in the coastal area while variety of length (9.3 cm – 19.6 cm) can be found in the three estuarine reaches. High number of individuals with variety of length ranges (9.3 cm – 19.6 cm) was observed in the middle estuary with polyhaline condition (6 – 31 ppt) with higher number of individuals was observed during wet season. The length-weight relationship and growth pattern is $W = 0.0117 L^{2.9989}$ (isometric) for all individuals, $W = 0.0157 L^{2.8787}$ (negative allometric) for males and $W = 0.0109 L^{3.0284}$ (isometric) for females. The female b value was higher than the male. There was no significant different detected for b values between wet and dry season. The mean relative condition factor of *L. subviridis* was 1.01 ± 0.13 . There was no significant different detected for Kn between wet and dry season and between male and female. From one way ANOVA test for in-situ parameters (i.e. TSS, turbidity, TDS, conductivity and salinity) were lowest while nutrient (i. e. orthophosphate) were highest in the upper estuary compared to the other three stations. T-test showed that

only ammonia, pH and TSS were significantly different ($p < 0.05$) between wet and dry seasons. Result from Principal component analysis (PCA) have classified the middle and upper estuary as having higher concentration of nutrients, organic matter and sand particle while the coastal and lower as having higher salinity, conductivity, TDS and percentage of silt and clay. Multiple regression analysis showed that number of individuals of *L. subviridis* was positively correlated with nutrient concentration, organic matter and sand particle composition and negatively correlated with salinity, TDS, conductivity and percentage of silt and clay.

CHAPTER 1

INTRODUCTION

Mangrove estuaries are complex environment due to its location which is influenced by three different elements of sea, river and land. They are mostly found in tropical warm water of Indo Pacific region (Alongi, 2002). Characteristically, an estuary can be defined as a semi enclosed body of water along the coastlines in which seawater is significantly diluted by freshwater from the river while mangroves are forest swamps or plant communities of salt-tolerant that grow along the coastline and estuaries of the tropic water.

Estuaries are important component of the coastal ecosystem yet these areas are among the most altered and endangered ecosystem on earth (Blaber, 2000). Natural fluctuation especially in salinity couple with anthropogenic activities faced by this area creates an extreme environmental condition. Mangrove exploitation for timber, land reclamation for agricultural activities and human settlement, dam construction and fishing jetty have resulted in a deterioration of the estuarine ecosystem (Ong et al., 1991; Alongi, 2002; Kamaruzaman, 2013; DasGupta and Shaw, 2013). The water quality of this area is also poor due to the direct discharge from aquaculture ponds, industrialization and improper human settlement along the area (Khairun et al., 2012; Nurul Ruhayu, 2010). Diversity of the fauna and flora has also decreased due to over harvesting and deterioration in habitat quality (Borja et al., 2012).

In evaluating quality and health of an estuary, the evaluator should cover important aspects such as shape, location, physical and chemical properties, weather

influence, biological community that inhabit or utilize the area, level of human interference and pollution received by the area (Borja et al., 2008).

Fishes are among the most suitable organism to be considered as a biological parameter for the health of the estuarine ecosystem (Borja et al., 2012). Fishes act as an indicator of the aquatic ecosystem degradation based on the principle that fish abundance and diversity are highly influenced by the changes of biotic and abiotic factors within their habitat (Fausch et al., 1990). This statement was agreed by Whitfield and Elliot (2002) who further listed fishes that were suitable as biological indicators of the estuarine health such as fishes that occupy all types of water body including the area that experience impacts from anthropogenic activities, their assemblage structure including from predator fishes (high trophic level) to their preys (low trophic level). Their lifespan is relatively longer than the other invertebrates thus they hold more information on aquatic ecosystem perturbation. Besides all the characteristics listed, being the main source for cheap protein especially for the bottom billion people, there are ample studies and information regarding the biology, ecology and economical value of fish that can be referred to.

Lately, the effect of anthropogenic activities and environmental variable on fish habitat and fish as a biological indicator in estuarine management has received more attention (Marshall and Elliot, 1998; Blaber, 2000; Mansor et al., 2012).

As a developing country, Malaysia also plays a part in contributing towards the degradation of this ecosystem. Ong et al. (1980) stated that 650,000 hectares of mangrove forest fringed the coastal area of Malaysia. However, about three decades later, the area decreased to 577,500 hectares representing a drop of about 11.2% or more than 2265.63 hectares/year (Kamaruzaman, 2013).

Penang Island is located on the north of the west coast of Peninsular Malaysia by the Straits of Malacca. With a population of 1, 647, 7000 as reported by the Department of Statistics Malaysia (2013), more than 50% of the people inhabit the island. The island is divided into two districts; the Northeast district where the city center is located and the Southwest district which is less developed. Similar to the other states in Peninsular Malaysia, the island experiences tropical climate with an average annual rainfall of 194 mm based on the Malaysian Meteorological Department, Bayan Lepas Station (2013). Despite the high population density of the Penang Island with a coverage area of 293 km², increasing pressure was continuously brought to the estuarine and coastal areas. As population increases with the rapid development of the island, further consideration was given to reclaim the estuaries and coastal areas which are suitable for industrial, urbanization, agriculture and aquaculture sectors.

Pinang River Estuary has been selected for this study due to its function and ecological importance in the fishery sector for the local community e.g. development of aquaculture site for shrimp and commercial fish, oil palm plantation and durian orchard, human settlement and fishing jetty. Most importantly, this estuarine area holds part of the valuable mangrove community in Peninsular Malaysia that provides natural protection against storms, hurricanes and violence waves. As stated by Teh et al. (2009), the mangrove forests that margin the northwest coastline of Peninsular Malaysia reduced the tsunami impact and had saved thousands of lives living in the area. It is located in the Southwest district of the Penang Island and hindered from the urbanized area of the island. Interestingly, the estuarine area acts as the second entrance to the world's smallest national park; the Penang National Park. Here, the scenario of the tropical mangrove estuary can be

experienced by boat cruising along the waterways. This short estuarine area is actually the downstream of the Pinang River of Balik Pulau which originated from Bukit Laksamana and Bukit Tiger Forest Reserve (Nurul Ruhayu, 2010).

Fishes that inhabit or utilize the estuarine area are definitely unique. As estuaries are not the only area where two different environments meet but tidal influence and the freshwater input from the river in addition of high and seasonal rainfall may cause fluctuation in most physicochemical parameters temporally and spatially. Anthropogenic activities by humans worsen the situation where it will then lead to high environmental stress conditions. Despite this harsh environmental condition, estuaries provide a safe breeding nursery ground for fin and finfish with abundance supply of food and less prey predator effect.

A study of Breine et al. (2008) categorized the distribution of estuarine fish fauna into six components based on salinity tolerance which are 1) freshwater species that occasionally enter oligohaline water; 2) truly dependent estuarine species; 3) diadromous (anadromous and catadromous species) 4) marine species that enter the estuary during adulthood when conditions are favorable; 5) marine species that utilize the estuary as a nursery and spawning area but most of their adult life at sea and 6) small number of marine species that pay a visit to the estuary during high tide to feed.

Mullet fish of family Mugilidae are among the most abundant and diverse fishes that were found schooling in the shallow coastal waters, estuaries and freshwaters of the subtropics and tropics (Oren, 1981). The general morphological characteristics that apply to all mullets are; elongated body, flattened head covered by scales, two short and well separated dorsal fins where the first fin is equipped

with spines while the second dorsal fin is made up of soft rays, vertebrae number between 24 or 25 and the position of fins of all genus's are relatively standardized (Schultz, 1946). Later, Harrison and Senou (1999) further listed the general characteristics of the mullet such as; elongate with subcylindrical body, broad and flattened head dorsally, eyes often partly covered by adipose tissue, terminal or inferior mouth with relatively small, hidden or absent teeth, well separated dorsal fin first with IV slender spines and the second dorsal fin with 9 to 10 soft rays, short anal fin with II or III spines and 7 to 12 soft rays in adult, emarginated or forked caudal fin, pectoral fins inserted high on body, pelvic fins with I spine and 5 soft rays, lateral line absent, cycloid or ctenoid scales covering head and body, pharyngeal pad and sulcus on pharyngobranchial organ, elongated and coiled intestine with muscular gizzard stomach with body coloration of dark blue, dark olive, greenish or greyish dorsally with silvery or pale yellowish ventrally, fins dusky or pale yellowish (particularly pelvic fins) perhaps with margins dusky, dark bar or spot sometimes dorsally at base of pectoral fins.

Most of the mullets are coastal species where they usually utilize the estuaries and brackish environment to feed or as a nursery ground (Blaber, 2000). This euryhaline fish is able to tolerate with the wide range of salinity, they are found swimming along the coastal water and estuarine area towards the tidal creek following the high tide. Spawning is likely to take place in the seawater as the egg hatches to larvae and somehow the larvae migrates and find their way towards the estuarine area and metamorphosed into juvenile (Oren, 1981).

Previous study by Blaber (1997) showed that mullet is not suitable as an indicator species because of their hardy feature. However, this fish is unique in their own way. This euryhaline species are highly dependent on the estuarine area as their

nursery ground (Wallace et al., 1984). Thus, any disturbance and decrease in the environmental quality certainly have a big impact on the fish population. As the fish swims across the estuarine and tidal river (e.g. dam construction) the space and food availability of the fish are reduced. Being a primary consumer, mullet is an important prey species for other piscivorous fishes in the estuarine area. Their omnivorous diet as stated by Fatema et al. (2013) includes algae, diatoms, desmids and plant materials floating in the water column which reduce the turbidity of the water and help in the survival of other species in the area (Oren, 1981). They are present in a large group and their position can be identified as they were observed to be leaping out of the water. This has made them as an easy target for life bait. Some parts of the world including the Southeast Asia, India and Bangladesh, consume this fish as a delicacy and concentrate on this species for aquaculture activities because of their hardy features and variety of food that they consume.

The greenback mullet, *Liza subviridis* (Valenciennes, 1836) is a euryhaline species that are found to be schooling in shallow coastal waters, estuaries, freshwaters and juvenile may be found searching for food in the mangrove and rice field areas (Mohd Azmi et al., 2010). The taxonomy status of this species is still under debate (Durand et al., 2012). For the new record, it is accepted as *Liza subviridis* based on Fishbase (<http://www.fishbase.org/>) World Register of Marine Species (<http://www.marinespecies.org/>), Integrated Taxonomic Information System (<http://www.itis.gov/>) (information retrieved on 4 April 2016), Eschmeyer and Fricke (2015) and Kottelat (2013). Schultz (1946) in his review on morphological character of the Mugilidae family stated that the taxonomy of this species is hard to be defined as the morphological characteristics of this family are remarkably similar and the difference between the juvenile and adult fish is large.

1.1 Objectives

The objectives of this study are as the following:

- a) To study the distribution of *Liza subviridis* along Pinang River estuary.
- b) To estimate the length-weight relationship and relative condition factors with relation to seasonal variation of male and female *Liza subviridis*.
- c) To determine the influence of environmental variables on number of individuals of *Liza subviridis* in Pinang River estuary.

CHAPTER 2

LITERATURE REVIEW

2.1 Utilization of mangrove estuaries by fish

The mangrove estuary is a unique ecosystem in which biotic and abiotic conditions are influenced by tidal effect of the seawater and also input from the freshwater system. The labyrinth mangrove root is not just for breathing; they accumulate sediment to form a suitable habitat for various small invertebrates and bacteria while providing a suitable hiding place for juvenile fishes and shrimps (Nagelkerken et al., 2008). Numerous studies worldwide have acknowledged the significance of mangroves and estuaries as habitat for fishes.

Fish assemblages studies was done throughout the world and have shown that estuaries contain variety of fish species representing larvae, juveniles and adults of estuarine resident, marine and freshwater origin with high juvenile abundance (Elliot and Dewailly, 1995; Albaret et al., 2004; Jaureguizar et al., 2004; Neves et al., 2011; Innal and Ozdemir, 2012; Yokoo et al., 2012).

The composition of fish is high especially in tropical countries; 80 species in 45 genera found in Merbok Estuary, Kedah (Mansor et al., 2012), while only 21 fish species were reported in the temperate estuary of Uruguayan Coast (Plavan et al., 2010).

Consequently, the studies conducted only focused on the species richness, composition and diversity of an estuary. They did not take into account some important ecological factors such as 1) some euryhaline species can thrive in the sea, estuarine and freshwater condition, 2) the area itself that function as a feeding ground

for some marine species during spring tide, refuge against predator and nursery for many commercial marine species and 3) some fish species experience ontogenetic shifts in food and habitat use (Faunce and Serafy, 2006).

Several studies throughout the world have proved that mangrove is an important nursery area for high number of juvenile fishes and invertebrates (Robertson and Duke, 1987; Laegdsgaard and Johnson, 1995; Abu El-Regal and Ibrahim, 2014). Preference of fish (especially juveniles) to this area is due to the presence of prop root and pneumatophores of mangrove trees that provide unique structure with ample food supply and low prey-predator effect (Laegdsgaard and Johnson, 2001).

Two strategies used by the fishes in utilizing the mangrove and estuary as a nursery as pointed by Beck et al. (2001) are:

1. Isolation of juvenile from the adult habitat (classic).

This type of strategy occurs only when the juveniles are found in the sheltered inshore area and then experience the ontogenetic shift (mainly in reproduction and food preference) towards the offshore habitat.

2. Overlapping of juvenile and adult habitat (general).

Those fish that experience this type of strategy can coexist in the nursery area but adult could move to offshore (mainly for spawning and food).

Serafy et al. (2007) reported that *Sphyraena barracuda* experience the classic strategy while juvenile and adult *Lutjanus griseus* coexist together indicating that they experience the later strategy.

2.2 Ecology of mangrove fishes

Majority of ecological research in estuarine area concentrates on spatial and/or temporal patterns of estuarine fish assemblage and their relation to environmental parameters collected during the sampling period.

In the subtropics region, Pombo et al. (2005) and Akin et al. (2005) reported that salinity, temperature and turbidity are the major abiotic factors shaping the assemblage structure of fish in the studied area. Not much difference with the tropics as Jaureguizar et al. (2004), Simier et al. (2006) and Mansor et al. (2012) reported that salinity, temperature, turbidity and pH showed strong correlation with the fish assemblage structure. According to Martino and Able (2003) and Plavan et al. (2010), the salinity of water is the key factor in structuring the fish community in the temperate region.

Those studies did not take into account that the influences of environmental parameters are species and size specific (Werner and Giliam, 1984; Loneragan, 1992). There is scarce information available on autecology study on fish species in the estuarine areas of Southeast Asia (Faunce and Serafy, 2006).

Mwandya et al. (2010) reported that abundance of *Mugil cephalus* in Zanzibar Island was affected by depth, turbidity, particle size distribution and food accessibility. They also found that higher densities of juvenile mullet were detected in the upper zone of the creeks.

2.3 The Mugilidae

Based on Berg (1940) as cited in Abu Khair and Mohd Azmi (1996), Mugilidae was previously placed in the order of Perciformes but because of the position of pelvic bones the order Mugiliformes were formed with two suborders: Sphyreanoidei and Mugiloidei. After decades of taxonomic revision, Mugilidae is now the sole family of the order Mugiliformes, but the order is still in need of revision as mostly done on morphological characteristics (Durand et al., 2012).

According to taxonomic revision in the Western Central Pacific area made by Harrison and Senou (1999), Mugilidae includes 11 genera and a total of 31 species where most of them are under the genera of *Liza* and *Valamugil*. The taxonomy status at the genera and species level of this family is still under debate (Durand et al., 2012).

2.4 The greenback mullet, *Liza subviridis*

The distribution of greenback mullet was reported in tropical and subtropical water. In the Pacific regions, they were reported from Fiji, Queensland, Samoa and New Caledonia while in Asia they are enormously found in Bangladesh and the neighboring coastal states including Indonesia, Philippines, Malaysia and Sri Lanka. A coastal species which can thrive in a wide range of salinity gradient schooling in shallow coastal waters, estuaries, freshwaters and juveniles can be found in rice field and mangroves to search for food (Mohd Azmi et al., 2010).

The maximum length of the greenback mullet is 40 cm but the common length is 25 cm in total (Harrison and Senou, 1999). Maturity is attained at 9 – 10.5 cm for males and 11.0 – 11.5 cm for females with corresponding age of 6 to 8 months (Abu Khair and Mohd Azmi, 1996). In the recent years, Nor Aziella (2012) found that the sexual maturity length for males is at 16.5 cm and 16.8 cm for females in Merbok estuary, in Kedah, Peninsular of Malaysia.

A medium sized species with fusiform body, broad head and dorsally flattened, small terminal mouth with thin lips; upper lip with an outer row of inconspicuous teeth and 1 or 2 inner rows of smaller ciliform teeth while lower lips with one row of fine ciliform teeth present or absent, short and blunt snout, adipose eye fold moderately developed, 1 spine and 14 – 16 soft rays at their pectoral fin, 3 spines and 9 or 8 soft rays at anal fin in adult, 40 – 68 gill rakers on lower limb of first gill arch and 9 – 11 transverse scales and 28 – 32 lateral scales, coloration in life; dark greenish dorsally, brownish overhead, white ventrally, 3 to 6 indistinct, dark stripes along upper rows of scales, first dorsal fin greyish, second dorsal fin yellowish grey with dusky margin, anal fin with dusky margin, caudal fin bluish with

black margin and pectoral fins yellowish and may have a blue spot at fin origin (Harrison and Senou, 1999).

Nor Aziella (2012) showed that morphometrics of *L. subviridis* have the highest value for standard length, body length and distance from snout to pelvic fin with the shortest snout length compared to *Liza vaigiensis*, *Valamugil engeli*, *Valamugil seheli* and *Valamugil speigleri*. On the other hand, through the meristic technique *L. subviridis* is in agreement with the result obtained by Harrison and Senou (1999).

Spawning is likely to take place in the sea as the egg hatches to larvae and in respond to their olfactory system the larvae will migrate and find their way towards the estuarine area and metamorphose into juvenile (Mohd Azmi et al., 2010). They exhibit heterosexuality and fertilization is external. The gonadosomatic index of tropical female's *L. subviridis* indicates that their spawning season is in September and December (Nor Aziella, 2012).

Mugilidae have been identified as iliophagous feeders with different particle size preference for each species (Blaber, 1976 and Almeida, 2003). Diet of *L. subviridis* undergoes changes from plankton based during post larva to bottom feeding in adult. Fatema et al. (2013) have identified diatoms, algae, desmids, plant materials, zooplankton, detritus, sand grains and fishes in the stomach of adult *Liza subviridis*.

Dankwa et al. (2005) reported that food preference of mugilid fishes do not depend on species, season and body size. They also reported that in *Liza dumerilii* the particle size of food preferred is between 41.2 – 1195.8 μm in the Volta estuary and 33.0 – 1649.0 μm in Pra estuary of Ghana.

The diverse diet and occupying the low trophic level in the food web benefit both the mullet and the ecosystem. The high abundance of mullet in an area presents no real threat to the environment as they are food sources for other piscivorous fish, birds and mammals and favorite bait for many spectacular marine game fish such as threadfin and barramundi.

Mullets are caught mainly with gill net and cast net. With excellent and tasty flesh and roe, mullet has become a popular food dish worldwide. Their roe commands exorbitant prices as a delicacy especially in Taiwan, Japan and Italy. With their hardy, fast growth rate, flesh high in energy and calorie content and variety of food consume, they have been extensively cultured worldwide e.g. Egypt and Bangladesh (Oren, 1981; Saleh, 2008).

2.4.1 Taxonomical classification of greenback mullet, *Liza subviridis*

Taxonomical classification of Mugilidae family has been done for the last several decades (Shultz, 1946; Abu Khair and Mohd Azmi, 1996; Harrison and Senou, 1999; Mohd Azmi et al., 2010). The taxonomical classification of the *Liza subviridis* as reported by Mohd Azmi et al. (2010):

Kingdom: Animalia

Phylum: Chordata

Subphylum: Craniata

Superclass: Gnathosmata

Class: Actinopterygii

Division: Teleostei

Subdivision: Euteleostei

Superorder: Acanthopterygii

Order: Mugiliformes

Family: Mugilidae

Genus: *Liza*

Species: *subviridis* (Valenciennes, 1836)

Greenback mullet was firstly described as *Mugil subviridis* by Valenciennes (1836) from India. Senou in Nakabo (2002) and Senou in Randall & Lim (2000) considered that *Liza* as a junior synonym of *Chelon*. A phylogenetic study through mitochondrial DNA also has proved that *Liza* and *Chelon* have close genetic relation and not monophyletic and suggested that they should be synonymized (Semina et al., 2007). This is further accentuated by Eschmeyer and Fong (2015) where there have verified that the available species of Mugilidae around the globe is 303 but the valid species is only 74. This is based on their monthly update since 1980 and so many unplaced available names leading to synonyms.

For the current taxonomic status of the greenback mullet, it is valid as *Liza subviridis* based on recent taxonomic revision in Fishbase (<http://www.fishbase.org/>), World Register of Marine Species (<http://www.marinespecies.org/>), Integrated Taxonomic Information System (<http://www.itis.gov/>) (information retrieved on 4 April 2016), Eschmeyer and Fricke (2015) and Kottelat (2013).



Plate 2.1 General appearance of *Liza subviridis* (Valenciennes, 1836).

2.5 Length-weight relationship, relative condition factor (K_n), length frequency distribution

The length and weight-relationship data is very useful in understanding the biology and ecology of fish. The relationship is usually used in fishery research for better development of stock assessment models for the sustainable management of fish in an aquatic ecosystem (Mendes et al., 2004). It is important in estimating the average weight corresponding to a known length group (Froese, 2006), and in evaluating the relative well-being of a fish population (Bolger and Connolly, 1989).

The relationship between length and weight for most of the fish species are expressed by the following equation: $W = a \times L^b$ (Le Cren, 1951). The b value define the fish growth pattern and may deviate from 3 as fish grow and change their shape. When $b = 3$, the weight of the fish growth is isometric. If the value is other than 3, then the fish growth is classified as allometric (i.e. $b > 3$ indicating positive allometric growth and $b < 3$ indicating negative allometric growth).

The length-weight relationship of Mugilidae have been extensively studied worldwide, e.g. by Moorthy et al. (2003), Lawson and Jimoh (2010), Renjini and Nandan (2011), Dankwa (2011) and Gondal et al. (2014). Although there were studies done on the same species in the Merbok estuary of Kedah by Nor Aziella (2012) and Vellar estuary of India by Rahman et al. (2013), the locality is different as both of the previous studies were done in a big estuarine system with less anthropogenic effect. The present study was undertaken to establish the growth pattern and the condition of this fish species in small microtidal with huge impact from anthropogenic activities.

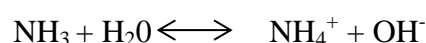
Relative condition factor (Kn) was calculated through formula $Kn = W/W'$ by Le Cren (1951) as cited by Froese (2006). The different between condition factor (K) and relative condition factor (Kn) is that the former measuring the deviation of an individual of fish from the cubic value $b = 3$ with the same length class while the later measure the deviation of an individual from an average weight for that length without the assumption of 'ideal fish $b = 3$ '. Relative condition factor was used in this study as it is more suitable for large amount of sample, variety of length ranges and the deviation from the isometric growth pattern (Clark, 1928) as cited in Froese (2006). Froese (2006) indicated that the coefficient of condition of fish value varied due to the spawning process as gonadal development decreases the feeding and muscle of the fish, the condition of the fish population in relation to its welfare, good nutritional value through abundant food supply and can compare two or more population of fish with different habitat condition.

Length frequency distribution is important in various aspects of fishery sciences. Through this, estimation on the status of the size structure of the fish population in their habitat can be known (Taiwo, 2010). Besides, the study also utter the conditon of the habitat health, wild stock and breeding season of the fish species (Ranjan et al., 2005).

2.6 Effect of water quality deterioration to fish diversity and abundance

Some studies have proven that environmental degradation resulting from human activities such as pollution and development are the main source of stressor for aquatic organisms. Alteration or degradation on water quality of an area may have negative effects on the biological and physical process of a fish which would lead to higher mortality, changes in behavior and changes of the fish assemblage of the area (Karr and Dudley, 1981; Borja et al., 2012). Araujo et al. (2000) stated that about 49 – 54 species of fish were found in Middle Thames estuary during 1980. However, a decade later, the number of species decreased to 35 – 44 with the mean of yearly abundance decreased about 3.94%.

Of all the water quality parameters, elevated ammonia level has major impact on the fish population. Experimental studies done showed that the toxicity of ammonia to seabass is 96-h LC50_s (lethal concentration for 50% of the population for 96 hours of exposure) by 1.7 mg/L NH₃ while higher concentration in seabream and turbot by 2.5 – 2.6 mg/L NH₃ (Person-Le Ruyet et al., 1995). Before that, it is important to differentiate the forms as NH₃ is harmful to aquatic organisms while NH₄⁺ is basically harmless to them (Körner et al., 2001; Randall and Tsui, 2002). When dissolved in water, un-ionized ammonia (NH₃) exists in equilibrium with ammonium ion (NH₄⁺). The proportion of forms is determined by the pH water (Randall and Tsui, 2002). As pH increases, hydroxyl ions also increase thus the equilibrium will be pushed to the left.



Durborow et al. (1997) showed that elevated nitrite concentration in a water body could lead to brown blood disease. Nitrite that enters the bloodstream will combine with haemoglobin to form methemoglobin that is incompetent in transporting oxygen to the cell. They suggested that coldwater fishes (e.g. trout and salmon) are the most sensitive to elevated nitrite concentration. Moderately affected are warm water fishes (e.g. catfish and tilapia) followed by goldfish and flathead minnows. Bass, bluegill and sunfish have high endurance towards elevated nitrite concentration.

Precipitation is the main factor that controls salinity measurement in a water body (Barletta et al., 2003). Salinity, conductivity and total dissolved solid are related to each other because these three factors are affected by dissolved ion concentration in the water sample. Ecological study by Barletta et al. (2003) and Blaber (2000) showed that fish assemblage structure is affected by salinity fluctuation received by the estuary. Marais (1978), Oren (1981) and Blaber (2000) also emphasized that salinity fluctuation may play an important role in metabolism and oxygen consumption rate of fish. Mullet are euryhaline fish and most of them spawn in seawater (Oren, 1981; Abu Khair and Mohd Azmi, 1996; Cardona, 2000; Blaber, 2000; Mohd Azmi et al., 2010; Chang and Iizuka, 2012).

Water temperature is affected by ambient temperature, shaded areas, altitude and spring or neap tides are reported to affect the temperature reading in river (Nurul Ruhayu, 2010). As reported by Kuthalingam (1959), Kutty and Murugapoopathy (1969), Kutty and Mohamed (1975) and Oren (1981), the suitable temperature for most of the mullets ranged between 15 - 35°C. Marais (1978) reported that increase in temperature regardless of salinity and body weight of *Mugil*

cephalus, *Liza dumerili*, and *Liza richardsonii* would increase the oxygen consumption rate and metabolic rate of these mullet.

Nurul Ruhayu (2010) reported that the range of dissolved oxygen in the downstream of Pinang River was less than 4.0 mg/L during low tide and can up to 10 mg/L during high tide of spring tide. The decrease of dissolved oxygen from upstream to downstream experiences in Pinang River was due to organic waste discharge from oil palm plantation and aquaculture site, tidal influence and rainfall received by the area (Nurul Ruhayu, 2010). Fish needs oxygen to generate energy for vital life process. McFarland and Moss (1967) reported that *M. cephalus* able to survive in low level oxygen water and factory waste polluted water. This showed that mullets are able to do water surface respiration (Odum, 1970; Cech and Wohlschlag, 1973; Narayanan, 1974).

Turbidity in water is affected by suspended solids and dissolved matters that obstruct the light from travelling in a straight line. The increase in turbidity reading has a strong linear relationship with the increase in total suspended solid concentration in the water column (Hannouche et al., 2011). The decrease in light penetration into water could disturb the food web of the ecosystem as the primary producers such as algae which need light for photosynthesis process. The effects of having high turbidity to fish are stunted growth and development that reduce the resistance towards disease and disturb the development of eggs and fish larvae (Robertson et al., 2006). The relationship of turbid water and fishes in estuarine area has long been recognized. Turbid waters provide a suitable hiding environment for the juveniles as predation in turbid waters is less as it reduce the visual effectiveness of the predators (Nagelkerken et al., 2008)

Fitzpatrick et al. (2012) researched in the tropical coral reef in Western Australia and revealed that depth was the key factor in shaping the demersal fish assemblage. The assemblage level pattern of shallow habitat was characterized by shorter fish length with higher species diversity and the reverse condition occurred in deeper waters. As iliophagus feeder, mullet prefer shallow area with rich organic matter sediment (Blaber, 2000).

CHAPTER 3

MATERIALS AND METHODS

3.1 Sampling location

This study was conducted in the Pinang River estuary (05° 23. 549' N, 100° 11. 546' E), which is a mangrove estuary located in the southwest side of Penang Island at northwest coast of Peninsular Malaysia. Pinang River is located at Balik Pulau and it is originated from Bukit Laksamana and Bukit Tiger in Pantai Aceh Forest Reserve.

Pinang River is a polluted river due to the presence of various anthropogenic activities (e.g.: durian plantation, domestic and aquaculture site, human settlement with fishing jetty in the middle and downstream) (Nurul Ruhayu, 2010). Pinang River estuary is classified as microtidal estuary where the area is subjected to semidiurnal tides with tidal range of 0.7 - 2.7 m during spring tide and 1.3 - 2.0 m during neap tide. Large intertidal mudflats that support a variety of crustaceans, mammals, fish and birds can be observed during low tides. The mangrove forest that fringes the estuarine area is composed of *Avicennia* sp., *Rhizophora* sp., *Bruguiera* sp. and *Nypa fruticans*.

The estuary stretches about 2 km long with a lower estuary width of 117 m measure during the high tide of neap tide. According to Nurul Ruhayu (2010) during spring tide, the tidal influence is perceptible up to the middle part (2 km from the lower estuary) of the Pinang River. During this study, the area experience wet season from June to November and dry season from December to May with dry months receive less than 150 mm precipitation in a month (Malaysian Meteorology Department, 2013).

Four sampling stations that experience tidal influence – at the upper, middle, lower estuaries and the coastal were established. The sampling stations were chosen based on different surface salinity gradient of the area measured during spring tide and the anthropogenic activities that occurred in the area. Plate 3.1 shows the map of the sampling stations.

3.1.1 Coastal

The first sampling station is the coastal area (N 05° 23.441' E 100° 10.993'). It is approximately 1 km from the mouth of the estuary (Plate 3.2). This shallow (mean depth = 1.2 m) coastal water is extensively being utilized by artisanal fishing activities.

3.1.2 Lower estuary

Lower estuary (N 05° 23.543' E 100° 11.223') is the second sampling station where mangrove vegetation forms an opening that connects the Pinang River estuary with the Straits of Malacca (Plate 3.3). The length measure for the mouth of the estuary is 117m during the high tide of neap tide. This area is affected by artisanal fishing activities and is the main entrance for boats into the estuarine area. During low tide, large areas of mud flat are exposed which support a variety of organisms (e.g. mudskippers, clamps, crabs and snakes) that made this estuarine area more unique and diverse.