NURUL HASYIMA BINTI ISMAIL MASTER OF SCIENCE 2018

# AGE AND GROWTH ESTIMATION USING GROWTH BAND COUNTS OF ORANGE MUD CRAB, *Scylla olivacea* (HERBST, 1796) FROM SETIU WETLANDS, PENINSULAR MALAYSIA

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UNIVERSITI MALAYSIA TERENGGANU

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**NURUL HASYIMA BINTI ISMAIL**

**Thesis Submitted in Fulfillment of the Requirement for the Degree of Master of Science in the Institute of Tropical Aquaculture Universiti Malaysia Terengganu.**

**MAY 2018**

## DEDICATION

*I dedicate this thesis to my beloved mother, late father and families for all their endless supports, encouragement and unconditional love throughout my studies and also to all my supervisors and beloved friends for all their supports, ideas and inspirations.*

*Thank you*

*"Knowledge doesn't come but you have to go to it" – Imam Malik.*

# ABSTRACT


Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Master of Science.

**AGE AND GROWTH ESTIMATION USING GROWTH BAND COUNTS OF ORANGE MUD CRAB, *Scylla olivacea* (HERBST, 1796) FROM SETIU WETLANDS, PENINSULAR MALAYSIA**

**NURUL HASYIMA BINTI ISMAIL**

**2018**

**Main Supervisor : Professor Mhd Ikhwanuddin bin Abdullah, Ph.D**

**Co-Supervisor : Professor Emeritus Mohd Azmi bin Ambak, Ph.D**

**Institute : Institute of Tropical Aquaculture, UMT**

Orange mud crab, *Scylla olivacea* (Herbst, 1796) is an important fishery products in Malaysia. Age study is important to generate the information on growth, recruitment, longevity, mortality for mud crab stock assessment. The aim of this study is to investigate the Growth Band Counts (GBC) deposited in thin sections of gastric mill of *S. olivacea* as age indicator for this species. A total of 90 wild caught *S. olivacea* were sampled from Setiu Wetlands, Peninsular Malaysia (February – October 2016) and 25 individuals of *S. olivacea* were reared in the hatchery of Institute of Tropical Aquaculture, Universiti Malaysia Terengganu for one year (March 2016 - March 2017). The periodicity of GBC in gastric mill of *S. olivacea* was validated using reared of known age samples. The results revealed that the presence of one GBC in reared *S. olivacea* were parallel to the age one year old of *S. olivacea* absolute age. One to three GBC were present in wild caught *S.*

*olivacea* which indicated the age of one to three years old, respectively. There was a significantly positive relationship between Carapace Width (CW) and age, as the number of growth bands increased with putative age. The accuracy of bands counting was assessed by lower coefficient of variation values of reared and wild caught *S. olivacea* with 6.00 – 6.23% and 8.52 – 8.40%, respectively. Using the von Bertalanffy Growth Model equation, the growth parameters of *S. olivacea* was estimated and growth curves was fitted to CW at age data. The growth parameters such as asymptotic CW ($CW_\infty$), growth coefficient (K year $^{-1}$) and initial condition parameter ($t_0$) of the known age *S. olivacea* were narrower at 20.07 cm, 0.31 year $^{-1}$ and - 0.20 than the wild samples at 28.01 cm, 0.36 year $^{-1}$ and - 0.73 for female and at 29.34 cm, 0.38 year $^{-1}$ and - 0.79 for male, respectively. The age composition suggested that *S. olivacea* population found in the Setiu Wetlands, Peninsular Malaysia has at least reached the age of 2 years old. The findings obtained from this study would substantially improve the direct age - based assessment for biological and ecological studies of mud crabs.

**ABSTRAK**

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains.

**PENENTUAN ANGGARAN UMUR DAN TUMBESARAN MENGGUNAKAN BILANGAN JALUR TUMBESARAN PADA KETAM NIPAH, *Scylla olivacea* (HERBST, 1796) DARI TANAH BENCAH SETIU, SEMENANJUNG MALAYSIA**

**NURUL HASYIMA BINTI ISMAIL**

**2018**

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Ketam nipah, *Scylla olivacea* (Herbst, 1796) adalah produk perikanan yang penting di Malaysia. Kajian umur adalah penting untuk menghasilkan maklumat mengenai tumbesaran, penambahan, jangka hayat dan kematian untuk penilaian stok ketam nipah. Tujuan kajian ini adalah untuk mengkaji bilangan jalur tumbesaran (GBC) yang tersimpan di dalam "gastric mill" *S. olivacea* bahagian yang nipis sebagai petunjuk umur untuk spesis ini. Sebanyak 90 ekor ketam liar *S. olivacea* ditangkap dari Tanah Bencah Setiu, Semenanjung Malaysia (Februari – Oktober 2016) dan 25 individu *S. olivacea* (umur diketahui) dipelihara di hatceri Institut Akuakultur Tropika, Universiti Malaysia Terengganu, (Mac 2016 – Mac 2017) selama satu tahun. Jangka masa GBC di dalam "gastric mill" *S. olivacea* telah disahkan menggunakan sampel yang diketahui umurnya. Keputusan kajian menunjukkan kehadiran satu GBC pada ketam peliharaan dan wujud

persamaan dengan satu tahun umur *S. olivacea*. Satu hingga tiga GBC dikenal pasti terdapat pada ketam liar *S. olivacea* yang juga berumur dari satu hingga tiga tahun. Didapati terdapat hubungan yang positif antara saiz karapas (CW) dan umur dimana terdapat peningkatan bilangan jalur tumbesaran dengan umur jangkaan. Ketetapan bilangan jalur tumbesaran dikira dengan "coefficient of variation" dan didapati *S. olivacea* peliharaan adalah lebih rendah berbanding *S. olivacea* liar iaitu masing-masing dengan 6.00 – 6.23% dan 8.52 – 8.40%. Menggunakan persamaan model tumbesaran von Bertalanffy, parameter pertumbuhan *S. olivacea* telah dianggarkan dan lengkung pertumbuhan telah sepadan dengan data CW pada umur. Anggaran parameter pertumbuhan seperti "asymptotic CW" ($CW_{\infty}$), "growth coefficient" (K $year^{-1}$) dan "initial condition parameter" ($t_0$) pada *S. olivacea* yang diketahui umurnya adalah lebih rendah pada 20.07 cm, 0.31 $tahun^{-1}$ dan - 0.20 jika berbanding dengan sampel *S. olivacea* liar pada 28.01 cm, 0.36 $tahun^{-1}$ dan - 0.73 bagi ketam betina dan pada 29.34 cm, 0.38 $tahun^{-1}$ dan - 0.79 bagi ketam jantan. Komposisi umur mencadangkan populasi ketam *S. olivacea* yang terdapat di Tanah Bencah Setiu, Semenanjung Malaysia adalah berusia 2 tahun lebih. Penemuan yang diperolehi daripada kajian ini akan membantu meningkatkan maklumat penilaian umur secara tepat untuk kajian biologi dan ekologi ketam nipah.

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بسم الله الرحمن الرحيم

*"In the name of ALLAH, the Most Gracious, the Most Merciful"*

Alhamdullilah, my deep reverence goes to Almighty ALLAH, my supreme, who enabled me to complete my study in Master of Science (Fisheries) at Universiti Malaysia Terengganu (UMT) only with ALLAH's will.

I want to express my special thanks of gratitude to my beloved mother, Salimah Binti Abdul Ghani and my family for their understanding and blessing. They have providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis.

I must express my very profound gratitude to my supervisors Prof. Emeritus Mohd Azmi Bin Ambak and Prof. Mhd. Ikhwanuddin Bin Abdullah for their valuable guidance, advice, suggestion, supervision and encouragement throughout my study period. They consistently allowed this paper to be my own work, but steered me in the right the direction whenever they thought I needed it.

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**APPROVAL**

I certify that an Examination Committee has met on 31st May 2018 to conduct the final examination of Nurul Hasyima Binti Ismail, on her Master of Science thesis entitled **"Age and growth estimation using growth band counts of orange mud crab, *Scylla olivacea* (Herbst, 1796) from Setiu Wetlands, Peninsular Malaysia"** in accordance with the regulations approved by the Senate of Universiti Malaysia Terengganu. The Committee recommends that the candidate be awarded the relevant degree. The members of the Examination Committee are as follows:

Marina Hassan, Ph.D.
Associate Professor
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Director of Institute of Tropical Aquaculture
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Date:

This thesis has been accepted by the Senate of University Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science.

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**PROF. ABOL MUNAFI AMBOK BOLONG, Ph.D.**
Director of Institute of Tropical Aquaculture
Universiti Malaysia Terengganu

Date:

**DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UMT or other institutions.

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**NURUL HASYIMA BINTI ISMAIL**

Date:

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| | | |
|---|---|---|
| BW | : | body weight |
| CV | : | Coefficient of variation |
| CW | : | carapace width |
| $CW_{\infty}$ | : | mean of asymptotic Carapace Width (CW) predicted by the equation |
| CWt | : | total carapace width at age |
| FAO | : | Food and Agriculture Organization |
| GBC | : | Growth band count |
| K | : | growth coefficient (per year $^{-1}$) |
| p value | : | probability |
| Ppt | : | part per thousand |
| $t_0$ | : | age of the crab would have at 0 cm carapace width |
| °C | : | degree Celsius |
| µm | : | micrometre |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the study

### 1.1.1 Mud crab production and demand

There are four mud crab species worldwide which are *Scylla serrata* (Forskal, 1775), *S. paramamosain* (Estampador, 1949), *S. tranquebarica* (Fabricius, 1798) and *S. olivacea* (Herbst, 1796) (Keenan et al., 1998). Researches on both the ecology and biology of the mud crab however, have mostly been focused on the *S. serrata* compared to the other 3 species (Keenan et al., 1995; Keenan, 1999; Moser et al., 2005; Shelley & Lovatelli, 2011).

Mud crab aquaculture has been practised for many years in some countries of the Southeast Asia (Vietnam, Philippine, Indonesia, Taiwan) which is primarily done by capturing and fattening juvenile crabs from the wild. There is a high demand for mud crabs that leads over-exploitation of wild stock in many areas (Allan & Fielder, 2003). From the year 2013 to 2014, the total mud crab production of the genus *Scylla* has markedly increased from 179, 536 tonnes to 183, 852 tonnes, respectively. A combined mud crab production of both Asia and Africa alone, recorded to be over 138, 000 tonnes totalled USD 377 million in 2008 (FAO, 2015).

Due to their commercial value, mud crabs are at threat of overfishing. In Malaysia, overfishing of genus *Scylla* was recorded in 1997. To make it worse, most of the caught crabs were also reported to be still at immature stage (Kosuge, 2001). Similar cases were also reported in other Southeast Asia countries such as Indonesia, Philippines, Vietnam, China, Taiwan, India and Sri Lanka. Such overfishing responsible to overexploitation of mud crabs species in the mentioned countries (Ikhwanuddin et al., 2011; Paterson & Mann, 2011; Azra & Ikhwanuddin, 2016).

### 1.1.2 Age and growth information

Accurate age information of mud crab species is crucial not only for regulation of commercial harvesting but also for future research application such as in conservation and management of mud crabs. The age information is fundamentally important to estimate growth, recruitment, fecundity and mortality in fish population (Campana, 2001; Maceina et al., 2007; Quist et al., 2012).

Age data in early life stages provide information on the effect of environmental factors on biological changes of mud crabs such as survival, growth and mortality. While, age data in adult stage provide information on the effects of fishing on stock, to enhance the understanding of the mud crabs life history and to exploit yield in sustainable manner (Jones, 1992; Charles, 2001; Humphries et al., 2013; Kilada & Ibrahim, 2016). In addition, mean length – at – age data provide growth information for whole population across species (Beamish et al., 2005; Rypel, 2009; Spurgeon et al., 2015). Accurate and precise age data are important to predict population response to climate changes, habitat fragmentation and conservation and management strategies (Campana, 2001; Quist et al., 2012).

## 1.2 Problem statement

Age estimation is a key point to assess the fishery stock under population dynamics models such as determination of age at maturity, growth and mortality rates and fecundity (Campana, 2001; Sherazul et al., 2010; Kilada & Ibrahim, 2016). However in crustacean, the use of age data is limited due to the loss of all hard structure during moulting events (Leland et al., 2011; Kilada & Acuña, 2015; Kilada & Driscoll, 2017).

Limited ageing studies on crustaceans resulted in the knowledge of mud crab age – based stock assessments remains inadequate. Crustaceans ageing information is understudied with less than 2% of the world's decapods longevity information available (Vogt, 2012; Leland et al., 2015). The information on mud crab stock assessment is crucial to enhance our understanding on fishery management and sustainability on mangrove area in Malaysia (Ikhwanuddin, 2001; Kosuge, 2001).

There are several indirect methods to assess age structure of these crustaceans such as observations of captive individuals and moulting increment data, mark – release – recapture method, tagging technique and size frequency distribution (Wiles & Guan, 1993; Hébert et al., 2002; Koeller et al., 2006). However, none of this method able to directly determine the age of individual crustaceans. One way to directly estimate the crustacean age is via growth band deposited on the hard structure. The method can be used to determine both the individual age structure and life stages (Leland et al., 2011; Kilada & Ibrahim, 2016). According to Leland et al. (2011), the hard structure of gastric ossicles in crustaceans contain concentric growth marks where the structure sequentially record past events and display annually growth record. Thus, this study is significant to provide essential and sufficient data of direct age estimation for captive rearing of known age individuals and wild population mud crab in Malaysia.

Until recently, most of the mud crab studies in Malaysia were focused on their taxonomic and biological aspect (Keenan et al, 1998; Ikhwanuddin, 2001; Ikhwanuddin et al., 2010; Sherazul et al., 2010; Ikhwanuddin et al., 2011). *Scylla olivacea* is the most common and most abundant mud crab species found in Setiu Wetlands, Terengganu. This species is also one of the important and high demand seafood product of the local area (Ikhwanuddin et al., 2010; Ikhwanuddin et al., 2011; Ikhwanuddin et al., 2014b). Due to the high demand of this species, a proper age estimation method are needed to improve mud crab stock assessments in Malaysia.

### 1.3 Significance of study

This study poses a new knowledge and important understanding on age and growth estimation of *S. olivacea* via direct age – determination method (Leland et al., 2011; Kilada et al., 2012). The implementation of direct ageing method on crustaceans especially for *S. olivacea* marks the first directly determining growth model and age documentation for this species. This study demonstrates the application of direct ageing and validation of the growth marks deposited annually in *S. olivacea.*

Accurate age estimation method is crucial for sustainable fisheries management plan and stock assessment (Smith et al., 1997; Campana, 2001; Stewart & Hughes, 2007). In fisheries, age information is closely related to population dynamic and also provide the selection on input control regulations for example, minimum legal size and size at sexual maturity. Validation of direct ageing method are fundamental and could substantially increase the resolution of age based data for mud crab management (Leland & Bucher, 2017).

## 1.4 Research objectives

Generally, this study aimed to estimate the age and growth of orange mud crab, *S. olivacea* from Setiu Wetlands, Peninsular Malaysia.

The specific objectives of this study are as follows:

i. To determine the growth band in gastric mill of reared *S. olivacea* and wild population.

ii. To validate the application of growth band count in gastric mill of reared *S. olivacea* in captivity to the wild caught *S. olivacea* from Setiu Wetlands, Peninsular Malaysia.

iii. To estimated age profile of *S. olivacea* population from Setiu Wetlands using size at age data of the growth band counts and Von Bertalanffy growth model.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Taxonomy status and classifications of *Scylla olivacea* (Herbst, 1796)

### 2.1.1 General taxonomy

Mud crabs belong to family Portunidae which consists of four distinct species, the *Scylla serrata*, *S. tranquebarica*, *S. paramamosain* and *S. olivacea* (Keenan et al., 1998). *Scylla olivacea*, *S. tranquebarica* and *S. paramamosain* are the species that commonly found in Malaysian mangrove waters (Ikhwanuddin et al., 2010; Ikhwanuddin et al., 2011; Ikhwanuddin et al., 2014b). Orange mud crab, *S. olivacea* that is locally known as "ketam nipah sepit merah" has high commercial value in Malaysia especially in the mangrove area of the east coast Peninsular Malaysia (Ikhwanuddin, 2001; Ikhwanuddin et al., 2011; Ikhwanuddin et al., 2014a; 2014b). The general classification of *S. olivacea* is as follow:

| | | |
|---|---|---|
| Kingdom | : | Animalia |
| Phylum | : | Arthropoda |
| Subphylum | : | Crustacea |
| Order | : | Decapoda |
| Family | : | Portunidae |
| Genus | : | *Scylla* |
| Species | : | *Scylla olivacea* (Herbst, 1796) |

### 2.1.2 General morphology

All four mud crabs species display different morphology characteristics of their frontal lobe shape, cheliped carpus spines, carapace size and shape and variations of coloration (Table 2.1). *Scylla olivacea* hallmark characteristics can be defined by its more rounded and low height frontal lobe, its small first cheliped carpus spine and reduced second spine (Keenan et al., 1995; Keenan et al., 1998; Trivedi & Vachhrajani, 2013). Absence of polygonal pattern on its cheliped legs and abdomen are another distinct characters that differentiate *S. olivacea* from other mud crabs species (Figure 2.1, 2.2). The species also uniquely displays shades of reddish to orange claw and brown to drack brown carapace (Ikhwanuddin et al., 2014b; Trivedi & Vachhrajani, 2013).

Table 2.1. General morphology as described by Keenan et al. (1998) for species identification of mud crab genus *Scylla*.

| **Species** | **Frontal lobe spines** | | **Cheliped** | |
|---|---|---|---|---|
| | **Shape** | **Height** | **Carpus spines** | **Propodus spines** |
| *S. serrata* | Blunted point | High | Both obvious | Obvious |
| *S. tranquebarica* | Blunted | Moderate | Both obvious | Obvious |
| *S. paramamosain* | Triangular | Moderately high | Inner absent, outer reduced | Obvious |
| *S. olivacea* | Rounded | Low | Inner absent, outer reduced | Reduced |


Figure 2.1: Variations in the shape of the frontal lobe morphology of mud crab species, genus *Scylla* (a) *S. serrata* (b) *S. tranquebarica* (c) *S. olivacea* (Source: Trivedi & Vachhrajani, 2013).

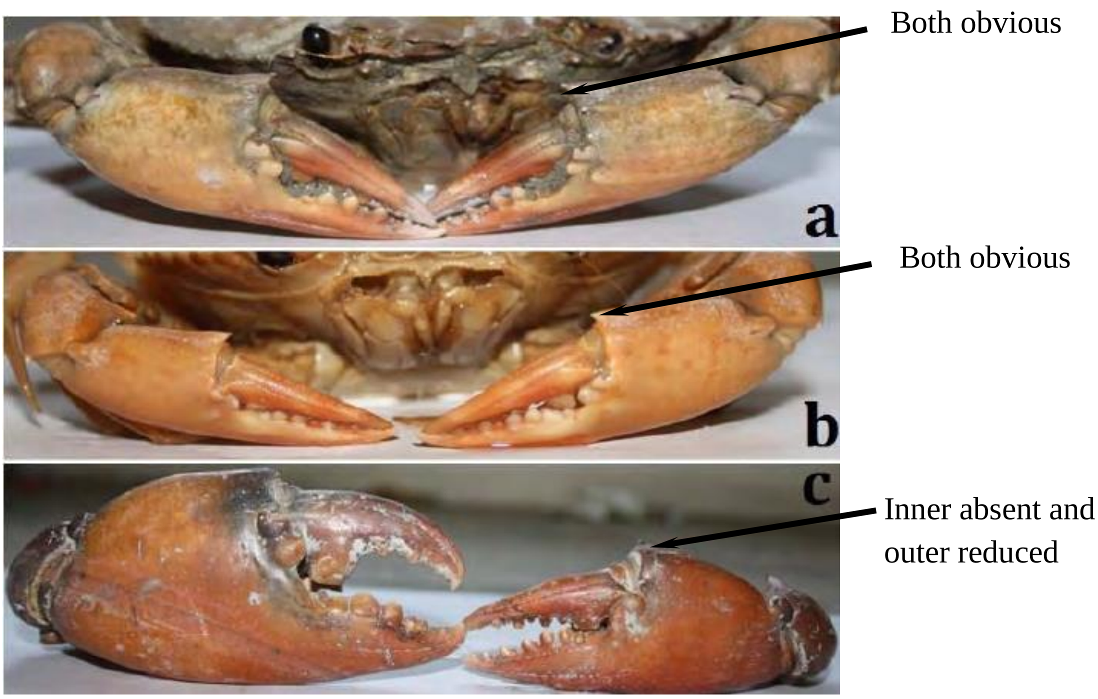


Figure 2.2: Variations of cheliped morphology of mud crab species, genus *Scylla* (a) *S. serrata* (b) *S. tranquebarica* (c) *S. olivacea* (Source: Trivedi & Vachhrajani, 2013).

## 2.2 Habitat and distribution of mud crabs

All mud crab species has a similar general life cycle, interrelating distribution and shares a similar morphological characteristics (Hill, 1994; Tongdee, 2001). Mud crabs normally inhabit mangrove forests and swamps areas. They typically associated with tropical to subtropical estuaries. The mangrove vegetation such as *Rhizophora* sp. provides conducive habitat and sufficient food supply for the mud crabs (Phelan & Grubert, 2007; Shelley & Lovatelli, 2011).

Mud crabs modified their metabolic functions when the salinity and temperature of the environment are in the optimum state for their excretion, respiration and moulting process. The suitable environment would help them maintaining their homeostasis and internal metabolic process (Koolkalya et al., 2006; Jirapunpipat et al., 2007). Mud crabs are known as salt tolerant species where they are able to live not only in freshwater but in hypersaline condition. Therefore, they able to effectively utilise their habitat and able to inhabit in various microhabitat of mangrove areas (Le Vay, 2001; Koolkalya et al., 2006; Le Vay et al., 2007; Lebata et al., 2007).

Mud crab species genus *Scylla* has wide geographical distribution across Indo – Pacific to Indian Oceans (Keenan et al., 1998; Ikhwanuddin et al., 2011; Azmie et al., 2012). Giant mud crab or *S. serrata* is widely distributed across South Africa to south Sydney, Australia waters except in South China Sea (Keenan et al., 1995; Keenan et al., 1998; Ikhwanuddin, 2001). However, *S. olivacea* and *S. tranquebarica* are restricted and have limited distribution within South China Sea and particular area within the Indo – Pacific regions. While *S. paramamosain* is an exclusive species to Java and South China Seas only (Table 2.2) (Chandrasekaran & Natarajan, 1994; Barnes et al., 2002; Macintosh et al., 2002; Moser et al., 2002; Fratini & Vannini, 2002; Moser et al., 2005; Le Vay et al., 2007; Bonine et al., 2008; Ikhwanuddin et al., 2011; Shelley & Lovatelli, 2011).

Table 2.2. Distribution and habitat of mud crab species genus *Scylla* according to Keenan et al. (1998).

| Species | Distributions | Habitat |
|---|---|---|
| *S. serrata* | The most widely distributions (Indian Ocean, Red Sea, Pacific Ocean) | Associated with mangrove full saline oceanic waters but still can tolerate reduce salinity. |
| *S. tranquebarica* | The most widespread species, often associated with *S. olivacea* (South China Sea, Pacific Ocean, India Ocean) | Associated with mangrove waters and coastlines with reduced salinity seawater. |
| *S. paramamosain* | The most abundant species where it occurs (South China Sea, Java Sea) | Associated with various habitats including mangrove forests and estuarine ponds. |
| *S. olivacea* | The moderately distributed, often associated with *S. tranquebarica.* (South China Sea, Indian Ocean, Pacific Ocean) | Associated with mangrove forests and coastlines with reduced salinity during monsoon seasons. |

Mud crabs tend to shed their exoskeleton via moulting events for growing (Mirera & Mtile, 2009; Alberts-Hubatsch et al., 2016). *Scylla serrata* Carapace Width (CW) can grow up to 300 mm and its weight can reach 2.5 kg. While the CW and the weight of *S. olivacea* can only reach up to 150 mm and 1.5 kg, respectively (Grubert & Phelan, 2007). Typical lifespan of mud crab is up to 3 to 4 years (Jirapunpipat et al., 2007; Moser et al., 2005; Alberts-Hubatsch et al., 2016). In general, size of the CW of a juvenile mud crab is about 40 mm wide whereas, the size range of young adult ranged between 90 - 110 mm and the size range for adult ranged between 110 - 200 mm (Shelly & Lovatelli, 2011).

## 2.3 Methods of age determination

For years, there are variety of approaches have been carried out to determine age of marine animals using direct and indirect age method. Age of fishes can be determined directly by using growth ring or band that is retained and deposited in the calcified or hard structures such as bones, spines, scales, vertebrae and otolith (Campana, 2001; Campana et al., 2006; Helfman et al., 2009). For invertebrates, the calcified or hard structure can be the statoliths and shell (Chatzinikolaou & Richardson, 2007; Kilada et al., 2007; Abele et al., 2009).

Recently, a new development in crustacean ageing studies where growth band were found to still remain deposited and retained in calcified structure of crustaceans even after moulting events (Leland et al., 2011; Kilada et al., 2012; Kilada & Acuña, 2015; Kilada & Ibrahim, 2016; Leland & Bucher, 2017; Kilada & Driscoll, 2017). The deposited bands accumulate in daily, monthly and annually basis, therefore could be used in predicting age over time (Campana, 2001; Matta & Kimura, 2012; Zavorka et al., 2014).

Alternative approaches via indirect age method could be applied to determine age of crustaceans. Some of the methods include the monitoring rear animals over period of time and by recording the moulting events of crustaceans (Sainte-Marie et al., 1995; Hébert et al., 2002), mark – recapture of specimens using Passive Integrated Transponder (PIT) tags or chemical tagging such as oxytetracycline (OTC) (Treble et al., 2005; Horká et al., 2010; Zavorka et al., 2014) and length / size frequency distribution analysis of animals (Smith et al., 1997; El-Haweet et al., 2005; Ayele & Ramakhrishna, 2015).

## 2.4 Growth and age studies in crustaceans

Limited studies on ageing crustaceans potentially related to their lack of permanent hard structures because of the moulting events (Bluhm, 2001; Hébert et al., 2002; Kilada et al., 2012). The most common indirect ageing methods of crustacean are by rearing individuals in captivity with provided moulting increments information (Sainte – Marie et al., 1995; Hébert et al., 2002; Leland et al., 2015; Kilada et al., 2017), by recapturing of tagged specimens using streamer tag or PIT tags (Wiles & Guan, 1993; Le Vay et al., 1999; Ikhwanuddin, 2001) and by analysing length frequency distribution data (Enin, 1995; Nandakumar, 2000; Koeller et al., 2006; Choi & Zisserson, 2007).

Another alternative direct ageing method is via the accumulation of lipofuscin in neural tissue. In this method, the lipofuscin pigment that accumulates over time in the eyestalk or brain of crustaceans are used as age marker (Sheehy et al., 1998; Bluhm, 2001; Bluhm & Brey, 2001; Kodama et al., 2006). This method successfully defined the age class of crustaceans by means of the lipofuscin accumulation rate in both tagged specimens or rear animal in the laboratory. Increased amount of lipofuscin concentration showed to positively correlate with age and positive linear relationship in chronological age (Ju et al., 2001; Islam et al., 2007; Islam & Kurokura, 2009). According to Bluhm (2001), the lipofuscin age cohort were applicable to crustaceans with well – defined annual recruitment events. Crustacean’s diet, temperature, individual circumstances and other factors influenced the lipofuscin accumulation, which consequently contributed to difficulties in assessing their age (Wahle et al., 1996; Crowley et al., 2014).

## 2.5 Growth band counts

Recently, a new technique has been developed to assess age of individual crustaceans via deposition of growth band in a calcified structure. The band found to be preserved in a calcified structure called gastric mill. Therefore, the growth bands were used as age marker (Leland et al., 2011; Kilada et al., 2012). Leland et al. (2015) identified the applicability of growth band retentions in the hard structures located in the gastric mill of crustaceans using calcein staining in post – moulting events. The result proved that the growth band were retained after moulting increment in several species of crustaceans such as redclaw crayfish, *Cherax quadricarinatus* (Figure 2.3), powerful crayfish, *Euastacus valentulus*, crab species, *S. serrata* and *Ranina ranina* and Moreton Bay bug lobster, *Thenus orientalis*.

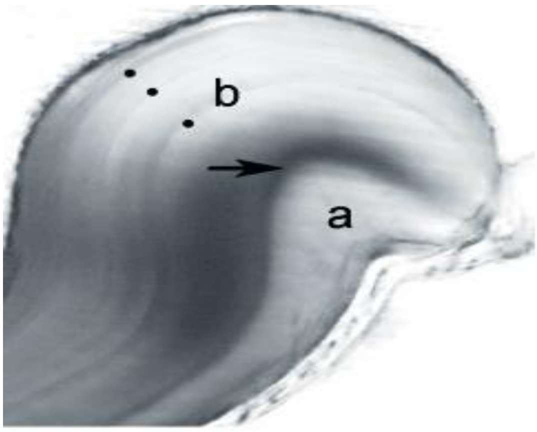


Figure 2.3: The exocuticular (a) and endocuticular (b) layers are divided by the cuticular boundary (indicated by the black arrow) and growth marks (indicated by black dots) were present within endocuticle layer of freshwater redclaw crayfish, *Cherax quadricarinatus* (Adopted from Leland et al., 2015).

The formation of growth band increment in the crustaceans however, is not completely understood (Gronkjaer, 2016; Leland & Bucher 2017). It is well known that crustaceans synthesize other materials such as muscle proteins during the inter-moult period (Grubert et al., 2012). Earlier study by Neville (1965) demonstrated the daily growth band deposition of paired endocuticle (lamellate and non-lamellate layers

– termed "growth layers") in the locusts where the endocuticular growth layer formation in the locust exoskeleton directly corresponded to temperature and light cycles, with experimental manipulation of circadian factors consistently altering normal deposition. A more recent study by Kilada et al. (2012) reported moult-independent of primary growth mark formation in snow crab eyestalks *(Chionoecetes opilio*).

Numerous studies have shown the strong evidence of the growth marks retention in the hard structures of gastric mill and eyestalk of temperate crustaceans species, that it positively correlated to the annually growth events of the crustaceans. Some examples are as in studies done on the American lobster juveniles, *Homarus americanus* (Tang et al., 2015), 4 species of northern Atlantic crustaceans (Kilada et al., 2015), 3 species of Chilean crustaceans (Kilada & Acuña, 2015), freshwater redclaw crayfish, *Cherax quadricarinatus* (Leland et al., 2015) and Antarctic krill, *Euphausia superba* (Kilada et al., 2017). However, not many ageing studies focused on the subtropical and tropical crustacean species except the blue swimming crab, *Portunus pelagicus* (Kilada & Ibrahim, 2016). Therefore, the intensive and comprehensive studies are needed to assess data on absolute age of individual crustaceans particularly *S. olivacea* using growth band deposition.

## 2.6 Von Bertalanffy Growth Model (VBGM)

Age and growth data are commonly interpreted in term of mathematical model. The growth rate in fishes can be described in the units of length and weight in body size over time (Silva et al., 2015; Hatakeyama et al., 2016). The age and growth of fishes can be estimated using growth model such as founded Von Bertalanffy growth model (Le Vay, 2001). The equation of Von Bertalanffy growth has been widely applied in fisheries studies. Estimated growth models were made by fitting the von Bertalanffy growth equation to the observed growth data by Von Bertalanffy (1983). The equation of VBGM are as follows:

$$\mathbf{Lt = L_{\infty} \,[1 - e^{-K\,(t - t_0)}]}$$

As crabs grow as width wise, their length is termed the carapace width (CW), so hereafter 'L' or length will be replaced by 'CW', then the VBG equation can be expressed as:

$$\mathbf{CWt = CW_{\infty} \,[1 - exp^{-K\,(t - t_0)}\,]}$$

Where:

CWt = total carapace width at age

$CW_{\infty}$ = mean of asymptotic carapace width predicted by the equation

K = growth coefficient (per year $^{-1}$)

$t_0$ = age of the crab would have at 0 cm CW

The VBGM commonly used to estimate and represent growth of entire life span of marine species (King, 2001) such as in fish (Newman et al., 2000; Essington et al., 2001; Lester et al., 2004), molluscs (Ridgway et al., 2011; Rosioru et al., 2012) and crustaceans (Sara, 2010; Zafar et al., 2006; Viswanathan et al., 2016).

Growth is defined as food intake changes into dietary energy and diverted into growth in size (King, 2001; Rosenlund et al., 2016). Growth in fishes also can be influenced in various factors such as biotic factor including sex (Imsland & Jonassen, 2003), abiotic factor such as water quality, photoperiod and oxygen level (Árnason et al, 2009), age (Von Bertalanffy, 1938) and stocking density (Maceina et al., 2007).

Growth of fishes is commonly expressed in two types of growth, firstly; a determine type of growth where short – lived species mostly inhabit in a warmer region and secondly; indeterminate type of growth which long – lived species inhabit in a colder region (Dutta, 1994; King, 2001, Hatakeyama et al., 2016). For a fast growing species, reaching a size early in life gives advantages, whereas larger size of individual less suffer to predation compared to smaller size individuals. Therefore, larger individuals have high immunity and increased the survival rate (King, 2001; Urban, 2002; Turker, 2006; Hatakeyama et al., 2016).

## 2.7 Age estimation in crab using Von Bertalanffy Growth Model (VBGM)

Studies in estimation of age and growth for crustaceans are rather difficult due to the absence of hard structure after moulting event (Essington et al., 2001; Lester et al., 2004). The limitations of growth parameters in crustaceans made it difficult to describe the growth model (e.g von Bertalanffy growth model, VBGM) because of the period of during moult and post – moult events. The complex sigmoid growth curve is suitable to describe the growth of crustaceans, which is in contrast to fish species, where growth of fish increasing continuously towards an asymptotic size (Ehrhardt, 2008; Moksnes et al., 2015).

Few studies have been attempted to fit the von Bertalanffy growth function for age and growth determination of crustaceans especially on mud crab species. Sara (2010) carried out a study on the growth parameters of *S. serrata* from Lawele Bay, southeast Sulawesi, Indonesia with the asymptotic CW, $CW_\infty$ = 21.14 cm, K values = 1.38 year $^{-1}$ for male and $CW_\infty$ = 21.02 cm, K values = 0.83 year $^{-1}$ for female. Based on the findings, author suggested that the population of *S. serrata* in Indonesia is under exploitation. Le Vay et al. (2007) described natural growth of *S. paramamosain* in Vietnam with $L_{inf}$ (150 mm) and K values (2.39 year $^{-1}$) via mark recapture technique. Recently, Viswanathan et al. (2016) studied the growth parameters of wild *S. olivacea* from Pichavaram mangroves, southeast India with $CW_\infty$, K and $t_0$ derived for males and females were 148.05 mm, 0.76 year $^{-1}$ and – 0.63 and 138.80 mm, 0.85 year $^{-1}$ and – 0.68, respectively.

# CHAPTER 3

# METHODOLOGY

## 3.1 Biosampling

### 3.1.1 Sampling site

Field sampling was carried out at Setiu Wetlands (5°40'47.93"N 102°43'45.04"E), Peninsular Malaysia, Malaysia (Figure 3.1) from February to October 2016. Setiu Wetland is located on the east coast of Peninsular Malaysia and southern part of South China Sea. It comprises several ecosystems such as sea, beach, mudflat, lagoon, river, estuary and mangrove forest which provide diversity and high utilization of natural resources (Nakisah & Fauziah, 2003; Suratman et al., 2014).

Mangrove forests in Setiu Wetlands consist of mangroves plants such as *Rhizophora* sp., *Avicennia* sp., and *Nypa frutican* along the coastlines. Therefore, the suitability of ecological functions provide optimum condition as nursery grounds for marine species, coastline protections and providing natural sources for local community (Mohd Azmi, 2014). The sediment of Setiu Wetland that comprised of high silt and clay provides suitable habitat for mud crab species (Ikhwanuddin et al., 2014a; 2014b).

Setiu Wetlands have become one of the major site for mariculture activities such as brackish water cage culture, pen culture, oyster culture and pond culture. Thus, Setiu Wetlands also known as active fishing sites and comprising several of aquaculture activities (Nakisah et al., 2008). Therefore due to its diversity, richness and also high demand of mud crab aquaculture, Setiu Wetlands, Peninsular Malaysia have been chosen as sampling site for this current study.

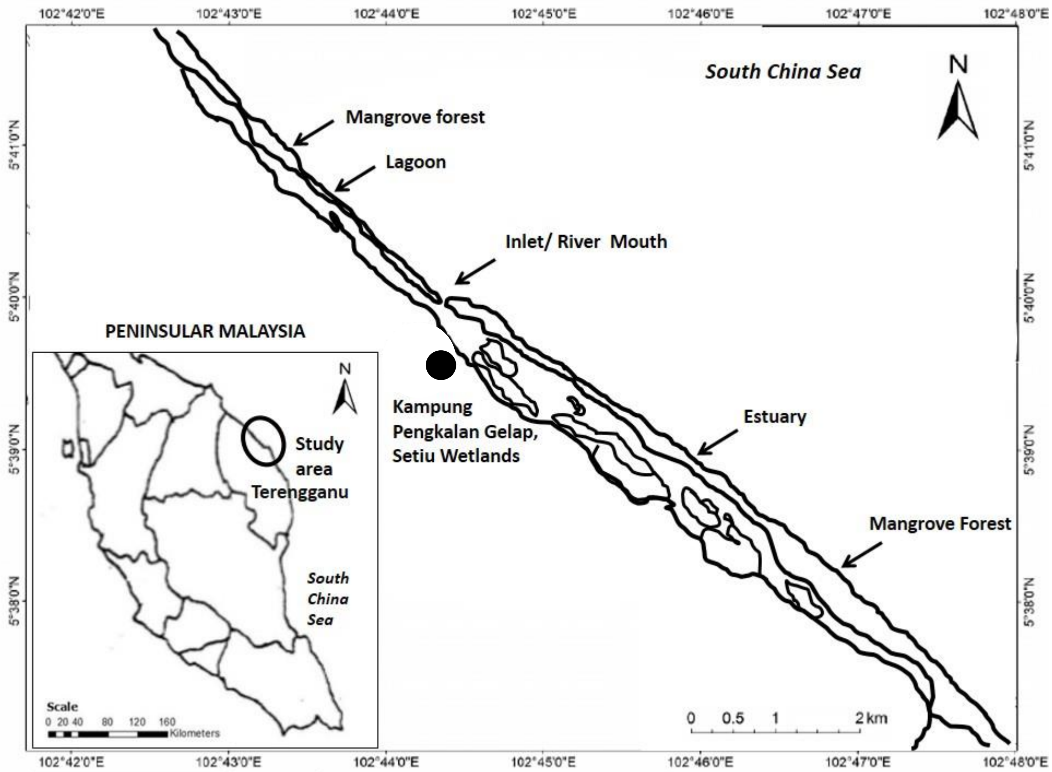


Figure 3.1: Sampling site of *S. olivacea* along Setiu Wetlands, Peninsular Malaysia, Malaysia.

### 3.1.2 Crab samples and identifications

Orange mud crab, *S. olivacea* (Figure 3.2a) was selected for this study due to its abundance and dominance in Setiu Wetlands compared to other mud crab species (Ikhwanuddin et al., 2014a; 2014b; Ihwan et al., 2014). *Scylla olivacea* can be identified by its own morphological characteristics including rounded shape and a low height frontal lobe spine, absence of carpus spine at the first spine and reduced at the second spine and also have reduced propodus spine (Figure 3.2b). In addition, there are also absence of polygonal pattern on cheliped, legs and abdomen of *S. olivacea.* The species also displayed varying colouration such as black or red depending on their habitat. The morphological description of *S. olivacea* was done based on Keenan et al. (1998).

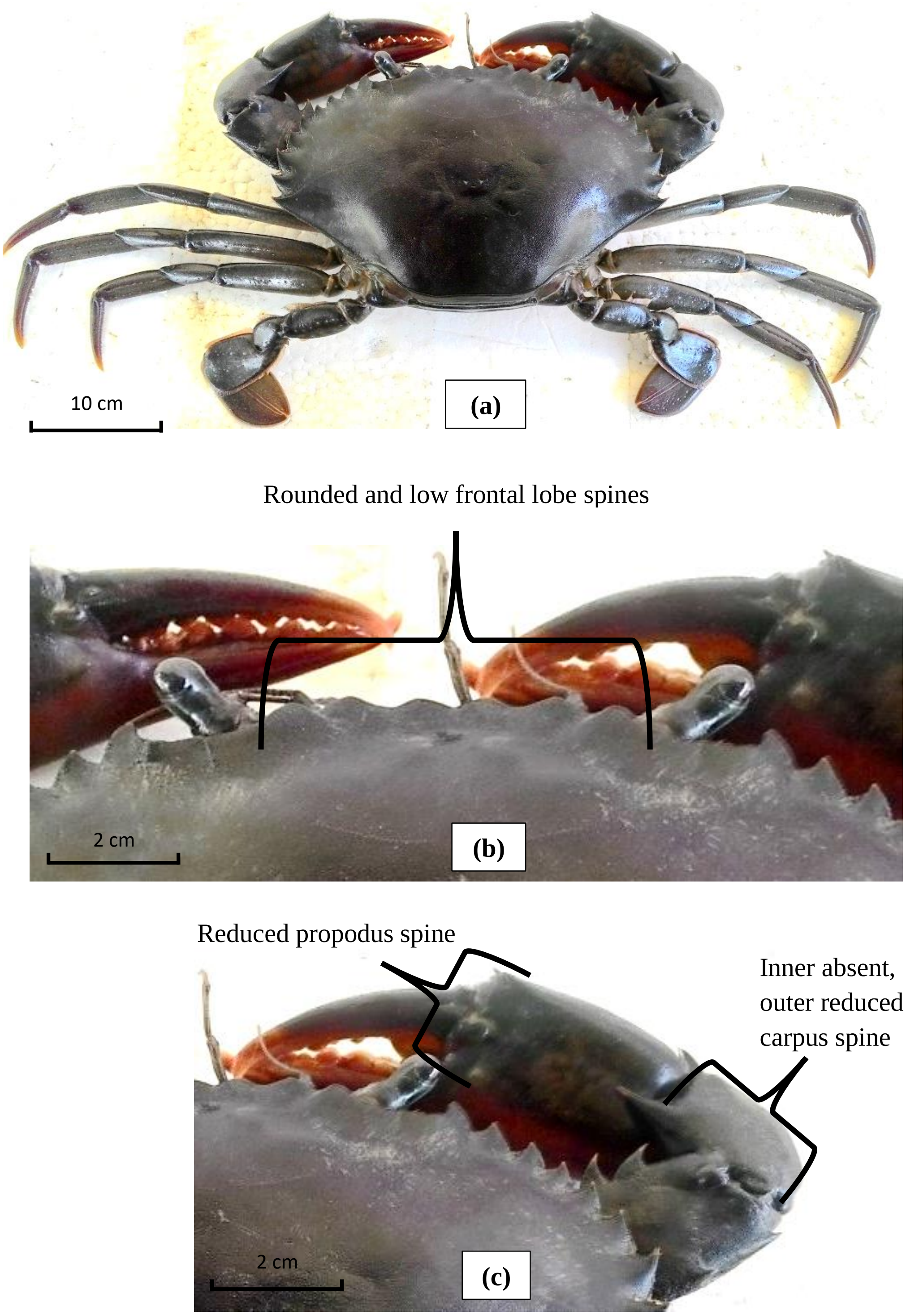


Figure 3.2: (a) *Scylla olivacea*, selected mud crab species for current study, (b) Frontal lobe, (c) Cheliped.

The sexes of mud crab were identified based on the shape of the abdomen. The female crab obtained wider, round and globular abdomen. An immature female crab has a small and pale abdominal flap (Figure 3.3a), while a matured female crab has a large round pigmented abdominal flap with more darkened colour (Figure 3.3b). A male mud crab has a narrow, straight and triangle shaped abdomen flap (Figure 3.3c) (Keenan et al., 1998).

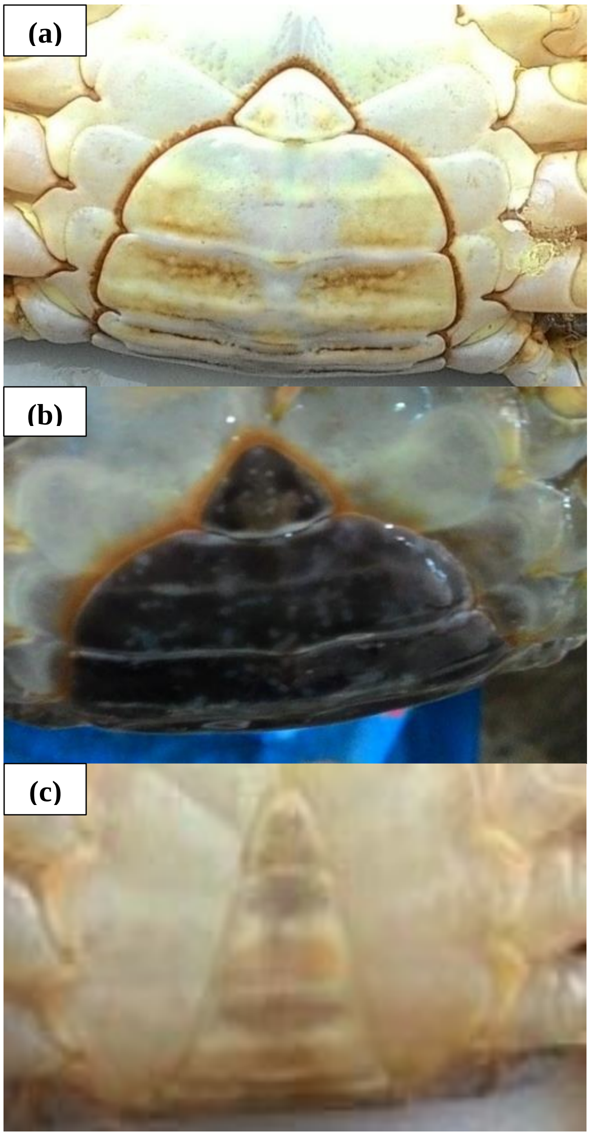


Figure 3.3: The differences of female and male of *S. olivacea* from Setiu Wetlands, Peninsular Malaysia. (a) Immature female *S. olivacea* with small and pale abdominal flap, (b) matured female *S. olivacea* with large round pigmented abdominal flap and with more darkened colour while (c) matured male with a narrow, straight and triangle shaped abdomen flap.

### 3.1.3 Sampling methods

*Scylla olivacea* samples were collected using a rectangular shaped collapsible trap or 'bubu' designed rectangular shaped with steel frame (16 x 50 x 30 cm) covered with 25 mm nets and entrance funnels length 15 cm at both ends (Figure 3.4). These traps were set up overnight for 12 hours underwater and were placed during low tide period and collected after subsequent high tide in the next day (Appendix 1). The traps were set up with bait (placed in the middle) such as chopped trash fish and raw chicken head.

Figure 3.4: Rectangular collapsible trap or 'bubu' with bait either chicken head or chopped fish were used to trap mud crab in Setiu Wetlands, Peninsular Malaysia, Malaysia.

*Scylla olivacea* Carapace Width (CW) and Body Weight (BW) were measured by using digital vernier caliper (Kern Micrometer digital caliper model, Germany) and digital electronic balance. The CW is the distance between the tips of the $9^{th}$ antero – lateral spines of the carapace (Figure 3.5a).The BW was measured with sensitivity to 0.1 grams using digital electronic balance (Shimadzu model, Japan) (Figure 3.5b).

### 3.1.4 Preparation of wild caught *S. olivacea* for growth band examination

During sampling period, 90 of wild *S. olivacea* that were caught from Setiu Wetlands were used in this study. The CW of wild caught *S. olivacea* ranged between 6.00 to 13.40 cm and BW ranged between 55.60 to 565.12 g. All samples were brought back to the Marine Hatchery of Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu (UMT) for quarantine. Then, all samples were dissected and underwent preparation and examination for growth band at Fisheries Science Laboratory, School of Fisheries Science and Aquaculture, UMT. The procedures of preparation and examination of growth band deposition can be referred in the sub - chapter 3.3.

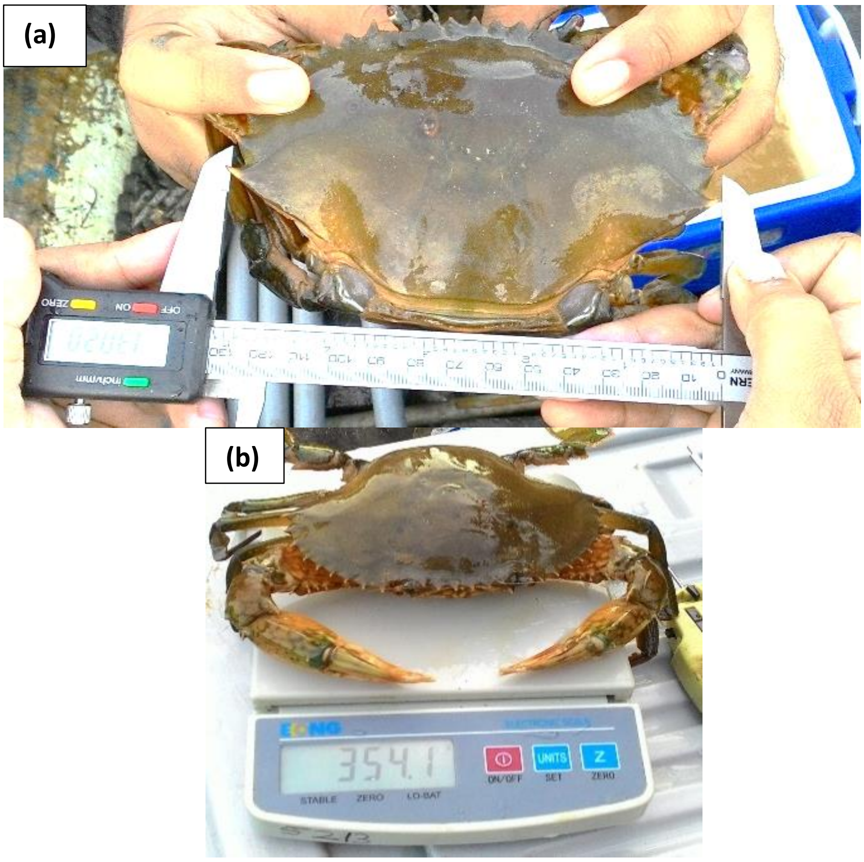


Figure 3.5: Measurement for *S. olivacea*. (a) Carapace Width, CW (cm) – is the distance between 9$^{th}$ tips of antero - lateral spines were measured using vernier caliper, (b) Body weight, BW (g) of *S. olivacea* were measured using digital electronic balance.

## 3.2 Preparation of captive *S. olivacea* for growth band examination

### 3.2.1 *S. olivacea* reared in captivity

#### 3.2.1.1 Broodstock management

The adult of *S. olivacea* from Setiu Wetlands and was quarantined for seven days to reduce the risk of pathogenic affection and poor hygiene during handling. After the quarantine, the female mud crab was subjected to eyestalk ablation procedure. The eyestalk ablation technique on broodstock crustacean is crucial to induce spawning and produced the berried female mud crab (Quinitio et al., 2001; Khazraeenia & Khazraiinia, 2009). The broodstock of *S. olivacea* was held in a fiberglass tank (320 cm x 138 cm x 60 cm) equipped with sand substrate and shelters (Appendix 2). The tank was covered by a black nylon net to minimize disturbance and to avoid physical contamination from insects and also to assist in temperature control.

The broodstock was fed with fresh diet of mixed seafood such as chopped raw mitre squid, *Loligo chinensis*, blood cockle, *Tegillarca granosa*, marsh clam or 'lokan', *Polymesoda expansa*, mud clam or 'kepah', *Polymesoda erosa* and chopped yellowstripe scad fish, *Selaroides leptolepis.* The crabs was fed twice daily in the morning (0900 hrs) and in the afternoon (1700 hrs) at 10% BW. The uneaten feed and waste material was removed regularly. The recirculating system was installed with continuously water quality maintenance. The water salinity in the broodstock tanks were maintained at 28 – 33 ppt at temperature of 25 – 32 °C. Once the matured female *S. olivacea* has carried egg mass, the berried female was put individually in 1.5 $m^3$ fiberglass tank with aerated seawater until the eggs hatched.

#### 3.2.1.2 Larvae rearing

The larvae of *S. olivacea* were reared from March 2016 until March 2017 (1 year culture period). Also, known age data were gathered for each of the individuals. Newly hatched *S. olivacea* larvae were transferred into larva rearing tanks (1.5 $m^3$ fiberglass tank). The zoea was carefully collected using siphon with a basin for subsampling and counting. The counted samples were then allocated to rearing tanks. The counting process was difficult due to the aggregating behaviour of zoea that caused bad estimation of the population.

Stocking density for *S. olivacea* zoea larvae was kept in a 1.5 $m^3$ (1,500 litre) rearing fiberglass tank that comprised of 90 - 110 individuals per litre. The water salinity in the larvae tanks was maintained within range 27 – 31 ppt and optimal temperature range 25 – 32 °C. The rearing tank was covered with plastic sheet to avoid temperature fluctuation. In addition, a sleeved water heater was also installed to maintain the water temperature. The aeration devices were supplied at mild aeration to keep larvae in the water column (Appendix 3).

*Scylla olivacea* larvae were fed with main live feed depending on their life stage. *Scylla olivacea* larvae were fed with rotifers at early zoea stage (z1 – z2) and artemia at z2 to z3 and through the megalopa stage to settlement. During larvae rearing, microalgae were also added in the larva rearing tank to improve water quality. *Scylla olivacea* larvae were fed 2 times a day (0900 and 1700 hrs) and waste of the live feeds were removed before adding the next feed.

*Scylla olivacea* larvae stage (z1 – megalopa) were reared within 12 to 20 days. Then, *S. olivacea* larvae at megalopa stage were harvested and transferred to another rearing tank. The transfer was done to reduce chances for cannibalism. *Scylla olivacea* larvae at megalopa stage developed into crablet between 10 to 14 days. Then, the crablets were collected and transferred into individual container for grow out process. The broodstock management and larvae rearing were carried out by referring method of Quinitio et al. (2001), Mann and Paterson (2004) and Shelly and Lovatelli (2011).

#### 3.2.1.3 Grow out

The crablets were counted and transferred into rectangular plastic containers (18.7cm x 15.6cm x 4.0cm) with 20 partitions (Figure 3.6a), covered with net and were placed in the fiberglass tank (320cm x 138cm x 60cm) at day 35. The CW and BW of *S. olivacea* crablets were recorded for individual allocation in plastic container. Size of most of the crablet ranged from 1.00 – 2.00 cm CW and were placed individually in the partition (4cm x 38cm). After moulting and when the size of crabs reached approximately 2.10 cm CW, each of the crab was labelled and placed in a rectangular plastic container (25cm x 19cm x 15cm) (Figure 3.6b). All crabs were held individually to avoid cannibalism and to increase the survival rate (Figure 3.6c; Appendix 4).

During grow out process, the fiberglass grow out tank was equipped with recirculating seawater system, aeration system and sleeved water heater. The fiberglass grow out tank was covered with black nylon net to reduce temperature fluctuation and physical contaminations. The water salinity and temperature in grow out tanks were maintained at 28 – 32 ppt and optimal range of temperature at 25 – 32 °C. The seawater in the tank were replaced 100% for every 2 days.

Water parameters in grow out tank such as dissolved oxygen, temperature and salinity were determined using a master refractometer (ATAGO, Japan) and YSI 556 multi probe meter (Appendix 5). Crabs were fed with raw mixed seafood such as chopped raw mitre squid, *Loligo chinensis* and chopped yellowstripe scad fish, *Selaroides leptolepis* during experiment at 10% BW and were given daily at 0900 hrs and 1700 hrs. The unconsumed food and waste material were removed to avoid contamination to the water body. The water and feeding management for grow out process were done by following the method of Quinitio et al. (2001) and Shelly and Lovatelli (2011).

During 1 year rearing period (March 2016 – March 2017), a preliminary experiment was done to identify the availability and visibility of growth band at different experimental age (8 and 10 months old) and remaining samples at age of 12 months old. In total, 25 *S. olivacea* were reared during the 1 year rearing experiment. Crabs of different size were weighted using digital electronic balance and the CW were measured using vernier caliper (Appendix 6). Afterwards, all the crabs were prepared for growth band examination at Fisheries Science Laboratory, School of Fisheries Science and Aquaculture, UMT (referred to sub-chapter 3.3). Figure 3.7 shows the tanks set up in the present study.

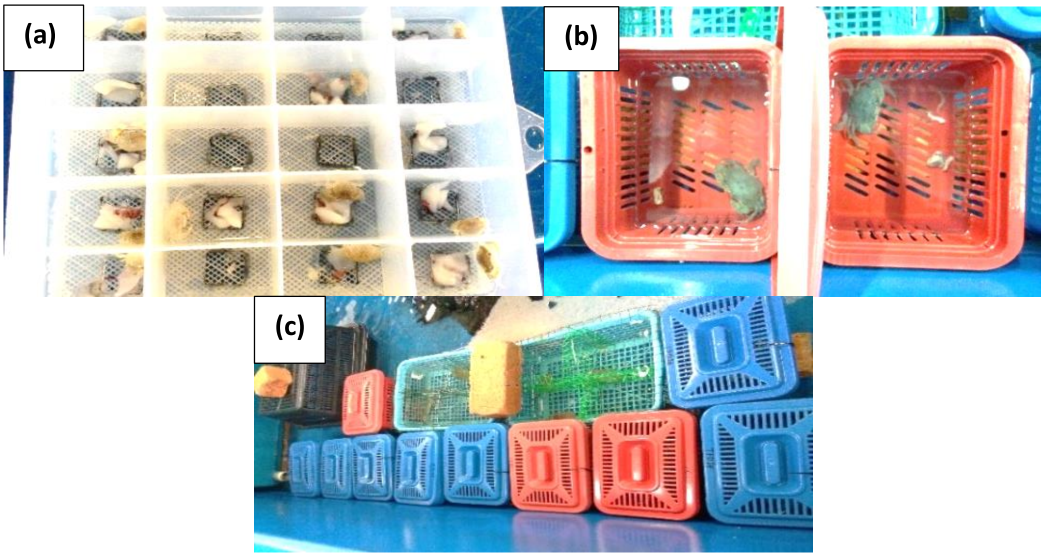


Figure 3.6: (a) Rectangular plastic container (18.7cm x 15.6cm x 4.0cm) with 20 partitions with crablet size 1.0 cm to 2.0 cm, (b) Rectangular plastic container (25cm x 19cm x 15cm) with crabs size (>2.0cm), (c) Crabs were placed individually labelled in fiberglass tank (320 cm x 138 cm x 60 cm).

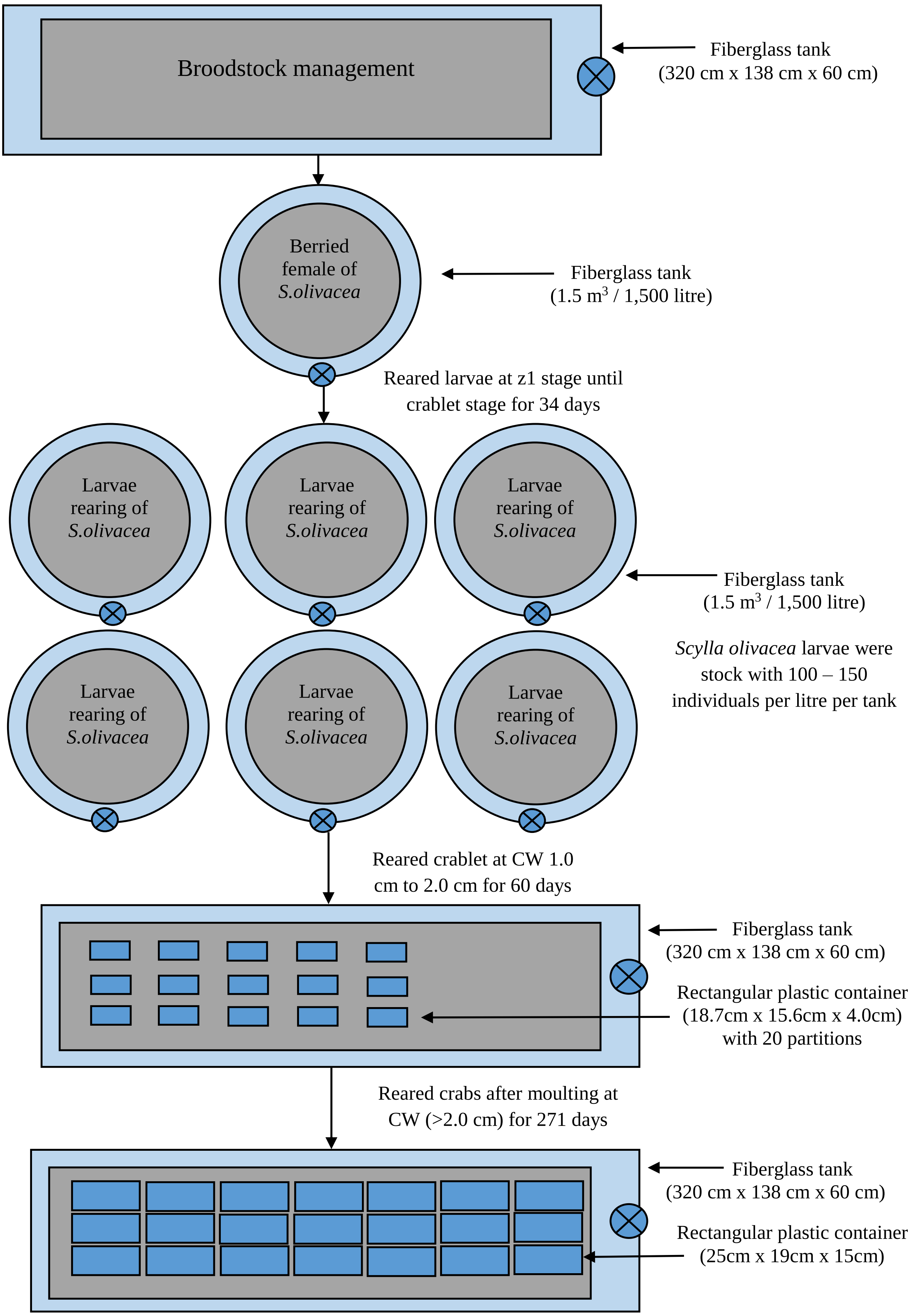


Figure 3.7: Tanks setup in the present study.

## 3.3 Preparation and examination of growth band

During the sampling period, a total of 90 wild crabs (6.00- 13.40 cm CW) were caught and 25 crabs (3.30 – 10.63 cm CW) from reared experiment. Collected crabs from the wild and reared in hatchery were used for growth band preparation and examination. This study has a different samples size of wild caught and captive rearing of known age individuals. For the wild *S. olivacea*, only 90 samples were available during sampling period. Hence, the sample size of wild *S. olivacea* was 90. However, the samples size still cater the mentioned size ranged for CW. For captive rearing known age individuals, the sample size of *S. olivacea* was limited due to the low survival rate. Also it is difficult to maintain the live specimens under unnatural and controlled conditions during 1 year rearing period.

### 3.3.1 Gastric mill extraction

All the anterior carapace of the dissected *S. olivacea* was removed and exposed their cardiac stomach. The gastric mill were extracted out from cardiac stomach and placed in preservation solution (70% glycerol, 4% ethanol and 26% water) for 4 days until all soft tissues could be removed under a dissecting microscope.

### 3.3.2 Thin cross sectioning and growth band counting

Gastric mill of the crabs consists of zygocardiac ossicles and mesocardiac ossicles (Figure 3.8a). The ossicles were separated after the cleaning and removing of all of the unwanted soft tissues. The ossicles were then embedded in epoxy resin EpoxiCure® 2 Epoxy System and EpoxiCure® 2 Hardener (Buehler, Illinois Tool Works Inc.) for 2 – 7 days cure time at room temperature (Appendix 7 and 8). The zygocardiac ossicles were prepared for thin transverse cutting while, the mesocardiac ossicles were prepared for thin longitudinal cutting (100 - 300 µm thickness). Both structure were cut using a Buehler IsoMet Precision Cutter (IsoMet® 1000 Precision Cutter, Buehler, Illinois Tool Works Inc.) (Appendix 9 and 10). Figure 3.8b shows the cutting axis for both the transverse and longitudinal cutting of zygocardiac and mesocardiac ossicles.

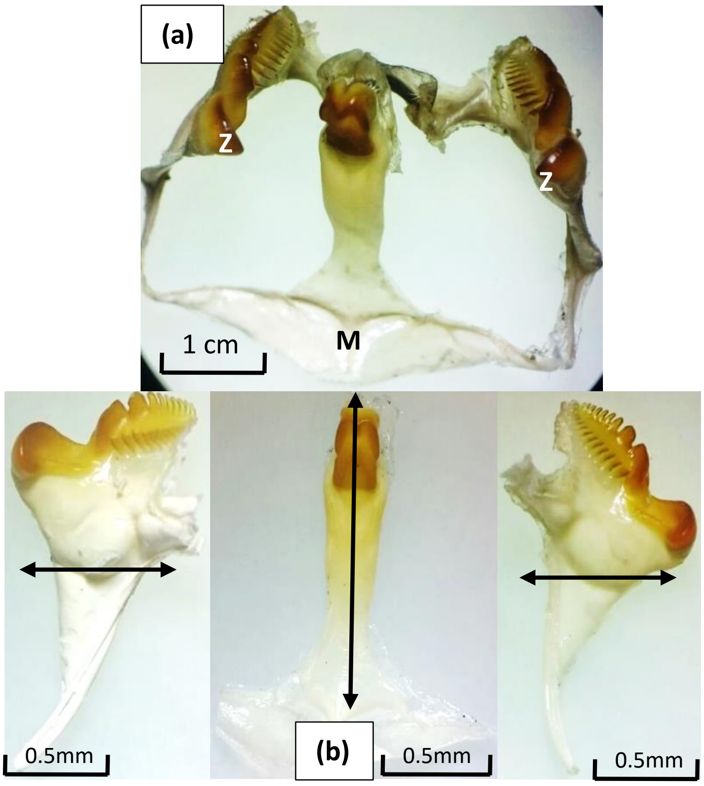


Figure 3.8: (a) The gastric mill structure consists of zygocardiac ossicles (Z) and mesocardiac ossicles (M) of *S. olivacea*, (b) the separated ossicle of zygocardiac and mesocardiac with transverse and longitudinal cutting axis (black line).

The thin section of zygocardiac and mesocardiac ossicles were mounted on a standard microscope slide, polished by hand when necessary using dry 0.3 µm grit lapping film and viewed under Olympus compound microscope (CX41, Olympus Group Companies) at 10x – 40x magnification (Appendix 11). Digital images were taken using DinoCapture 2.0 software version 1.4.3 (BigC Dino-Lite Digital Microscope, AnMo Electronics Corporation) attached to the compound microscope. Images were digitally enhanced using Adobe Photoshop CS6 version 12.0.4 x 64

(Adobe System Incorporated) to increase the contrast between adjacent bands (Appendix 12).

The growth bands were identified as paired (bipartite) light and dark zones in endocuticle. They were counted from the basal (between membranous layer and hypodermis) to the distal region of the endocuticle of the structure. The method on the preparation of the growth band examination was carried out by following the method of Leland et al. (2011) and Kilada et al. (2012). Figure 3.9 shows the growth band identifications in the zygocardiac ossicles after thin cross sectioning.

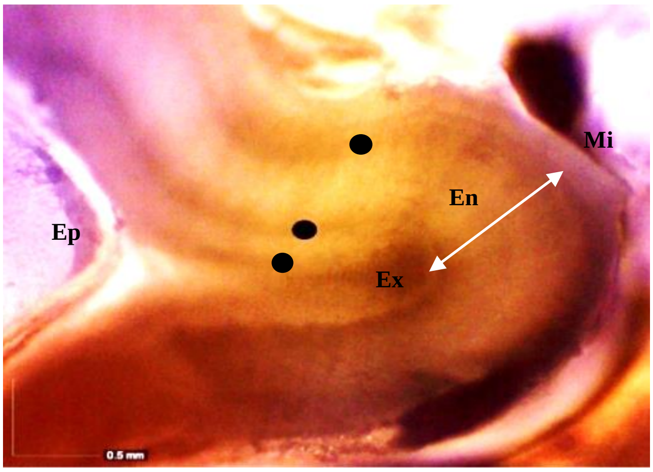


Figure 3.9: The growth band (black dots) can be identified as paired (bipartite) light and dark zones in endocuticle. They were counted from the basal (between membranous layer and hypodermis) to the distal region of the endocuticle of the structure.

### 3.4 Data collections analysis

The length – weight relationship or the CW – BW relationship were determined by employing the Le Cren’s (1951) non – linear equation by least squares method using the logarithmic forms of the exponential equation. The equation of length – weight relationship is as follow:

$$\mathbf{W = a\,L^{b}}$$

Where:

W = Body Weight; BW (g)

L = length or Carapace Width; CW (cm)

a = is the intercept of the regression curve

b = the regression coefficient

The observed values of length or CW and BW were transformed into logarithmic values and were expressed as regressions equation of Log BW = a + b Log CW. The regression relationship and Student t-test was calculated with the aid of Microsoft Excel (MS – Excel 2013) and SPSS version 22 software (IBM® SPSS® Statistics V22.0). The Student t-test was performed to test the differences in the regression relationship between sexes of *S. olivacea.*

The relationship between CW, BW, GBC and Age relationship were calculated using the equation as follow:

$$\mathbf{y = a\,X^{b}}$$

Where:

y = dependent variable

X = independent variable

a and b = model parameters

Values of the exponent b provide information on fish growth. Calculated b value of 3 were defined as being in the isometric range (b = 3) while b < 3 was defined as negative allometry and b > 3 as positive allometry (Ricker, 1973; Silva et al., 2015; Kilada & Ibrahim, 2016).

In the present study, the reliable age information was based on the growth band examination data and the precision of band count were analyzed using coefficient of variation (CV) by Campana (2001). The bands were counted independently by two readers or two persons to assess the accuracy of the band counts, minimizing the bias of the counting and reproduced the reproducibility of repeated measurements. The equation of CV as follows:

$$\mathbf{CV}_j = \mathbf{100\%\ x} \left( \frac{\sqrt{\sum_{i=1}^{R} (X i\, j - \overline{X}\, j)^2}}{\overline{X}\, j} \right)$$

Where:

$CV_j$ = the age precision estimate for the $j$ th individual. The CV values of all individuals are averaged across the whole sample to produce a mean CV.

$X_{ij}$ = represents the $i$ th age of the $j$ th individual

$\overline{X}$ = represents the average age of the $j^{th}$ individuals

In order to identify the growth parameters of *S. olivacea* from wild caught and those reared in hatchery, the CW at age data based on growth band data were fitted to the Von Bertalanffy Growth Model (VBGM). The growth parameter such as $L_\infty$, K and $t_0$ value were estimated by Von Bertalanffy Growth (VBG) equation (Von

Bertalanffy, 1983) based on CW at age data. The explanation of the VBGM equation as referred to the subchapter 2.6.

The growth parameters such as $CW_\infty$, K and $t_0$ values were estimated by VBGM equation based on CW – Growth Band Counts data (GBC). Then, the growth curve were estimated and interpreted based on VBGM equation. The interpretation of growth curve using Microsoft Excel 2013 and the growth parameters were obtained from SPSS version 22 software (IBM® SPSS® Statistics V22.0).

### 3.5 Experimental design

Generally, the experiment was done to determine the age and growth of *S. olivacea* from Setiu Wetlands, Peninsular Malaysia using GBC data as age indicator. During sampling period, a total of 90 wild *S. olivacea* (6.00 – 13.40 cm CW) were caught from Setiu Wetlands, Peninsular Malaysia from February 2016 until October 2016 and 25 of *S. olivacea* (3.30 – 10.63 cm CW) were reared for 1 years from March 2016 to March 2017 in the AKUATROP's Marine Hatchery, UMT.

The wild caught of *S. olivacea* provide unknown age information and reared *S. olivacea* provide known age information as age validation method (1 year rearing period). The age validation of *S. olivacea* began from the larvae stage and reared until they reached young adult stage. During age validation experiment, the preliminary experiment of growth band examination was done at age of 8 months old (n=4) and 10 months old (n=4) in order to identify the presentable of growth band in *S. olivacea* when age of *S. olivacea* below 1 year old. The selected samples for preliminary experiment were based in their minimum size of CW at that time. The remaining reared *S. olivacea* (n=17) were retained until they reached the age of 12 months old.

All the wild caught crabs and reared samples were measured based on CW, BW and sexes according to Keenan et al. (1998). Then, all samples were prepared for growth band examination at Fisheries Science Laboratory, School of Fisheries Science and Aquaculture, UMT. The growth bands were identified according to Leland et al. (2011) and Kilada et al. (2012). The growth bands were counted, recorded and analysed in order to assess the age and the growth estimation of both the wild caught and reared *S. olivacea*.

The Growth Band Count (GBC) data and size at age data from wild caught and reared samples were assessed to determine age and growth of *S. olivacea*. The accuracy of band count were calculated using CV formula. The CW, BW and sexes were described by means of the size frequency distribution and identified correlation between CW, BW, sexes and GBC data through linear relationship. The growth parameters and VBGM were applied based on GBC data to estimate the age and growth of *S. olivacea* in Setiu Wetlands. Work-flow and experimental design of the present study are as in Figure 3.10.

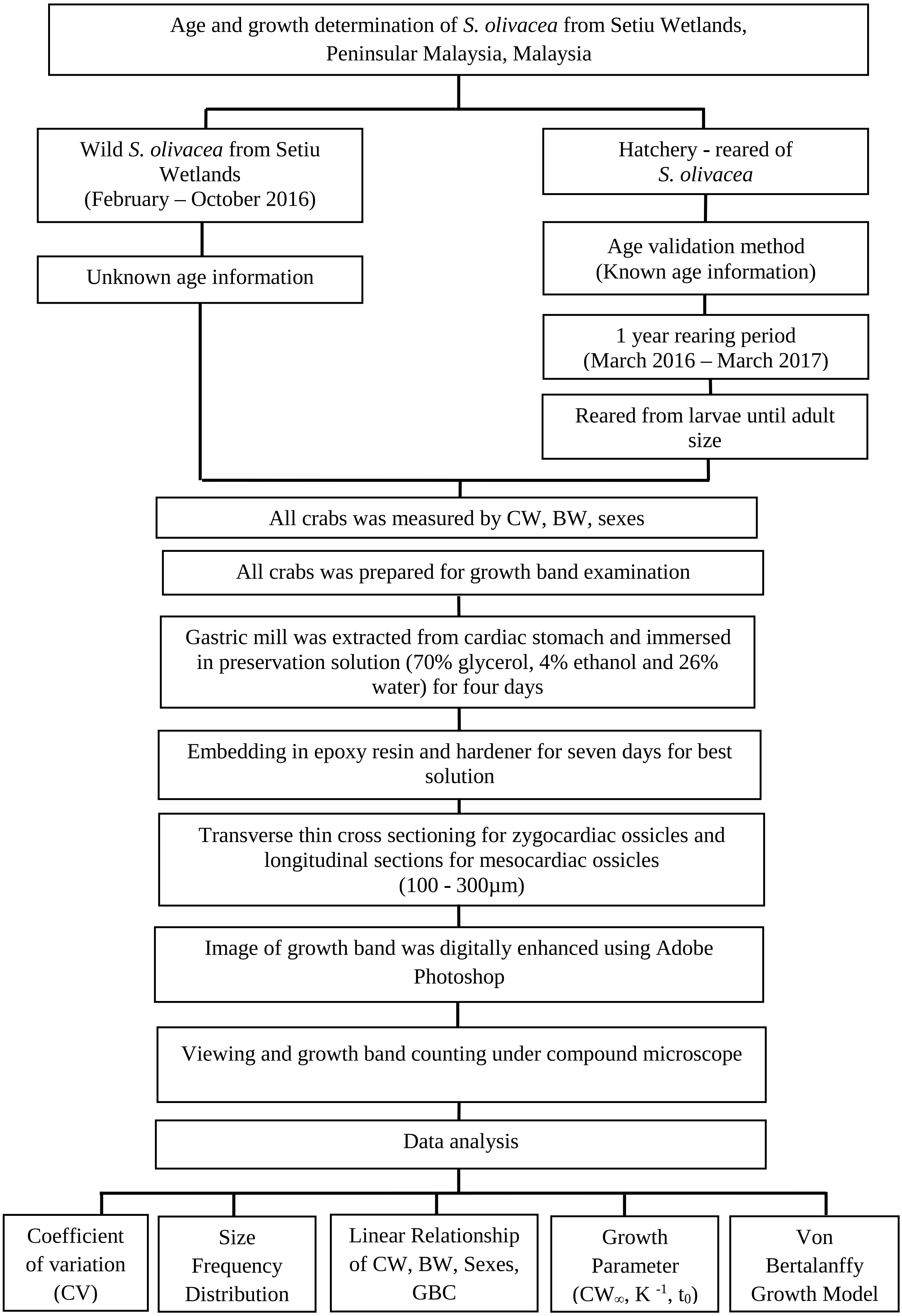

Figure 3.10: Work-flow and experimental design of the present study.

# CHAPTER 4

# RESULTS

## 4.1 Validation age data from *S. olivacea* in captivity

### 4.1.1 Analysis of size frequency distribution

A total of 25 individuals of *S. olivacea* were successfully reared during the 1 year cultured period from March 2016 to March 2017. The reared *S. olivacea* was comprised of females with 48% (n = 12) and males with 52% (n = 13) of the total individuals. The minimum, maximum, mean of the CW are shown in Table 4.1. The mean CW ± SD of female was 4.80 ± 0.70 cm and male was 6.03 ± 1.86 cm. The mean BW ± SD of female was 27.92 ± 15.28 g and male 48.58 ± 51.19 g.

Table 4.1. The mean carapace width (CW) and body weight (BW) of reared *S. olivacea* (n = 25) at AKUATROP's Marine Hatchery UMT.

| Parameter | Carapace Width (cm) | | Body Weight (g) | |
|---|---|---|---|---|
| | **Female** | **Male** | **Female** | **Male** |
| N | 12 | 13 | 12 | 13 |
| Range | 3.30 - 5.94 | 3.32 - 10.63 | 5.70 - 61.50 | 5.60 - 202.80 |
| Mean ± SD | 4.80 ± 0.70 | 6.03 ± 1.86 | 27.92 ± 15.28 | 48.58 ± 51.19 |

The size distribution of the CW of overall reared *S. olivacea* ranged between 3.30 - 10.63 cm CW and the mode ranged between 4.00 - 4.90 cm CW. The size distribution of female *S. olivacea* was ranged between 3.30 - 5.94 cm CW with mean 4.80 ± 0.70 cm CW. The CW size range of male *S. olivacea* ranged between 3.32 - 10.63 cm with mean 6.03 ± 1.86 cm. The BW of female *S. olivacea* ranged from 5.70

to 61.50 g and 5.60 to 202.80 g for male *S. olivacea*. (Figure 4.1). Male *S. olivacea* were heavier in BW and bigger in CW compared to female *S. olivacea*.

There was a significant different in the size of CW of the crabs between sexes, where females *S. olivacea* (4.80 ± 0.70 cm CW) and males *S. olivacea* (6.03 ± 1.86 cm CW), ($t\ (23) = 2.15$, $\rho = 0.04$). Contrarily to the CW size, the BW between females (27.92 ± 15.28 g) and males (48.58 ± 51.19 g), showed no significant different (Appendix 13a).

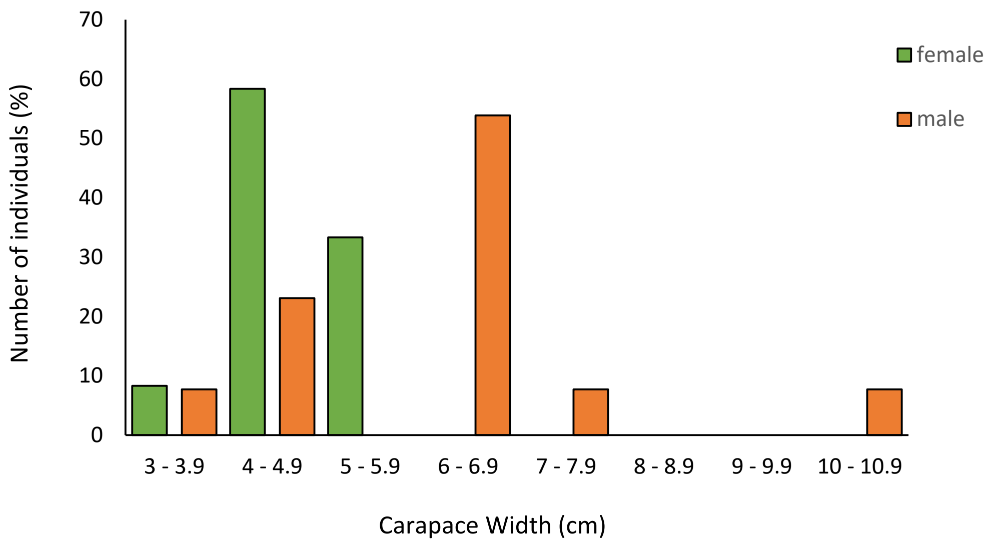


Figure 4.1: The CW frequency distribution of female and male *S. olivacea* reared in captivity for 1 year cultured period (female; n= 12, male; n = 13).

### 4.1.2 The CW and BW relationship of *S. olivacea* reared in captivity and coefficient of variation (CV) values

Relationship between CW and BW data was investigated separately for both the male and female *S. olivacea*. The CW and BW of *S. olivacea* showed to have positively relationship. A linear regression was carried out to find out whether the CW could be used to predict the BW of the reared *S. olivacea*. A significant regression equation was found ($F (1, 10) = 6.58$, $\rho = 0.028$), with $R^2 = 0.61$ and regression equation of female reared *S. olivacea* was log BW = 0.35 + 2.48 (log CW). A significant regression was also found in male reared *S. olivacea* ($F (1, 11) = 8.03$, $\rho = 0.001$, with $R^2 = 0.98$ and regression equation is log BW = 0.71 + 2.93 (log CW) (Table 4.2, Appendix 13b).

Table 4.2. The regression linear information of the carapace width (CW) – body weight (BW) of *S. olivacea* reared in captivity for 1 year culture period (female; n = 12, male; n= 13).

| Sex | Regression Log BW = ***a*** + ***b*** Log CW | $R^2$ | ρ – value |
|---|---|---|---|
| **Female** | Log BW = 0.35+ 2.48 Log CW | 0.61 | 0.028 |
| **Male** | Log BW = 0.71 + 2.93 Log CW | 0.98 | 0.001 |

The coefficient of determination ($R^2$) of log CW – Log BW in females and males showed positive correlation between variables. There was an exponential values (b) of the log CW – log BW relationship of female and male with the values of 2.48 and 2.93, respectively. The relationship did obey the cube law with the values of ‘b’ varied from 2.5 to 3.0. The b values that were closed to 3, indicates the allometric growth for both sexes where the mud crabs grows faster in size than in weight.

### 4.1.3 The CW and Growth Band Count (GBC) of *S. olivacea* reared in captivity

The result showed the presence of growth band in *S. olivacea* reared in captivity. The growth band was presented in the gastric mill structures consisted of both zygorcardiac ossicles (Z) and mesocardiac ossicles (M). The growth band formation can be identified as narrow dark band that was deposited in the broad translucent zone called the endocuticle layer comprising of exocuticle layer. The epicuticle layer is the most outer layer of the ossicles and covered with membranous layer (Figure 4.2). The identification of growth band was carried out by referring to the method of Kilada et al. (2012). The preparation of growth band examination as referred to the subchapter 3.3.

The observation of growth bands for reared samples was done based on the capability to count and to read the growth band. The capability was categorized by good readability under band 1.0, intermediate readability (0.83) band and poor readability (0.66) band. The value of the growth band readability was obtained from a simple mathematical calculation that was done on the growth band counting.

The growth bands were interpreted by having one clear narrow dark band (1.0) deposited in the ossicles while intermediate readability, contained 0.83 band, where the bands was available but unable to be counted as complete narrow dark band. The poor readability, 0.66 band, explained the unavailability of growth band deposited in the gastric ossciles of reared *S. olivacea.* Each of the growth band readability also represented the known age information of reared *S. olivacea,* 1.0 band represented 12 months old (n = 17), 0.83 band represented 10 months old (n = 4) and 0.66 represented

8 months old (n = 4). The uneven numbers of the samples size explained that some of the samples was brittle and fractured during sectioning and cleaning process.

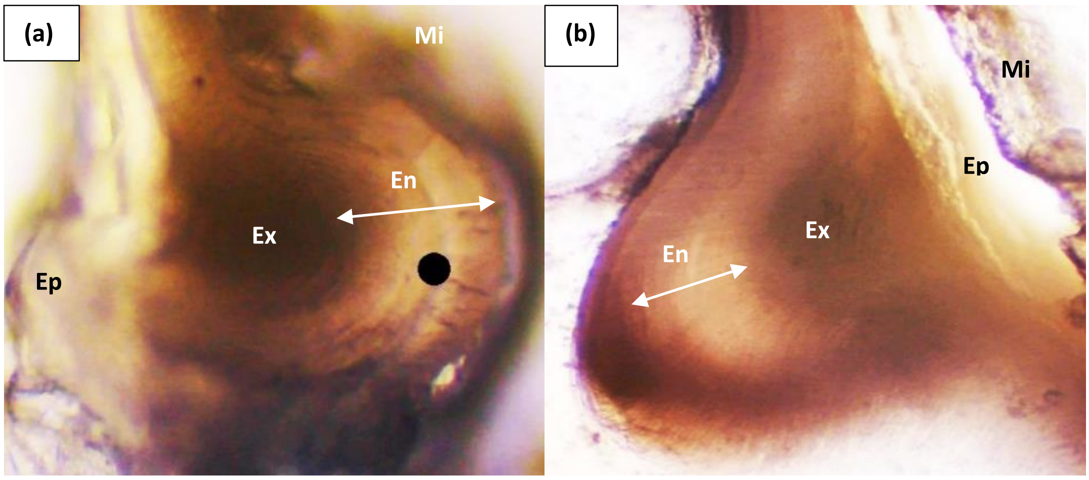


Figure 4.2: Growth band (black dots) shown in transverse sectioned (200 µm) of zygocardiac ossciles in (a) *S. olivacea* reared in captivity with CW 5.34 cm showed one clear primary growth band (1.0) while poor readability (0.66) of growth band in (b) with CW 4.40 cm (b).

All growth bands were counted directly using a compound microscope and data were taken carefully and consistently by two independent readers to minimize biases in the counting procedure. Counting the growth band in the zygocardiac ossicles was easier due to the visibility and presence of clear primary growth band in the structure compared to band identification in mesocardiac ossicles. The coefficient of variation (CV) was calculated using the CV formula. The CV values assessed the bias of reading and counting the growth band by the two independent readers. Further estimation linear regression of CW, GBC and CV values is shown in Table 4.3.

Table 4.3. The equation describing the CW and GBC relationship of *S. olivacea* reared in captivity for 1 year cultured period and the CV values for female and male *S. olivacea.*

| **Sex** | **N** | **CW = *a* x growth band count *b*** | **$R^2$** | **Coefficient of variation, CV (%)** | **ρ - value** |
|---|---|---|---|---|---|
| Female | 12 | CW = 5.15 GBC $^{0.81}$ | 0.74 | 6.00 % | 0.001 |
| Male | 13 | CW = 6.70 GBC $^{1.96}$ | 0.69 | 6.23 % | 0.004 |

A positive interaction was displayed in CW and GBC relationship of female and male *S. olivacea* with r = 0.84, $\rho < 0.05$ and r = 0.73, $\rho < 0.05$ respectively. The GBC was increased as the CW increased in both female and male of reared *S. olivacea*. Student t – test showed that there was no significant different in GBC between female (0.91 ± 0.14 GBC) and male (0.92 ± 0.13 GBC) of reared *S. olivacea.* This indicates that the growth band counts were similar in both female and male of reared *S. olivacea* (Appendix 13c).

A linear regression was calculated to predict CW based on GBC. A significant regression equation in female *S. olivacea* was found CW = 5.15 GBC $^{0.81}$, $\rho = 0.008$, with $R^2 = 0.74$. In male reared *S. olivacea,* a significant regression was also found with CW = 6.70 GBC $^{1.96}$, $\rho = 0.02$ with $R^2 = 0.69$. This indicates that the changes in the CW were related to changes in the GBC. The CV values of female and males was 6.00 % and 6.23 %, respectively. There was a slightly different CV values between sexes at the percentage of 0.23%. The CV values that showed lower values with less than 10%, demonstrated a good consistency of the band counting or reading.

### 4.1.4 The GBC – known age relationships and the CW – Known age relationships of *S. olivacea* reared in captivity

The GBC – known age relationship were investigated based on the validation data of the reared *S. olivacea.* Presence of growth band showed to positively correlate with the age and size of individuals. The GBC at known age data observed 0.66 growth band at 8 months old, 0.83 growth band at 10 months and 1.0 growth band at 12 months old. Both female and male of the reared *S. olivacea* were at the same known age, with the mean age value of 0.91 ± 0.13 month and 0.92 ± 0.13 month, respectively. This was also further supported by a non – significant different output of the t – test between sexes of the reared *S. olivacea* (Appendix 13d).

GBC showed strongly and positively correlated with the known age of both female and male *S. olivacea* at r = 0.99, $\rho < 0.01$ and r = 0.99, $\rho < 0.01$, respectively. This confirms that as age increased, the GBC also significantly increased. Further estimation of linear regression using power equation for GBC and known age is shown in Table 4.4.

Table 4.4. The power equation describing the GBC – known age (Months) relationship of *S. olivacea* reared in captivity.

| **Sex** | **N** | **GBC = *a* x known age (months) *b*** | **$R^2$** | **ρ – value** |
|---|---|---|---|---|
| Female | 12 | GBC = 0.078 x known age $^{1.024}$ | 0.996 | 0.06 |
| Male | 13 | GBC = 0.079 x known age $^{1.023}$ | 0.996 | 0.06 |

The regression equation of female showed to be significant with GBC = 0.078 x known age $^{1.024}$, $\rho = 0.001$ with high coefficient at $R^2 = 0.99$. Similarly, the regression equation of male also displayed significant regression with GBC = 0.079 x known age $^{1.023}$, $\rho = 0.001$ with high coefficient $R^2 = 0.99$. The changes in the GBC were related to changes in the known age (months) in both female and male of reared *S. olivacea.*

The CW – known age relationship of reared female and male *S. olivacea* were revealed. Student t – test revealed that there was a significant different in the CW between sexes of reared female (4.80 ± 0.70 cm CW) and in the CW of reared male (6.03 ± 1. 86 cm CW) *S. olivacea* with t (23) = 2.15, $\rho = 0.04$, $\rho < 0.05$ (Appendix 13e). For known age, Student t – test showed that there was no significant between sexes of reared female (11.00 ± 1.59 month) and reared male (11.07 ± 1.55 month) of *S. olivacea.*

The correlation of CW and known age relationship were found to be positively significant in female with r = 0.74, $\rho < 0.05$ and in male with r = 0.69, $\rho < 0.05$. There was a significant regression between CW and known age of female with equation CW = 0.64 known age $^{0.83}$, $\rho < 0.05$, $R^2 = 0.70$. Similar in males, there was a significant regression between CW and known age with equation CW = 0.11 known age $^{1.63}$, $\rho < 0.05$, $R^2 = 0.54$ (Table 4.5). The changes in the CW were related to the changes in the known age in both female and male of reared *S. olivacea.*

Table 4.5. The power equation describing the CW (cm) – known age (months) of reared *S. olivacea* in captivity.

| **Sex** | **CW = *a* x known age (months)** $^{b}$ | $R^2$ | **ρ value** |
|---|---|---|---|
| Female | CW = 0.64 x known age $^{0.83}$ | 0.70 | 0.001 |
| Male | CW = 0.11 x known age $^{1.63}$ | 0.54 | 0.001 |

### 4.1.5 Estimation of growth parameters

The results of the CW at age data of reared *S. olivacea* based on GBC data were fitted to the von Bertalanffy growth model. The growth parameters were used to estimate the age of reared *S. olivacea* based on CW at age data. The growth band that was validated annually indicated 1 growth band at the age of 1 year old of *S. olivacea* with average CW of 6.11 cm. Therefore, the CW at age data were used to estimate the growth parameters, such as $CW_\infty$, K and $t_0$. The three growth parameters were derived from inverse linear regression that was plot and fitted using the VBGM equation ($CWt = CW_\infty [1 - \exp^{-K(t - t0)}]$) (Appendix 13f and 13g). The explanation of the VBGM equation can be referred to the subchapter 2.6. Summarized estimated growth parameters of reared *S. olivacea* is shown in Table 4.6.

Table 4.6. Summary of estimated growth parameter of reared *S. olivacea* in captivity for 1 year culture period.

| Growth parameters | Nonlinear regression procedure |
|---|---|
| $CW_{\infty}$ (cm) | 20.075 |
| K year $^{-1}$ | 0.3130 |
| $t_0$ | - 0.202 |

The growth parameters of $CW_{\infty}$, K year $^{-1}$ and $t_0$ values were 20.075 cm CW, 0.313 year $^{-1}$ and - 0.202, respectively. The VBGM estimated the age of 1 year old with CW 5.38 cm and was corresponded to the observed CW at age relationship based on GBC data (the average size of reared *S. olivacea* is 6.11 cm CW at age of 1 year old). The VBGM showed to be the best model to describe the relationship of CW and age of reared *S. olivacea* in captivity (Figure 4.3).

The VBGM successfully predicted the age of reared *S. olivacea* at age of 1 year old to 3 year old in the size range of 5.38 to 12.21 cm. The estimated age of 1 year old CW of 5.38 cm was correlated to the absolute CW – known age data where the size of CW was 6.11 cm. The VBGM further estimated age from 4 year old to 20 year old, where the maximum CW reached to 20.00 cm. The slower growth period began at age of 10 year old to 20 year old at size ranged between 19.00 – 20.00 cm CW, indicating that the growth are getting slower with older age. The growth parameters obtained from VBGM showed the estimation values of age and CW of reared *S. olivacea* could reach to asymptotic size by years.

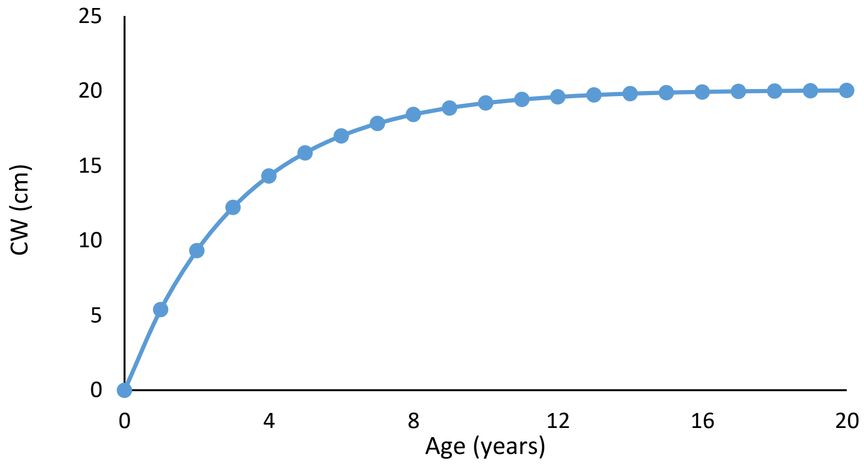


Figure 4.3: Von Bertalanffy growth model using nonlinear regression fitted to CW (cm) at age data of reared *S. olivacea*.

## 4.2 Growth and age estimation of wild caught *S. olivacea* from Setiu Wetlands, Peninsular Malaysia, Malaysia

### 4.2.1 Analysis of size frequency distribution

A total of 39 females and 51 males *S. olivacea,* were caught from Setiu Wetlands, Peninsular Malaysia from February to October 2016. A percentage of 56.7 % of the caught individuals were male, which indicated higher occurrence of male compared to female with only 43.3%. The mean value of female CW ± SD was 10.12 ± 1.85 cm, while the male had slightly smaller CW mean value of 9.32 ± 1.78 cm. Female BW ± SD was at mean value of 201.64 ± 127.46 g, while the mean value of male was 183.01 ± 140.91 g (Table 4.7).

Table 4.7. The Carapace Width (CW) and Body Weight (BW) measurement of wild caught *S. olivacea* from Setiu Wetlands, Peninsular Malaysia.

| Parameter | Carapace Width (cm) | | Body Weight (g) | |
|---|---|---|---|---|
| | Female | Male | Female | Male |
| N | 39 | 51 | 39 | 51 |
| Range | 7.30 -13.40 | 6.55-13.20 | 55.60-532.51 | 57.00-565.12 |
| Mean ± SD | 10.12±1.85 | 9.32±1.78 | 201.64±127.46 | 183.01±140.91 |

The frequency distribution of wild caught *S. olivacea* CW is presented in Figure 4.4. The CW of wild caught *S. olivacea* ranged between 6.55 – 13.40 cm whilst the BW of wild caught *S. olivacea* ranged between 55.60 – 565.12 g. A Student t-test was conducted to compare the mean of CW between females and males of wild caught *S. olivacea*. There was a significant different in the CW between females *S. olivacea* (10.12 ± 1.85 cm) and males *S. olivacea* (9.33 ± 1.78 cm), (t (88) = 2.06, ρ = 0.04, ρ < 0.05). Therefore indicates that the size of wild caught *S. olivacea* CW was different between sexes. The BW of *S. olivacea* however, did not significantly vary between sexes (Appendix 14a).

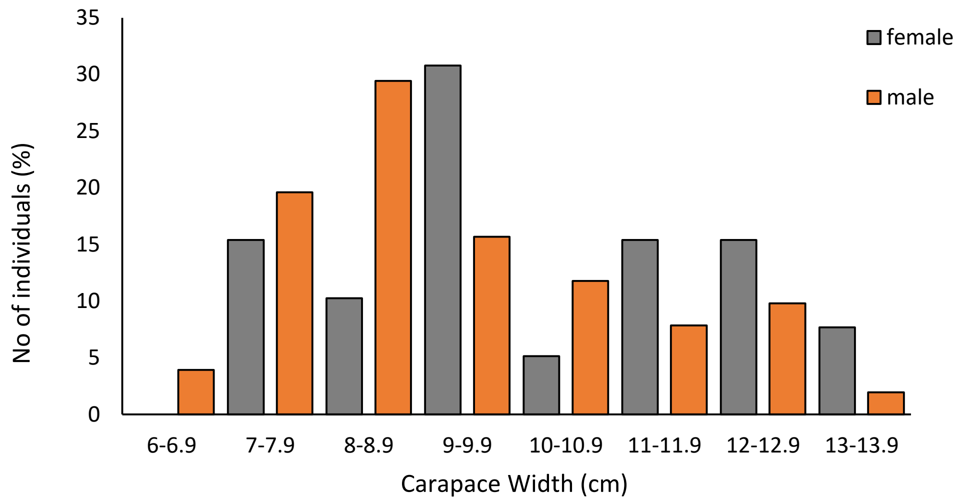


Figure 4.4: The CW frequency distribution of wild caught *S. olivacea* from Setiu Wetlands, Peninsular Malaysia (female; n = 39, male; n =51).

### 4.2.2 The CW and BW relationship of wild caught of *S. olivacea* from Setiu Wetlands, Peninsular Malaysia

The correlation between CW and BW was separately done according to sex. There was a strong significantly positive correlation between CW and BW of both female ($r = 0.92$, $\rho < 0.01$) and male ($r = 0.94$, $\rho < 0.01$) *S. olivacea*. The results of CW – BW linear regression of wild caught *S. olivacea* according to sex are presented in Table 4.8.

Table 4.8. The linear regression information of Carapace Width (CW) – Body Weight (BW) of wild caught of *S. olivacea* from Setiu Wetlands, Peninsular Malaysia (female; n = 39, male; n= 51).

| Sex | Regression Equation<br>Log BW = *a* + *b* Log CW | $R^2$ | ρ value |
|---|---|---|---|
| **Female** | Log BW = 1.11 + 3.34 Log CW | 0.94 | 0.001 |
| **Male** | Log BW = 1.18 + 3.47 Log CW | 0.95 | 0.001 |

The linear regression relationship of log CW – Log BW in female and male was done. A linear regression was calculated separately to predict BW based on CW of both sexes. The significant regression was ($F$ (1, 37) = 578.29, $\rho = 0.001$) with $R^2 = 0.94$ and regression equation of wild caught female *S. olivacea* is log BW = 1.11 + 3.34 (log CW). The significant regression was also found in wild caught male *S. olivacea* ($F$ (1, 49) = 957.12, $\rho = 0.001$) with $R^2 = 0.95$ and regression equation of log BW = 1.18 + 3. 47 (log CW) (Appendix 14b).

The coefficient of determination obtained for the log CW – Log BW of both females and males supported that there was positive correlation existed between the CW and BW in these crabs. The exponential values (b) of the log CW – log BW relationship of female and male were 3.34 and 3.47, respectively and the values does obey the cube law with b value more than 3. The b values that were slightly larger than 3 indicated a positive allometric growth for both sexes, where the mud crabs grows faster in weight than in size.

#### 4.2.3 The CW and GBC relationship of wild caught *S. olivacea* and Coefficient of variation (CV) value

The results showed the presence of growth band in wild caught *S. olivacea* from Setiu Wetlands. The GBC was ranged from 1 to 3 band in zygocardiac and mesocardiac ossicles of wild caught *S. olivacea* (Figure 4.5). All growth band were counted directly and consistently between two independent readers to minimize biases in counting. The growth bands were viewed using compound microscope and the image were taken carefully. Counting the growth band in the zygocardiac ossicles was easier due to the clear visibility and presence of complete band compared to poor visibility and incomplete growth band in the mesocardiac ossicles. The identification of growth band was carried out by referring to the method of Kilada et al. (2012). The preparation of growth band examination can be referred to the subchapter 3.3.

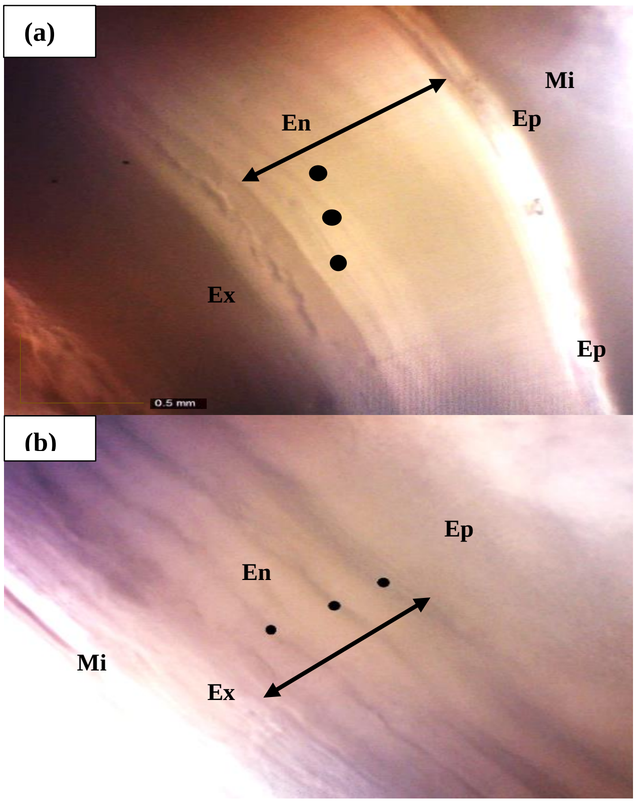


Figure 4.5: The growth band (black dots) shown in transverse thin cross sectioning (200 µm) of zygocardiac ossicles (Z). Three growth band (black dots) is shown in wild male *S. olivacea* with CW 12.8 cm and (b) is wild female *S. olivacea* with CW 9.73 cm.

The CW between sexes showed to have significant different, with female (10.12 ± 10.12 cm) and males (9.33 ± 1.78 cm) at (t (88) = 2.06, $\rho = 0.04$, $\rho < 0.05$ of wild caught *S. olivacea*. GBC data between sexes of wild caught *S. olivacea* did not display any significant different (Appendix 14c). This indicates that the CW in female and male were different while, the increases number of GBC in both sexes was similar.

The power equation of CW and GBC relationship is shown in Table 4.9. The results showed that the CV values were varied from 8.64 % to 8.52 % for female and male of wild caught *S. olivacea*, respectively. The CV values that showed lower values with less than 10%, demonstrated a good consistency of the band counting or reading.

Table 4.9. The equation describing the CW and GBC relationship of wild caught *S. olivacea* and the Coefficient of variation (CV) values.

| **Sex** | **N** | **CW = *a* x growth band count *b*** | **$R^2$** | **Coefficient of variation CV (%)** | **ρ value** |
|---|---|---|---|---|---|
| Female | 39 | $CW = 7.30\ GBC^{0.35}$ | 0.51 | 8.64 % | 0.001 |
| Male | 51 | $CW = 6.99\ GBC^{0.36}$ | 0.66 | 8.52 % | 0.004 |

The linear regression information of CW – GBC of wild caught *S. olivacea* was done. A linear regression was carried out to find out whether the CW could be used to predict the GBC of wild caught *S. olivacea*. There was a significant regression in female ($\rho = 0.001$) with $R^2 = 0.51$ and regression equation $CW = 7.30\ GBC^{0.35}$ (CW). The regression analysis showed to be statistically significant ($\rho = 0.004$) with $R^2 = 0.66$ and regression equation of $CW = 6.99\ GBC^{0.36}$ (CW) in wild caught male *S. olivacea*.

### 4.2.4 The GBC – Estimated age relationship and the CW – Estimated age relationship of wild caught *S. olivacea*

The GBC and estimated age data of wild caught *S. olivacea* was based on validation data obtained from reared *S. olivacea* with 1 band represent age of 1 year old at mean CW of 6.11 cm. A Student t – test was done to compare the estimated age between sexes of wild caught *S. olivacea* .There was no significant different in the estimated age between sexes, where the females mean estimated age was 1.34 ± 0.67 year and males was 1.89 ± 0.94 year (Appendix 14d).

The GBC and estimated age relationship of female and male of wild caught *S. olivacea* was done. A strong significant positive relationship was found between GBC and estimated age of female and male wild caught *S. olivacea* with r = 0.97, $\rho < 0.01$ and r = 0.98, $\rho < 0.01$, respectively. This confirms that as estimated age increased, the GBC also significantly increased. The linear regression of GBC and estimated age of wild caught *S. olivacea* is shown in Table 4.10.

Table 4.10. The linear regression of GBC – Estimated age of wild caught *S. olivacea* from Setiu Wetlands, Peninsular Malaysia.

| **Sex** | **N** | **GBC = *a* x estimated age (years)** $^{b}$ | $\mathbf{R^2}$ | **ρ value** |
|---|---|---|---|---|
| Female | 39 | GBC = 1.49 x estimated age $^{1.02}$ | 0.97 | 0.001 |
| Male | 51 | GBC = 1.05 x estimated age $^{1.01}$ | 0.98 | 0.002 |

The regression relationship between GBC and estimated age showed high value of correlation determination with $R^2 = 0.97$, $R^2 = 0.98$, comprising of female and male respectively. There was also significant regression in female ($\rho = 0.001$, $\rho < 0.01$) with regression equation GBC = 1.49 x estimated age $^{1.02}$ and in male ($\rho = 0.002$, $\rho < 0.01$) with regression equation of GBC = 1.05 x estimated age $^{1.01}$.

Estimated age of females was 0.67, 1.33 and 2.00 years old while estimated age of males was 0.94, 1.89 and 2.84 years old comprising of 1 band, 2 bands and 3 bands, respectively. The differences between GBC and estimated age were always only few months of or identical to the estimated age. For example, 2 bands were presence in the gastric ossicles of wild caught female and male *S. olivacea* at age of 1.33, 1.89 and 1.63 years old, respectively. In this case, although the second band was not fully deposited, it was visible and wide enough to be read and considered as the second band.

The equations describing the relationship of CW and estimated age of wild caught females and males *S. olivacea* (Table 4.11). A Student t-test was done to compared means of CW between sexes where females (10.12 ± 1.85 cm) and males (9.32 ± 1.78 cm). The CW between sexes showed to be significantly different at t (88) = 2.06, $\rho = 0.04$, $\rho < 0.05$. Similarly, the estimated age between females (1.71 ± 0.48 years) and males (2.12 ± 0.73 years) also displayed significant different at t (88) = 3.13, $\rho = 0.02$, $\rho < 0.05$. (Appendix 14e). There was significant strong positive correlation between the CW and estimated age of both females and males at r = 0.72, $\rho < 0.05$ and r = 0.81, $\rho < 0.05$, respectively. The linear regression of CW and estimated age is shown in Table 4.11.

Table 4.11. Power equation describing the CW (cm) – estimated age (year) of wild caught *S. olivacea* from Setiu Wetlands, Peninsular Malaysia.

| **Sex** | **N** | **CW = *a* x estimated age *b*** | **R2** | **ρ value** |
|---|---|---|---|---|
| Female | 39 | CW = 8.40 estimated age $^{0.35}$ | 0.52 | 0.001 |
| Male | 51 | CW = 7.15 estimated age $^{0.36}$ | 0.66 | 0.001 |

The regression relationship between CW and estimated age in female showed a significant regression coefficient with $R^2 = 0.52$, $\rho = 0.001$ and regression equation CW = 8.40 estimated age $^{0.35}$. There was also significant regression coefficient in males with $R^2 = 0.66$, $\rho = 0.001$ and regression equation CW = 7.15 estimated age $^{0.36}$. The regression coefficient output supported that the estimated aged were influenced by the increased of size (CW) of wild caught *S. olivacea*.

### 4.2.5 Estimation of growth parameters

Age of wild caught *S. olivacea* from Setiu Wetlands was estimated using GBC data. One growth band indicated the age of 1 year old of *S. olivacea*. The growth parameters were used to estimated age of wild caught *S. olivacea* based on CW at age data. The growth parameters estimated for female and male wild caught *S. olivacea* are shown in Table 4.12. The growth parameters were $CW_\infty$, K and $t_0$ and were derived from inverse linear regression plot and fitted the Von Bertalanffy model using VBGM equation "$CWt = CW_\infty [1 - \exp^{-K(t-t0)}]$". The explanation of the VBGM equation as has been detailed in the subchapter 2.6.

Table 4.12. Summary of estimated growth parameter of wild caught *S. olivacea* from Setiu Wetlands, Peninsular Malaysia, Malaysia.

| **Sex** | **Growth parameters** | | |
|---|---|---|---|
| | **$CW_\infty$ (cm)** | **K year $^{-1}$** | **$t_0$** |
| Female | 28.011 | 0.369 | -0.734 |
| Male | 29.340 | 0.380 | -0.792 |

The von Bertalanffy growth parameters estimated that males had higher $CW_\infty$ = 29.34 cm CW than females ($CW_\infty$ = 28.01 cm CW). The growth coefficient value (K) in males was 0.38 year$^{-1}$ and in female was 0.36 year$^{-1}$. The hypothetical age ($t_0$) of mud crab when the CW at zero cm for female and male were – 0.73 and 0.79, respectively. The VBGM using nonlinear procedure showed to be the best model to describe the growth curve wild caught *S. olivacea* (Figure 4.7, Appendix 14f and 14g).

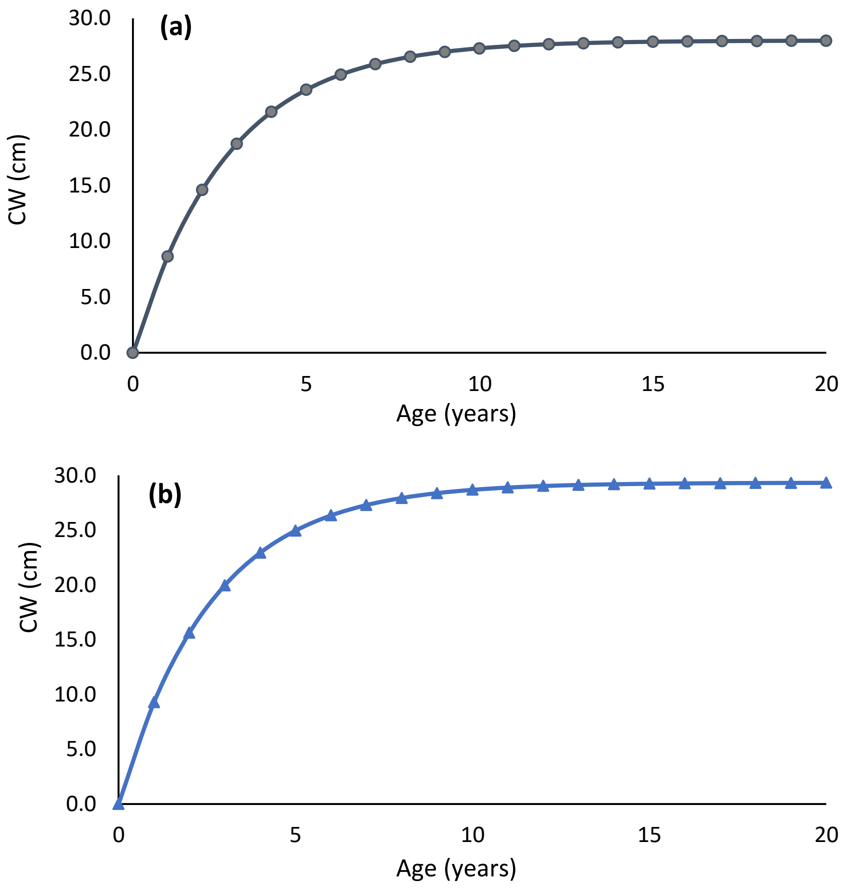


Figure 4.6: Von Bertalanffy growth model using nonlinear regression fitted to the CW at age data of wild caught *S. olivacea* (a) female crab, (b) male crab from Setiu Wetlands, Peninsular Malaysia.

Since the lifespan of mud crabs was 3 years, the VBGM estimated that the age of 1 to 3 years old were correlated to the observed value obtained from CW and GBC data. The size range of observed values at age of 1 to 3 years old females was 6.00 to 13.40 cm CW whereas, the VBGM estimated age of 1 to 3 years old were slightly higher in size ranged from 9.00 to 20.00 cm CW. The CW at age data of wild female *S. olivacea* recorded to be in range of 7.00 – 8.00 cm at age of 1 year old, CW range of 7.50 – 9.10 cm at 2 years old and CW range of 9.00 – 13.40 cm at 3 years old. On the other hand, VBGM estimated the CW females ranged between 8.00 – 14.60 cm at age of 1 year old, 14.60 – 18.70 cm at age of 2 years old and 18.70 – 20.00 cm at age of 3 years old.

The CW at age data based on GBC data of wild male *S. olivacea* showed that the CW ranged between 6.50 – 7.80 cm at age of 1 year old, 7.40 – 9.20 cm at age of 2 years old and 8.80 – 13.20 cm at age of 3 years old. While, the VBGM estimated that the CW of males ranged between 9.20 – 15.00 cm at age of 1 year old, 15.63 – 19.90 cm at age of 2 years old and 19.96 – 22.90 cm at age of 3 years old.

The VBGM predicted larger size of CW even compared to the true age data of wild *S. olivacea*. The VBGM even extrapolated the growth model up to 20 years of *S. olivacea* lifespan. Slow growth was observed in later years until maximum CW is approached. The slow growth period was displayed at age of 9 years (27.00 cm CW) for females and 10 years old (28.86 cm CW) for males (Table 4.13).

Table 4.13. The summary of CW at age data and VBGM estimation of wild caught *S. olivacea* from Setiu Wetlands, Peninsular Malaysia.

| **Procedure** | **CW at age data** | | **VBGM estimation** | |
|---|---|---|---|---|
| | **CW (cm)** | | | |
| **Sex / Age (years)** | **Female** | **Male** | **Female** | **Male** |
| 1 | 7.00 – 8.00 | 6.50 – 7.80 | 8.00 – 14.60 | 9.20 – 15.00 |
| 2 | 7.50 – 9.10 | 7.40 – 9.20 | 14.60 – 18.70 | 15.63 – 19.90 |
| 3 | 9.00 – 13.40 | 8.80 – 13.20 | 18.70 – 20.00 | 19.96 – 22.90 |

### 4.2.6 Age composition of wild *S. olivacea* in Setiu Wetlands, Peninsular Malaysia

The age composition of *S. olivacea* varied from 1+ to 3+ age groups. The present study observed that the highest occurrence of female and male *S. olivacea* were at 2+ age group with 56.86 % and 46.15 %, respectively. The age composition of male *S. olivacea* at 1+ age group showed high occurrence with 23.53% compared to female *S. olivacea* with 15.38 % at same age group. While, female *S. olivacea* revealed the highest occurrence with 38.46 % compared to male *S. olivacea* with 19.61 % at 3+ age group. From overall observation, the highest occurrence of all individuals was at the 2+ age group. The frequency distribution of *S. olivacea* age composition according to sex is shown in Figure 4.8.

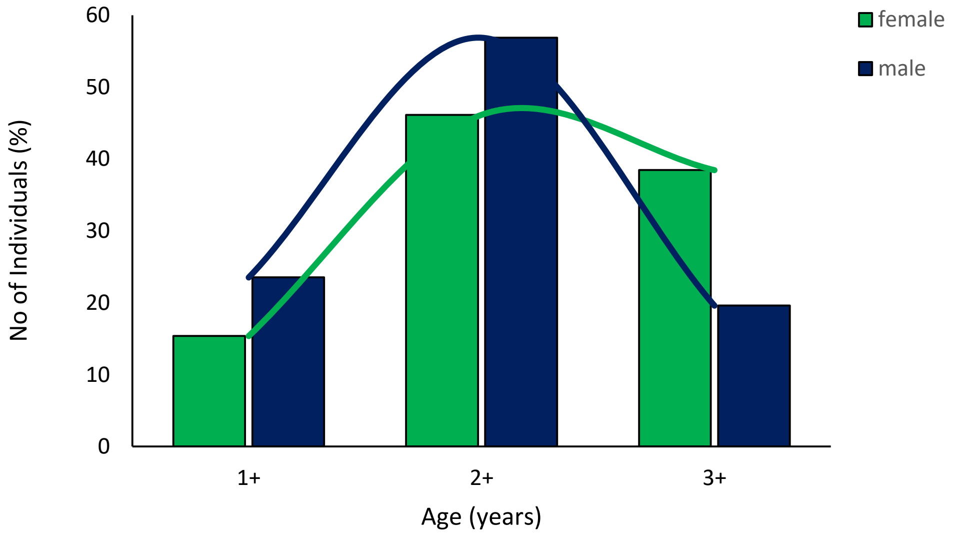


Figure 4.7: The frequency distribution of age composition of wild caught *S. olivacea* according to sex (female; n= 39, male; n= 51) from Setiu Wetlands, Peninsular Malaysia, Malaysia.

# CHAPTER 5

# DISCUSSIONS

## 5.1 Validation of growth band deposition

The present study is the first work that was carried out to determine the growth and age estimation of *S. olivacea* using growth band counts as age indicator. Direct age method was newly introduced by this present study for age determination of crustaceans using growth band deposition in their gastric mill. Several studies have reported the application of growth bands count for age determination in crustaceans (Leland et al., 2011; Kilada et al., 2012; Kilada & Acuña, 2015; Kilada & Ibrahim, 2016; Sheridan et al., 2016). However, no study has ever been done on *S. olivacea.*

Validation method was done to confirm the periodicity of growth band in *S. olivacea* that reared in captivity for one year cultured period with known age data. In this study, presence of annual growth band in the thin section of gastric mill ossicles of *S. olivacea* showed to complement the known age of reared *S. olivacea* (1 growth band indicating the absolute age of 1 year old of *S. olivacea*). The validation investigation on band interpretation of the known age animals provides the most accurate age information (Campana, 2001; Stewart & Hughes, 2007; Spurgeon et al., 2015).

The results of this present study were mostly obtained from the growth band deposition in the gastric mill ossicles of *S. olivacea* with CW size of more than 5.00 cm at the age of 12 months old. It was difficult to obtain the age information from the growth band deposition in the small size *S. olivacea* (with CW size < 5.00 cm). Their small size potentially caused incomplete and unclear primary growth band formation. Jantrarotai et al. (2006) explained that the development of gastric mill in the cardiac stomach may not be found at zoeal stages of *S. olivacea* and suggested that the development of cardiac stomach is related to the size of the individual crabs.

The low number of juvenile crabs numbers (2.00 – 5.00 cm CW) encountered in this study due to high mortality rate (80%) during rearing period, made it difficult to assess the proper age of below 1 year old. According to Mirera and Moksnes (2013), small size *S. serrata* (2.00 to 8.00 cm CW) has the highest rates of mortalities with the average rate of 42% per week due to the high cannibalism activity under high stock density in the nursery cages.

## 5.2 The CW – BW relationship of *S. olivacea*

The CW – BW relationship of reared *S. olivacea* with exponent ‘b’ value was found to be 2.48 and 2.93 in female and male, respectively. While, the ‘b’ value of CW – BW relationship of wild caught *S. olivacea* was 3.34 and 3.47 in female and male, respectively. In general, the exponent ‘b’ value often lies between the ranges of 2.50 to 4.00. According to Karachle and Stergiou (2012) and Silva et al. (2015), an ideal ‘b’ value should be closed to 3. The ideal values indicate isometric growth and values other than 3, indicate an allometric growth. Therefore, the recorded exponent ‘b’ values that were not equivalent to 3, confirmed the allometric growth of the mud crabs in this present study. The different in exponential ‘b’ value was potentially influenced by various physiological, biological and environmental factors such as temperature, salinity, seasons, food intake, sex and stage of maturity (Tongdee, 2001; Mhapatra et al., 2010; Susanto & Irnawati, 2014).

The CW – BW relationship data for both male and female *S. olivacea* showed an allometric growth with the recorded 'b' value of reared samples was <3, while the values were > 3 for wild caught samples. The lower 'b' value of reared *S. olivacea* indicated that the increased in its CW was faster than its weight. On the other hand, the 'b' values of > 3 in the wild caught *S. olivacea* explained that its weight increased faster than its CW. The differences in the 'b' value and growth performance of the reared and wild caught mud crabs potentially caused by their ability to compete for food and resist cannibalism (Ut et al., 2007).

Overall 'b' value data of the male *S. olivacea* from both reared (2.93) and wild caught (3.47) showed higher exponential values than the female. The 'b' value of both reared and wild caught female was 2.48 and 3.34, respectively. The result of this present study, therefore, supported that males *S. olivacea* had higher tendency to be heavier than the females. There are numbers of researchers reported stronger CW and BW relationship in males rather than in females. One of the research is the one carried out by Ikhwanuddin et al. (2010), who concluded that the males *S. olivacea* from Setiu Wetlands were heavier than females with BW = $3.91e^{0.37}$ and BW = $5.00e^{0.33}$, respectively. Similarly, Jirapunpipat et al. (2007) found that the 'b' values were higher in males compared to females of *S. olivacea* from Klong Ngao mangrove swamp, Thailand with the 'b' value of 3.45 and 2.86, respectively.

A recent study by Viswanathan et al. (2016) also found that the 'b' values obtained in males *S. olivacea* from Pichavaram mangroves, South – East India were higher than females with 3.80 and 3.59, respectively. The CW – BW relationship of *S. tranquebarica* collected from Parangipettai coast, India revealed higher 'b' values in males (3.27) than in females (3.02) (Thirunavukkarasu & Shanmugam, 2011). Siahainenia et al. (2016) observed the 'b' values of 2.99 of *S. serrata* from pooled data from four locations in coastal waters of Western Seram, Maluku, Indonesia. The males mud crabs must be heavier than females due to their different diet influenced, the size difference, metabolic rate, cheliped strength and foraging behaviour (Sukumaran & Neelakantan, 1997; Josileen & Menon, 2005; Babu et al., 2007; Susanto & Irnawati, 2014).

## 5.3 CW Frequency distribution of *S. olivacea*

In this present study, the results described the natural growth of *S. olivacea* reared in captivity and wild from Setiu Wetlands. Most of the wild *S. olivacea* samples from Setiu Wetlands were larger in size (> 6.00 cm CW) compared to the reared *S. olivacea* (< 6.00 cm CW). This study has covered various size of CW of *S. olivacea* to ensure the representative of the different age classes in the population.

Similar results were also reported by Ikhwanuddin et al. (2010), who found that the wild *S. olivacea* caught from Setiu Wetlands ranged from 9.00 to 9.90 cm CW has the size frequency of 35.2%. Zaidi et al. (2011) found that the *S. olivacea* CW from Setiu Wetlands ranged between 4.00 to 14.50 cm with the record on individuals at size interval between 9.00 to 9.50 cm were also at high occurrence. Data from both studies are in agreement with our finding on the common size range of wild caught *S. olivacea* from Setiu Wetlands that ranged from 8.00 to 10.00 cm CW.

The size of reared *S. olivacea* ranged from 3.00 to 10.90 cm CW, while the BW ranged between 5.60 to 202.80 g. There was high occurrence of reared *S. olivacea* at class interval ranged between 4.00 to 4.90 cm CW. Reared *S. olivacea* were smaller in size and lighter in weight compared to wild caught *S. olivacea* in this present study. A few studies have been carried out to estimate growth increment of cultured mud crab collected from East Africa. For instance, study by Moksnes et al. (2015) discovered the size of *S. serrata* reared in laboratory and pond for 45 days was ranged from 0.40 to 4.00 cm CW, respectively. Earlier study done by Ut et al. (2007) estimated the natural growth and size of hatchery reared juvenile *S. paramamosain* in stocking pond, Vietnam, for 60 days to be ranged from 20.00 to 40.00 mm CW. When Hamad (2012) investigated the growth increment of juvenile *S. serrata* at moulting stage in laboratory tanks for 40 days, they found the CW size ranged between 1.80 to 2.50 cm. While, Mirera and Moksnes (2013) found that the size range of *S. serrata* was in the range 2.20 to 5.60 cm CW during 20 to 30 days grow out period in laboratory tanks.

The present study revealed the size distribution of CW of *S. olivacea* reared for 1 year (365 days) with the average of CW was 6.11 cm can be equivalent to or higher than those other reported genus *Scylla* species. This is due to possibilities that significant growth and survival of reared mud crabs under a controlled environment are likely to be influenced by the nutritional and environmental factors (Ut et al., 2007) together with some uncertainties and limitation occurred during grow out process (Moksnes et al., 2015).

## 5.4 Reading and Growth band interpretation

There is limitation in estimating the age and the growth of other crustacean species using growth band data which make it difficult to compare their age via direct method (Kilada & Driscoll, 2017). Therefore, the age estimation of *S. olivacea* was corroborated with validation data in this study. The growth band formation of hatchery – reared *S. olivacea* discovered the presence of 1 clear primary growth band that was concurrent with the rearing period of 1 year. This indicates that 1 growth band was equivalent to the age of 1 year old of *S. olivacea.* The growth band in the cross sectioned gastric mill of wild *S. olivacea* discovered the presence of 1 to 3 growth bands suggesting that the age of *S. olivacea* was 3 years old and in agreement with CW at age and validation data of this study.

The age estimation of wild blue swimming crab, *Portunus pelagicus* using band count and validated with length frequency analysis was carried out by Kilada and Ibrahim (2016). They observed the longevity of 3 years with clear visibility of 3 growth bands at mean CW ranged between 10.00 to 12.00 cm (first mode) and 7.60 cm to 9.70 cm (second mode) for samples from Lakes Bardaweel and Lakes Timsah, Eastern Mediterranean, respectively. The validation of age estimation of juvenile American lobster, *Homarus americanus* is 4 years old similar to the 4 band counts and known age samples (reared in the hatchery for 4 years period) (Tang et al., 2015).

Similar to this present work, Islam et al. (2007) and Islam and Kurokura (2009) also distinguished three age classes (0+, 1+ and 3+ years) that indicate the three years lifespan of wild caught *S. olivacea* and *S. paramomasain* at Pak Phanang Bay, Thailand, however by using lipofuscin concentration as age indicator. Recently, Viswanathan et al. (2016) discovered the age estimation of wild caught *S. olivacea* at the Pichavaram mangroves, south – east India with the longevity of 3 years through length frequency analysis and estimated growth by ELEFAN I and Von Bertalanffy plot with values ranged from 2.1 and 3.9 years, respectively. This indicates the variation of growth and age estimation of the same species in the different location can be described by several direct age estimation methods.

The precision in reading the growth band in the ossicles structure was assessed by calculating the Coefficient of variation (CV) of two independent readers. The values obtained from the CV estimation indicated the accuracy of the estimated age to the true age (Campana, 2001). The estimation of CV from this present study ranged from 6.00 to 6.23% of reared *S. olivacea* and 8.52 to 8.64% of wild caught *S. olivacea.* The lower values of CV in the present work proved that there was minimum bias in the interpretation of band counting in individual crabs (Kilada & Ibrahim, 2016).

The CV values calculated from band count in the gastric mill ossicles of *Portunus pelagicus* ranged from 6.3 to 7.2% (Kilada & Ibrahim, 2016), 6.0, 6.3 and 7.2% for the Chilean red shrimp, *Pleuroncodes monodon*, yellow squat lobsters, *Curvimunida johni* and nylon shrimp, *Heterocarpus reedi*, respectively (Kilada & Acuña, 2015). Other species such as northern shrimp, *Pandalus borealis* and sculptured shrimp, *Sclerocrangon boreas* had CV values that ranged from 8.0 to 11.0%, 15% in snow crab, *Chionoecetes opilio* and American lobster, *Homarus americanus* ranged from 7.0 to 10% of band counting in the gastric mill and eyestalk ossicles of these species (Kilada et al., 2012).

## 5.5 Growth parameters fitted to Von Bertalanffy Growth Model (VBGM)

This present work revealed the age estimation of early life stages of *S. olivacea* (known age) and adult wild caught *S. olivacea* from Setiu Wetland using VBGM. The VBGM is a mathematical growth model of body length as a function of age that is considered as the base of growth model and has been shown to conform the observed growth of most aquatic animals (Sparre & Venema, 1998). The VBGM is widely used in the fisheries biology and commonly applied in the aquaculture studies (Hopins, 1992; Campana, 2001). However, the literature on the growth function are understudied and not commonly applied to assess growth of crustaceans (Moksnes et al., 2015; Christensen et al., 2004) due to moulting event under natural condition (Zafar et al., 2006; Sheridan et al., 2016).

In this present study, the growth curve was derived from nonlinear regression and fitted to the Von Bertalanffy growth equation. Apparently, fitting the growth in size of CW of mud crab to the growth curve (VBGM) showed a relatively good fit (Sara, 2010; Moksnes et al., 2015; Viswanathan et al., 2016). Findings from this study, successfully revealed the age and growth estimation using VBGM for reared and wild caught *S. olivacea.* The growth parameters for reared *S. olivacea,* the $CW_\infty$ was 20.07 cm, K was 0.31 and $t_0$ was -0.20. The growth parameters for female and male wild caught *S. olivacea* was $CW_\infty$ of 28.01 cm, K of 0.36 year $^{-1}$ and $t_0$ of -0.73 and $CW_\infty$ of 29.34 cm, K of 0.38 year$^{-1}$ and $t_0$ of -0.79, respectively. The growth curve showed that when the asymptotic carapace width reached the maximum values, it caused the growth rate defined by K (year $^{-1}$) to decline (Sparre & Venema, 1998; Cailliet et al., 2006; Enberg et al., 2008).

Sara (2010) described the K value of wild caught *S. olivacea* using ELEFAN I analysis was higher (1.38 year $^{-1}$ (males) and 0.83 year $^{-1}$ (females)) compared to the growth parameters in this study and indicated that *S. olivacea* at Lawele Bay, south east Sulawesi, Indonesia is a fast growth mud crab species. Finding by Sara (2010) was contradicts to this study, where the K values were lower (0.31 year $^{-1}$, 0.36 year $^{-1}$ (female) and 0.38 year $^{-1}$ (male)), indicating that the *S. olivacea* from Setiu Wetlands was a slow growth mud crab species. A much more recent study by Viswanathan et al. (2016) reported similar findings as this present study. They also reported low growth parameters values of male and female wild *S. olivacea* collected from south east India using FISAT II software with $CW_\infty$ of 14.80 cm , K of 0.762 year$^{-1}$ and $t_0$ of – 0.637 and $CW_\infty$ of 13.80 cm, K of 0.856 year $^{-1}$ and $t_0$ of 0.681, respectively.

The VBGM is also used to predict the growth parameters of other crustacean species that were sampled from different location around Asian and Australian waters. For instances, works by Zafar et al. (2006) and Ward et al. (2008) successfully detailed the $CW_\infty$ of 10.59 cm, K of 0.28 year $^{-1}$ for male and $CW_\infty$ of 10.50 cm, K of 0.36 year $^{-1}$ for female and $CW_\infty$ of 15.25 cm, K of 1.46 year $^{-1}$ (male) and $CW_\infty$ of 18.54 cm, K of 0.81 year $^{-1}$ (female) of *S. serrata* sampled from Chakaria Sundarban, India and Australia, respectively, using VBGM.

The results from the mentioned previous study are in agreement with the results of this study even though the researches were done on different variations of growth method. This was also depending on the variances of growing conditions, environmental, physiological and biological influences and also sampling sites species – specific differences (Moksnes et al., 2015).

## 5.6 Age estimation of *S. olivacea* based on growth band count data

In this study, there was a linear relationship between size and GBC of reared (known age) and wild caught *S. olivacea* from Setiu Wetlands. Size at age data based on GBC showed a positive correlation. This findings supported that the growth and age of *S. olivacea* were similar to others crustacean species (Kilada et al, 2012; Kilada & Acuña, 2015; Kilada et al, 2015; Leland et al, 2015; Kilada & Ibrahim, 2016). In the regression analysis, a higher regression coefficient was observed in the GBC data, CW and estimated age relationship. Therefore, indicated that the values had a similar interval with the known age of *S. olivacea* (validation data). The results obtained in this present study were similar to the findings by Leland et al. (2011), Kilada et al. (2012), Kilada and Acuña (2015), Tang et al. (2015) and Kilada and Ibrahim (2016), which have proven that the direct method (growth band deposition) is applicable to determine the age of crustaceans.

This present study successfully estimated 3 years lifespan of *S. olivacea* with aid from growth curve (VBGM). From the growth curve, we recorded that the average CW size of reared *S. olivacea* of 5.40 cm represented 1 year old age, 9.32 cm represented 2 years old and 12.21 cm represented 3 years old. From the wild caught growth curve, we found that female *S. olivacea* with 8.56 cm, 14. 63 cm and 18. 76 cm CW estimated to be at the age of 1, 2 and 3 years old, respectively.

Study that carried out by Moksnes et al. (2005) suggested that the *S. serrata* from wild and cultured would reach maturity after 9.9 months with approximate CW of 12.00 cm and reached commercial size (BW; 500 g to 1 kg) after 12.4 and 17.3 months, respectively. The authors predicted the time frame for average size (BW; 300 g, 500 g and 1 kg) would take about 7.3, 9.8 and 14.6 months to reach suitable weight.

Similarly, Josileen and Menon (2005) predicted the maximum CW of male *P. pelagicus* can be reached at the age of 1, 2 and years old with the size of attained at 17.6 cm CW, 20.3 cm CW and 20.7 cm CW, respectively. While the female with size of 15.1 cm CW, 18.1 cm CW and 18.7 cm CW at the age of 1, 2 and 3 years old, respectively.

The wild caught *S. olivacea* showed higher growth increment compared to the reared *S. olivacea* due to molting event under different natural and controlled environments condition (Moksnes et al, 2015). The suitable and optimal condition provided by natural environment does encourage the growth of the species (Josileen & Menon, 2005; Enberg et al., 2008) compared to the under controlled environment (Ut et al., 2007). Findings from this study revealed that the age estimation of *S. olivacea* using GBC data as age indicator provides significant results for assessment and management of *S. olivacea* population in Setiu Wetlands. The age data would contribute to the input control regulations, for example minimum legal size and size at sexual maturity of *S. olivacea.*

The present study also showed the age composition between size distribution and age estimation based on growth band data. The analysis suggested that most of the *S. olivacea* live in the mangroves ecosystem of Setiu Wetlands at the age of 2 years old age group. As reported in studies by Moser et al. (2002), Josileen and Menon (2005), Sara (2010), Shelley and Lovatelli (2011), Moksnes et al. (2015) and Viswanathan et al. (2016), this present study also found that the lifespan of *S. olivacea* was 3 years.

# CHAPTER 6

# CONCLUSION AND RECOMMENDATION

Findings from this study showed that the evaluation of primary growth mark periodicity via long term rearing period. This study also managed to prove that the growth band formation was deposited annually in the gastric mill ossicles of *S. olivacea*. This present study also the first attempt to estimate growth parameters using VBGM, CW at age data and growth band deposition as age indicator.

The direct ageing method was validated by means of the known – age of the reared *S. olivacea* which later was used to estimate the age of wild *S. olivacea.* For reared *S. olivacea,* the periodicity of growth band deposition indicated that a single primary growth mark was deposited in 12 months grow out period while for wild caught *S. olivacea*, the growth band deposited from 1 to 3 growth band. Findings from VBGM was concurrent to the positive correlation between size and putative age.

The accurate age information from this study would substantially improve the developing age – based assessment model for stock structure, catch limits and environmental changes of crustaceans. The direct age determinations also showed to be corresponded to the biological factors such as temperature, salinity and density that are known to influence the growth of crustaceans. The accuracy age estimation of slow – growing crustaceans can be improved by carrying out the ecological studies for conservation management such as enhancing the knowledge of current growth models while decreasing research costs. The age validation method will allow specific growth assessments and provide more accurate longevity estimates.

Therefore, this study will stand as a steady reference to facilitate future research on population dynamics using direct crustacean ageing method and implementation for sustainable utilizing of mud crabs. In addition, further direct age development is beneficial to locate specific differences in the growth of mud crabs. Further studies are necessary to gain understanding in the age information such as prolong the grow out period (e.g. 2 years and above), rearing and maintain the larger samples size of juveniles and adult mud crabs. Also more studies are crucial to assess the age of individuals via several of age determination method. The finding of this work is relevant to initiate further specific stock recommendations that will form the basis of crustaceans ageing research and development applications.

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# APPENDICES

Appendix 1: Sampling activity

Appendix 2: Fiberglass tank (320cm x 138cm x 60cm)

Appendix 3: The aeration devices were supplied.

Appendix 4: Rectangular plastic container (25cm x 19cm x 15cm)

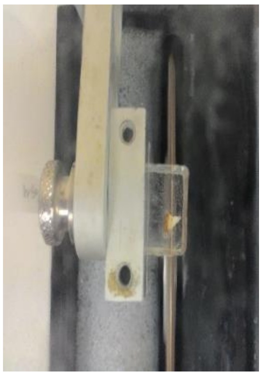

Appendix 5: Refractometer and YSI multiprobe meter

Appendix 6: Crab measurements using vernier caliper.

Appendix 7: Epoxy resin EpoxiCure® 2 Epoxy System and EpoxiCure® 2 Hardener

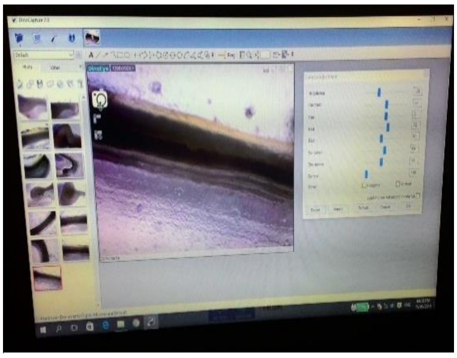
Appendix 8: Samples were embed for 2 – 7 days at room temperature


Appendix 9: Buehler IsoMet Precision Cutter (IsoMet® 1000 Precision Cutter.



Appendix 10: Sectioned samples cutting axis

Appendix 11: Thin samples were mounted on a standard microscope slide.



Appendix 12: Digital images were taken using DinoCapture 2.0 software and enhanced using Adobe Photoshop CS6 version.

# Appendix 13

A. Independent Samples t –test analysis of between CW and BW of reared *S. olivacea.*

**Group Statistics**

| Model | Sex | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| CW | female | 12 | 4.80 | 0.70 | 0.20 |
| | male | 13 | 6.04 | 1.86 | 0.52 |
| BW | female | 12 | 27.92 | 15.28 | 4.41 |
| | male | 13 | 48.58 | 51.19 | 14.20 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| CW | Equal variances assumed | 3.42 | 0.08 | 2.15 | 23.00 | 0.04 | 1.24 | 0.57 | 2.42 | 0.05 |
| | Equal variances not assumed | | | 2.23 | 15.56 | 0.04 | 1.24 | 0.55 | 2.42 | 0.06 |
| BW | Equal variances assumed | 2.57 | 0.12 | 1.34 | 23.00 | 0.19 | 20.66 | 15.39 | 52.51 | 11.18 |
| | Equal variances not assumed | | | 1.39 | 14.28 | 0.19 | 20.66 | 14.87 | 52.49 | 11.16 |

*. Significant value at the 0.05 level (2-tailed)

B. Regression information of Log CW and Log BW of reared *S. olivacea* between sexes.

**Model Summary**

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| female | 0.84 | 0.61 | 0.56 | 0.29 |
| male | 0.99 | 0.98 | 0.98 | 0.06 |

**ANOVA[a]**

| Model | | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| female | Regression | 0.32 | 1.00 | 0.32 | 6.58 | 0.028[b] |
| | Residual | 0.48 | 10.00 | 0.05 | | |
| | Total | 0.80 | 11.00 | | | |
| male | Regression | 1.81 | 1.00 | 1.81 | 8.03 | 0.000[b] |
| | Residual | 0.04 | 11.00 | 0.00 | | |
| | Total | 1.85 | 12.00 | | | |

**Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| female | (Constant) | -0.35 | 0.67 | | -.50 | 0.63 |
| | logCW_F | 2.48 | 0.99 | 0.63 | 2.56 | 0.03 |
| male | (Constant) | -0.71 | 0.10 | | -7.31 | 0.00 |
| | logCW_M | 2.93 | 0.13 | 0.99 | 23.20 | 0.00 |

C. Independent Samples t –test analysis of between CW and GBC of reared *S. olivacea.*

**Group Statistics**

| | Sex | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| CW | Female | 12 | 4.80 | 0.70 | 0.20 |
| | Male | 13 | 6.04 | 1.86 | 0.52 |
| GBC | female | 12 | 0.91 | 0.14 | 0.04 |
| | Male | 13 | 0.92 | 0.13 | 0.04 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| CW | Equal variances assumed | 3.42 | 0.08 | 2.16 | 23.00 | 0.04 | 1.24 | 0.57 | 2.42 | 0.05 |
| | Equal variances not assumed | | | 2.23 | 15.56 | 0.04 | 1.24 | 0.55 | 2.42 | 0.06 |
| GBC | Equal variances assumed | 0.03 | 0.86 | 0.12 | 23.00 | 0.90 | 0.01 | 0.05 | 0.12 | 0.10 |
| | Equal variances not assumed | | | 0.12 | 22.72 | 0.90 | 0.01 | 0.05 | 0.12 | 0.10 |

*. Significant value at the 0.05 level (2-tailed)

D. Independent Samples t –test analysis of between GBC and Known age of reared *S. olivacea.*

**Group Statistics**

| Model | Sex | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| GBC | Female | 12 | 11.00 | 1.60 | 0.46 |
| | Male | 13 | 11.08 | 1.55 | 0.43 |
| AGE | Female | 12 | 0.91 | 0.13 | 0.04 |
| | Male | 13 | 0.92 | 0.13 | 0.04 |

*. Significant value at the 0.05 level (2-tailed)

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference: Lower | Upper |
| GBC | Equal variances assumed | 0.03 | 0.86 | 0.12 | 23.00 | 0.90 | 0.08 | 0.63 | 1.38 | 1.23 |
| | Equal variances not assumed | | | 0.12 | 22.72 | 0.90 | 0.08 | 0.63 | 1.38 | 1.23 |
| AGE | Equal variances assumed | 0.03 | 0.86 | 0.12 | 23.00 | 0.90 | 0.01 | 0.05 | 0.12 | 0.10 |
| | Equal variances not assumed | | | 0.12 | 22.72 | 0.90 | 0.01 | 0.05 | 0.12 | 0.10 |

E. Independent Samples t –test analysis of between CW and Known age of reared *S. olivacea.*

**Group Statistics**

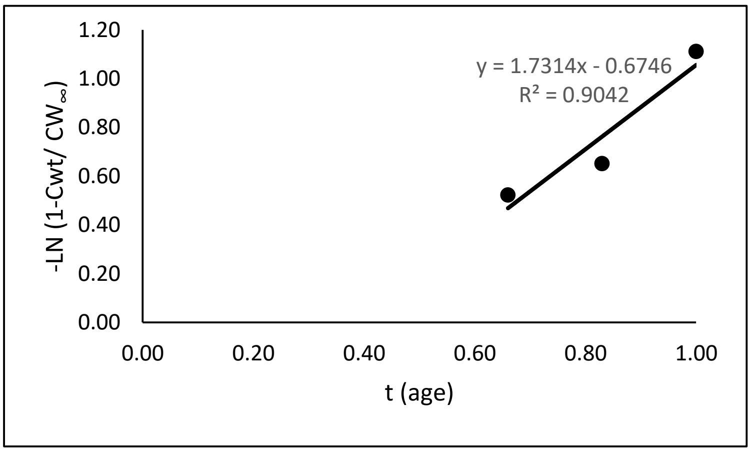
| Sex | | N | Mean | Std. Deviation | Std. Error mean |
|---|---|---|---|---|---|
| CW | male | 13 | 6.04 | 1.86 | 0.52 |
| | female | 12 | 4.80 | 0.70 | 0.20 |
| KnownAge | male | 13 | 11.08 | 1.55 | 0.43 |
| | female | 12 | 11.00 | 1.60 | 0.46 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | 95% Confidence Interval of the Difference | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| CW | Equal variances assumed | 3.42 | 0.08 | 2.16 | 23.00 | 0.04 | 1.24 | 0.57 | 0.05 | 2.42 |
| | Equal variances not assumed | | | 2.23 | 15.55 | 0.04 | 1.24 | 0.56 | 0.06 | 2.42 |
| KnownAge | Equal variances assumed | 0.03 | 0.86 | 0.12 | 23.00 | 0.90 | 0.08 | 0.63 | -1.23 | 1.38 |
| | Equal variances not assumed | | | 0.12 | 22.72 | 0.90 | 0.08 | 0.63 | -1.23 | 1.38 |

*. Significant value at the 0.05 level (2-tailed)

F. The inverse linear regression plot were describe in Excel using equation $-\text{Ln}\,(1 - CW_t / CW_\infty)$ against t (age) in month for known age of reared *S. olivacea.*




G. Growth parameters interpret by SPSS software output using von Bertalanffy equation $CW_t = CW_\infty\,[1 - \exp^{-K\,(t - t0)}]$ for known age of reared *S. olivacea.*

**Parameter Estimates**

| Parameter | Estimate | Std. Error | 95% Confidence Interval Lower Bound | Upper Bound |
|---|---|---|---|---|
| $CW_\infty$ | 20.07 | 76.81 | -135.74 | 175.89 |
| K | 0.31 | 3.99 | -1.54 | 2.16 |
| $t_0$ | 0.20 | 0.68 | -1.23 | 1.64 |

## Appendix 14

A. Independent Samples t –test analysis of between CW and BW of wild caught *S. olivacea* from Setiu Wetlands, Peninsular Malaysia, Malaysia.

**Group Statistics**

| Model | Sex | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| CW | female | 39 | 10.12 | 1.85 | 0.30 |
| | male | 51 | 9.33 | 1.78 | 0.25 |
| BW | female | 39 | 201.64 | 127.46 | 20.41 |
| | male | 51 | 183.01 | 140.91 | 19.73 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| CW | Equal variances assumed | 0.77 | 0.38 | 2.06 | 88.00 | 0.04 | 0.79 | 0.39 | 0.03 | 1.56 |
| | Equal variances not assumed | | | 2.05 | 80.28 | 0.04 | 0.79 | 0.39 | 0.02 | 1.56 |
| BW | Equal variances assumed | 0.10 | 0.76 | 0.65 | 88.00 | 0.51 | 18.62 | 28.77 | -38.56 | 75.81 |
| | Equal variances not assumed | | | 0.66 | 85.48 | 0.51 | 18.62 | 28.39 | -37.82 | 75.06 |

*. Significant value at the 0.05 level (2-tailed)

B. Regression information of Log CW and Log BW of wild caught *S. olivacea* between sexes.

**Model Summary**

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| female | 0.96 | 0.94 | 0.94 | 0.07 |
| male | 0.97 | 0.95 | 0.95 | 0.06 |

**ANOVA[a]**

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| female | Regression | 2.69 | 1.00 | 2.69 | 578.29 | .000[b] |
| | Residual | 0.17 | 37.00 | 0.00 | | |
| | Total | 2.86 | 38.00 | | | |
| male | Regression | 3.91 | 1.00 | 3.91 | 957.12 | .000[b] |
| | Residual | 0.20 | 49.00 | 0.00 | | |
| | Total | 4.11 | 50.00 | | | |

**Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. | 95.0% Confidence Interval for B | |
|---|---|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | | Lower Bound | Upper Bound |
| female | (Constant) | 1.11 | 0.14 | | 8.01 | 0.00 | 1.40 | 0.83 |
| | LogCW_F | 3.34 | 0.14 | 0.97 | 24.05 | 0.00 | 3.06 | 3.62 |
| male | (Constant) | 1.18 | 0.11 | | 10.93 | 0.00 | 1.41 | 0.97 |
| | LogCW_M | 3.47 | 0.11 | 0.98 | 30.94 | 0.00 | 3.25 | 3.71 |

C. Independent Samples t –test analysis of between CW and GBC of wild caught *S. olivacea* samples from Setiu Wetlands, Terengganu.

**Group Statistics**

| Model | sex | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| CW | Female | 39 | 10.12 | 1.85 | 0.30 |
| | Male | 51 | 9.33 | 1.78 | 0.25 |
| GBC | Female | 39 | 2.56 | 0.71 | 0.11 |
| | male | 51 | 2.25 | 0.77 | 0.11 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | T | Df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| CW | Equal variances assumed | 0.77 | 0.38 | 2.06 | 88.00 | 0.04 | 0.79 | 0.39 | 0.03 | 1.56 |
| | Equal variances not assumed | | | 2.05 | 80.28 | 0.04 | 0.79 | 0.39 | 0.02 | 1.56 |
| GBC | Equal variances assumed | 0.76 | 0.39 | 1.94 | 88.00 | 0.06 | 0.31 | 0.16 | -0.01 | 0.63 |
| | Equal variances not assumed | | | 1.96 | 84.56 | 0.05 | 0.31 | 0.16 | 0.00 | 0.62 |

*. Significant value at the 0.05 level (2-tailed)

D. Independent Samples t –test analysis of between GBC and Estimated age of wild caught *S. olivacea* from Setiu Wetlands, Terengganu.

**Group Statistics**

| Model | Sex | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| GBC | Female | 3 | 2.00 | 1.00 | 0.58 |
| | Male | 3 | 2.00 | 1.00 | 0.58 |
| EstiAge | Female | 3 | 1.34 | 0.67 | 0.39 |
| | male | 3 | 1.89 | 0.94 | 0.55 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| GBC | Equal variances assumed | 0.00 | 1.00 | 0.00 | 4.00 | 0.94 | 0.00 | 0.82 | -2.27 | 2.27 |
| | Equal variances not assumed | | | 0.00 | 4.00 | 0.94 | 0.00 | 0.82 | -2.27 | 2.27 |
| EstiAge | Equal variances assumed | 0.23 | 0.66 | -0.82 | 4.00 | 0.45 | -0.55 | 0.67 | -2.41 | 1.30 |
| | Equal variances not assumed | | | -0.83 | 3.61 | 0.46 | -0.55 | 0.67 | -2.49 | 1.39 |

*. Significant value at the 0.05 level (2-tailed)

E. Independent Samples t –test analysis of between CW and Estimated age of wild caught *S. olivacea* from Setiu Wetlands, Terengganu.

**Group Statistics**

| Sex | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| CW | male | 51 | 9.33 | 1.78 | 0.25 |
| | female | 39 | 10.12 | 1.85 | 0.30 |
| EstiAge | male | 51 | 2.13 | 0.73 | 0.10 |
| | female | 39 | 1.71 | 0.48 | 0.08 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference Lower | Upper |
| CW | Equal variances assumed | 0.77 | 0.38 | 2.06 | 88.00 | 0.04 | 0.79 | 0.39 | 1.56 | 0.03 |
| | Equal variances not assumed | | | 2.05 | 80.28 | 0.04 | 0.79 | 0.39 | 1.56 | 0.02 |
| EstiAge | Equal variances assumed | 12.92 | 0.00 | 3.13 | 88.00 | 0.02 | 0.42 | 0.14 | 0.15 | 0.69 |
| | Equal variances not assumed | | | 3.31 | 86.10 | 0.02 | 0.42 | 0.13 | 0.17 | 0.68 |

*. Significant value at the 0.05 level (2-tailed)

F. Growth parameters interpret by SPSS software output using von Bertalanffy equation $CW_t = CW_\infty [1 - \exp^{-K(t-t0)}]$ of female and male wild caught *S. olivacea* from Setiu Wetlands, Terengganu.

**Parameter Estimates**

| Parameter | Estimate | Std. Error | 95% Confidence Interval Lower Bound | Upper Bound |
|---|---|---|---|---|
| $CW_\infty$ | 28.011 | 198.042 | -247.854 | 303.875 |
| K | 0.369 | .609 | -1.158 | 1.897 |
| $t_0$ | 0.734 | 4.618 | -4.857 | 6.325 |

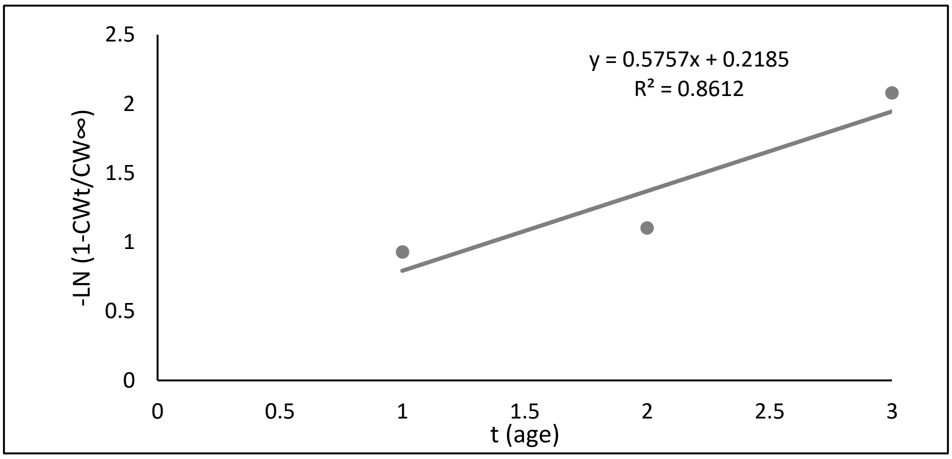
Female wild caught *S. olivacea*

**Parameter Estimates**

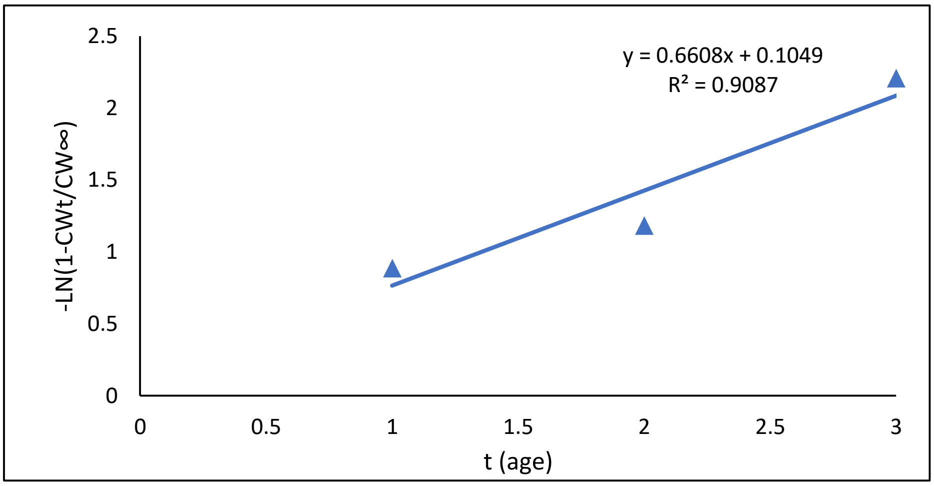
| Parameter | Estimate | Std. Error | 95% Confidence Interval Lower Bound | Upper Bound |
|---|---|---|---|---|
| b1 | 29.340 | 149.297 | -260.574 | 319.254 |
| b2 | 0.380 | .309 | -.557 | 1.318 |
| b3 | 0.792 | 2.277 | -6.572 | 8.156 |

Male wild caught *S. olivacea*

G. Von Bertalanffy plot describe in Excel using equation –Ln (1 – $CW_t$ / $CW_\infty$) against t (age) in of female and male wild caught *S. olivacea* from Setiu Wetlands, Terengganu.




Female wild caught *S. olivacea*



Male wild caught *S. olivacea*

## BIODATA OF THE AUTHOR

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Nurul Hasyima Binti Ismail was born in Hospital Besar, Kuala Terengganu, Malaysia, in 1990 and raised in a Kampung Seberang Baroh, Chabang Tiga, Kuala Terengganu, Terengganu. Her mother, Salimah Binti Abdul Ghani is now 4 years government retiree and her father was 20 years passed away. She was the younger and only daughter in a family. Therefore, she become an apple of the eye in her family.

Nurul Hasyima attended secondary school at Sekolah Menengah Kebangsaan Sultan Ahmad, Terengganu and after SPM, she enrolled Universiti Malaysia Terengganu and received her Diploma in Fisheries on 2011. In 2011, she continued her study and received the Bsc. of Applied Science (Conservation & Management of Biodiversity) in 2014. Her final year project were focussed on fish diversity and ecology. For one year, she started in several of career such as part time school teacher and also tour consultant at travels and Tour Company. Through working experiences, she has gained such a valuable experiences and has improved her soft skills in working environment.

In September 2015, she decided to continue her master's in major Fisheries Management and Aquaculture under supervision of Prof. Emeritus Dr. Mohd Azmi bin Ambak and Prof. Dr. Mhd Ikhwanuddin bin Abdullah until present day. She hopes to pass with flying colour in her master's research.

She reflect a varied personality including ambition and the qualities of generosity and thoughtfulness. She's also a well determined and vigorous individual, yet pleasantly calm. She encourage fighting for what she desire and believe in and doing it through God because nothing great comes easy and with God everything is possible. Since she a full-time student, motivated by her love for learning and succeeding as she strive to become an outstanding and successful person in the future.

## PUBLICATION

1. Published
   **N. Hasyima-Ismail,** A. Amin-Safwan, N. Fairuz-Fozi, F.H. Megat, H. Muhd- Farouk, S.A. Kamaruddin, M. Ikhwanuddin and M.A. Ambak. (2017). Study on carapace width growth band counts relationship of orange mud crab, *Scylla olivacea* (Herbst, 1796) from Peninsular Malaysia, Malaysia. Pak. J. Biol. Sci., 20: 140-146.

2. Submitted
   **Nurul Hasyima-Ismail,** Siti Azrina Kamaruddin, Adnan Amin-Safwan, Fadhlul Hashim Megat, Nur Fairuz-Fozi, Mat Piah Rumieaida, Mhd Ikhwanuddin and Mohd Azmi Ambak. Validation of growth band counts and size of carapace width relationship for age determination of orange mud crab reared in captivity. Journal of Marine and Coastal Fisheries, Taylor & Francis. Currently under reviewed.

3. Submitted
   Mhd Ikhwanuddin, Huda Abdullah Nurul, Ambok Bolong Abol-Munafi, Mohd Pauzi Mardhiyyah, **Nurul Hasyim-Ismail**, Adnan Amin-Safwan and Harman Muhd-Farouk. Development of ovarian maturation in orange mud crab, *Scylla olivacea* (Herbst, 1796) through induction of eyestalk ablation and methyl farnesoate. Aquaculture International. Currently under reviewed.

## PROCEDDING

1. Poster presenter
   **Nurul Hasyima-Ismail**, Mohd Azmi Ambak and Mhd Ikhwanuddin. Growth and age estimation of orange mud crab, *S. olivacea* (Herbst, 1796) by growth band analysis from Setiu Wetlands, Terengganu. 6$^{th}$ International Conference on Biotechnology for the Wellness Industry, "Wellness for Health and Productivity: Recent Trends and Oppotunities", Equatorial Hotel Melaka, Malaysia. 16$^{th}$ – 17$^{th}$ August 2016.

2. Poster presenter
   **Nurul Hasyima-Ismail**, Mohd Azmi Ambak and Mhd Ikhwanuddin. Age determination of orange mud crab from Setiu Wetlands, Terengganu, *Scylla olivacea* (Herbst, 1796) in captivity. International Fisheries Symposium (IFS 2016) "Promoting Healthier Aquaculture and Fisheries for Food Safety and Security", Phu Quoc Island, Vietnam, 31st October- 2nd November 2016.

3. Oral presenter
   **Nurul Hasyima- Ismail**, Mohd Azmi Ambak, Mhd Ikhwanuddin, Amin – Safwan Adnan, Megat Fadhlul Hashim, Muhd- Farouk Harman. A preliminary investigation on age estimation of orange mud crab *Scylla olivacea* (Herbst, 1796) using growth band from Setiu Wetlands, Terengganu. 13$^{th}$ Universiti Malaysia Terengganu International Annual Symposium on Sustaninability Science and Management (UMTAS 2016), Primula Beach Hotel, Terengganu, 13$^{th}$ – 15$^{th}$ December 2016.

## ACTIVITIES

1. Secretariat
   Program Identity Ekonomi Nelayan Besut, Aqua – Tech 2016. Universiti Malaysia Terengganu (UMT). 22 January 2016.
2. Secretariat
   International Workshop on Portunid Crabs Aquaculture and Sustainable Fisheries (2016). Universiti Malaysia Terengganu. 28 – 29 March 2016.
3. Secretariat
   Seminar Ekosistem Setiu 2016. Sains Marin dan Sumber Akuatik untuk kelangsungan hidup. Universiti Malaysia Terengganu. 25 July 2016.
4. Participant
   Bengkel Sehari Penulisan Tesis Pantas dan Pengurusan Artikel Mendeley. Malaysian Postgraduate Workshop Series. Hotel Grand Puteri, Terengganu. 12 March 2016.
5. Participant
   Bengkel Pemantapan Ilmu 'Boost up Your Writing Skills' 2017. Institute Topical Aquaculture, Universiti Malaysia Terengganu. 14$^{th}$ – 16$^{th}$ August 2017.
6. Participant
   Bengkel Penerbitan Artikel Jurnal Terindeks 2017. Institute Topical Aquaculture, Universiti Malaysia Terengganu. 3th – 4$^{th}$ October 2017 and 14$^{th}$ – 17$^{th}$ November 2017.