NOR HAZIRAH BINTI MOHD ZUKI DOCTOR OF PHILOSOPHY 2024

MATURATION PHASES OF MALE PARROTFISH (*Scarus rivulatus*, *S. qouyi* AND *S. ghobban*) IN RELATION TO REPRODUCTIVE INDEX, SEX STEROID HORMONES AND FATTY ACID PROFILE AT PULAU BIDONG, TERENGGANU.

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Thesis Submitted in Fulfilment of the Requirement for the Degree of Philosophy
In the Institute of Oceanography and Environment
Universiti Malaysia Terengganu

2024

DEDICATION

Alhamdulillah, all praises to Allah for granting me a good life and great health up until now.
I am so grateful to Allah for giving me the strength to overcome every single obstacle during this journey.
Thank you Allah

Special dedication to my Mom and Dad…
Norhashimah Bt Ismail & Mohd Zuki Bin Abdul Rahman
Your duas, blessings, and sacrifices meant so much to me.
Your kindness, attention and care toward my little one cannot be repaid.

To my dearest beloved husband, Ahmad Shahril Bin Rosli….
Your abundance of love, sincerities and blessings keep me sturdy through this journey.

To my little handsome man, Hadif Naufal…
Thank you for being such an understanding boy.
Just like the fireflies, your presence is like the glow that in spite of all darkness and sorrows.

To my siblings and in-laws…
Along, my brother Haziq and Izzat, Mira & Umi…
Thank you for being there for me and never-ending support.

Special dedication toward my supervisors…
Thank you Prof and Dr. for the endless support, guidance and patience in finishing this PhD journey.

To all my friends
I am very lucky to be able to experience life alongside you.

My success is the greatest gift to all especially Mom and Dad,
The man of my life
and son.

May Allah grant His best reward and ease every hardship to all.
Aamiin.

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirements for the degree of Doctor of Philosophy

MATURATION PHASES OF MALE PARROTFISH (*Scarus rivulatus*, *S. qouyi* AND *S. ghobban*) IN RELATION TO REPRODUCTIVE INDEX, SEX STEROID HORMONES AND FATTY ACID PROFILE AT PULAU BIDONG, TERENGGANU

NOR HAZIRAH BINTI MOHD ZUKI

2022

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Parrotfish play a significant role in tropical coral reef ecosystems. This study investigates maturation phases in three male parrotfish species; Surf parrotfish (*Scarus rivulatus*), Quoy's parrotfish (*S. qouyi*) and Yellowscale parrotfish (*S. ghobban*). Fish specimens were collected from Pulau Bidong, Terengganu, South China Sea. Parrotfish are hermaphroditic, with gonad development in males starting from transition phase (sex change phase) to male adult phases (initial and terminal). The stages of gonad development were differentiated through a histological examination procedure, during which the spermatogenic cells were identified and categorized. For the initial gonad development phase, there are four stages: developing, spawning, regressing, and regenerating. In contrast, the terminal phase only includes developing, spawning, and regressing stages. After the transition phase, the gonads in all parrotfish species developed according to similar microcharacteristic cycle criteria. Study found significantly high mean values of spermatozoa cells that positively correlated with reproduction index (gonadosomatic and hepatosomatic) during spawning stages of both initial and terminal phases, as well as constant higher level of 11-ketotestosterone hormone, compared to estradiol. Fatty acid analysis identified a total of 27 fatty acids (FAs), which were classified into saturated FAs, monounsaturated FAs, and polyunsaturated FAs. There were

similarities in FA profiles among parrotfish species. The mean concentration did not differ significantly across maturation phases ($p>0.05$), as the SIMPER analysis revealed that the changes in mean values between gonad phases in parrotfish showed a lower dissimilarity percentage of dissimilarity than similarities. The high average values of saturated FAs (C14:0, C16:0 and C18:0) during transition phase indicate that parrotfish require high energy reserves during the sex change phase to male individuals. There were fluctuations of polyunsaturated FAs concentration from the initial to terminal phases following the transition to male parrotfish. The mean concentration increases from the developing to the regressing stages, indicating that the polyunsaturated FAs are essential for the reproductive performance of male parrotfish.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

FASA KEMATANGAN IKAN BAYAN JANTAN (*Scarus rivulatus, S. qouyi* DAN *S. ghobban*) DAN KAITANNYA DENGAN INDEKS PEMBIAKAN, HORMON STEROID PEMBIAKAN DAN PROFIL ASID LEMAK DI PULAU BIDONG, TERENGGANU

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Professor Takaomi Arai, PhD
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Ikan Bayan memainkan peranan yang penting dalam ekosistem terumbu karang tropika. Kajian ini mengenal pasti fasa pematangan bagi tiga spesis ikan Bayan jantan; Surf parrotfish (*Scarus rivulatus*), Quoy's parrotfish (*S. qouyi*) dan Yellowscale parrotfish (*S. ghobban*) yang diperoleh dari Pulau Bidong, Terengganu, Laut China Selatan. Ikan Bayan adalah hermafrodit, yang mana perkembangan gonad jantan bermula daripada fasa peralihan (pertukaran jantina) kepada jantan (awal dan terminal). Peringkat perkembangan gonad dibezakan melalui prosedur pemeriksaan histologi di mana sel spermatogenik dikenal pasti dan dibezakan. Untuk fasa pembangunan gonad awal, mereka mempunyai empat peringkat; pengembangan, pembenihan, penerimaan dan regenerasi, manakala untuk fasa terminal hanya mengalami perkembangan, pembenihan dan regresif. Keputusan menunjukkan bahawa selepas fasa peralihan, gonad dalam semua spesis ikan Bayan mempunyai kriteria kitaran ciri mikro yang serupa. Kajian mendapati nilai purata sel spermatozoa yang ketara tinggi berkorelasi secara positif dengan indeks pembiakan (gonadosomatik dan hepatosomatik) semasa peringkat peralihan kedua-dua fasa awal

dan terminal, serta tahap hormon 11-ketotestosteron yang lebih tinggi berbanding estradiol. Analisis asid lemak mengenal pasti sejumlah 27 asid lemak (FA), yang dikelaskan kepada FA tepu, FA tak tepu tunggal dan FA poli tak tepu. Terdapat persamaan dalam profil FA di kalangan spesies ikan Bayan. Purata kepekatan adalah tidak berbeza dengan ketara mengikut fasa pematangan ($p>0.05$) kerana melalui SIMPER analisis, perubahan nilai purata antara fasa perkembangan gonad dalam ikan Bayan mempunyai nilai peratusan ketidaksamaan yang rendah berbanding persamaan. Nilai purata FA tepu yang sangat tinggi (C14:0, C16:0 dan C18:0) semasa fasa peralihan gonad menunjukkan bahawa simpanan tenaga yang tinggi diperlukan ikan Bayan semasa fasa peralihan jantina kepada individu jantan. Terdapat turun naik kepekatan FA poli tak tepu dari fasa awal kepada fasa terminal selepas peralihan kepada ikan Bayan jantan. Purata kepekatan meningkat dengan ketara daripada peringkat pengembangan kepada peringkat regresif, yang menunjukkan bahawa FA poli tak tepu adalah penting untuk prestasi pembiakan ikan Bayan jantan.

ACKNOWLEDGEMENTS

My PhD research would not have been possible without the blessings of Allah the Almighty. It is with His blessings that, after years of bending my efforts here at the UMT, I'm finally able to complete this research. My sincerest appreciation goes to my main supervisors, Prof. Dr. Zainudin Bachok, Dr Siti Ariza, and Prof. Takaomi Arai, who have been providing me with guidance and new ideas that never failed to pique my curiosity throughout the duration of this research. These special individuals have always been understanding and helpful to ensure that my research achieved its objectives and stayed on track. Not to be forgotten, to the late Dr. Safiah, whose assistance had helped me throughout the year before she passed on to meet her Creator. Her passing was indeed a heart-breaking loss to me and a grief I carried until the end of my PhD journey. She was kind and remained committed to helping me despite her illness. My memories with her will forever stay in my heart.

In the years I spent conducting my PhD research, I have faced and walked through a series of challenges. Brazing with me through this taxing course was my beloved husband Ahmad Shahril, who has been very understanding and extremely supportive of my studies. I do not think I could thank him enough for his endless support and his words of encouragement, especially during times when I hit hard bottom. I would also like to express my gratitude to my parents and siblings for the sacrifices they made to look after my wee prince, Hadif Naufal, from the moment when he was just three-month old to when he was four. Thank you, *Mak* and *Abah,* for staying up late to put Hadif to bed and showering him with love no less than the one you gave me when I was a child. I would not have survived this journey if my parents had not been behind my back. May Allah reward my husband and parents with continuous blessings for their kindness and generous assistance in my moment of need.

I would like to take this opportunity to mention several names that played significant roles in every stage of my PhD research, particularly to the wonderful people who have lent me their time, energy, and skills with my fish sampling work on Bidong Island. The first group on the list are my co-workers – Siti Raudhah,

Amalina, Atiq, and a few of my friends – who have stayed with me burning the midnight oil to complete the process of fish sampling on the island. I would also like to extend my special thanks to the all officers from INOS, Puan Azwarina and Puan Atikah, for their guidance. Also, to the group of scuba-divers from UMT – the dive leader, En. Shahrul, followed by his fellow divers, En. Shahrul Idham, En.Ahmad Nazila, En. Andrew, En. Nasir and En. Uda, and others – for the hard work and dedication that they had put in to find the correct fish sample. I would also like to express my words of appreciation to the laboratory officers – En. Mat Embong, Pn. Atiyyah, Pn. Farizan, and Pn. Wahidah, to name a few – whom I have frequently consulted to for advice whilst conducting the experiments.

Additionally, I would also like to extend my warmest gratitude to the people outside of my research and professional communities who have been equally helpful throughout my PhD journey. To my housemates: Dr. Alia Syafiqah, Dr. Hafizah, Nassoriah, and Asmidar, Zahidah – thank you for always driving me, back and forth, from our campus to MBKT. Another group of people whom I would like to thank to are my relatives – *Along, Angah, Chu Yon*, my uncle, and late aunt – for their words of encouragement that have always added that extra push to help me move forward in my PhD studies.

I am aware that these acknowledgments will still fall small to the appreciation that they deserve. My PhD research would not have been successful if I had undertaken this research without the payers of a mother, a father, a husband and supervisors. Where I am today is due to their endless guidance and support. They were with me through my ups and downs, guiding me through every hurdle, and offering me with the best solution available. I am grateful to Allah for facilitating me at every step of my research journey, giving me the opportunity to cross path with some of the amazing people whose help and contributions I will forever be indebted to. To these people, I pray that Allah will make your lives, be they may here in this world or in the Hereafter, easy and successful.

APPROVALS

I certify that an Examination Committee has met on 16th June 2021 to conduct the final examination of Nor Hazirah Binti Mohd Zuki, on her Doctor of Philosophy thesis entitled **"Maturation Phases of Males Parrotfish (*Scarus rivulatus, S. qouyi* and *S. ghobban*) In Relation to Reproductive Index, Sex Steroid Hormone And Fatty Acid Profile At Pulau Bidong, Terengganu"** 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:

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This thesis has been accepted by the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Doctor of Philosophy.

MOHD FADZIL MOHD AKHIR,
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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.

_____

NOR HAZIRAH BINTI MOHD ZUKI

Date:

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>	More than
<	Less than
±	Plus minus
º	Degree
%	Percentage
µm	Micrometer
Mg	miligram
Cm	Centimeter
FAs	Fatty acids
G	Gram
Pg	picogram
µl	Microliter
GSI	Gonasomatic index
HIS	Hepatosomatic index
K factor	Condition factor
IP	Initial phase
TP	Terminal phase
Dvp	Developing
Spw	Spawning
Rgs	Regressing
Rgn	Regenerating
SW	Southwest
NE	Northeast
Sg	Spermatogonia
St	Spermatid
Sc	Spermatocyte
Sz	Spermatozoa
SAFA	Saturated fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
TL	Total length
BW	Body weight
SL	Standard length
SR	*Scarus rivulatus*
SQ	*Scarus qouyi*
SG	*Scarus ghobban*
SPSS	Statistical Package for the Social Sciences
PRIMER	Plymouth Routines in Multivariate Ecological Research
ANOVA	Analysis of variance
PERMANOVA	Permutational Multivariate ANOVA
SIMPER	Similarity Percentage
FAME	Fatty acid methyl ester
GCFID	Gas Chromatography with Flame Ionisation Detection
ANCOVA	Analysis of covariance

Spp.	Species
IUCN	International Union for Conservation of Nature
EAM	Epilithic algal matrix
11KT	11-ketotestosterone
E2	Estradiol
CF	Condition factor

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the Study

Parrotfish are among coral reef fishes from family Scaridae known as an important functional group within herbivorous fish. Most parrotfish are diandric protogynous hermaphrodite, which has a complex adult phase (initial & terminal). Parrotfish are commonly found near to the coral reef area in large schooling group during the day (Meyer et al., 2010). Species aggressions of parrotfish may occur either through intraspecific (same species) or interspecific (different species) interaction (Mumby & Wabnitz 2002).

As diandric hermaphrodite, male parrotfish may derive into two pathways; from matured females or directly from juvenile (Fennessy & Sodavy, 2002). There are several factors that may influent the gonad maturation in parrotfish such as growth rate via length-weight relationship (Amin et al., 2019), nutrition from food intake, and secretion of reproductive hormones (Volkoff & London, 2018). Besides that, parrotfish are dichromatic fish where a drab colour female will turn into a bright colour male after sex change had occurred (Povlowich et al., 2018; Choat & Robertson, 1975).Male fish is highly demanded as food fish or sport fishing (Reef Check Malaysia, 2018).

Generally, parrotfish have multiple functions in the coral ecosystem, especially to balance macroalgae population. A study done by Holbrook et al., (2016) had shown a strong relationship between herbivorous fish with macroalgae effect. They also revealed that the decreased number of herbivorous fishes greatly influences the surge of biomass algae. Apart from that, parrotfish has the ability to remove and clean the reef substrate directly open the new space for coral larvae to

settle down on the substratum, thus increase the survival of coral planulae (Streelman et al., 2002 ;Bonaldo & Bellwood, 2009). Another function of parrotfish within reef areas is to facilitate bioerosion process and sediment production (Comeros-raynal et al., 2012; Hoey & Bellwood, 2008; Schonberg et al., 2017). This emphasises the significant of parrotfish in coral reef areas and how important it is to conserve their populations in reef areas.

Up to now, most studies discussed where parrotfish have haremic mating system (Munday et al., 2006). The terminal male becomes as dominant male in the harem group spawn with multiple females within the territory (van Rooij et al., 1996a). Spawning in parrotfish occur along the year where spawning pattern of female and male positively correlated with GSI and HSI and morphometric index was found at each month and lunar phase (Gonzalex-Felez et al., 2019; Tailor and Cruz, 2017; El-Sayedah et al., 2012; Kokokiris et al., 2006). Besides that, during gonad maturation, sex steroid hormone such as testosterone, estradiol and 11-keto testosterone was released which is closely related to the sex change event from female to male yet possibly has significant different at different gonad maturation stages (El-Sayedah et al., 2012).

With regard to fish reproduction, nutrient requirement at each different gonad phase might be changed due to energy demand and gametogenesis (Babatunde et al., 2020; Simat et al., 2020; Kacar et al., 2016). These changes were recognized through the lipid content by fatty acid analysis (Gonzalez-Felix et al., 2019; Anido et al., 2015). Previous studies show that fatty acid SAFAs, MUFAs, and PUFAs was associated with the initiation of eggs release (Saudant et al., 1996; Bachok et al., 2009), fish metabolism (Tocher, 2003) and essential for growth in an organism (Tazbozan & Ali, 2017). The composition of this fatty acid in reef fish individuals may be varied due to fish diet (Fey et al., 2021). In relation to reproduction index, hormone and fatty acid composition, maturation of male gonads were also determined through characteristic of spermatogenic cells present in the testis via histological examination (Abdel-Aziz et al., 2006; Ebisawa et al., 1999). However, until now there was no literal study on the male gonad maturation phase of parrotfish at the East coast of Malaysia.

In Peninsular Malaysia parrotfish are typically found to inhabit most coral reef ecosystems. A little study on the parrotfish in Malaysia merely focuses on fish abundant and distribution with a selected island at East Coast and West Coast of Peninsular Malaysia (Reef Check Malaysia, 2019), fish diversity checklist at Redang Island (Du et al., 2019) and a single study on habitat ecology (Arai et al., 2015a) and moisture content (Arai et al., 2016). The abundance of parrotfish was lower in East Malaysia than in West Malaysia (Arai, 2014). However, according to Reef Check Malaysia (2019) the population of parrotfish in Malaysia is still considered in good condition, but their number starts to slowly reduce from 5.39 to 2.33 per $500m^3$ from 2010 to 2019 yet bumphead parrotfish are completely absent from most surveys. This was suggested that the fishing pressure of parrotfish species in Malaysia is quite high (Arai, 2014).

A study regarding parrotfish reproduction is essential which may provide a better understanding about how the changes of reproductive biology and the relationship between physiological factors and gonad development. It was known that the reproduction of parrotfish is complicated to describe (Clifton & Rogers, 2007; Munoz & Warner, 2004). However, the combination of biological aspects such as the maturation phase, reproductive index, food source and reproductive physiology like sex hormone with the comparisons from some previous studies may strengthen the existence data or provide better comprehensive data respectively.

1.2 Problem Statement and Justification of the Study

Recently parrotfish abundance was reported to keep decreasing in Malaysian waters (Reef Check Report 2021). Despite parrotfish having multiple functions such as macroalgae controllers in coral reef ecosystems, however, up to date there is limited published data related to parrotfish reproduction conducted at tropical regions especially at the East Coast of Malaysia, Terengganu. This deficiency leads to a poor understanding in the reproductive profile of diandric hermaphrodite parrotfish in relation to gonad maturation phases.

As a diandric protogynous hermaphrodite, male parrotfish have more size advantage and are targeted as food fish (Reef Check Report 2021). This can be seen where more male parrotfish were sold in wet markets in Sabah such as at Kota Kinabalu, Sandakan, Semporna and Kudat (Reef Check Malaysia, 2022). Furthermore, parrotfish are known as dimorphism where male can be differentiated by colour. Therefore, surely male parrotfish can be easily identified and confirmed from the external observation of their body colour. In contrast, female are less commonly found at wet markets. A study revealed that female parrotfish had smaller size than male (Tuwo et al., 2021). Possibly this is one of the reasons why females less targeted or caught, also might be due to less market value compared to male.

There is no experimental study literally explains the relation between the maturation phase of male individuals with length-weight relationship, reproductive index (gonadosomatic index, GSI, Hepasomatic index, HSI and condition factor, CF), sex steroid hormone pattern and fatty acid profiles in parrotfish at the East Coast of Malaysia. Fatty acid profile may change to fulfill adequate nutrition requirements for gonad development. Whether there is any change in fatty acid profile of parrotfish at different maturation phases of Scarus genus is still unknown.

Other than that, releasing of sex steroid hormones such as estradiol and 11-ketotestosterone may be associate with the maturation of gonad phase. There might be a different pattern of hormone at different gonad phases. However, the pattern of this hormone profile at different maturation phases in parrotfish of *Scarus* genus at East Coast of Malaysia has still not been discovered and investigated. Hence, in general, this study will provide new baseline data for three species of parrotfish of the genus Scarus in the East coast of Malaysia, specifically in Pulau Bidong, Terengganu, in terms of differentiating microcharacteristic criteria of the gonadal developmental phase of male parrotfish, the relationship of length and weight to reproductive index, the change in gonadal maturation phase in relation to spermatogenic cell count, reproductive hormone pattern and fatty acid profile in fish reproduction.

1.3 Significant of the Study

This study will contribute to a better understanding on the gonad reproductive phase in parrotfish reproduction at the East Coast of Malaysia specifically Pulau Bidong, Terengganu. Male adult phase in parrotfish is divided into two phases (initial and terminal). Therefore, other than body color differentiation, histological study may investigate the difference between these two adult phases from the microscopic examination aspect. Eventually, this study also provides the first compilation of male gonad maturation phases in *Scarus* genus at East Coast of Malaysia.

Besides understanding parrotfish maturation phase criteria, the relation between reproductive index which were GSI and HSI, reproductive hormone and fatty acid profile will provide information data to relate to parrotfish reproduction. Therefore, the study of the maturation phase in conjunction with the reproductive index will provide information on the extent to which GSI and HSI of male parrotfish are involved during gonadal maturation. This is because GSI and HSI are commonly used as a sign of the spawning phase that occurs during fish reproduction

Other than that, change of hormone profile of two important sex steroid hormones which are estradiol and 11-ketotestosterone will indicate their relation to gonad maturation phase in male parrotfish. It is either estradiol and 11-ketotestosterone has a significant role during gonad maturation in fish could be answered.

Meanwhile, study of fatty acid composition will contribute to understanding any changes in fatty acid profile of male parrotfish at different gonad development phases of *Scarus* genus. Thus, this present study aim to explain the relation of reproductive parameters specifically length-weight relationship, reproductive index, sex steroid hormone and fatty acid profile towards the gonad maturation phase in male parrotfish of three *Scarus* genus at coral reef ecosystem at Pulau Bidong, Terengganu, East Coast of Malaysia.

Perhaps the present study could improve the understanding on differentiation of gonad phase between initial and terminal phase of male parrotfish. Also, provide additional data and further reference to understand the male gonad maturation phase, especially for diandric protogynous hermaphrodite fish.

1.4 Objectives of the Study

This study aims to provide a better understanding of the gonad maturation phase of male parrotfish in relation to the reproductive index, hormone and fatty acid profile at Pulau Bidong, Terengganu, Malaysia. The specific objectives were:

1. To determine the length-weight relationship of male of three parrotfish species *Scarus* genus (*Scarus rivulatus*, *S. qouyi* and *S. ghobban*) in relation to their reproduction (GSI, HSI & CF) at Pulau Bidong, Terengganu.

2. To determine the maturation phases of the male parrotfish of *Scarus* genus (*S. rivulatus*, *S. qouyi* and *S. ghobban*) through histological examination and its relation to reproductive hormone profile.

3. To study the relation of fatty acid profile at gonad maturation phases of male parrotfish of *Scarus* genus (*S. rivulatus*, *S. qouyi* and *S. ghobban*).

1.5 Hypothesis

All the objectives stated above were hypothesized as below:

1. Length-weight relationship is related to the reproductive index in male parrotfish of *Scarus* genus (*S. rivulatus, S. qouyi* and *S. ghobban*).

2. Gonad maturation phases show a similar micro-characteristic pattern for all three parrotfish species of *Scarus* genus, significantly relation to reproductive hormone (estradiol & 11-ketotestosterone) profiles, spermatogenic cell numbers, and reproductive index (GSI and HSI).

3. The pattern of fatty acid profiles in male parrotfish of *Scarus* genus is associated toward gonad maturation phases.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of Parrotfish

Parrotfish was significantly classified into two clades (seagrass or coral reef) based on the phylogenetic DNA study by Streelman et al., (2002). He points out that the divergence of these clades in parrotfish occurred nearly 42 million years ago. From his analysis, Seagrass genera composed of genus *Colomus*, *Cryptotomus*, *Sparisoma*, *Leptoscarus* and *Nicholsina* while coral reef genera composed of genus *Scarus*, *Chlorurus*, *Hipposcarus*, *Cetoscarus* and *Bolbometopon*. It has also been revealed that parrotfish from genus Scarus and Chlorurus were identified to monopolize the species at 76% from 80% of reef clade compared to seagrass. Additionally, Scaridae was among the largest parrotfish family composed of 90 species with about 51 species for genus Scarus and 17 species for Chlorurus.

Generally, *Scarus* genus was shown to be the major species among parrotfish. Three male parrotfish in the present study were coming from the same genus and family as shown in Figure 2.1. The colour variation among the species is distinctly different. Clearly, the orange patches of *S. rivulatus* are on the cheek and *S. qouyi* with bright blue-green patches. *S. ghobban* has a dull orange colour with blue body line bars. Scarus has strong teeth with beak-like tooth plates on jaws that experience scrapping feeding mode (Streelman et al., 2002). According to Matsunuma et al., 2011, Scaridae typically scrap algae from the dead substrate of the coral. The bitten crushed rock with the algae is released into the sand and ground, indeed this parrotfish family is known as an important producer of sand in coral reef areas.

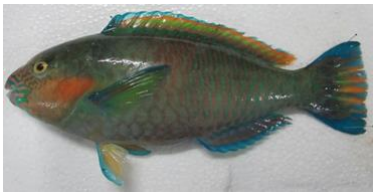	 (Matsunuma et al., 2011)	
Kingdom: Animalia Phylum: Chordata Class: Actinopterygii Oder: Perciformes Family: Scaridae Genus: Scarus Species: *S. rivulatus* Valenciennes, (1840)	Kingdom: Animalia Phylum: Chordata Class: Actinopterygii Oder: Perciformes Family: Scaridae Genus: Scarus Species: *S. qouyi* Valenciennes, (1840)	Kingdom: Animalia Phylum: Chordata Class: Actinopterygii Oder: Perciformes Family: Scaridae Genus: Scarus Species: *S. ghobban* Forsskål, (1775)

Figure 2.1 Taxonomy of three parrotfish species at Pulau Bidong

Scaridae family was a foraging group of parrotfish actively observed during the day (Robertson and Warner 1978). At night times, adult parrotfish commonly slept under the sheltered coral at shallow water depth 6-16 meters (van Rooij et al., 1996a). Parrotfish individuals of *Sparisoma viride* were frequently observed to have the same sleeping site. Uniquely, some parrotfish species *S. vertula*, *S. iserti* and *S. taeniopteris* sleep in the cocoon during the night (van Rooij et al., 1996a). This sleeping behaviour by parrotfish indirectly could also protect them from any predation encounter in the reef community.

Species	Initial phase (IP)	Terminal phase (TP)
S. rivulatus	 Lioa et al., (2004)	
S. qouyi	 Matsunuma et al., (2011)	 Lioa et al., (2004)
S. ghobban		 Lioa et al., (2004)

Figure 2.2: The color differentiation of initial and and terminal phase of three parrotfish species *S. rivulatus, S. qouyi* and *S. ghobban.*

Apart from that, parrotfish in the Scaridae family were known to experience two complex adult phases after the juvenile which were termed as initial phase (IP) and terminal phase (TP) (El-Sayed et al., 2011). Both of these adult phases could be recognized externally by body colour as shown in Figure 2.2. Based on the previous study, Choat et al., (1996), has revealed that, at the same age, Scaridae TP males were larger than IP males and females with bright colours (El-Sayed et al., 2011). Naturally, stoplight parrotfish *Sparisoma viride* is considered matured when they enter the initial phase followed by terminal phase (Pavlowich et al., 2018). This perception was adapted from a previous study by van Rooij et al., 1996a in Scaridae. A study on sexual patterns in Scaridae found that the range size between the initial phase and terminal phase was overlapped, and also species dependent (Robertson & Warner 1978). The occurrence of this condition might be correlated with the different lengths at age (Choat et al., 1996), size at maturity (Barba, 2010; NOAA, 2008), types of social interaction (i.e group vs territories) (van Rooij et al., 1996a) and growth strategy (Amin, 2019) of Scaridae family. From these complexity factors,

the initial phase and terminal phase were impossible to be classified or differentiate by using size structure.

2.2 Function of Parrotfish at Reef Ecosystem

Parrotfishes are a significant group of reef herbivores recognized for their numerous functional roles on coral reefs (Roberta, 2010). Parrotfish are responsible for algal growth control, sediment ingestion and production, bioerosion, and coral predation on coral reefs (Choat 1991; Hoey & Bellwood 2008; Bellwood et al., 2003; Alwany et al., 2009). Previous research had established that herbivorous fish like parrotfish belonging to various families, groups, and species served different roles in coral reef ecosystems (Hoey & Bellwood 2009).

Besides that, parrotfish feed on almost all coral reef substratum types, including volcanic rock, live coral colonies and dead coral surfaces covered by algal turfs, hence causing a significant impact on the benthic substratum of coral reefs (Bellwood & Choat 1990; Bruggemann et al., 1994; Bonaldo et al., 2006). Numerous functional groups within parrotfishes have been classified based on morphology and feeding behaviour which are excavators, scrapers and browsers. (Bellwood & Choat, 1990; Streelman et al., 2002). The classification of species into functional groupings is essential for recognizing their influence on bioerosion, coral fitness and survival, habitat alteration, and ecosystem dynamics (Bellwood & Choat, 1990; Bellwood et al., 2004).

Excavators have a deep shape and thick cement covering of jaws. The fish show short, powerful bites and a greater degree of grazing which remove large pieces of the substratum leaving a prominent scar (Nanami et al., 2016). The most common excavators consist of genus *Bolbometopon*, *Cetoscarus*, *Chlorurus* and some species of *Sparisoma* (Bellwood & Choat, 1990; Bellwood, 1994). In comparison, scrapers have a shallow shape and thin cement covering jaws which show shallow bites and graze less per bite unit (Lokrantz et al., 2008; Bonaldo et al., 2011). Several

Sparisoma species and genus *Scarus* were classified into scrapers group (Bellwood & Choat, 1990; Bellwood, 1994). Scar characteristics differed between excavators and scrapers based on jaw morphology (Nanami et al., 2016). According to Bellwood and Choat (1990), excavators have slow bites and feed at higher rates while scrapers take rapid bites and feed at relatively lower rates. Browsers have relatively weak jaws and extract food from the substrata using discrete teeth without scarring it (Bellwood & Choat, 1990; Streelman et al., 2002).

Parrotfish are vital to the reef's ecosystem survival due to the cleaning function of the fish which consume algae on the coral reef. Hence, the coral is able to grow and become more resilient to local stressors such as pollution and global warming (Gallagher, 2018). Besides that, some species of parrotfish graze the corals and excrete the undigested material as sand contributing to the tropical white sandy beaches. According to Perry et al. (2012), parrotfish are the dominant contributors to bioerosion on coral reefs and are the main determinants in total reef carbonate budgets. The parrotfish community in the coral reef contributes to the higher amount of coral cover which is proportionally related to the net positive carbonate balance (Januchowski-Hartley et al., 2017). Jackson et al. (2014) stated that parrotfish are crucial for coral recovery in temperate countries and become an important component to protect the marine ecosystem in Malaysia.

In the previous study by Gallagher (2018), reported that 55% of the world's coral reefs are affected by overfishing activity, hence reducing the coral cover in the area whereas it wiped out the population of parrotfish. The reduction in the parrotfish population contributes to the increased growth of macroalgae and eventually decreases in coral cover (McManus et al., 2000; Valentine & Heck 2005; Edwards et al., 2014). Thus, maintaining the parrotfish population is important for the resilience of reefs and supporting reef recovery.

2.3 Coral Reef Ecosystem of Pulau Bidong

In the east coast of Peninsular Malaysia, Bidong Archipelago was an unpopulated place consists of several islands which have much coral diversity (Reef Check Malaysia, 2015). One of the islands is Pulau Bidong, known as a research-based center for University Malaysia Terengganu. Pulau Bidong was a Vietnamese refugee until the late 1980s (Grismer et al., 2014). There were several islands and sandy beaches surrounding Pulau Bidong such as Pulau Karah, Pulau Gelok, Pulau Tengkorak, Pantai Pasir Cina, Pantai Pasir Tenggara and Pantai Vietnam (Afiq, 2021). There was less human, fishing and coastal development impact on the coral reef at this area (Safuan, 2021).

Even though Pulau Bidong was not gazetted as Marine Park, it has a well-developed coral reef ecosystem (Matsunama et al., 2011). Coral reef at Pulau Bidong was dominated by branching, foliose and massive coral cover (Afiq, 2021). The previous study revealed that, Pulau Bidong is rich with 20 genera of coral reef and the most dominant genus were Acropora, Fungia and Montipora (Safuan et al., 2020). The other coral taxa which can be found at Pulau Bidong such as Platygyra, Ctetanics, Favia, Herpolitha, Pavona, Porites and Pacillopora (Bachok et al., 2020). However, at January 2019 coral reef ecosystem at Pulau Bidong was affected by a tropical storm known as Pabuk which cause a high reduction or live coral cover around Pantai Pasir China about 19% and dead of coral cover from 26.3% to 65.09% (Safuan et al., 2020). Up to date, the coral reef condition around Pulau Bidong and Yu is considered as “Good” condition where the live coral cover was 55.52.%. (Reef Check Report, 2021). The major reef covered was hard coral with 39.06% (Reef Check Report, 2021). Other than that, around the Pulau Bidong area, the fish diversity was equally diverse, even and rich (Mat Piah et al., 2014).

There were several studies of reef fishes at Pulau Bidong such as the fatty acid composition of reef fishes (Arai et al., 2015b; Arai et al., 2015c) and fish diversity (Afiq et al., 2021; Mat Piah et al., 2014; Arai, 2014) had been done at reef ecosystem in Pulau Bidong. The fatty acids composition of several coral reef fish families (e.g. Lutjanidae, Labridae, Nemipteridae, Pomacentridae, Scaridae,

Serranidae) at Pulau Bidong indicated that, the diet of fish species at Pulau Bidong was from the same food source but might be different between species due to habitat use and migration (Arai et al., 2015a). The previous studies also revealed that, changes of fatty acids SAFAs and MUFAs possibly be affected by physiological condition, sexual development and recent feeding events near the reef areas (Arai et al., 2015d).

Recently, a study had showed that there was high fish diversity with a total 30 families, 61 genera and 101 species was identified at Pulau Bidong via underwater visual census (Afiq et al., 2021). According to a fish diversity study at Pulau Bidong, the dominant fish species were *Dascyllus trimaculatus*, *D. reticulatus*, *Pomacentrus moluccensis*, *Scolopsis monogramma* and *Nemipterus furcosus* (Mat Piah et al., 2014). On the other hand, the most diverse family was Pomacentridaes (damselfish) with a total of 19 species while Scaridae (parrotfish), Labridae (wrasse) and Serranidae (grouper) were identified as less diverse (Afiq et al., 2021). Additionally, according to the Reef Check Malaysia report surveyed in 2019, the highest abundance of fish at Pulau Bidong and Yu archipelago was Snapper, followed by Butterflyfish and Parrotfish. These undoubtedly show Pulau Bidong was rich with fish abundance and diversity.

2.4 Distribution of Parrotfish

Matsunuma et al., (2011) mentioned that, Scaridae are abundantly found at a shallow water region depth of less than 30 meters and has a wide spread of distribution. In worldwide there were 100 species and ten genera of parrotfish identified (Kulbicki et al., 2018). Among these ten genera, Scarus genus was able to be found in all oceans, Sparisoma and Cryptotomus were restricted to the Atlantic and five genera such as Bolbometopon, Cetoscarus, Calotomus, Hipposcarus, and Chlorurus were found at the Indo-Pacific region. Parrotfish species abundantly found in tropical reefs (Bellwood et al., 2004). For instance, *S. ghobban* was distributed in the Indo-Pacific and tropical eastern Pacific while *S. qouyi* was distributed in the eastern Indian Ocean and West Pacific (Matsunuma et al., 2011).

In Malaysia, according to Reef Check Malaysia (2019), the national average of parrotfish abundance showed a slight decrease from 2.4 individuals per 500 m^3 in 2018 to 2.33 in 2019. Besides, this reduction in parrotfish abundance started to occur in 2010 at Peninsular and East of Malaysia. This number of parrotfish may reduce due to high fishing pressure in Malaysia suggested by Arai et al., (2014), especially for males might be due to size advantage.

At Pulau Bidong, east coast of Peninsular Malaysia, the Scarus genus was found at Pantai Pasir Cina, Vietnam Jetty, Underwater Gallery, Pantai Tenggara, Batu Menangis, Christmas Garden, Teluk Air, Karang Tengah, Bayu Payung and Dinding Laut. *Bolbometopon muricatum* was only found at three locations and *Hipposcarus longiceps* at four locations (Afiq et al., 2021). There were five species of parrotfish from three genera such as Scarus, Bolbometopon and Hipposcarus have been identified at ten locations around Pulau Bidong (Afiq et al., 2021). Five species were *S. ghobban, S. psittacus*, *S. qouyi*, *Bolbometopon muricatum* and *Hipposcarus longiceps* (Afiq et al., 2021). Among these five species *S. psittacus*, *S. qouyi* and *S. ghobban* almost found at all eight stations of Pulau Bidong (Afiq et al., 2021). All these five parrotfish species were found at reef associated zones and undergo territorial and gregarious social behaviour. This study also found that, most reef fish at Pulau Bidong were herbivorous (50%) including all five parrotfish species, carnivore (30%) and omnivore (15%). Since the parrotfish contribute to a significant proportion of coral reef communities in Pulau Bidong, are easily available and have many ecological importance, thus it could be a good model to further study their reproductive criteria at Pulau Bidong.

2.5 Parrotfish Reproduction

2.5.1 Reproductive Behaviour of Parrotfish Species

The reproductive biology of parrotfish is very complicated to understand. Parrotfish is sex changing species commonly known as hermaphrodite fish. According to Munday et al., (2006), there are two types of sex change in hermaphrodite fish which are protogynous and protandrous. Protogynous is defined as a group of fish changing their sex from female to male while protandrous changes sex from male to female. Most parrotfish species (Scaridae) are categorized as sequential protogynous hermaphrodites (Matsunuma et al., 2011). A sequential hermaphrodite is an individual which is born as one sex and then changes into the opposite sex. Other fish families which include as protogynous groups are Labridae, Goblidae and Serranidae while Sparidae, Pomacentridae, Clupeidae and Latidae are protandrous groups (Allops and West, 2003). However, some parrotfish species were diandric protogynous hermaphrodites, presenting both primary and secondary male individuals (Robertson & Warner, 1978). An example of these species are *S. sordidus, S. globiceps* and *S. fasciatus* (Choat & Robertson 1975), *S. croicencis* (Robertson & Warner, 1978), *S. ferrugineus* (El-Sayedah et al., 2012), *Hipposcarus Harid* and *Chlorurus sordidus* (El-Sayed et al., 2011) and *S. rivulatus* (Choat et al., 1996). In a randomised controlled study at *S. viride* (van Rooij et al., 1995b), *S. trispinosus* (Freitas et al., 2019) and most Scaridae at Western Carribean (Robertson & Warner 1978) had found that, spawning in parrotfish occurred throughout the year. This indicates that changes in the seasonal variation may not disrupt their sexual reproduction activity.

The sexual identity in diandric hermaphrodites is divided into four categories. First is from undifferentiated juvenile parrotfish become IP female first (secondary male) then change sex into TP male (Choat & Robertson 1975) while second is parrotfish directly become as male (primary male) from juvenile without past through female as first sex (Munday et al., 2006). Another two are female and primary male which originate from mature male (primary male) (Choat & Robertson 1975). In other words, the primary male was divided into two adult phases which is

IP primary male and TP primary male. IP Primary male changes to TP primary male through colour transitions without undergoing sex change (Taylor & Choat, 2014) and retains as male but TP fish unable to return to become IP (Robertson & Warner 1978). In contrast, IP female will change to become TP male via sexual transition at the variation of sizes (Choat &Robertson 1975) which found to be completed within 10 days (Robertson & Warmer 1978). Meanwhile, the previous study of sex structure of the Scaridae family suggests that IP primary male change in TP phase in smaller mean size compared to changes of IP female to secondary male, but if female individuals had to remain as females they might do so as primary male (Choat & Robertson 1975).

The proportion of sex ratio in Scaridae is unpredictable mainly because parrotfish have complex social class and also a high capability to adapt to various social conditions (Robertson & Warner 1978). Previous studies showed that the sex ratio of nine Caribbean parrotfish species skewed more towards female than male and colour phase ratio more towards IP male than TP (Robertson & Warner 1978). Similarly, Frietas et al., (2019) also demonstrated that, the sex ratio of *S. trispinosus* at South Atlantic is also strongly skewed toward females than male, possibly due to selective fishing pressure. Besides, Barba (2010) identified primary males as slightly more abundant in species with indeterminate growth than asymptotic growth. A number of studies have found that, the proportion of sex ratio between primary males and females of Scaridae could also be affected by social group categories and depth range (van Rooij et al., 1996a), fishing pressure (Freitas et al., 2019, Choat et al., 2003) and shelf reefs area (Barba, 2010). Therefore, in order to describe the sex ratio between primary males and females in both adult phase populations, it is necessary to focus on one factor while other parameters should be negligible to avoid sex bias.

Sex change in parrotfish is quite complex. To date, the main factors which trigger the occurrence of sex change in parrotfish are still unclear (Pavlowich et al., 2018). A few studies have attempted to explain the sex change in Scaridae through social and mating strategies (Munday et al., 2006; van Rooij et al., 1996a; Robertson and Warner, 1978). According to van Rooij et al., (1996a), *Sparisoma viride* was involved in two types of social interaction which are territories and groups. Both of

this group present adult IP and TP. In territorial social behaviour, there may have one single TP male, IP male with multiple IP female while in the group there consist of multiple TP male, IP female, IP male and transitional fish. The territory terminal male (TTP) male commonly showed strong defensive behaviour towards their territory area although during the spawning period not like territory IP (TIP). Individuals in the territory are usually observed chasing each other while the individual of group IP (GIP) and group TP (GTP) tolerate each other. TTP male experience pair spawning mode with TIP females more than 13 times daily at the border or in the territory area. There was no group mating observed in the territory. Also TTP male rarely mate with GIP females or roving individuals. On the other hand, for large TIP females (>25 cm), spawning was observed to occur two times daily. The TIP female spawn at a deep site for the first time with super-GTP (largest male), then with TTP male in the territory. In contrast, a group of multiple male and female spawning observed only larger GIP females (>20 cm) and larger GTP males at a deeper site or close to their home range. The rest of the GTP male with smaller sizes experienced spawning only when temporary absence of larger TP group members. Spawning occurred 16 times daily in super-GTP while two times daily in larger GIP females. From both social behaviour in *S. viride*, clearly that parrotfish may have haremic mating system inline as reported by Streelman et al., (2002) in the genus of *Scarus*, *Chlorurus*, *Hipposcarus*, *Cetoscarus* and *Bolbometopan*.

Up to now, most previous studies on the reproductive behaviour of parrotfish unable to recognize the specific key for sex change in parrotfish (Freitas et al., 2019, Pavlowich et al., 2018, van Rooij et al., 1996a, Warner 1984, Robertson & Warner 1978, Choat & Robertson 1975). However, review literature suggests that haremic mating systems or mating group structure could influence the relative timing of sex change in protogynous fish (Munday et al., 2006). This evidence supports the prediction in previous studies where the removal of a dominant large male in haremic protogynous species may trigger the next largest individual to change their sex to become male to replace the former resident (Warner, 1984). Moreover, according to Munday et al., (2006), from a size advantage hypothesis in Protogyny, the female-male sex change occurred when there is a sharper increase in average fertility of male than female at a specific intersection point with body size. The expectation

through this theory is, large male individuals could increase their reproductive value yet produce more offspring and efficiently breed with more females than small males. Detailed examination of a sex-change pathway by Benvenuto et al., 2017, also confirmed the hypothesis that the presence of a few larger males in haremic protogynous species, showed significantly higher reproductive success after mating with the majority of the females. This theoretical study indicates that the larger dominant male is important in a haremic mating system to maximize the fertilization success and the effectiveness of population size. Therefore, the haremic pattern of the mating system shown in parrotfish becomes a significant criterion to understand the cue for their sex change. Generally, the further study which emphasizes the correlation of sex change between females and primary males to become dominant male in the territory would be interesting, and directly could strengthen the recent evidence on the reproductive behaviour of parrotfish.

In terms of growth trend, the length-weight relationship of 13 Scaridae species from the genus *Scarus, Chlorurus, Calotomus, Cetoscarus* and *Hipposcarus* showed negative allometric growth (Amin, 2019). In other words, the rise in body weight makes the fish slimmer (Riedal et al., 2007). This probably was correlated with spawning behaviour in parrotfish (van Rooij et al., 1995a). The reallocation of energy more towards territory defence than growth during spawning or non-spawning period of adult TP or IP male is ascribed as the close influence factor excluding food limiting factor. This evidence was also described by El-Sayed et al., (2011) where the increment of annual scale in Scaridae was inferred due to the slower somatic growth during the spawning period. Meanwhile, based on growth modeling, three Scaridae species *Hipposcarus harid, S. ferrugineus, Chlorurus sordidus* are generally categorized as slow growing fish with long-life spans (El-Sayed et al., 2011). In contrast, McLawin and Taylor (2009) discussed parrotfish *Chlorurus sordidus* is short-livedspans. Even so, Choat et al., (1996) revealed that, three parrotfish species *S. psittacus, S. schelgeli* and *S rivulatus* showed short-life spans while C. *sordidus, S. frenatus* and *S. niger* showed long-life spans. Practically, through these previous studies, it clearly highlights that the growth pattern of parrotfish Scaridae can be different or similar between species. This difference may characterize the size at maturation of fish individuals either at a small or large size.

In the assumption, those parrotfish species that reach gonad maturation at small size have a high tendency to experience a faster growth rate with a short-life span opposite to the long-life spans trend (Choat et al., 1996). Therefore, significantly allometric growth has a close correlation with reproduction in parrotfish.

2.5.2 Gonadal Maturation Phase in Parrotfish

Classification of gonad maturity in fish is commonly applied through histological characteristics. Until now, there were only a few studies describing the histological phase of male parrotfish. DeMartini and Howards (2016) revealed the gonad phase of the parrotfish genus Scarus, Chlorurus and Colotomus with the modifying of a standard protocol for the adaptation of monoandric species shown some subdivision stage at undeveloped and ripe testis through microscopic examination. Classification of gonads focussing on primary and secondary male microscopic characteristics was done by Girolomo et al., (1999) for monoandric protogynous species, *Sparisoma critense*.

In meanwhile, Abdel-Aziz et al., (2006) described some ultrastructure and microscopic criteria of *Scarus ferruginus* at several gonad phases that were on going spermatogenesis (stage III), ripe (stage IV) and spent (stage v), also histologically distinguished the microscopic differences between primary and secondary male comprehensively. The difference observed between primary and secondary males was present or absent of central vas deferens, the remnant of ovarian tissue or ovarian lumen. There was also presence of tunica albuginea around the testis of primary male while abundant of matured sperms present at efferent sperm ducts along testicular wall of secondary male (Abdel-Aziz et al., 2006). Besides, the most distinct of Leydig cells between initial and terminal phase was observed in male testis through microscopic examination in parrotfish (El-Sayedah et al., 2012). This indicates that, from histological examination, primary and secondary male with initial and terminal phase have some macroscopic difference

Although there were several studies (De Martini and Howards 2016, Abdel-Aziz et al., 2006, El-Sayedah et al., 2012, Hamilton et al., 2008, Girolamo et al., 1999, Robertson et al., 1982) on gonad phase of male parrotfish, unfortunately, some of these previous studies do not meet the consistency in term of phase terminology, but still has same description and criteria to each other in term of germ cell present in the gonad. However, the universal standard terminology has been well explained by Brown-Peterson et al., (2013) which is suitable to be applied in all teleost fish including the hermaphrodite fish either diandric or monoandric. Four major phases such as immature, developing, and spawning capable, regressing and regenerating were taken as the main gonad phase while some sub-phase or previous term was specifically sorted into the major characteristic criteria. This standard terminology by Brown-Peterson et al., 2013 also was applied in the present study species *S. rivulatus*, *S. qouyi* and *S. ghobban*.

Development of major germ cells from spermatogonia to spermatozoa via spermatogenesis in the testis was accessed as the main criterion to determine the different maturity phases of the male gonad (Uribe et al., 2014). Change of male gonad due to hormone treatment also quantitatively refers to the number of germinal cells present at each gonad phase (Wolf et al., 2004). This is because there was a close correlation between the spermatocytes and spermatozoa during spermatogenesis in Pacific Yellowtail emperor fish (Ebisawa et al., 1999). In their study, the germ cell count method was applied and confirmed that spermatocyte and spermatozoa were dominantly present during the developing and spawning phase respectively. Also, a study on gonad maturity of Pacific steephead parrotfish at Okinawa Island observed the different percentage of spermatogonia, spermatocyte, spermatid and spermatozoa at every different gonad phase. This indicates that, applying the cell count technique to the major germinal cells at each gonad phase was another alternative way to confirm or determine the gonad maturity and provide strong support to the existence evident.

2.6 Sex Steroid Hormone in Parrotfish

Gonad development in teleost fish involves a complex endocrine system, especially for sex change fish or known as hermaphrodite. One of the physiological mechanisms lately been studied to understand the initiation of sex change is, a change in sex steroid hormone known as estradiol and 11-ketotestosterone (Godwin 2009; Lam et al.,2015; Liu et al., 2017; Todd et al., 2016). Estradiol (E2) and 11-ketotestosterone (11KT) are the most powerful estrogen and androgen which involved in sex change fish (Todd et al., 2016; Jiang et al., 2003), gonad restructuring (Bhandari et al., 2003), also mainly responsible for vitellogenesis and spermatogenesis process respectively (Alvarado et al., 2016). Both of these sex steroid hormones produced in the gonad and other tissue (e.g. brain, muscle) (Liu et al., 2017) were found significantly higher in bluebaneded goby (*Lythrypnus dalli*) during sex change events (Lorenzi et al., 2012).

In the understanding mechanism of sex change in hermaphrodite, four important coral reefs family has been given more attention such as wrasses (Labridae), parrotfishes (Scaridae), damselfishes (Pomacentridae) and gobies (Gobiidae) due to their conspicuous on coral reefs and their adaptability to captive conditions (Godwin 2009). For example change in E2 and 11KT in relation to sex change has been studied in honeycomb grouper, *Epinephelus merra* (Bhandari et al., 2003; Bhandari et al., 2006; Nakamura et al., 2007), bluebanded goby, *Lythrypnus dalli* (Lorenzi et al., 2012), red porgy, *Pagrus pagrus* (Kokokiris et al., 2006), wrasse, *Thalassoma duperrey* (Nakamura et al., 2005) and bluehead wrasse, *Thalassoma bifasciatum* (Perry and Grober 2003). Even though the sex change of several fish species had widely been elucidated, proximate cues of initiation of sex change from female to male (protogynous) or male to female (protandrous) still remain largely elusive (Todd et al., 2016). In the meantime, cues of sex change in protogynous hermaphrodites had been experimentally demonstrated to be affected by an external and internal factors such as a change in the social structure (Thomas et al., 2019; Solomon et al., 2013; Nakamura et al., 1989; Warner and Swearer 1991) and sex steroid hormone (Bhandari et al., 2006)

Stimulations of external and internal cues toward sex change in fish usually cause a cascade change in behaviour, physiology and morphology after a sex change event occurred (Solomon et al., 2013). For example, the removal of the dominant male (TP male) of bluehead wrasse (*Thalassoma bifasciatum*) from a stable social group immediately causes a change in aggregation, courtship and spawning behaviour of dominant (i.e. largest individual) yellow female, then turn into bluish temporal spawning colour of head and darken body after two days, followed by degeneration of vitellogenic oocytes (Thomas et al., 2019; Warner and Swearer 1991) and lastly fully turn into the permanent blue colour of TP male after 28 days maximally (Godwin et al., 1996; Warner and Swearer 1991). A similar condition had been shown in honeycomb grouper, *Epinephelus merra* (Bhandari et al., 2003) and wrasse, *Thalassoma duperrey* (Nakamura et al., 1989), where loss of TP male in social structure positively triggers a sudden drop of sex steroid hormone profile of E2 and increases 11KT at point sex change began at stage 2 where vitellogenic oocytes had degenerated. This indicates that environmental cues such as a change in the social structure (loss of dominant male) are one of the important keys to the initiation of sex change from female to TP male of protogynous hermaphrodite.

Another potent factor believed could trigger the sex change in protogynous hermaphrodite was the change of sex steroid levels E2 and 11KT. A few studies try to investigate the relationship of E2 and 11KT to the initiation of sex change in protogynous hermaphrodite fish (Nakamura et al 2007; Bhandari et al., 2006; Kokokiris et al., 2006). A study on honeycomb grouper (*Epinephelus merra*) found that injection of 11KT to the 15 mature female grouper had triggered the initiation of sex change from female to male. It was observed that, injection of 11KT caused a sharp decrease of E2 and elevation of 11KT and testosterone significantly, concomitant with full transformation of gonad into testis after 75 days of treatments (Bhandari et al., 2006). In the meantime, treated grouper *E. merra* also had shown significantly lower GSI than untreated fish (Bhandari et al., 2006). Other than that, the study of gonad restructuring during natural sex inversion in relation to sex steroid hormone (i.e. estradiol, 11-ketotestosterone, testosterone) in Mediterranean red porgy, *Pagrus pagrus* (Kokokiris et al., 2006) had found significant changes of E2 and 11KT as same as Bhandari et al., (2006) and Nakamura et al., (2007). These show

that, sex change cues in protogynous hermaphrodite fish can be evaluated by changes in sex steroid level E2 and 11KT in relation to the gonad maturation phase and GSI. In contrast, it was seen that the change of testosterone was not consistent, and probably may have another physiological function in gonad maturation, thus not directly involved in sex change fish (El-sayedah et al., 2012).

Releasing of sex steroid hormone (testosterone, 11KT, E2) occurs in the testis which is mainly controlled by the hypothalamus in the brain (Liu et al., 2017). Any environmental signal received by the brain such as a change of social structure commonly transduced via two pathways either HPG (hypothalamic-pituitary-gonadal) or HPI (hypothalamic-pituitary-interrenal) axis (Liu et al., 2017; Todd et al.2016; Goikoetixea et al., 2017). Basically HPG axis play a role in steroid-hormone synthesis and sex change (Lam et al., 2015), meanwhile HPI axis frequently refers to the stress axis where cortisol is synthesis and released (Goikoetixea et al., 2017). LH and FSH play important roles in male fish to regulate spermatogenesis (Lamm et al., 2015) and the production sex steroid hormones including testosterone, 11KT and E2 (Goikoetxea et al., 2017; Liu et al., 2017). The concentration between 11KT and E2 production in the gonad is believed as a crucial parameter to control the sexual fate of the gonad during sex change (Liu et al., 2017). Therefore, identification of minimal and maximal sex steroid levels 11-KT and E2 or their threshold level in gonads is necessary for further study neither to correlate nor understand the different between cues of natural sex change respond and cues of stress respond during sex change events in hermaphrodite fish.

2.7 Fatty Acid Profile in Relation to Reproduction

Fatty acid is the main lipid component which consists of a methyl group and has long hydrocarbon chains at the end of its structure (Tazbozan and Ali, 2017). Fatty acids are classified into saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) (Tazbozan and Ali, 2017). PUFA is an essential fatty acid that cannot be synthesized by the human body. They are important in human health (Pulz & Guss, 2004; Schmitz & Ecker, 2007), have

antibacterial properties (Huang & Ebersole 2010) and are used to inhibit tumor progression (Field & Schley, 2004; Das et al., 2009). PUFA must be consumed from the diet. Marine fish consist of high essential fatty acid PUFA and MUFA but their composition might be different for different fish species (Parzanini et al., 2021)

. Fatty acid analysis was currently used to determine the food web structure from the fatty acid profile and this diet could be confirmed through fatty acids biomarkers (Imbs et al., 2010; Imbs & Yakovleva 2012). Several studies showed the different composition of fatty acid in each individual within the same species and food source might be affected by physical and biological factors such as seasons (Aroyehun et al., 2019; Babatunde et al., 2020), growth (Amalina et al., 2016), food source (Clements et al., 2016 ; Pereire et al., 2012) and reproduction status (Anido et al., 2015, Gonzáléz-Félix et al., 2019). All these factors may influence the difference of fatty acid profiles in fish (Tazbozan & Ali, 2017). Parrotfish were herbivorous fish that evidently identified as primary consumers of macroscopic algae (Clements et al., 2016). Previous study on Surf parrotfish, *S. rivulatus* at Great Barrier Reef, Australia found that, most of this species feed on macroalgae (largest strand algae >5 cm), epilithic algal matrix, sands, soft coral and hard coral (Bonaldo & Bellwood, 2008). Each of these diets could be identified through the lipid content in the fish liver and then compared with fatty acids biomarkers (Arai et al., 2015a).

Most of the parrotfish in the Scarus genus were grouped as scraper and excavator feeding types (Clements et al., 2016). They scrapped the epilithic algal matrix (EAM) on the hard coral surface with their beak-like teeth (Clement et al., 2016). The EAM is defined as a ubiquitous component of coral reefs including turf algae (small algae <1 cm grow on reef substratum), macroalgae spores, microalgae, sediment and detritus (Wilson et al., 2003; Bonaldo et al., 2014). A study on the fatty acid profile of hard coral Acroporidae at the South China Sea revealed that the composition of PUFAs was varied between species and significantly different between the coral genera (Safuan, 2021). In this study, fatty acid biomarkers 18:4n-3, 20:5n-3, 22:6n-3, and 20:4n-6 were identified in Anocropora, *Astreopora* and *Acropora* whereas 22:4n-6 and 18:3n-6 in Montifora genera. A similar result was also shown on fatty biomarkers in hard corals at Nha Trang Bay, the South China

Sea, Vietnam (Imbs & Yakovlova, 2012). However, since the coral has symbiosis relationships with the zooxanthellae, it was suggested that the translocation of PUFA from their symbionts to the host occurred (Imbs & Yakovlova, 2012). Besides PUFAs, C14:0, C16:0, and C18:0 of SAFAs also showed the important fatty acid component in the hard coral (Imbs & Yakovlova, 2012).

The composition of fatty acid SAFAs, MUFAs and PUFAs was also affected due to the reproduction status and seasons. For example study on gonad maturation of male and female Limbaugh's damselfish, *Chromis limbaughi* at the Gulf of California, Mexico demonstrated that, the changes in fatty acids profile were related to the change of maturation phase, GSI, HSI and K factor, especially during the spawning capable (González-Félix et al., 2019). Besides that, Kacar et al., (2016) revealed the variations of fatty acid concentration in male and female catfish, *Silurus triostegus* at Ataturk Damlake, Turkey were highly dependent on reproduction period, temperature and seasons. This study showed that C20 PUFA decreased in spawning period might be due to gamete formation (Kacar et al., 2016). The influence of seasonal changes on the fatty acids in fish during reproduction was demonstrated at the temperate season, however in tropical water change of fatty acid profile in fish due to different seasons is scarce. Only a study on the influence of seasons and feeding towards fatty acid composition in wild cobia, *Rachycention canadum* was found (Babatunde et al., 2020). With regard to the changes in fatty acid, another study on lipid content of several coral reef fishes at the South China Sea showed, different growth of reef fish had also resulted in significant difference for fatty acid composition between the species due to diet and size class difference (Arai et al., 2016). This implies possibly that the change of fatty acid profile was influenced by the changes in reproduction, diet, seasons and growth in reef fish.

CHAPTER 3

GENERAL METHODOLOGY

3.1 Overview of Methodology

The general methodology for this recent study was summarized in Figure 3.1. All of the parrotfish samples for the three objectives were taken at the same place which is at Pulau Bidong, Terengganu. The dominant parrotfish species at the study area were selected after primary survey was conducted two days before sampling during day and night. This survey was done to identify species present within the sampling area. The current study selected *S. rivulatus, S. qouyi* and *S. ghobban* as the criterion due to their common occurrence in the study region. The parrotfish sample was taken from April to November 2014.

The fish samples were collected by SCUBA diving technique. Three species of parrotfish had been caught manually by using a long standard scoop net bought from the aquarium shop. Diving and sample collection were done at night when the fish was in inactive mode. The fish sample was randomly taken during the sampling, so there was variation in size. After sample has been collected, live parrotfish were immediately taken to the UMT research based station to be aerated in the storage tank. Then, body weight, standard length, gonad weight and liver weight were measured to meet the thesis first objective (length-weight relationship). The live fish sample is needed for blood collection. Blood plasma was used for hormone analysis for the second objective The gonad of parrotfish was fixed in 10% buffered formalin which was used in histological analysis to achieve the second objective (maturation phase). Lastly, parrotfish liver was kept at -20 to meet the third objective (fatty acid profile) in this study. All samples collected for the various studies (length-weight relationship, histological examination, hormone, fatty acid) were obtained from the same fish individuals, including samples from the gonad, liver, blood, and morphometric.

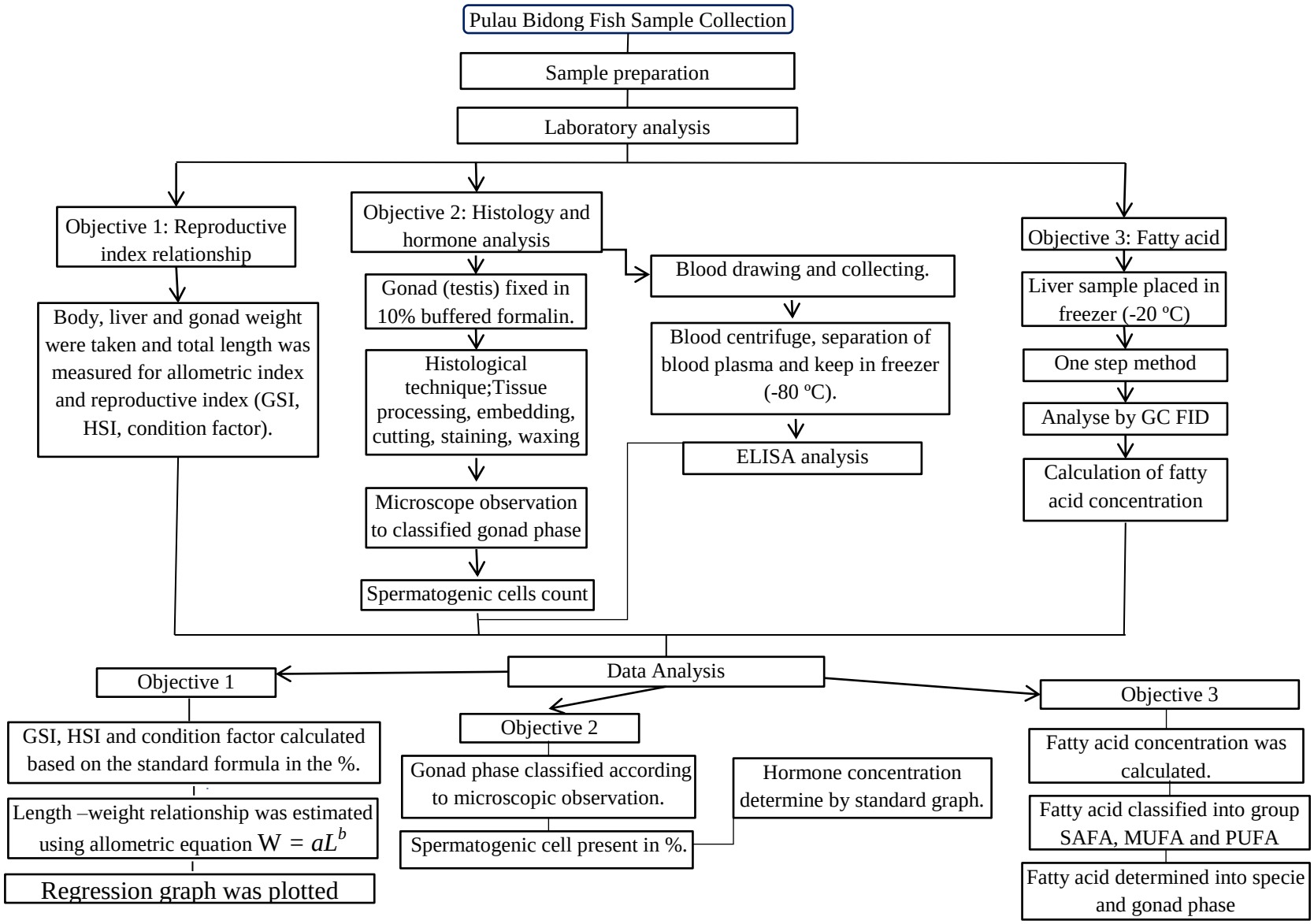

Figure 3.1 Schematic diagram of general methodology procedure in the study.

3.2 Study Location

This study was conducted at Pulau Bidong (5°37'7.79" N, 103°3'49.01" E), Terengganu at the east coast of Peninsular Malaysia. Pulau Bidong is located in the southern South China Sea. In the late 70s until early 90s, Pulau Bidong was inhabited by Vietnamese refugees (Grismer et al., 2014). Prior to this point in time, Pulau Bidong had no local communitites residing on it, and there was a lack of any coastline development, minimal local activity, and absence of waste. Only UMT marine research station was constructed on the western coast of Pulau Bidong, often referred to as Pantai Pasir Cina where the fish sample was obtained.

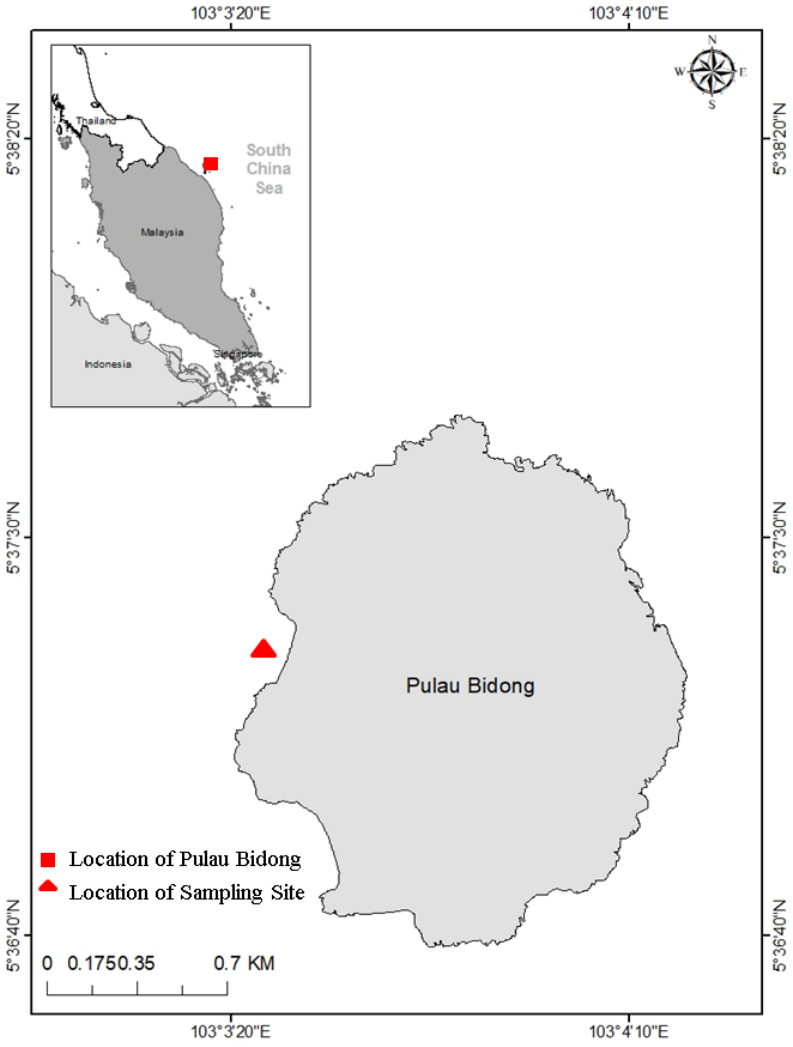

Figure 3.2 Study area of parrotfish in the Pulau Bidong, Terengganu, South China Sea.

Pantai Pasir Cina was dominated by branching coral based on the underwater survey. The major coral reefs found were from genus *Acropora*, *Fungia* and *Porites* at the study area.

3.3 Sample Collection

In this study, sample collection involved three predominant parrotfish species *S. rivulatus*, *S. qouyi* and *S. ghobban* at Pantai Pasir Cina, Pulau Bidong. The reef structure at Pantai Pasir Cina was dominated by branching coral (Afiq-Firdaus et al., 2021) while the dominant genera were *Acropora, Fungia* and *Montifora* (Safuan et al., 2020). The coral condition at Pantai Pasir Cina was excellent with 78.32% coral cover (Afiq-Firdaus et al., 2023).

Fish sample was taken between April – November 2014. The fish sample was caught using a standard scoop net with 14, 16 and 18 inches by 10-14 divers for each sampling. Night diving technique was done for sample collection because based on observation parrotfish was inactive during this period. Diving was done for around one to nearly two hours at a depth range 5-10 meters by UMT divers.

All male parrotfish species were caught randomly in order to fulfil the complete gonad maturation phase. Fish samples were carefully placed in the storage tank with oxygen support. Fish samples need to be aerated immediately after fish was caught by divers. The live fish sample is necessary for blood collection. The blood plasma was used to fulfill the second objective. Blood could not be drawn out in die fish. Clove oil 100 mg/L was used as an anesthetic to anesthetize the parrotfish prior to blood sampling to minimise pain or distress to the fish according to Coyle et al., (2004). The weight and length of fish body, gonad and liver were taken for first objective. The blood sample was taken directly at UMT research based station near the sampling area using a heparinized syringe and needle at the caudal vein at lateral line of the fish body. The liver and gonad from dissected fish sample were kept in

cool storage and preserved in 10% buffered formalin respectively for second and third objectives.

The overall number of parrotfish species collected was 134 for all seasons. There were 46 individuals of *S. rivulatus* TP male, 52 individuals of *S. qouyi* IP male and 36 individuals of *S. ghobban* with 33 IP male and 3 TP male. All fish samples were primarily identified through histology (present any remnants of the membrane bound cavity or lumen and yellow-brown body) according to Abdel-Aziz et al., (2006). However samples data were pooled because based on the ultrastructure study there were no any peculiarities observed and were generally similar to other teleosts (Abdel-Aziz et al., 2006).

3.4 Species Identification

The male parrotfish was only selected in this study. The sex identification of male parrotfish was selected through the external colouration according to Munoz and Warner, (2003) and validate through histologically. In previous study, IP and TP (initial and terminal) in parrotfish were usually distinguished by colour as an indicator (DeMartini & Howard, 2016; El-Sayed et al., 2011; Abdel-Aziz et al., 2006; Munday et al., 2006). Therefore, the adult phase of three parrotfish species were identified by major colour differentiation described by Matsunuma et al., 2011 and Lioa et al., 2004. The differentiation colour of adult parrotfish was shown in Figure 3.3.

Species	Initial phase	Terminal phase
S. rivulatus	 Lioa et al., 2004	
S. qouyi	 Matsunuma et al., 2011	 Lioa et al., 2004
S. ghobban		 Lioa et al., 2004

Figure 3.3 The colour differentiation of initial and and terminal phase of three parrotfish species *S. rivulatus*, *S. qouyi* and *S. ghobban*.

First, according to Matsunuma et al., (2011), TP male of *S. qouyi* are recognized by a blue-green body near the dorsal fin, narrow pink edges at scales, followed by violet-pink at central abdomen, slightly pinkish or magenta body colour near the anal pore. The dorsal head of *S. qouyi* was violet-grey with the large bright blue-green irregular patch on the cheek, followed by dorsal orange operculum mixed with violet-pink at the ventral. The caudal and pectoral fin had large deep blue with light brown dorsally striped.

For the second species *S. ghobban*, according to Matsunuma et al., (2011) the IP male was mainly covered with a dull orange-yellow body, mixed with 5 vertical irregular blue bars and had yellowish colour with slightly horizontal blue at the edge of the caudal and pectoral fin. Mouth and eyes of IP male *S. ghobban* had blue bars. Meanwhile, the TP male was dorsally green, followed by salmon pink scales rimmed, ventrally shaded to pale green with pale salmon pink bar on each scale. Head colour was dorsally green, cheek and chin shade to pale salmon. Also TP *S. ghobban* chin

had two transverse blue bands and three narrow irregular green bands extending posteriorly from the eye.

In the third species, according to Lioa et al., (2004) *S. rivulatus* had several blue-green irregular stripes around the eye, gill covered with orange patches at the cheek, and lower part of the eye without a green area. A part of the body colour was blue-purple ventrally near approaches to the head until the anal fin, followed by blue-green at the abdomen, narrow pink at the edge of scales and had pectoral fin with bright green colour.

3.5 Laboratory and Data Analysis

In the first objective (Chapter 4), total length and body weight were determined to study the size-frequency distribution generally and length-weight relationship of male parrotfish species samples. The total number of samples used for the first objective was 134 individuals. Regression analysis was applied to determine the length-weight relationship. The length-weight relationship of male parrotfish was then determined according to species with 46, 52 and 36 from *S. rivulatus*, *S. qouyi* and *S. ghobban* respectively. Gonad and liver weight relationship with the total length of three parrotfish also was determined by regression analysis. The reproductive pattern was observed through the reproductive index (GSI, HSI & CF) at different species. Data were analysed by using one-ways ANOVA and Tukey HSD of Posthoc test. The fractional rank transformation was applied for GSI, HSI and CF data to meet the normal distribution graph before proceed with one-ways ANOVA and Posthoc test (Tukey HSD).

In the second objective (Chapter 5), the gonad (testis) samples of male parrotfish were analysed using the standard histological method (Humason, 1962). For the second objective, a minimum of 8 to a maximum of 23 gonad samples were analysed in each maturation phase. Classification of male gonad maturation phases for diandric protogynous hermaphrodite was described according to Brown-Peterson

et al., (2011). A microscopic criterion for each gonad phase was adapted by modification characteristics from previous studies (Abdel-Aziz et al., 2006; Brown-Peterson et al., 2011; DeMartini & Howard, 2016). In hormone analysis (Chapter 5), estradiol and 11-ketotestosterone hormone were analysed using enzyme-linked immunosorbent assay (ELISA) test method (El-Sayed et al., 2011). For the analysis of the hormones 11-ketotestosterone (11-KT) and estradiol (E2), 120 and 96 blood plasma samples respectively were taken from three parrotfish species.

In third objective (Chapter 6), dried liver tissue used for fatty acid analysis was analysed through one-step extraction method based on Abdulkadir and Tsuchiya (2008). In the fatty acid analysis 12 to 17 samples were used to determine the fatty acid profile for each parrotfish species and each of three samples were used for maturation phases. According to previous studies of fatty acid analysis at least three sample minimum was reliable for the data analysis (Berneira et al., 2020; Khosravi et al., 2015; Arai et al., 2105a; Arai et al., 2015c). More details method for sample analysis was explained at each chapter. Then the fatty acid concentration was pooled according to species and maturation phases due to the same fatty acid profile and gonad macro-characteristic criteria to avoid the large variation of data. The fatty acid profile was analysed using PERMANOVA analysis using PRIMER-E version 6 and the significant difference was proceeded with Posthoc pair wise test of PERMANOVA. The dissimilarity and similarity percentage contribution was analysed by SIMPER analysis.

CHAPTER 4

LENGTH-WEIGHT RELATIONSHIP IN VARIETY SIZE CLASSES PARROTFISH OF THREE SPECIES (*Scarus rivulatus, S. qouyi* & *S. ghobban*) AT PULAU BIDONG, TERENGGANU

4.1 Introduction

Pulau Bidong was surrounded by coral communities around the island. There was a fringing reef with diverse fish species including parrotfish (Azlani, 2017). Parrotfish have a significant role in the coral population in Pulau Bidong. This is because parrotfish could increase the survival of coral planulae in the marine ecosystem. Conserving the parrotfish species in Pulau Bidong could indirectly maintain the healthy coral reef population. The knowledge regarding the relationship between body length and weight has connection to parrotfish reproduction aspect is necessary to be known. Therefore, the study on fish reproduction through the length-weight relationship is essential as the basic understanding.

Length-weight relationship is the index that has been used to evaluate the change in parts or organs of an organism in relation to the proportional change in body size and directly provides information about the growth pattern of fish (Mehanna & Farouk, 2021). The length-weight relationship has been very important for fisheries management as it can basically be used to estimate the average weight of a specific length group in fish (Jan & Ahmed, 2021). This was commonly applied in fish ecology (Shin et al., 2005), reproduction (Brouwer & Griffiths, 2005), feeding habits (Kallianiotis et al., 2005), and fish health (Lemos, 2012). Apart from that, evidence shows that the length-weight relationship also had a close relationship with fish growth pattern (Farooq et al., 2017), gonad maturation (Somarakis et al., 2004) and reproductive dynamics (Nunes et al., 2011). In the meantime, preliminary study had shown that length-weight relationship was possibly used in order to describe fish reproductive patterns and life history strategies (McCann & Shuter, 1997).

In terms of reproductive biological studies, gonadosomatic index (GSI), hepatosomatic index (HSI) and size frequency distribution are three indices that have been used as indicators of fish reproduction yet considered as low-cost measurements of reproductive conditions (Somarakis et al., 2004). GSI is also known as the ratio of gonad weight towards body weight. Commonly during juvenile, the size of fish gonads is small then the weight will increase slowly as the fish grows up to adult stage. Previous research shows that the increment of gonad weight especially near to spawning was due to the swelling and ripening of oocytes (Taylor & MclIwan, 2012).

Normally changes in the HSI value are associated with changes of GSI trend during fish reproduction (Nunes et al., 2011). This is because the liver is an important source of energy and provides nutrients in fish growth and reproduction. However, these changes can be affected by external factors such as habitat variability (Lloret et al., 2002), food availability (Dominguez-Petit et al., 2010) and seasonal changes (Ganias et al., 2007). Besides biological indexes such as GSI and HSI, condition factor (K index) is another reproductive physiological index that can act as a supportive indicator towards fish reproduction by using length-weight relationship principle (Froese, 2006). K index has been defined as the energy availability in fish, especially during spawning season (Dutil et al., 2003). As revealed by Mello & Rose, (2005), fish allocate a large amount of energy for gonad development, spawning behaviour and migration in the reproduction process. Previous studies had shown that condition factors have positive correlation with the reproduction of Asian catfish, European hake and Atlantic cod (Hossain et al., 2006; Koops et al., 2004; Mello & Rose, 2005).

Although the relationship between length and weight may provide useful data in the context of the reproductive index GSI, HSI and CF, but either this pattern will have any significant within different parrotfish species is still unknown. In fact, parrotfish within the same family have the possibility to create different growth patterns. In world wide, most research on the reproductive index pattern of coral reef fish is well explained within clownfish (pomacentidae) (Gordon & Bok 2001 ; Dhaneesh et al., 2009; Casas et al., 2016), groupers (serranidae) (Adams et al., 2000; Rhodes & Sadovy, 2002; Erisman et al., 2007; Renones et al., 2010) and anglefish

(pomacantidae) (Baensch & Tamaru, 2009; Feitosa et al., 2016; Obota et al., 2016). However, none has been conducted on the reproductive trend of parrotfish. As parrotfish were a part of the important community at Pulau Bidong, thus, it is necessary to understand their length and weight relationship which is associated with reproductive index GSI, HSI and CF. Perhaps it could provide beneficial data regarding how body weight with length might be related with parrotfish reproduction index. Therefore, the objectives of this chapter were to determine the size frequency distribution of three parrotfish species, to determine the relationship between length and weight of three parrotfish species, and to determine the relationship between fish length and reproductive index (GSI, HSI and CF) of three parrotfish species (*S. rivulatus, S. qouyi* and *S. ghobban*) in Pulau Bidong, Terengganu

4.2 Materials and Methods

4.2.1 Sample Collection

The study area was located at Pulau Bidong (5°37'7.79" N, 103°3'49.01" E) of the South China Sea. The sampling area was estimated around 19,969 m^2 (Figure 3.1). Three species of parrotfish *S. rivulatus*, *S. qouyi* and *S. ghobban* were randomly sampled. From 134 parrotfish samples, it was observed that *S. rivulatus* and *S. qouyi* are more dominant at the sampling area compared to *S. ghobban.* The fish sample was collected during night time around 8.00 pm until 10.00 pm. This is because parrotfish were inactive during this period. Step of fish sampling was according to the step which has been explained at Chapter 3 section 3.3 paragraphs 2-3.

4.2.2 Species Identification

Parrotfish have two adult colour phases which are the initial phase (IP) and terminal phase (TP). Only TP was found in *S. qouyi* and *S. rivulatus*. For *S. ghobban*, the main samples were all IP except three samples from TP. Two parrotfish species

(*S. qouyi* & *S. ghobban*) in Pulau Bidong were identified through colour differentiation according to Matsunuma et al., (2011) while *S. rivulatus* was referred to Liao et al., (2004). These differentiations of colour for species identification were explained in Chapter 3 at Section 3.4.

4.2.3 Size-frequency Distribution

The total length (cm) of each parrotfish was taken by using a measurement ruler nearest to 0.1 cm. Then, fish samples were classified into 14 size classes based on total length (minimum = 12 cm and maximum = 39 cm) to balance the same number of size classes where each size class had two different sizes. A size interval of 2 cm was decided for all species according to Choat et al., 2012. Lastly, the frequencies of each size class was calculated and used to plot the size-frequency distribution graph.

4.2.4 Gonadosomatic Index (GSI)

The fish sampled was dissected after the total length (cm) and body weight (g) was taken. The fish gonad was weighed (g) by using an analytical balance nearest to 0.0000 (g). Gonadosomatic index (GSI) was calculated to show the relationship between gonad weight and body weight in relation to reproduction. The GSI of each individual of parrotfish spp. was calculated by using the formula as below (Munoz & Warner, 2004; Li et al., 2019) :

GSI = 100 x [gonad weight (g) / total body weight (g)]

4.2.5 Hepasomatic Index (HSI)

After a total length (cm) and body weight (g) was taken, the fish sampled was dissected. The fish liver was weighed (g) by using an analytical balance nearest to 0.0001 (g). Hepatosomatic index (HSI) was calculated to show the changes in liver weight in relation to body weight. The HSI of parrotfish spp. was calculated as follows (Li et al., 2019):

$$HSI = 100 \times [\text{ liver weight (g)} / \text{total body weight (g)}]$$

4.2.6 Condition Factor (CF)

The total length and body weight of the fish sampled was measured by using the measuring ruler nearest to 0.1 (cm) and electrical/weighing balanced nearest to 0.01 (g) respectively. Condition factors (CF), which have a relationship towards the reproductive physiology of fish was calculated by using formula bellow (Gonzalex et al., 2019):

$$CF = 100 \times [\text{ total body weight (g)} / \text{total length}^3 \text{ (cm)}]$$

4.2.7 Data Analysis

To plot the size–frequency of the parrotfish distribution graph, the midlength (cm) of each size class was selected as the x-axis. Then at the y-axis, the percentage frequency of each parrotfish species at each group of size interval to the total number of each month was calculated. The graph of the size-frequency distribution was plotted separately by different species.

The regression analysis was used to determine the relationship between total length (cm), body weight (g), gonad weight (g) and liver weight (g) by using

Microsoft Excel 2010. Length-weight relationships were determined separately by species for *S. rivulatus* (TP) male, *S. qouyi* (IP) male, and *S. ghobban* combination of IP with TP male due to a small sample size (<5) for TP. The length-weight relationships were expressed as power logarithmic relationship equation $W = aL^b$ (Ricker, 1975), where W is the body weight (g), L is the total length (mm), '*a*' is the intercept and '*b*' is the allometric coefficient. The relationship is considered isometric when the '*b*' coefficient is approximately equal to three ('*b*' ≈3) (Froese, 2006). '*a*' and '*b*' parameters were transformed into logarithms as $\log_{10} W = \log_{10} a + b \log_{10} L$ and a regression line was plotted. The length-weight relationship was compared among male parrotfish species by correlation coefficient (r^2) and '*b*' slope value respectively.

The histogram graph of GSI, HSI and CF was presented by using the mean value according to their midlength to compare the graph pattern between the three parrotfish species. One-way ANOVA was used to determine the significant difference between the species and pairwise test was used the Tukey HSD in post hoc test samples.

4.3 Results

4.3.1 Size-frequency Distribution of Three Species Parrotfish

Figure 4.1 shows the total sample of three species of parrotfish at Pulau Bidong that had been analyzed using a percentage frequency graph with midlength (cm) from standard length (SL) of fish as the x-axis. There were various size class samples with a minimum value 12.5 cm (SL) to a maximum value of 38.5 cm (SL). The total number of three parrotfish fish species samples was 134 individuals. Based on total samples, the number of *S. rivulatus*, *S. qouyi* and *S. ghobban* were 46, 52, and 36 individuals respectively. From this total sample, *S. qouyi* had shown as the highest number of individuals, followed by *S. rivulatus* then followed by *S. ghobban*. *S. qouyi* had the smallest fish sample at 12.5 cm size class whereas for *S. rivulatus* and *S. ghobban*, fish samples of this range size class were absent. Meanwhile, the biggest fish sample was present in *S. ghobban* species in the size class of 38.5 cm midlength.

Within the three fish species the minimum percentage for *S. rivulatus* was 2% in range size 14.5 (cm) and 30.5 (cm) midlength while the maximum was 35% in range size 18.5 (cm) midlength. For *S. qouyi* the minimum value was 4% in range size 12.5 (cm) and the maximum was 35% in the range size 16.5 (cm). For *S. ghobban* the minimum percentage was 3% in midlength which was in range size 36.5 (cm), while their maximum value was 14% in range size 24.5 (cm). In general, from three parrotfish species, it was observed that the highest frequency in the graph was located in size class 18.5 cm, 16.5 cm and 22.5 cm midlength for *S. rivulatus*, *S. qouyi* and *S. ghobban* respectively. Among three species of parrotfish, *S. rivulatus* and *S. qouyi* had shown almost all normal distribution patterns compared to *S. ghobban*. Even so, *S. ghobban* was dominated at larger size more than 22.5 cm midlength compared to *S. rivulatus* and *S. qouyi.*

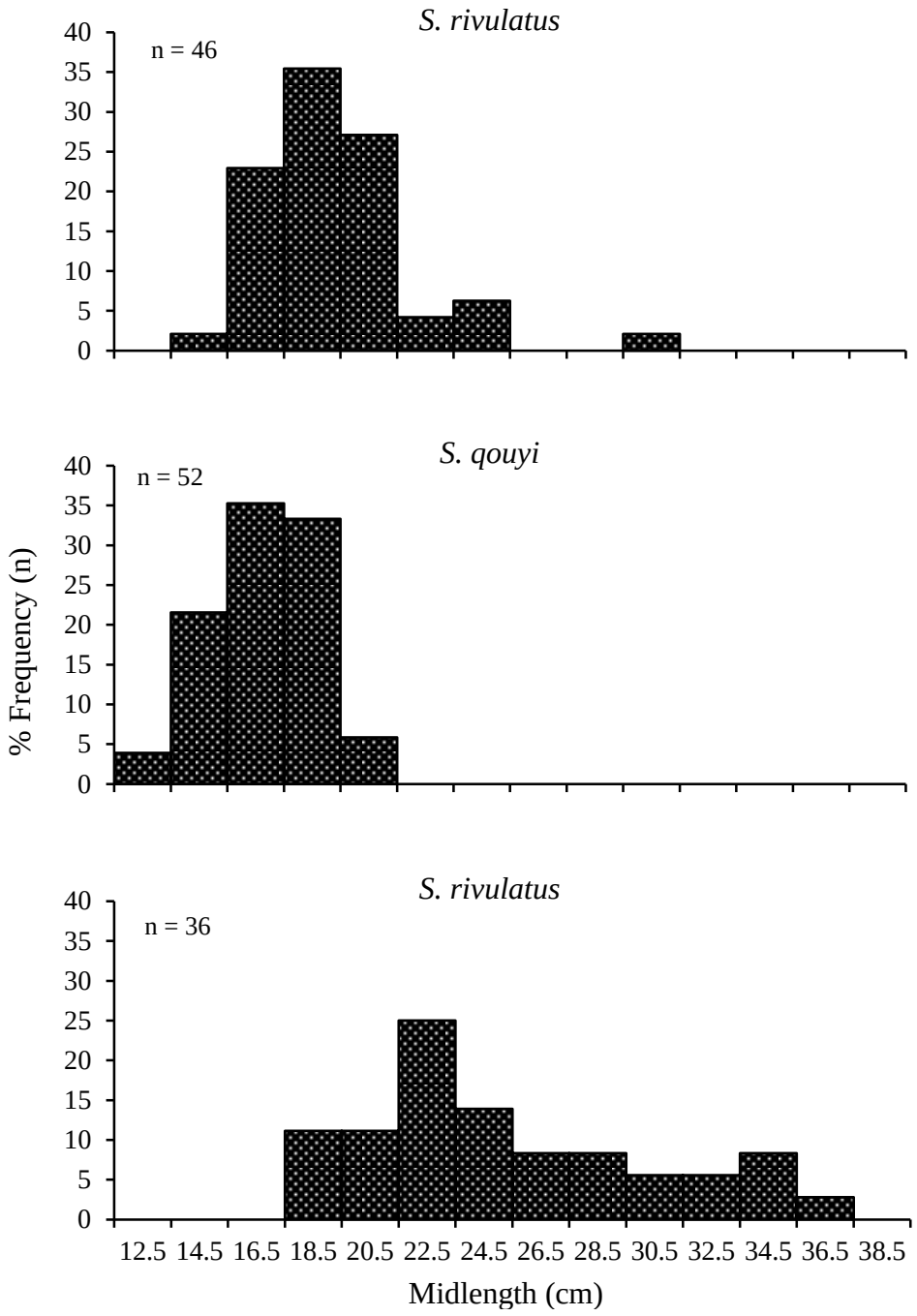

Figure 4.1: Size-frequency distributions of parrotfish *S. rivulatus*, *S. qouyi* and *S. ghobban* at Pulau Bidong. [(n) represent as a total number of individuals in each species].

4.3.2 Length-weight Relationship of Three Parrotfish Species at Pulau Bidong

Length-weight relationship of three parrotfish species in Figure 4.2 showed a positive growth pattern. From three parrotfish species, there was a significant relationship between total length (cm) and body weight (g) ($p<0.05$). Generally, *S. ghobban* IP male showed an ideal growth as r^2 =0.933 (n=36) not for by *S. qouyi* IP male r^2 =0.8782, (n=51) and *S. rivulatus* TP male r^2 =0.8714, (n=46). The '*b*' slope lies between values 2.6441 to 3.2626. IP male of *S. ghobban* showed the highest '*b*' slope 3.2626, followed by IP male *S. qouyi* ('*b*' =2.7552) and lowest was TP male *S. rivulatus* ('*b*' =2.6441).

Meanwhile, the linear regression lines by 'r^2' between each species were not significantly different (ANCOVA $p>0.05$). The calculated logarithmic linear equation for length-weight relationship for *S. rivulatus* was Log W = -1.4168 + 2.7552 Log L_{TL}, *S. qouyi* was W = -1.2942 + 2.6441 Log L_{TL} and *S. ghobban* was W = -2.138 + 3.2626 Log $L_{TL.}$. From the calculated linear equation, had resulted the allometric growth equation $W = 0.0383L_{TL}^{2.7552}$ for *S. rivulatus*, $W = 0.0508L_{TL}^{2.6441}$ for *S. qouyi* and $W = 0.00728L_{TL}^{3.2626}$ for *S. ghobban.*

Table 4.1 Length-weight relationship of three parrotfish species *S. rivulatus*, *S. qouyi* and *S. ghobban* at Pulau Bidong, Terengganu in 2014.

Species	*S. rivulatus*	*S. qouyi*	*S. ghobban*
n	46	52	36
TL range (cm)	15.5 – 30.0	13.2 – 20.1	14.4 – 38.7
Intercept (a)	1.4168	1.2942	2.1380
Slope (b)	2.7552	2.6441	3.2626
R^2	0.8714	0.8782	0.9331
Equation ($W=aL^b$)	$W = 0.0383L_{TL}^{2.7552}$	$W = 0.0508L_{TL}^{2.6441}$	$W = 0.00728L_{TL}^{3.2626}$

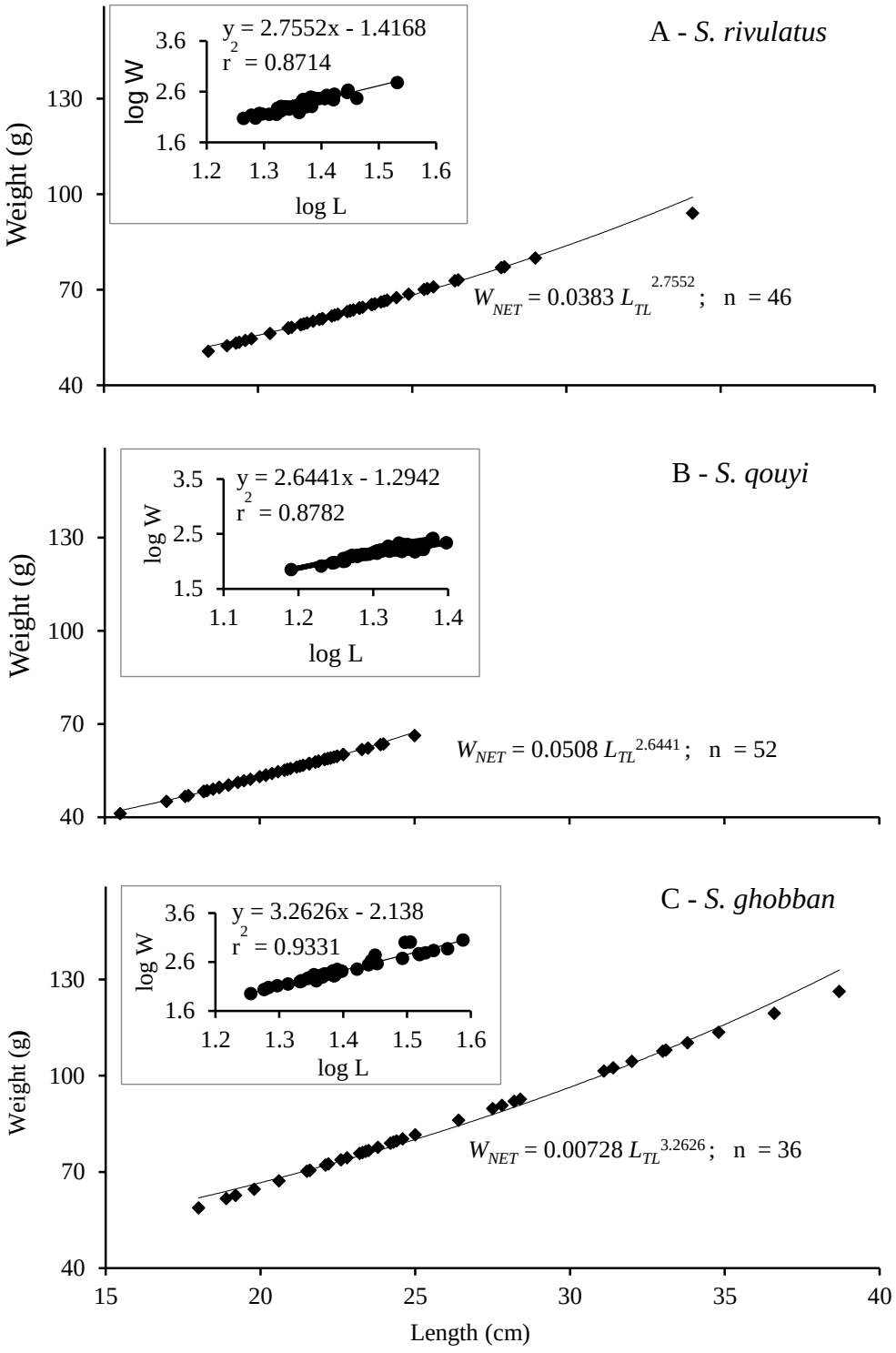

Figure 4.2: Relationship between body weight and total length of three parrotfish species from Pulau Bidong at 2014. A – *S. rivulatus*, B – *S. qouyi* and C – *S. ghobban*

4.3.3 Reproductive Relationship of Gonad and Liver Weight with Total Length of Three Parrotfish at Pulau Bidong

Gonad weight (g) with total length (mm) showed a weak relationship with the r^2 was 0.0449 to 0.2863 for all three parrotfish species (Table 4.2). The regression analysis for gonad weight showed significantly different ($p< 0.05$) for *S. qouyi* and S. *ghobban* compared to *S. rivulatus* ($p> 0.05$). In contrast, there was no significant difference in the liver weight relationship for both *S. qouyi* and *S. rivulatus* ($p> 0.05$). Meanwhile, the liver weight (g) also showed a weak relationship with total length (mm) with minimum $r^2 = 0.0072$ for *S. qouyi* and maximum $r^2 = 0.4993$ for *S. ghobban*.

Table 4.2: Linear regression analysis for gonad and liver weight relationship with total length of three parrot fish species at Pulau Bidong.(n = number of specimen; S.E = standard error; SR = *S. rivulatus*; SQ = *S. qouyi*; SG = *S. ghobban*. Notes: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns = not significant.

				Intercept		**Slope**			**Hypotesis test**		
Items	n	r^2	a	p-values	b	S.E	t-stat	p-values	F	p-values	Mark
Gonad weight-total length relationship											
SR	47	0.045	-4.475	0.039	2.745	1.543	1.78	0.082	3.17	0.082	ns
SQ	51	0.108	-6.068	0.009	4.099	1.686	2.43	0.019	5.91	0.019	*
SG	36	0.286	-7.011	0.0002	4.4710	1.210	3.69	0.0008	13.64	0.0008	***
Liver weight-total length relationship											
SR	47	0.032	-1.525	0.429	1.724	1.404	1.23	0.226	1.51	0.226	ns
SQ	51	0.007	-0.293	0.859	0.746	1.250	0.60	0.553	0.36	0.553	ns
SG	36	0.499	-5.573	$1.51x10^{-5}$	4.571	0.785	5.82	$1.47x10^{-6}$	33.90	$1.47x10^{-6}$	***

4.3.4 GSI Pattern with Midlength Graph of Three Parrotfish Species

Figure 4.3 shows the mean GSI pattern of three parrotfish species at Pulau Bidong. The total numbers of parrotfish in all sampling periods were n = 46 for *S. rivulatus*, n = 52 for *S. qouyi* and n = 36 for *S. ghobban*. Generally based on the graph, most of the GSI value starts from the lower value at a smaller size and then slowly increases until a certain size, after that slowly reduces as the size of the fish becomes more larger which is roughly like a bell-shaped pattern curve.

In *S. rivulatus*, GSI values start from 0.05 ± 0.02 at 14.5 cm midlength. Then the GSI increased at 16.5 cm midlength which rich the highest GSI with 0.33 ± 0.18 before slowly decreasing from 18.5 cm to 22.5 cm midlength. The GSI of *S. rivulatus* was increased again at 30.5 cm with GSI value 0.25 ± 0.02. The mean values of GSI for *S. rivulatus* range from 0.04 ± 0.02 to 0.33 ± 0.18. For *S. qouyi* the GSI value showed a steady increase from 0.03 ± 0.01 at 12.5 cm and reached the peak value at 18.5 cm midlength with 0.32 ± 0.14. Then the GSI of *S. qouyi* had decreased to 0.13 ± 0.02 at 20.5 cm. The range of GSI for *S. qouyi* was between 0.03 ± 0.01 to 0.32 ± 0.14. Meanwhile, for *S. ghobban* the GSI pattern was slightly different. The GSI showed repeated increased and decreased pattern at range size 16.5 cm to 20.5 cm, 20.5 cm to 24.5 cm and 24.5 cm to 34.5 cm midlength. The highest GSI for *S. ghobban* was at 26.5 cm with 0.68 ± 0.34. The mean GSI values of *S. ghobban* range between 0.68 ± 0.34 to 0.01 ± 0.003.

Mostly from three parrotfish species of *Scarus* genus, the GSI trend according to their midlength was almost similar pattern. Among three parrotfish species the highest GSI were observed at different midlength where for *S. rivulatus* reached the maximum GSI at 16.5 cm, *S. qouyi* at 18.5 cm and *S. ghobban* at 26.5 cm midlength. From one-way ANOVA, the GSI value of three parrotfish species was significantly different between *S. qouyi* with *S. rivulatus* and *S.ghobban* species with $p<0.05$. This study involved random fish sampling the length of fish was uncontrollable and their diversion of range size was unpredicted.

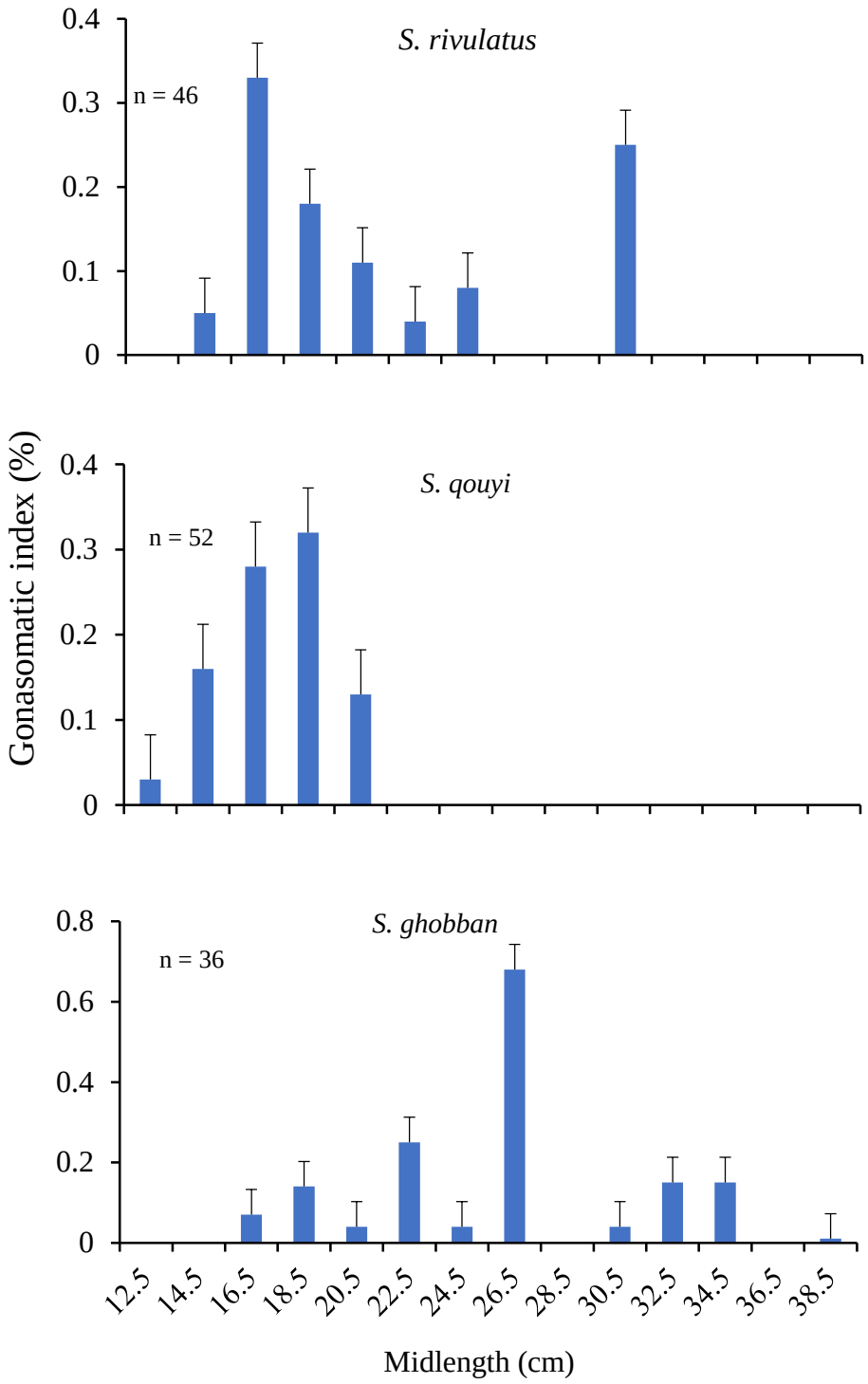

Figure 4.3: The mean ± SD of gonadosomatic index (GSI) pattern of parrotfish *S. rivulatus*, *S. qouyi* and *S. ghobban* at Pulau Bidong. [n : total number of fish samples]

4.3.5 HSI Pattern with Midlength Graph of Three Parrotfish Species.

The mean of Hepatosomatic index (HSI) of three parrotfish species *S. rivulatus* (n = 46), *S. qouyi* (n = 52) and *S. ghobban* (n = 36) were shown in Figure 4.4 according to fish midlength. From the results, the mean HSI value showed an increased and decreased pattern across the midlength for all three parrotfish species. Generally, the graph pattern for *S. qouyi* and *S. ghobban* were almost same while for *S. rivulatus* the HSI pattern was slightly higher at two earlier midlengths which were at 12.5 cm and 14.5 cm.

Based on the graph in Figure 4.4, HSI for *S. rivulatus* had been slowly reduced from 12.5 cm until 16.5cm with HSI value 6.45 ± 0.35 and 3.69 ± 0.63 respectively. Then the HSI was increased at 18.5 cm and 20.5 at 4.72 ± 0.97 before a sharp decrease to 1.74 ± 0.35 at 22.5 cm. After that, HSI value of *S. rivulatus* was increased again at maximum value 6.96 ± 1.40 at 24.5 cm before decreased at 28.5 cm midlength. For *S. qouyi* there was steady increase of HSI from 12.5 cm until 16.5 cm midlength, then decreased at 18.5 cm midlength. The HSI value of *S. qouyi* reached peak at both 14.5 cm and 16.5 cm with almost the same HSI value 4.77 ± 1.32 and 4.77 ± 1.27 respectively.

In *S. ghobban* there was roughly formed like bell shaped curve where the HSI was slowly increased from minimum midlength 16.5 cm with 1.98 ± 0.09, then arrived at the peak at range size 24.5 cm with 6.07 ± 0.41 to 28.5 cm with 6.87 ± 0.32. Then the HSI value of *S. ghobban* was reduced from 30.5 cm to 32.5 cm midlength with HSI value 4.54 ± 0.61 and 4.48 ± 0.32 respectively. On the whole, it was observed that from three parrotfish species *S. rivulatus*, *S. qouyi* and *S. ghobban* the highest and lowest HSI value located at different midlength. The lowest HSI value was at 22.5 cm, 18.5 cm and 16.5 cm for *S. rivulatus*, *S. qouyi* and *S. ghobban* respectively. However, HSI value between this three parrotfish species was not significantly different with $p>0.05$. The clear HSI pattern was shown in *S. ghobban* due to the variety range size present from smaller range size to maximum range size.

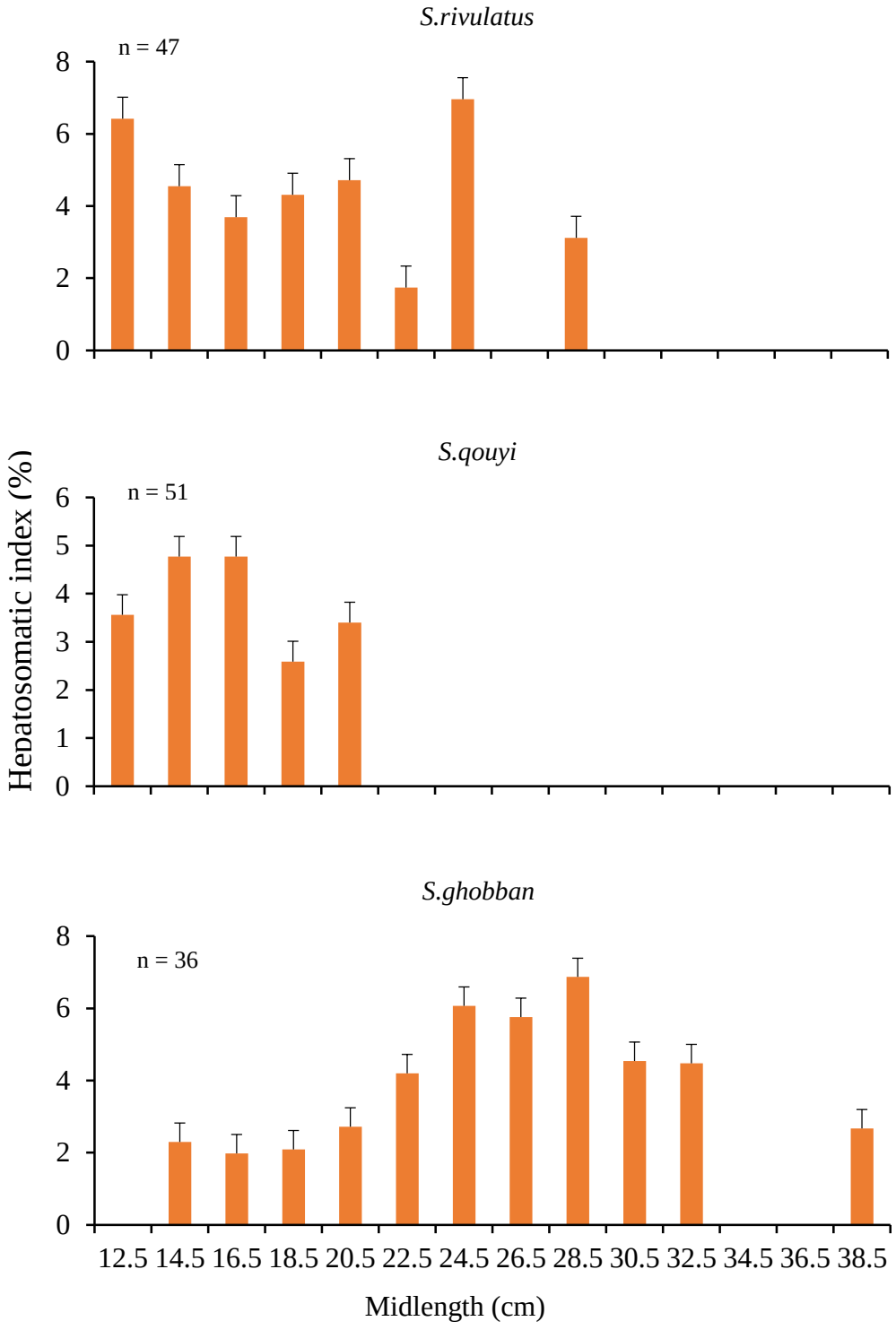

Figure 4.4: The mean ± SD value of HSI pattern of parrot fish *S. rivulatus*, *S. qouyi* and *S. ghobban* at Pulau Bidong.[n: total number of fish sample]

4.3.6 Condition Factor with Midlength Graph of Three Parrotfish Species.

Figure 4.5 shows the mean of condition factor (CF) pattern of three parrotfish species at Pulau Bidong. From the graph trend, the change of CF was slightly small between the midlengths. In *S. rivulatus* CF showed a slowly increased value from 16.5 cm with 1.71 ± 0.08 to 22.5 cm midlength with highest value 1.95 ± 0.03. After that, the CF value of *S. rivulatus* had decreased to 1.64 ± 0.14. The lowest CF value for *S. rivulatus* was at 30.5 cm with 1.52 ± 0.38.

The graph pattern had shown by *S. qouyi* also nearly the same as *S.* r*ivulatus*. In *S. qouyi*, there was a slightly increased of CF from 12.5 cm with 1.79 ± 0.08 cm to 14.5 cm midlength with 2.06 ± 0.48. Then the value had slowly reduced from 1.74 ± 0.10 to 1.71 ± 0.14 at 16.5 cm until 20.5 cm midlength. For *S. ghobban* the CF value showed a steady increased from 1.53 ± 0.31 at 14.5 cm until 1.71 ± 0.05 at 20.5 cm. Then the CF value showed a slight decreased at 22.5 cm and reached the peak value at 24.5 cm midlength with 2.26 ± 0.09. After 24.5 cm midlength the CF value of *S. ghobban* had showed a slight increased and decreased trend from 26.5 cm until 38.5 cm.

Briefly, from three parrotfish species *S. rivulatus*, *S. qouyi* and *S. ghobban*, the most of CF values range between 1.52 ± 0.38 to 1.95 ± 0.03 except at two midlength 14.5 cm and 24.5 cm for *S. qouyi* (2.06 ± 0.48) and *S. ghobban* (2.26 ± 0.09) respectively which their value exceed than 2.0. From one-way ANOVA, CF value had shown significantly different between *S. ghobban* with *S. rivulatus* and *S. qouyi* with $p<0.05$.

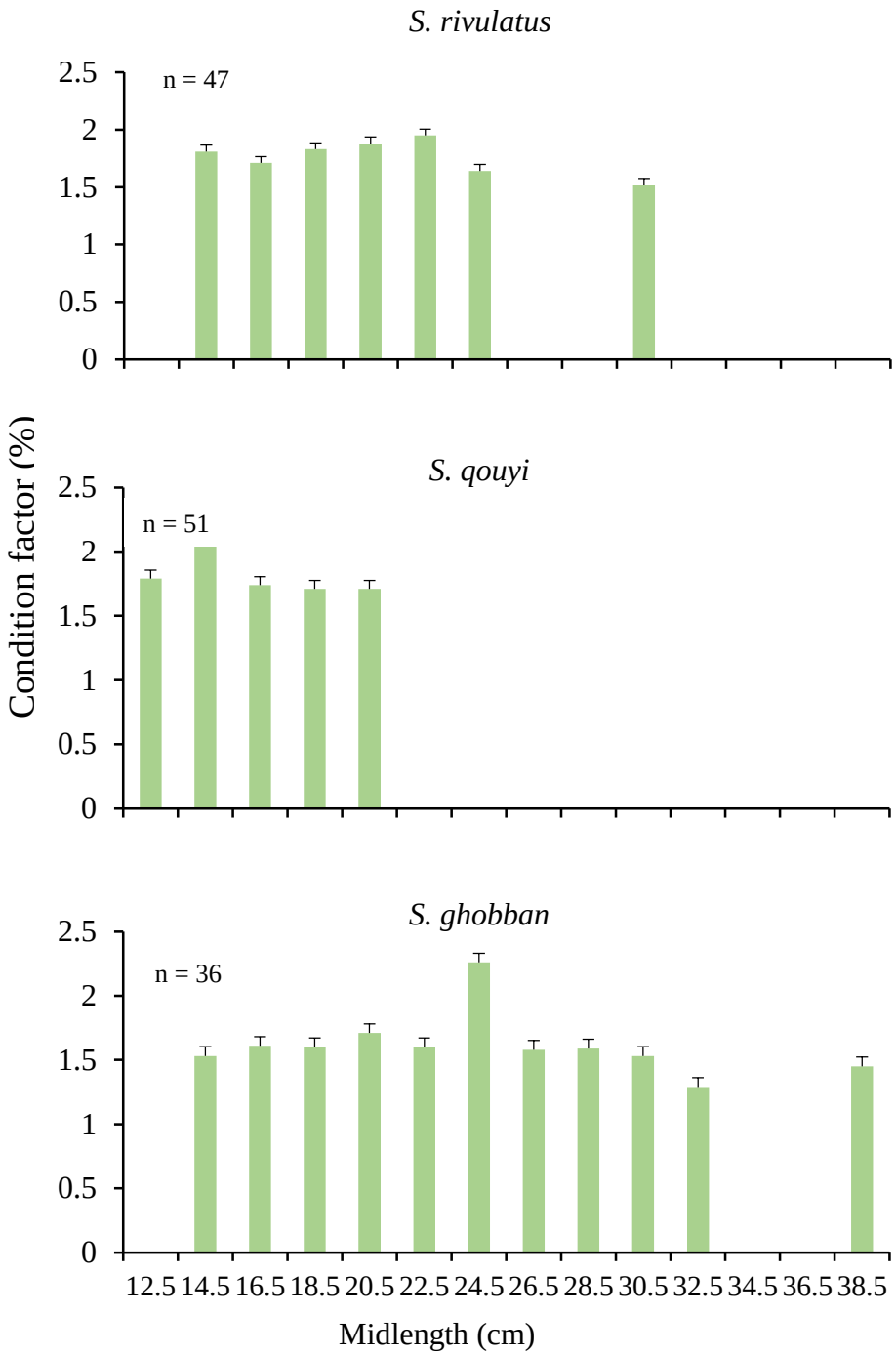

Figure 4.5: The mean ± SD of condition factor (CF) pattern of parrotfish *S. rivulatus*, *S. qouyi* and *S. ghobban* at Pulau Bidong. [n : total number of fish samples]

4.4 Discussion

4.4.1 Size – frequency Distribution

At Pulau Bidong, male parrotfish was dominated by *S. qouyi* IP and there was the least number of *S. ghobban* IP and TP. In the meanwhile, it was observed that their size distribution pattern varied according to species where the highest frequencies were located at different midlength. This may be caused by the different range sizes to reach the adult phase (TP) between the species also link with the reproductive strategy in parrotfish. Previous studies had showed different parrotfish species may have different minimum and maximum lengths to reach their initial and terminal phase (Choat & Robertson, 1975). Additionally, the size of the initial phase and terminal phase was overlap in parrotfish species.

In other aspects, the different size also might be related to the size at maturity of parrotfish species. Choat et al., (1996) reveal that several parrotfish species have shown sex-specific growth patterns. Possibly male parrotfish species which have reached TP phase more early, maybe matured at a small size could result in less number of large fish. The variation of size-at-age found from these previous studies in Scaridae might be one of the important factors that lead to the different size distribution patterns between three male parrotfish *S. qouyi*, *S. rivulatus* and *S. ghobban* at Pulau Bidong.

The large parrotfish dominated by *S. ghobban* is also less in number compared with *S. rivulatus* and *S. qouyi* in the present study, probably influenced by the different social behaviour of parrotfish at Pulau Bidong. Group versus territoriality behaviour in parrotfish, *Sparisoma viride* had shown a different effect on growth and reproduction (van Rooij et al., 1995a). They have found a high significant growth in terminal phase fish, yet from their observation, almost no spawning activity occurred in group fish compared to territorial fish (van Rooij et al., 1995a).

Besides, Mumby and Wabnitz (2002) found that the interaction of five different parrotfish species also showed a significant effect on territory size toward reproductive behaviour. They also state that the greater fish (parrotfish) tend to have larger territories with a lower population density. Moreover, naturally fish with a dominant adult group could chase the smaller fish size from the territory area (Abesamis & Russ, 2005). Having low population density could reduce competition for food yet fish will focus on reproductive growth. In contrast Adams (2001) reviewed the territory size was independent toward food resource but more dependent on the social behaviour of the species. Therefore, by this species-specific social behaviour impact, it was not impossible if the least number of *S. ghobban*, more diversion in range size and the presence of larger size compared with the other two species (*S. rivulatus* & *S. qouyi*) at Pulau Bidong.

4.4.2 Length-weight Relationship of Three Parrotfish Species at Pulau Bidong.

Length-weight relationships are essential in fisheries studies where they could provide important information regarding fish's growth, condition and suitability in its habitat (Clarito, 2021). Information about the length-weight relationship of coral reef fish especially parrotfish species in the East Coast of Malaysia is very scarce. This was the first report to study the length-weight relationship of three parrotfish species of Scarus genus which were *S. rivulatus*, *S. qouyi* and *S. ghobban* in the coral reef ecosystem at Pulau Bidong, Terengganu. From three parrotfish species of the Scarus genus, results showed the total length (cm) and body weight (g) had a significant relationship ($p<0.05$).

The present study revealed that the calculated 'b' value in *S. rivulatus* and *S. qouyi* exhibited negative allometric growth ($b<3$). This indicates that the growth pattern of these two species become slender or thinner as it increases in length (Tagarao et al., 2020). Similar results have been found in other parrotfish species *S. ghobban* (Veeramani et al., 2010), *Hipposcarus harid* and *Chlorurus sordidus* (El-Sayed et al., 2011) at other tropical country. On the other hand, *S. ghobban* had shown positive allometric growth ($b>3$) which meant that when the fish weight

increased, the body size would also increase (Tagaroa et al., 2020). Positive allometric growth also was shown in parrotfish *S. rivulatus*, *S. schelgeli* and *Chlorurus gibbus* from Great Barrier Reef, Australia (Choat et al., 1996).

According to the previous studies, When coefficient of 'b'=3 the growth type is identified as isometric growth which indicates the similar growth pattern from small to large body size (Santoz et al., 2002; Hanif et al., 2020). Meanwhile, if 'b'<3 fish growth pattern was defined as negative allometric growth (Jones et al., 1999; Ragheb, 2023) and 'b'>3 indicates positive allometric growth pattern (Mazumder et al., 2016; Hanif et al., 2020; Ragheb, 2023). The 'b' slop in the present study ranges between 2.64 to 3.27 that lies between 2.5 and 3.5 (Froese, 2006) which describe the normal dimension for every fish. Results also of showed that the value of coefficient of determination r^2 for *S. rivulatus* and *S. qouyi* was close to each other with 0.87 and 0.88 respectively but for *S. ghobban* was 0.93 which indicates ideal growth of fish (Hanif et al., 2020) also signifying the increased mass of fish attributed to the body length increased (Komba et al., 2020). Previous study in length-weight relationship of parrotfish species *S. rivulatus*, *S. schelgeli*, *S. niger* and *S. frenatus* demonstrated the same r^2 which exceed 9.0 (Choat et al., 1996) while for *S. ferruginus* and *Chlorurus sordidus*, their r^2 value range between 0.87-0.84 (El-Sayed et al., 2011).

The variation of 'b' value in length-weight relationships in the same species maybe due to one or more factors including availability of food, physicochemical parameters of the environment, reproductive cycles, sexual maturity, number of sample examined, growth phase and maturation of gonad (Clarito, 2021; Hanif et al., 2020; Tagaroa et al., 2020; Hanif et al., 2017; Hossain et al., 2014). From the reproductive aspect growth can be affected by the reproduction phase. Study in Sharpnose Hammer Croaker, *Johnius borneensis* demonstrated that, different male gonad phase occurred at different length and weight, also resulting in different GSI value associated with variation of growth pattern (Tagarao et al., 2020). Besides, near to spawning, growth might be reduced due to loss of energy for sexual activity such that mating behaviour, chasing opposite partner, accumulation of egg yolk in

the gonad, sperm production and territory defence (Munday et al., 2006; van Rooij et al., 1996).

From growth phase aspect, parrotfish *Sparisoma viride* had shown a different growth in TP male than IP was attributed to the different reallocation of energy in reproduction and territorial defence (van Rooij et al, 1996) also had been discussed at three species of parrotfish where the slower growth of initial phase than terminal phase may reflect the higher gonadosomatic index (El-Sayed et al., 2011). Some of the previous evident was in line with the present study *S. rivulatus*, *S. qouyi* and *S. ghobban* where this three study species exhibited different adult phase (IP & TP), gonad phase (Table 5.1) and showed change of GSI pattern at various length among three species (Figure 4.3). Therefore, possibly these might be two influence factors which contribute to the different growth pattern shown in three parrotfish species in the coral reef ecosystem at Pulau Bidong, Terengganu. Besides, the uncontrollable sample size and number of fish samples during random sampling may be one of contributing factors to this differentiation. Thus, since parrotfish was among important herbivorous fish in the reef ecosystem, perhaps this present result could be as baseline information for further studies on the monitoring and conservation aspects of parrotfish population at Terengganu, East Coast of Malaysia region.

Table 4.3 Length-weight relationship and size range of different parrotfish species from several regions.

Region	**n**	**Species**	**TL/SL range (cm)**	**a**	**b**	**R^2**	**Source**
Saudi Arabia	175	*Hipposcarus harid*	15.9 - 31.0	2.50	2.99	0.954	El-Sayed Ali et al., 2011
	245	*Scarus ferrugineus*	12.5 - 31.6	1.92	3.09	0.872	
	115	*Chlorurus sordidus*	13.6 - 29.1	3.09	2.94	0.838	
India	78	*Scarus ghobban*	15.0 - 82.0	0.19	2.54	0.953	Veeramani et al., 2010
Guam	376	*Chlorurus sordidus*	12.4 - 26.5	$1x10^{-5}$	3.13	0.970	MclLwain & Taylor 2009
Australia	374	*Scarus rivulatus*	18.0 - 29.0	1.73	3.14	0.982	Choat et al., 1996
	384	*Scarus schelgeli*	22.0 - 24.8	1.86	3.12	0.992	
	40	*Scarus psittacus*	10.4 - 19.3	6.08	2.90	0.981	
	60	*Scarus niger*	11.2 - 27.8	2.57	3.09	0.993	

	45	*Scarus frenatus*	10.6 - 29.5	2.79	3.06	0.990	
	67	*Chlorurus sordidus*	7.7 - 22.4	1.82	3.15	0.990	
	85	*Chlorurus gibbbus*	10.5 - 49.9	9.25	2.85	0.9974	
Malaysia (Pulau Bidong)	46	*Scarus rivulatus*	15.5 - 30.0	1.42	2.76	0.8714	Current study
	52	*Scarus qouyi*	13.2 – 20.1	1.29	2.64	0.8782	
	36	*Scarus ghobban*	14.4 – 38.7	2.14	3.26	0.9331	

4.4.3 Reproductive Relationship of Gonad and Liver Weight with Total Length of Three Parrotfish at Pulau Bidong

As fish grow, the increment in body length could influence the timing of gonad maturation phase. In the present study, the total length had shown a significantly positive correlation with body weight, gonad weight and liver weight (Table 4.2). Body weight showed the strongest correlation compared to gonad and liver weight. A similar result was obtained by Choat et al., (1996) and Veeramani et al., (2010) in body weight coefficient but slightly differs from MclLwain and Taylor, (2009) in gonad weight might be due to different fish sex and variation maturation ratio.

On the other hand, the previous study had found the growth of somatic cells and reproductive cells was independent (Rijnsdorp, 1990). In the meanwhile, weight of the liver is also associated with fish reproduction. Moreover, the amount of energy produced in fish will be dispersed throughout the body at different amounts depending on the requirement for tissue growth, gonad development and spawning activity (Smith et al., 1990). This indicates that the correlation between these three indexes (gonad weight, liver weight & body weight) could be related to parrotfish reproduction at Pulau Bidong.

There was the existence of co-relationship but negatively correlated (Table 4.2). Gonad and liver weight basically express into the gonadosomatic index, hepatosomatic index and condition factor. From previous studies, inversely

relationships were shown in reef fish (Palazon-fernandez, 2007; Mat Piah & Bucher, 2014) and Atlantic sardine (Nunes et al., 2011) due to the used of reserve energy from the muscle and liver for the gonadal growth, especially during the pre-spawning and spawning season (Eliassen & Vahl, 1982). The insignificant correlation of GSI with CF was probably related to the less or indirect contribution of energy use from the muscle in reproduction (Ganias et al., 2007; Nunes et al., 2011). Therefore, the trend of each reproductive index might have a relationship with their single role specifically toward parrotfish reproduction.

4.4.4 Reproductive Index Pattern with Midlength of Three Parrotfish Species Associate to Reproduction at Pulau Bidong

In fish reproductive biology the GSI, HSI and CF value were widely used to reflect the fish maturation state and status of gonad development (Hasan et al., 2020; Yanti et al., 2019; Johnson & Tamatamah 2013). To date there was an absence of study regarding parrotfish reproductive biology on the East Coast of Peninsular Malaysia. The present study was the first attempt to study parrotfish reproductive index in term of GSI, HSI and CF associated with gonad maturation in the coral reef ecosystem at Pulau Bidong, Terengganu. There is only one study regarding moisture content in the liver of coral reef fishes which provides information on the biological factor (GSI, HSI, CF) at Malaysia South China Sea (Arai et al., 2016). GSI, HSI and CF had some correlation to each other during fish spawning (Nunes et al., 2013.). However, this condition might be different with different fish species.

Based on the current study, the change of GSI and CF with midlength in parrotfish showed significantly different between species. The GSI was used to show the maturation state during gonad development (Yanti et al., 2019; Vitale et al., 2015) while CF was used to measure the energy reserve in fish reproduction (Sudarsham & Kulkarni, 2013). The high accumulation of reserve energy starts to occur in immature fish as preparation for maturing stages usually results in higher condition factors (Sudarsham & Kulkarni, 2013). Besides, studies also reported that changes of CF along the reproductive cycle in white grunt, *Haemulon plumieri* (Palazon-

fernandez, 2007) and *Notopterus notopterus* (Sudarsham & Kulkarni, 2013) was caused by passage transfer of somatic energy for gonad development and spawning activity. This may indicate the correlation between GSI and CF during the process of gonad maturation in fish.

A study in river catfish *Clupisoma garua* had reported that the maturation of fish was correlated with the increase of GSI until the peak value which was the highest degree of gonad maturity (Hasan et al., 2020). There was an inverse pattern relationship observed between the GSI and CF in European sprat *Sprattus sprattus* (Vitale et al., 2015). This reproductive trend was also shown in the study of reproductive biology of pufferfish, *Marilyna pluerostica* and *Tetractenos hamiltoni* (Matpiah & Bucher, 2014). In *S. rivulatus, S. qouyi* and *S. ghobban* a similar pattern between GSI and CF were observed at Pulau Bidong in this current study where the increase of GSI (Figure 4.3) with length was oppositely to the trend of CF (Figure 4.5). Previous study demonstrated that, there was less energy consumed during spawning derived from somatic tissue mainly axial muscle was reported (Smith et al., 1990), coincident with no significant changes observed in biochemical composition (total lipid) in the muscle on several commercial fishes during gonad maturation (Blanchard et al., 2005; Dominguez-Petit et al., 2010; Sutharshiny et al., 2013; Dhurmeea et al., 2018). Therefore, CF could not be a strong indicator to reflect the reproductive activity that occurs in fish.

HSI was associated with lipid composition in the fish liver (Sutharshiny et al., 2013). The depletion of lipid in the fish liver could be supported by several studies through the biochemical change that occur in liver accesses by the lipid content (Blanchard et al., 2005; Zudaire et al., 2014) which is closely associated with the changes in HSI value in relation to reproduction. Sutharshiny et al., (2013) reported that, lipid in liver had shown double increased at early maturation stages (stage 2 – 4) before drastically decline at advanced stages (stage 5 – 6) in both sex *of Scomberoides lysan* at Sri Lanka, India. Study of gonad maturation in dusky parrotfish *S. niger* confirmed that HSI and GSI were slowly increased from early maturation until reached peak of spawning phase (Yanti et al., 2019). In the present study, the change of HSI pattern between *S. rivulatus* and *S. ghobban* with length

was similar. For *S. qouyi* the beginning of HSI pattern was slightly different. However, from statistical analysis the different of HSI was not significant between three parrotfish species at Pulau Bidong. This indicates that size of parrotfish may or may not influence the maturation phase between fish species.

The difference of GSI, HSI and CF pattern at midlength between the parrotfish might be due the different reproductive strategy, specifically their spawning behaviour among the species and energy allocation strategy. Previous study on reproductive biology of parrotfish fish *Sparisoma cretense* was observed that parrotfish had two type of spawning behaviour which was territory and group mating systems (Afonso et al., 2008). Pair spawning was frequently observed in territory group mating system (Afonso et al., 2008). The range size of male adults in the territory group was significantly larger than group mating system in parrotfish *S. cretense* (Van Rooij et al., 1996a). The large terminal male spawned with multiple females within the territory area and had high reproductive success (Girolamo et al., 1999). Conversely, in the group spawning system, sperm competition between multiple male occurred in bucktooth parrotfish and had low reproductive success (Marconato and Shapiro, 1996). A study also revealed that parrotfish individual in group spawning spawn at small range size compared to territory group mating system (Van Rooij et al., 1996a). The differences of these spawning system criteria had resulted the large variability in relative gonad size of ripe males in parrotfish *S. cretense* (Afonso et al., 2008). Meaning that, the difference of spawning behaviour between parrotfish species can possibly influence the size of gonads during gonad maturation phase and may directly affect the reproductive index (GSI, HSI & CF) in fish reproduction.

Another factor was energy allocation strategies. There are two types of energy allocation strategies in fish reproduction known as a capital breeder and income breeder. In the capital, breeder fish depend on the energy reserve in the liver mainly for reproduction success. Fish are usually characterized by less food intake during gonad development, accumulation of total lipids occurred before gametogenesis, also almost showed the significant changes in HSI value (Dhurmeea et al., 2018; Mat Piah & Bucher, 2014). In contrast, for income breeder gamete

development relies on concurrent food intake to fulfill the energy demand for reproduction. Opposite to capital breeder, food was continuously taken during the gonad reproductive cycle in income breeder also only little or no significant changes of lipid content with HSI value was observed, assuming energy reserve will be used for other purposes (mating, feeding & increase egg production) (Blanchard et al., 2005; Domingues-Petit et al., 2010). For parrotfish species they are suggested as income breeders for *S. iseri* which food was taken continuously during reproduction, thus HSI was assumed not directly affected by reproduction while *S. ferruginus* was characterized as a capital breeder (Hoey & Bonaldo, 2014). Therefore, from this evidence it shows that, three parrotfish species in this present study might be categorized as income breeder where changes of HSI pattern was not affected the by the significant value. However, further study on the stomach content at gonad maturation is necessary to confirm this reproductive behaviour in parrotfish species.

4.5 Conclusion

From size-frequency distribution *S. ghobban* had a wider range sizes. A recent study had shown there was a positive linear relationship between total length and body weight in three parrotfish species (*S. rivulatus, S. qouyi* & *S. ghobban*) where there was an increase of length and weight as along the fish growth. Among three parrotfish species, two species (*S. rivulatus & S. qouyi*) showed negative allometric growth pattern while another one (*S. ghobban*) showed positive allometric growth. This means that different parrotfish species in the reef ecosystem at Pulau Bidong possibly have different growth pattern. There were changes of reproductive index such as GSI, HSI and CF value of parrotfish with length. The changes of this reproduction index occurred almost with the same graph pattern but reached their peak value at different lengths. This study hypothesizes that the spawning phase occurs when GSI reaches its peak level, followed by a dramatic reduction in values as the sperm is expelled, known as the regressing phase. Thus, through these conditions length of fish may reflect the reproductive activity that occurs during gonad maturation of three species of parrotfish in Pulau Bidong, Terengganu.

CHAPTER 5

GONAD HISTOLOGICAL EXAMINATION AND REPRODUCTIVE HORMONE PROFILE IN MALE PARROTFISH FOR GENUS SCARUS (*S. rivulatus, S. qouyi* & *S. ghobban*) AT PULAU BIDONG, TERENGGANU

5.1 Introduction

Generally, a microscopic study in reproduction is significant to classify the differentiation of gonad structure at the cellular level in teleost fish. Histological study in gonad maturation of parrotfish could provide information based on the progression of gonad development as supplementary support towards the mechanism involved in sex change in protogynous hermaphrodites. Nevertheless, the study of male parrotfish gonad phase was slightly complex due to the different adult phase (IP & TP) involved in diandric species (Godwin, 2009). This is one of the possible reasons only a little study has been done for parrotfish.

However, there is some limitation information found within the previous study. DeMartini and Howard (2016) described microscopic criteria of parrotfish at each gonad phase, however, there was no further discussed of their histological examination result, absent of gonad phase figure and used some previous terminology which different from as standardized by Brown-Peterson et al., (2011) for hermaphrodite fish. Likewise, Abdel-Aziz et al., (2006) have conclusively revealed the ultrastructure study on male parrotfish, *S. ferrugineus,* yet still not applied a clear conceptual model of fish reproductive cycle as shown by Brown-Peterson et al., (2011). The differentiation in the terminology used by the previous study could become a little problematic for comparison or reference purposes when applied in further study. Also, relatively few studies emphasize one sex of gonad characteristics either male or female with a lack of information specifically on the different criteria between IP and TP gonad characteristics (El-sayedah et al., 2012; Girolamo et al., 1999; Hamilton et al., 2008; Adams & Choat, 2008; Robertson et al.,

1982). Most important to highlight from these several studies is that none of them could completely find the complete gonad maturation phase from earlier (developing) to the final phase (regenerating) at IP and TP parrotfish.

Regarding to this matter, some difficulty encountered through the study of parrotfish gonad development was to find size of fish completely accomplish their gonad development phase cycle (developing, spawning, regressing, regenerating) for both IP and TP (Brown-Peterson et al., 2011). This may be because protogynous hermaphrodites experience different sizes at sex change and different sizes at initial maturity (Benvenuto et al., 2017; DeMartini & Howard, 2016) also overlapping size between IP and TP.

The environment and social interaction stimulus are anticipated as the key factors to the sex change that occurs in hermaphrodite fish (Todd et al., 2016). In recent years, researchers have investigated a few approaches to understand the pathway involved in this hermaphroditism sex change through the hormone study (Jiang et al., 2003; Solomon-lane, Crespi, & Grober, 2013) and molecular regulation (Baker, 2004; Horiguchi et al., 2013; Tsakogiannis et al., 2018). As an example, there was two main reproductive hormones estradiol and 11-ketotestosterone in hermaphrodite fish currently known to have some correlation with the sex change factor (Bhandari et al., 2006; Lone et al., 2001) and gonad maturation. Meanwhile, matured gonads were generally associated with the spawning phase where the changes of GSI and HSI were usually evaluated in fish at the same time (Bhandari et al., 2003). This study was important to demonstrate the changes in the pattern of spermatogenic cells, hormone profile, GSI and HSI in relation to gonadal maturation in male parrotfish for three species of the genus Scarus.

Returning to the issue of complexity involved in the histology of parrotfish and a little information from the previous study, it is necessary to improve the existing understanding in order to support what are the criteria to differentiate the difference of gonad maturation phase at adult male parrotfish completely. Therefore, the objective of this chapter focuses on the classification of male gonadal maturation phases for the genus Scarus (*S. rivulatus, S. qouyi* & *S. ghobban*) in the adult phase

(IP & TP), the determination of the differentiation of the number of spermatogenic cells in the maturation phases, and the determination of gonadal indices (GSI & HSI) and reproductive hormone pattern of parrotfishes during gonadal maturation. It was hypothesized that there are some different criteria of gonad maturation phase of male parrotfish. Also, the spermatogenic cell present and hormone pattern may have significantly different at different maturation phases and predicted that, GSI and HSI trends might be higher during spawning stages compare at Pulau Bidong, Terengganu.

5.2 Materials and Method

5.2.1 Sample Collection and Species Identification

Parrotfish from the Scarus genus of three species *S. rivulatus*, S. *qouyi* and *S. ghobban* were collected. Parrotfish samples were collected at Pulau Bidong, Terengganu. There were 134 of male parrotfish which consisted of 88 IP and 49 TP individuals. For histological analysis and spermatogenic cell count data were pooled since there was no differentiation in histological characteristics between the three *Scarus* species. The species identification was explained in detail at Chapter 3 Section 3.4 and the different colours of adult phases (initial & terminal) were shown in chapter 3 at Figure 3.3. All live fish samples were directly processed at UMT base research station near the sampling location at Pulau Bidong. Step of fish sampling was according to the step which has been explained at Chapter 3 section 3.3 paragraphs 2-3.

5.2.2 Histological Examination

A total of 118 male gonad samples of *S. rivulatus* (n = 43), *S. qouyi* (n = 43), and *S. ghobban* (n = 32) were collected for histological examination. Testis was taken out *in situ* after the blood sample has been collected for hormone analysis

purposes. The histological examination of the male gonad was done according to the standard histological procedure (Humason, 1962). Testes were fixed in 10% buffered formalin. To avoid any bias against variation in gonad tissue, cutting was carried out at three sections (anterior, middle, and posterior). The results obtained from several samples (n = 30) indicated that no differences were found between the three sections. Therefore, the best section was selected for observation purposes. After fixation in buffered formalin, testis in the cassettes was placed in the tissue processor equipment. Once the process was completed, fixed testes were removed from tissue processors, thus embedded with paraffin wax. Samples cutting were made at one micrometer and were processed by standard method procedure. For the staining method, Haematoxylin-eosin method was used. Then, all testes were observed under the advanced microscope (Nikon eclipse 70-I).

Male gonad maturation phases were identified based on the modification criteria from previous studies (Abdel-Aziz et al., 2006; Brown-Peterson et al., 2011; DeMartini & Howard, 2016) (Table 5.1), standard phase of gonad maturation of hermaphrodite fish was adapted from Brown-Peterson et al., 2011 and the different of spermatogenic structure was referred to Marquez and Ferrierra, (2011), Vergillo et al., 2012 and Abdel-Aziz et al., 2006.

5.2.3 Spermatogenic Cell Count

Spermatogenic cells (spermatocyte, spermatid, spermatozoa, Leydig cell, atretic oocyte, primary growth oocyte, previtellogenic oocyte, primary growth spermatocyte) present at different gonad phases was counted under an advanced microscope (Nikon eclipse 70-I). For each species, three gonad samples were taken from each gonadal phase (Aripin, 2015). Three parts or spots at each replicate was counted at 40x magnifications and the total number of spermatogenic cells present at each gonad phase was analysed by mean values (Aripin, 2015).

The spermatogenic cells were differentiated through their organization or its location associated with the developing phase in the testis according to Abdel-Aziz et al., 2006, Brown-Peterson et al., 2011, El-Sayedah et al., 2012 and Vergilio et al., 2012. Generally, male gonads are composed of connective fibers which form the interstitial tissue. The interstitial tissue contains of blood cells, tissue fibers and Leydig cells (Vergilio et al., 2012 & El-Sayedah et al., 2012). This connective tissue extrudes septa to the interior organ which formed the seminiferous lobules (El-Sayedah et al., 2012). Seminiferous tubules consist of spermatogenic cells (Vergilio et al., 2012). Leydig cells located between lobes and blood vessels in the interstitial tissue (Vergilio et al., 2012). Spermatogonia were found at the edge of the seminiferous lobules (Abdel-Aziz et al., 2006) while mature spermatozoa were found in the lumen of cysts in males gonads (Vergilio et al., 2012).

At the gonad developing phase, spermatocytes were clearly seen along the lobules (Brown-Peterson et al., 2011) while spermatozoa were present in the lumen of seminiferous lobules (Abdel-Aziz et al., 2006). During spawning phase spermatozoa were released and gonad was loaded with spermatozoa in the lumen of testicular tubules, Leydig cells was scared in interstitial tissue (El-Sayedah et al., 2012) while spermatocytes found throughout the testis (Brown-Peterson et al., 2011). The number of spermatozoa stores depleted at the regressing phase with only leftover spermatozoa was present in lumen of lobules and in sperm ducts. Meanwhile, there was a rapid increase of spermatogonia seen in the periphery of testis (Brown-Peterson et al., 2011). The proliferation of spermatogonia continuously occurred at all over the testis during the regenerating phase and only a small amount of spermatozoa seen in lumen of lobules (Brown-Peterson et al., 2011).

5.2.4 Reproductive Hormone Analysis (11-ketotestosterone & Estradiol)

Blood plasma was used for hormone analysis. Blood was taken from the live fish sample at the sampling site. Parrotfish sample taken from the divers was immediately aerated in the large storage tank. Clove oil was used as an anaesthetic purpose to immobilize fish samples for blood drawing procedures. Blood was taken

at the caudal fin at lateral line of the fish sample with a heparinized syringe (1 ml or 3 ml) and needle (25 or 23 gauge) then transfer into EDTA (ethylenediaminetetraacetic acid) test tube (2 ml). Next, blood was centrifuged with the centrifuge machine (Eppendorf Centrifuge) at 12000 rpm for 2 minutes to separate the blood plasma. Blood plasma was taken out then placed in the 2 ml centrifuge tube and stored in the ice box temporarily before being transferred to the deep freezer at -80 ºC until the hormone analysis. From three parrotfish species, 120 and 96 blood plasma samples were collected for analysis of the hormones 11-ketotestosterone (11-KT) and estradiol (E2), respectively. Each sample of blood plasma was analysed with three replicates. The blood plasma sample was diluted first into 1000× serial dilution during the analysis for both hormones. The 11-KT and E2 were analysed using the 11-ketotestosterone EIA Kit and estradiol EIA Kit by Cayman Chemical Company respectively. The standard ELISA procedure was followed according to the provided protocol in the EIA kit for both 11-KT and E2 (El-Sayed et al., 2011).

5.2.5 Data Analysis

The mean of the total spermatogenic cell count was present by percentage value according to the maturation phase (Aripin, 2015). The statistical significance of two spermatogenic cells (spermatocyte & spermatozoa) and mean of the reproductive index (GSI & HSI) at different gonad maturation phases were analysed using log transformation to normalize the data set then proceed to an analysis of variance by One Way ANOVA using SPSS (Statistical Package for Social Science) IBM statistical software. Then, followed by a Post Hoc test with Duncan multiple tests in order to compare the difference between the subjected groups. For reproductive hormones, 11-KT and E2, rank transforms were applied to normalize concentration data and correct the skewness. Then the mean value was analysed by using One Way ANOVA. Post Hoc with Tukey HSD test was applied to compare the difference of hormone concentrations between the group (maturation phases).

5.3 Results

5.3.1 Classification of Gonad Stages of Parrotfish Species

As can be seen from Table 5.1 there were five phases of IP and TP gonad maturation in male parrotfish found in the current study including the transitional phase. From Table 5.1, the gonad phase was separated into two life phases which were the initial phase (IP) and terminal phase (TP). The difference between IP and TP was mainly determined based on the body colour as described in Chapter 4. Other than that, more comparable could be made with the present of more numerous Leydig cells (Lc) at the developing (Figure 5.2F), spawning (Figure 5.2G) and regressing (Figure 5.2H) in TP compare to IP at developing phase (Figure 5.1B) which has matched with the characteristic describe by Abdel-Aziz et al., (2016). Following to this, in TP gonad, the yellow-brown body (Figure 5.2 F-H) was obviously appear compared to IP

Table 5.1 Microscopic descriptions of the phases of initial phase (IP) and terminal phase (TP) of three male parrotfish species (*S. rivulatus*, *S. qouyi*, *S. ghobban*) in reproductive cycle at Pulau Bidong, Terengganu. Note: absence of TP male regenerating phase; the descriptions modified from Abdel-Aziz et al., 2006, Brown-Peterson et al., 2011& DeMartini & Howards 2016.

Phases	**Microscopic examinations/characteristic**
Transition	Present of resting ovarian and primitive spermatic tissue (e.g. inactive sperm crypts).
Initial phase	
Developing	Spermatocytes (Sc) and spermatozoa (Sz) present within seminiferous lobules. Present of spermatogonia (Sg). Interstitial tissue contains less number of Leydig cells. Spermatogenesis is gradually present in the testis.
Spawning	Present of active spermatogenesis. Enlargement of seminiferous lobules full with spermatozoa (Sz). Numbers of spermatid (St) and spermatocytes (Sc) reduced.
Regressing	Spermatogenesis in male testes reduced. Present some residual spermatozoa (Sz). Rapid increase of spermatogonia (Sg) at periphery of the testis.
Regenerating	Present some empty lobule in the testis. Present of thicken interstitial tissue and probably vacuolated Leydig cells.

Terminal phase	
Developing	Testis may contain a former membrane bound ovarian cavity (Ao). Leydig cells (L) group appear in the interstitial tissue between seminiferous lobules. Male testis consists of Spermatogonia (Sg), spermatids (St) and spermatozoa (Sz).
Spawning	Spermatogenesis actively occurred in male lamellae and may be present in the previous ovarian cavity. Leydig cell (L) group with distinct nucleus present abundantly in the interstitial tissue between seminiferous lobules. Abundant spermatozoa (Sz) present in seminiferous lobules. Some lobules filled with spermatocytes (Sc).
Regressing	Present some residual spermatozoa (Sz). Rapid increase of spermatogonia at periphery of the testis.

Figure 5.1(A) shows the transition phase of gonad development. Meaning that, parrotfish experience a transition phase also generally known as phase where the sex change mostly occurs. Interestingly, although no ovarian tissue residue such as atretic oocyte was found at IP and TP male, but remains of previtellogenic (Pv) and residual of primary growth oocyte was observed at transition phase (Figure 5.1A). This illustrate that some fish had differentiated from female during IP before change sex into TP male known as secondary male. Their body colour had completely transformed as TP male even though transition phase still incomplete. A few Leydig cell also clearly can be observed near to interstitial tissue.

Figure 5.1(B-D) and Figure 5.2(E) shows the results of gonad characteristic in IP male. Male testis at the developing phase of IP shows numerous spermatogonia, less number of spermatozoa and slightly present of spermatocytes (Figure 5.1B). Within the IP, Leydig cells can only be seen at the developing phase compared to others. At the spawning phase, obviously abundant spermatozoa were observed due to the enlargement of the seminiferous lobule (5.1C). Spermatozoa become dominated at this stage, where spermatocyte and spermatid are markedly reduced. Numbers of spermatogonia increase vigorously at the regressing phase and only a few remain of spermatozoa observed (5.1D). Followed by the occurrence of a regenerating phase at IP with numerous empty lobules was obviously present compared to the three earlier stages of gonad maturation (5.2E).

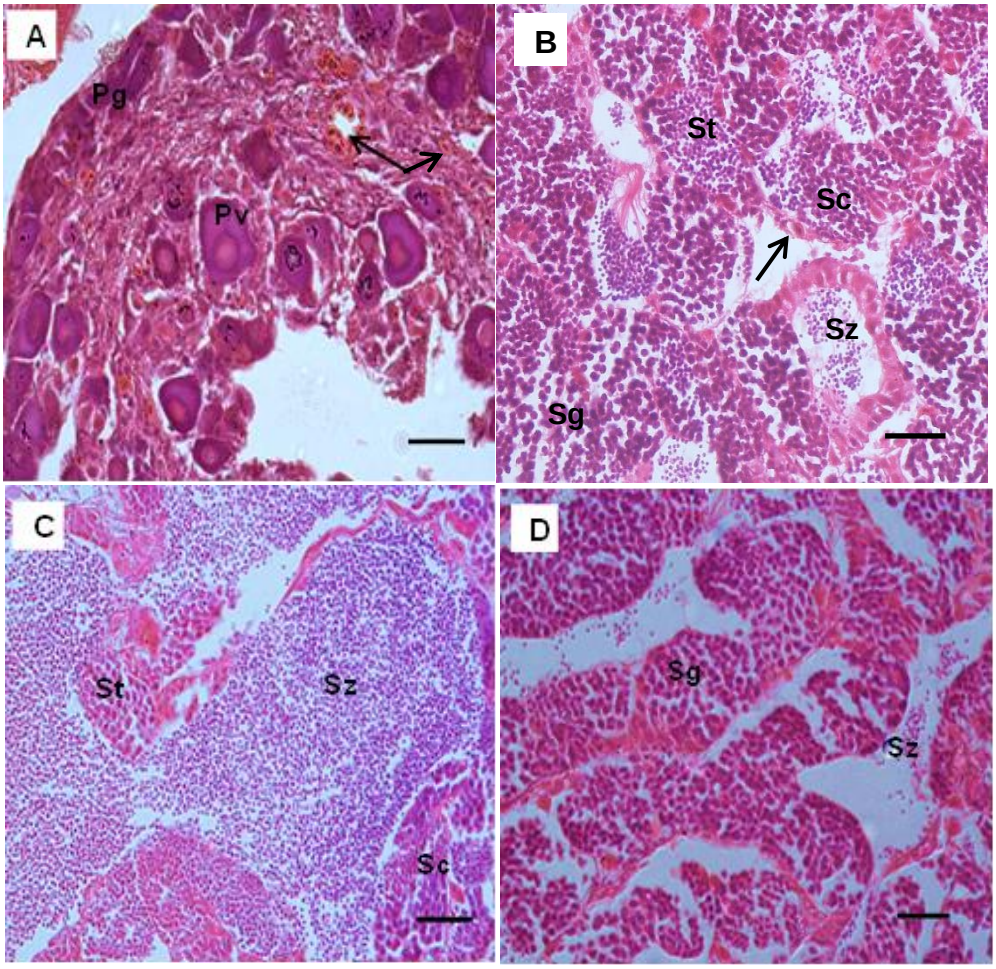

Figure 5.1 Transverse section of initial phase of three male parrotfish species (*S. rivulatus*, *S. qouyi* & *S. ghobban*) at Pulau Bidong, Terengganu: **A** transition stage of parrotfish (Pv = previtellogenic; Pg = residual primary growth oocyte; black errow = Leydig cell); **B** initial phase of parrotfish at developing stage (St = spermatocyte; Sg = spermatogonia; Sz = spermatozoa; black arrow = Leydig cell); **C** initial phase of parrotfish at spawning stage (St = spermatid; Sc = spermatocyte; Sz = spermatozoa **D**); initial phase of parrotfish at regressing stage (Sg = spermatogonia; Sz = spermatozoa);. Notes: *H&E magnification 40x; scale 10 µm*

As same as IP, Figure 5.2(F-H) showed the TP gonad phase begins by developing followed by spawning then regressing. Unfortunately, the TP regenerating phase sample was absent or not found in this study. Compared to IP, the number of Leydig cells in TP was definitely different. Leydig cells were observed in all stages of gonad maturation phases in TP which was in developing (Figure 5.2F), spawning (Figure 5.2G) and regressing (5.2H) compared to IP only in developing was present (Figure 5.1B). Another difference between IP and TP gonads was the presence of a yellow-brown body. The other spermatogenic cell (spermatogonia,

spermatocyte, spermatid, spermatozoa) present at each phase of TP was at the same development pattern and criteria as described in IP.

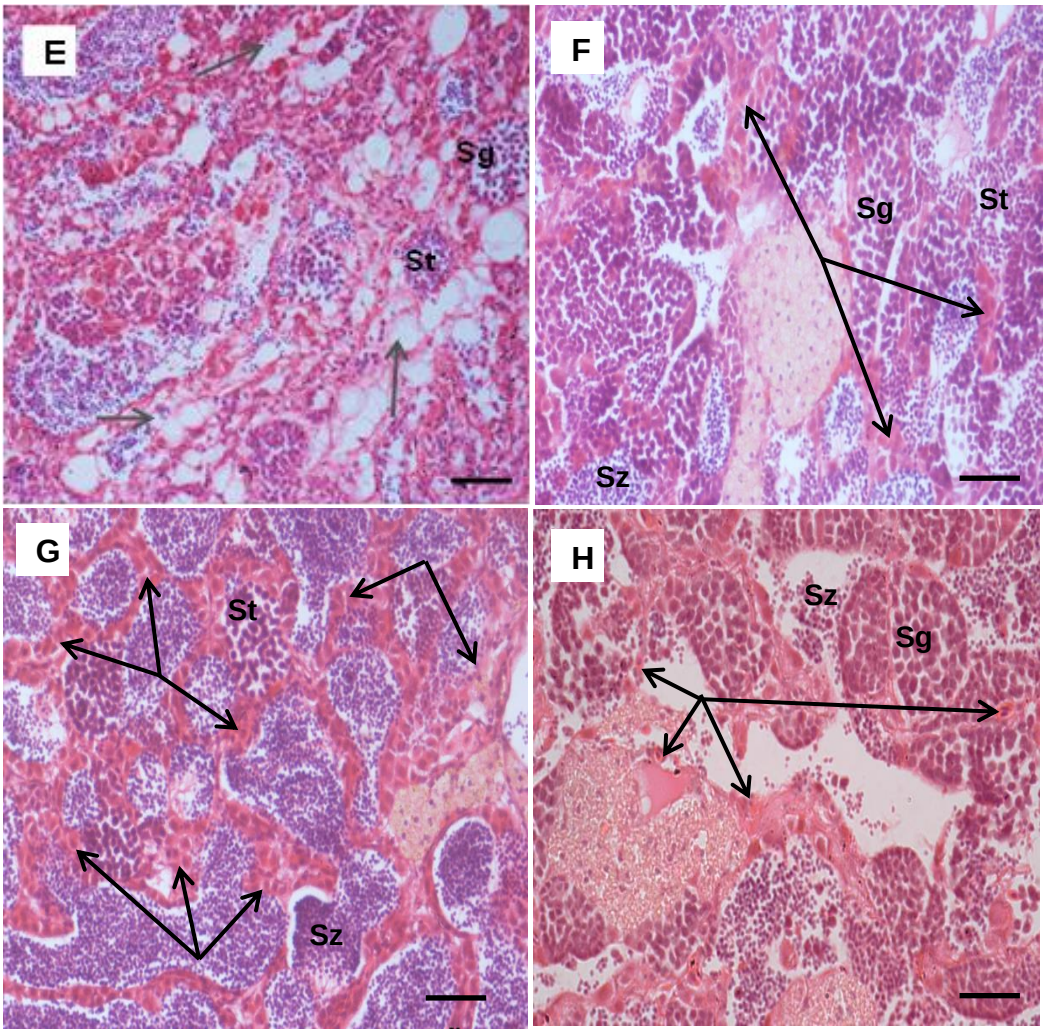

Figure 5.2 Transverse section of initial and terminal phase of three male parrotfish species (*S. rivulatus*, *S. qouyi* & *S. ghobban*) at Pulau Bidong, Terengganu: **E** initial phase of parrotfish at regenerating stage (Sg = spermatogonia; St = spermatocyte; green errow = empty lobule) **F** terminal phase of parrotfish at developing stage (Sg = spermatogonia; St = spermatid; Sz = spermatozoa); and **G** terminal phase of parrotfish at spawning stage (St = spermatid; Sz = spermatozoa); **H** terminal phase of parrotfish at regressing stage (Sg = spermatogonia; Sz = spermatozoa; black (errow) = Leydig cell). Notes: *H&E magnification 40x; scale = 10µm*

5.3.2 Spermatogenic Cell Count in Male Testis of Parrotfish Species (*S. rivulatus, S. qouyi* and *S. ghobban*)

Based on figure 5.3, spermatocyte (Sc) is present in all gonad phases except at the transition (Trans) phase. Spermatozoa (Sz) monopoly the highest percentage with 52%, followed by spermatocyte (Sc) 21% compared with all spermatogenic cell spermatid (St), spermatozoa (Sz), Leydig cell (Lc), primary growth oocyte (Pgo), previtellogenic oocyte (Pv) and primary growth spermatocyte (Psc). In the meanwhile Pgo, Psc and Pv remained below 1% which was only present during Trans and Ao was present only at the TP phase with 0.05%. Lc was observed highest at Trans with 43% while between adult phases, TP was higher than IP with 41% and 16% respectively.

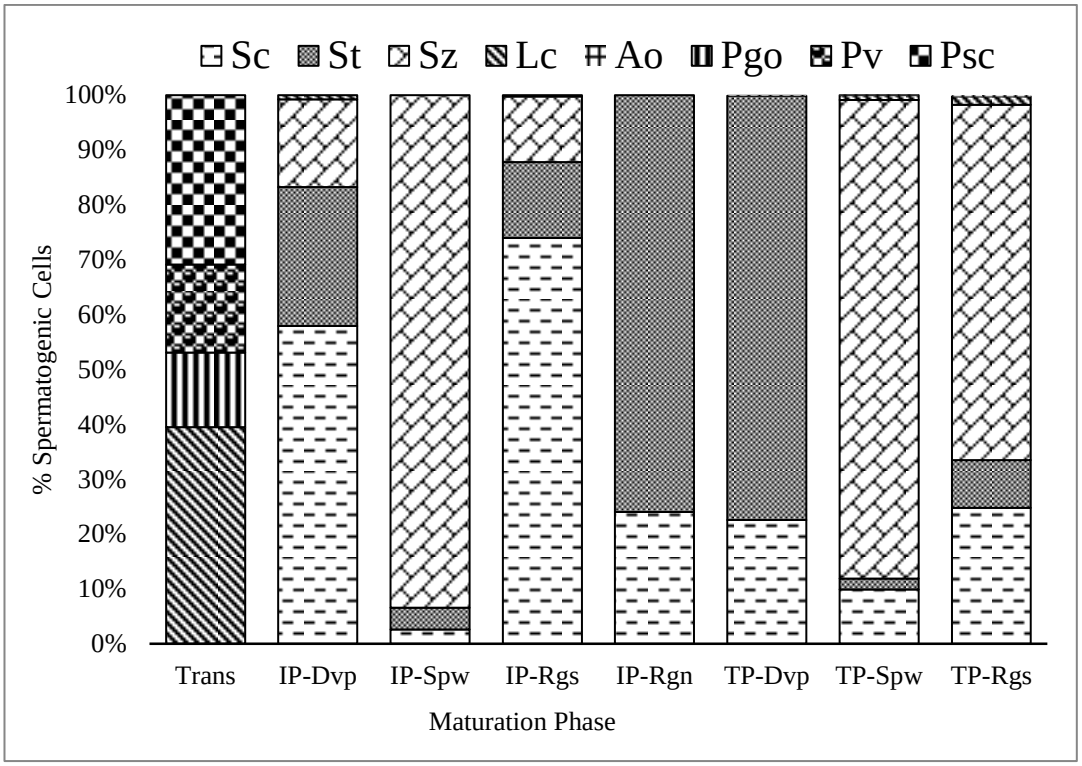

Figure 5.3: Overall total number of spermatogenic cell in male testis by five stages of gonad development in parrot fish species (*S. rivulatus*, *S. qouyi* and *S. ghobban*) at Pulau Bidong, Terengganu (2014). [Note: IP = initial phase; TP = terminal phase; Dvp = developing; Spw = spawning; Rgs = regressing; Rgn = regenerating; Sc = spermatocyte; St = spermatid; Sz = spermatozoa; Lc = Leydig cell; Ao = atretic oocyte; Pgo = residual primary growth oocyte; Pv = previtellogenic oocyte; Psc = primary growth spermatocyte]

As parrotfish consist of two distinct adult phases (initial and terminal), a separate interpretation was applied (Figure 5.3). The spermatogenic cell observed according to the gonad phase found that the highest percentage Sc was at IP-Dvp 51% and TP-Dvp 43%. St dominantly located at IP-Dvp = 43% and TP-Dvp = 91%. Sz was obviously highest at spawning (Spw) phase at both IP = 91% and TP = 81%. For Lc, IP male contain two times lower than TP with 28% and 67% respectively. This result clearly showed that there was some different pattern of spermatogenic cells present at each maturation phase.

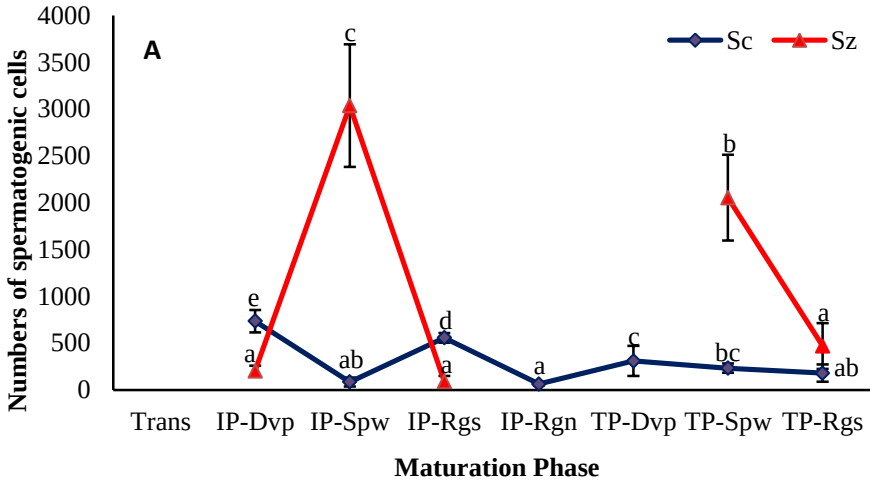

Figure 5.4 Changes of spermatogenic cells count of three parrotfish species (*S. rivulatus*, *S. qouyi* and *S. ghobban*) at different gonad maturation phase at Pulau Bidong, Terengganu (2014). Abbreviations: Sc = spermatocyte; Sz = spermatozoa; Trans = transition; IP = initial phase; TP = terminal phase; Dvp = developing; Spw = spawning; Rgs = regressing and Rgn = regenerating Data given as mean±SD.

Meanwhile, Figure 5.4 shows the changes pattern of two dominant spermatogenic cells according to the maturation phase. The composition of spermatocyte (Sc) and spermatozoa (Sz) were selected as the key to distinguish the degree of gonad maturation. It can be seen from the one-way ANOVA, the number of Sc and Sz were statistically different ($p<0.05$) at different maturation phases. On average, Dunken multiple tests revealed that, Sc was significantly highest at the developing phase of both IP and TP male where IP = 735 and TP = 310. For Sz, the greatest increase of cells significantly observed at IP (3037) and TP (2054) spawning phase while absent at IP-Rgn, Trans and TP-Dvp.

5.3.3 Hormone Profile in Male Parrotfish Species (*S. rivulatus*, *S. qouyi* and *S. ghobban*)

Changes of reproductive hormone concentration 11-KT and E2 were significantly different at different gonad phases where $p<0.05$ (Figure 5.5). Concentration of 11-KT and E2 was almost the same at the Trans phase before a sharp decrease of E2 at IP-Dvp occurred. Meanwhile, the concentration of 11-KT was gradually increased from Trans phase until IP-Rgs, then showed a sudden drop at IP-Rgn continuously with slight increased and decreased pattern until TP-Rgs. As same as E2, hormone concentration showed an inconsistent pattern which was slight up and down from IP-Spw until TP-Rgs. For the 11-KT pattern, it was observed that IP male has a slightly higher concentration than TP at different gonad maturation phases.

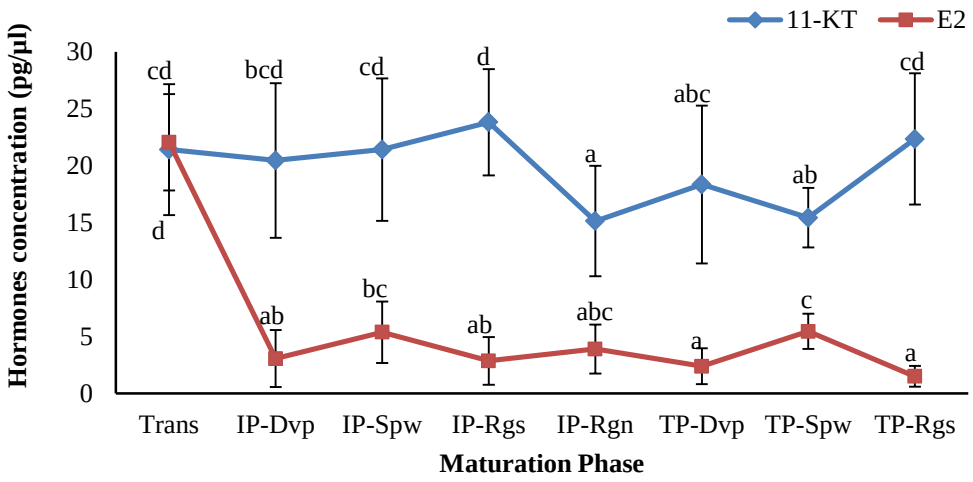

Figure 5.5 Changes of plasma level of reproductive hormone of three parrotfish species (*S. rivulatus*, *S. qouyi* and *S. ghobban*) at different gonad maturation phase at Pulau Bidong, Terengganu (2014). Abbreviations: 11-KT = 11-ketotestosterone; E2 = estradiol; Trans = transition; IP = initial phase; TP = terminal phase; Dvp = developing; Spw = spawning; Rgs = regressing and Rgn = regenerating. Data given as mean±SD.

5.3.4 Reproductive Index Pattern of Male Parrotfish Species (*S. rivulatus*, *S. qouyi* and *S. ghobban*) at Pulau Bidong

The trend of GSI and HSI was shown in figure 5.6 according to gonad phase and species then the graph was combined in order to have more clearly pattern. In *S. rivulatus* GSI and HSI varied from 0.02±0.01 to 0.34±0.29 and 2.99±0.06 to 4.86 ±0.31 respectively, with GSI showed a significant difference between the gonad phase ($p<0.05$) but not occurred for HSI. A rising trend in GSI was found, starting at a low value of Trans (0.02±0.01), to its highest point TP-Spw (0.34±0.29), and then declining until TP-Rgs (0.14±0.24). HSI trend was seen in relation to GSI.

The GSI trend for *S. qouyi* shows the same pattern as *S. rivulatus*, however, a slight difference was shown by HSI with two peaks present but not significantly different. GSI shows the primary peak at IP-Spw (0.35±0.25) same as HSI (4.57±2.52), while secondary peak of HSI was observed during IP-Rgn with 4.45±2.22. There was a slightly increase in GSI from Trans (0.01±0.009) to IP-Spw (0.35±0.25), followed by a sharp decline till reaching lowest value (0.18±0.11) during the IP-Rgn phase which was statistically significant. As well as GSI, HSI also presents the same pattern but is slightly different by having two times peak value with average minimum value range from 2.63±1.01 to 4.57±2.52 maximum values.

In *S. ghobban*, GSI had shown a steadily increased trend from Trans (0.03±0.02) to IP-Spw (0.52±1.09), reached maximum average value at IP-Spw, then slowly decreased until IP-Rgn (0.16±0.15) with significant difference between the gonad phases ($p<0.05$). For HSI, increased trend was shown from Trans (4.14±2.00) to IP-Spw (4.89±1.05), drastically decreased afterward until IP-Rgs (1.75±1.06) and increased again to TP-Spw (4.28±1.22) with no significance different was shown ($p>0.05$).

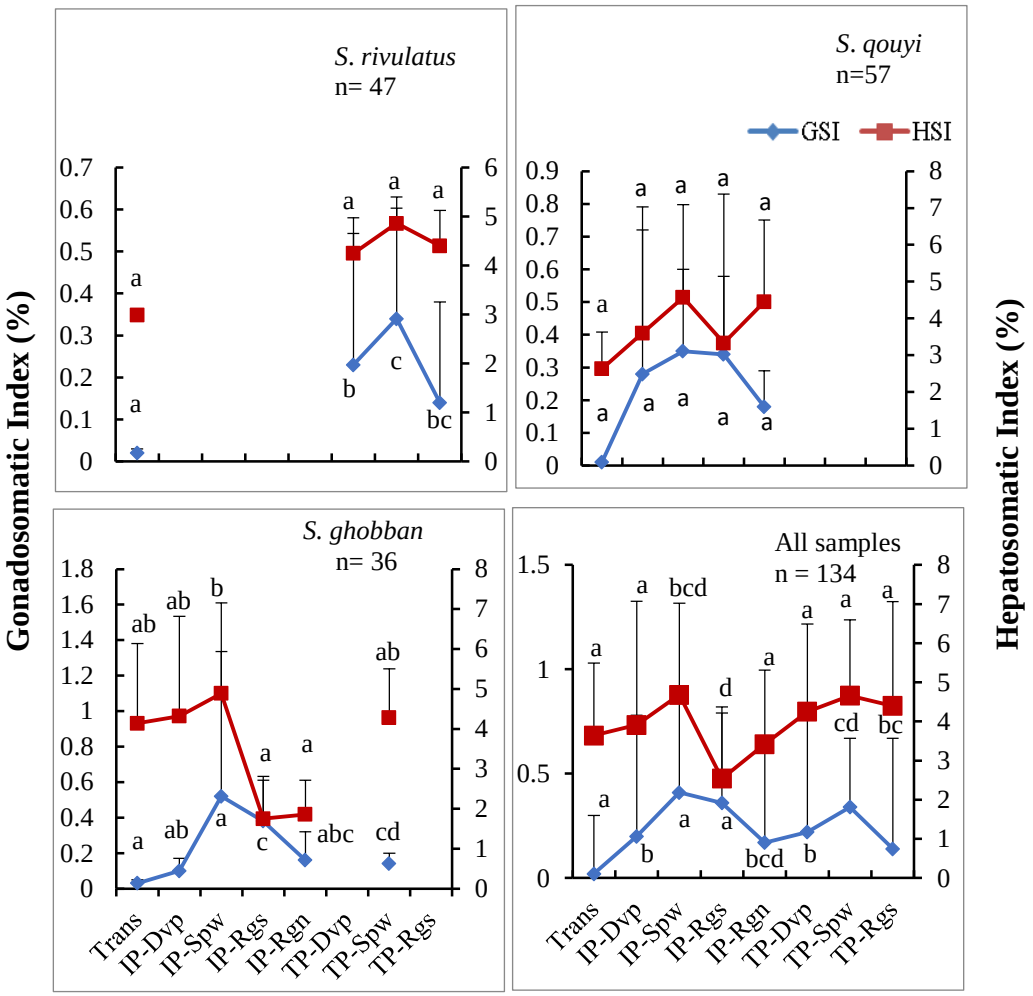

Figure 5.6: GSI and HSI trend of three male parrotfish species (*S. rivulatus*, *S. qouyi*, *S. ghobban*) from Pulau Bidong, Terengganu (2014) at different gonad maturation phases. (IP = initial phase of male; TP = terminal phase of male; Dvp = developing; Spw = spawning; Rgs = regressing; Rgn = regenerating). Note: absence of TP-Rgn; value are mean ± S.E; ($p<0.05$).

For the combination of species overall, both GSI and HSI were significantly different ($p<0.05$). Increased GSI begin at Trans (0.02±0.28) to IP-Spw (0.41±0.46), slowly decreased until IP-Rgn (0.17±0.48), again steadily increased to TP-Spw (0.34±0.33), and then decreased to TP-Rgs (0.14±0.53). The primary peak of GSI was at Ip-Spw (0.41±0.46) while the secondary peak was at TP-Spw (0.34±0.33). Meanwhile, the increased pattern was shown at the early phase of HSI from Trans (3.64±1.85) to IP-Spw (4.68±2.34), followed by a sharply decreased value at IP-Rgs (2.54±1.68). Again, the HSI value slowly increased until Tp-Spw (4.66±1.94) and then secondly decreased at TP-Rgs (4.40±2.66). Generally, although HSI of three

mean parrotfish species was not significant compared to GSI, however, some trends were still identified within the different gonad development phases.

5.4 Discussion

5.4.1 Classification of Gonad Phase

Referring to the first hypothesis in this chapter, results had shown five phases of male gonad maturation present in the genus *Scarus* (*S. rivulatus, S. qouyi, S. ghobban*) such as transition (Trans), developing (Dvp), spawning (Spw), regressing (Rgs) and regenerating (Rgn including both IP and TP. There was no different in microscopic criteria within the three *Scarus* genus. Most significantly, this result serves as the first compilation of microscopic characteristics of male gonad maturation phase within Scarus genus at South China Sea, making a complete distinction between IP and TP with description and characterization which unable to be found in previous studies (Abdel-Aziz et al., 2006; Barba, 2010; DeMartini & Howard, 2016; Ebisawa et al., 2016; El-Sayedah et al., 2012; Hamilton et al., 2008; Robertson et al., 1982). Nonetheless, the current study was unable to find a regenerating testis sample for TP male. Therefore, the TP regenerating phase was not included in the present study. One of the possible reasons, may be the size of TP regenerating phase larger which is outside the range of body size in the current study. A study of *Scarus ferrugineus* revealed that, the resting TP males present within the range size 20 – 47 (cm) SL (El-Sayedah et al., 2012). Also, DeMartini and Howards (2016) clearly demonstrate 50% sex change in the five parrotfish range was 22.6 - 47.3 (cm) FL where resting TP was observed. Thus, in this case, the evidence presented from the previous study could support the possible reason, as stated earlier. It was suggested that to complete the TP male gonad phase large fish samples are needed in further study.

As described in the result with respect to IP and TP, there is a slight difference in gonad criterion between IP and TP male which was the presence of yellow-brown body and Leydig cell more at TP than IP. This also has been seen in

parrotfish, *S. ferrugineus* (Abdel-Aziz et al., 2016). A clear difference in the number of Leydig cells might be attributed to the increase of testosterone and 11-ketotestosterone in the TP male after sex changes (El-Sayedah et al., 2012). This is because testosterone might play a significant role in maintaining secondary characteristics (body colour & spawning behaviour) in parrotfish (El-Sayedah et al., 2012; Godwin 2009) where 11-ketotestosterone was related to the tissue differentiation of male gonad in wrasse (Nakamura et al., 2003). This was supported by a review study on natural sex change, which has been clearly described through neuroendocrine control of hermaphrodite fish (Todd et al., 2016). Again, the previous study in rodents reveals that, the concentration of testosterone was very vital to maintain the masculinization of the male fetus yet a lack of testosterone in adult Leydig cells would significantly affect the steroidogenic pathway in their testis (Teerds & Huhtaniemi, 2015). Meanwhile, over past few decades yellow-brown body was pointed as a sign of sex changes in hermaphrodite fish, slinger seabream (Garratt, 1986) and a few Sparidae species (Lissia-Frau et al., 1977) meaning that fish considered as secondary male (from female to male). This criterion was in line with the current study, where some yellow-brown was observed through histology in TP male and a little in IP. In another case of protogynous grouper, Kline et al., 2011 identified that a yellow-brown body was found in the male. Being that, it could potentially indicate either the yellow-brown body present at TP male was a sign of sex change or a result of the metabolism process during tissue differentiation in the gonad (Sadovy and Shapiro, 1987). Referring to this acceptably evident, it could soon be assumed that, in the TP parrotfish, the presence of Leydig cells plays a critical role in the steroidogenesis function or hormone release. However, the explanation for the appearance of the yellow-brown body in TP male still remains unclear. Additionally, the origin of spermatogenic cells and testicular somatic cells in the female sex change fish is still not clearly known (Nakamura et al., 2015). Therefore further study on the correlation of yellow-brown bodies in sex change fish and the relationship between the numbers of Leydig cells present with steroidogenesis production in the reproduction of parrotfish is recommended.

Generally, there was some differentiation of spermatogenic cells occurs during the gonad maturation phase. At the developing phase, all germ cells start to

develop, where spermatogonia were the largest germ cell in the testes (Kahwa, 2013) same as observed in the current study (Figure 5.1B). Through ultrastructure examination, there was a presence of spherical nucleus, mitochondria and saturated chromatin in the spermatogonia (Kahwa, 2013). As fish enter the late developing phase, spermatogonia is transformed into primary spermatocytes through the replication of chromosomes and meiosis (Uribe, Grier, & Mejía-Roa, 2014). This transformation mainly cause less spermatogonia could be observed after developing phase in the present study. Additionally, the presence of spermatids at this stage is also known as a sign for the beginning of spermiogenesis (Kahwa, 2013). After the gonad enter the spawning phase at IP and TP male, dense of spermatozoa were observed in the current study cause by the expansion of seminiferous lobule in the ripe testis (El-Sayedah et al., 2012). It was also a result of spermiogenesis where spermatid was differentiating into spermatozoa (Kahwa, 2013). This characterization was also been observed in *S. ferrugineus* (Abdel-Aziz et al., 2006). When fish undergoes regressing and regenerating, the necrosis of sperm cell occurred. Followed with phagocytosis of all necrotic sperm cells including spermatogonia by Sertoli cells in the lobule lumen of the male testis (Kahwa, 2013). Briefly, all of these were the explanations in the present study for the different characterization cells present at each phase of male gonad development. Therefore, obviously, each phase of the gonad development can be identified by the microscopic characteristic present in the parrotfish testis. In addition, each phase also contributed to the different number of spermatogenic cells which will be explained in the following subtopic.

Based on the finding, the majority of the gonad were identified as IP (initial phase) and TP (terminal phase) primary males, with a smaller proportion being secondary males. This is because certain fish individuals had undergone a transformation from female to male, which often referred as secondary male (Devlin & Nagahama, 2002). In Western Carribean, from two parrotfish species *S. croicensis* and *S. vertula*, only 10% of diandric parrotfish was observed in their natural habitat as primary male. An almost similar result were obtained in *S. ghobban* at Okinawa Island (Ebisawa et al., 2016). This might be depending on the reproductive strategy of the species. According to Pandian (2013), in order to sustain higher reproductive success in hermaphrodite fish, the presence of different sexual changes pathways

such that active male or female likely primary or secondary or either IP or TP male hermaphrodite was very crucial. Also, these two pathways combined as one reproductive strategy to ensure less competition for a mating partner and genetic diversity sustainability.

There is still no literal explanation of the typically natural initiation of sex change in parrotfish. The nearest species was zebrafish due to the undifferentiated gonochorist (Wang et al., 2017). Previous study in Eurasian perch found that genetic control of the sex differentiation was induced by sex steroid balanced in the brain (Piferrer & Guiguen, 2008) during embryogenesis (Rougeot, Krim, Mandiki, Kestemont, & Melard, 2007). This has been confirmed by Sawyer et al., 2006 in which enzyme (Cyp11b) responsible for the synthesis of testosterone and 11-deoxycortisol (Goikoetxea, Todd, & Gemmell, 2017) was identified in the development of zebrafish embryos. In addition, Fujimoto et al., (2010) also clearly demonstrated the present of sex differentiation gene markers (i.e *foxl2*, *p450* arom, *dmrt1* & *vasa*) in germ cells during the embryonic stage of Pond loach, *Misgunus anguillicaudatus*. This indicates that the natural initiation of sex differentiation in parrotfish is likely to occur after egg fertilization, either being as primary or secondary male. Further study in identification of gene expression where differentiations of adult stage stat occur in diandric hermaphrodite fish would be very valuable.

Focussing on neuroendocrine regulation, there are two axes or pathways involved in sex change of secondary male. This has been illustrated by Liu et al., (2017). For the first pathway, in normal reproduction or stable social interaction sex change occurred after follicular atresia in the female ovary through hypothalamic-pituitary-gonadal (HPG) axis control by GnRH (Devlin & Nagahama, 2002). Second, the sex change was triggered by social cues when the loss or removal of the dominant male in the haremic system (Kline et al., 2011; Munday, White, & Warner, 2006) thus, activate the hypothalamic-pituitary-interrenal (HPI) axis pathway. This condition causes elevation of arginine vasotocin (AVT) which triggers the sudden increase of cortisol release by an interrenal gland in the kidney (Liu et al., 2017) indirectly disrupting the HPG axis. A study by Solomon-Lane et al., (2013)

demonstrated that prolonged (3 days) elevation of cortisol in a large dominant female when the dominant male was removed from the social group directly triggered the initiation of sex change. Similarly mode of prolonged (32 days) stress was observed during onset of sex change in grouper, *Epinephelus adscencionis* due to removal of male (Kline et al., 2011). Therefore, results from previous study suggests that alteration of social interaction such that loosening dominant male is one of the important key factors triggering the timing of sex changes in diandric hermaphrodite via the alternative pathway (HPI axis).

As a whole, returning to the hypothesis at the earlier introduction on histological examination of parrotfish strongly indicates that three parrotfish species studied of gonad maturation phase of *Scarus* genus had shown the same microscopic criteria at Pulau Bidong, South China Sea. Indeed, perhaps this present study could be very useful and as an informative reference on the maturation phase of the male gonad in the parrotfish *Scarus* genus in the future as the basic knowledge or guideline.

5.4.2 Spermatogenic Cells Count in Gonad Development Phase

There was a different number of spermatogenic cells present at each gonad phase. Result from the present study has revealed that spermatocytes and spermatozoa were the majority that appeared at all phases of gonad development, certainly illustrating they have a significantly important role during the gonad maturation in parrotfish. Besides, from the results, clearly, the peak number of spermatozoa was during the spawning phase at both IP and TP meaning that at this stage male parrotfish gonad was fully matured and might be ready to spawn. The previous study by Ebisawa et al., (1999) acknowledged the presence of spermatocyte and spermatid in Pacific yellowtail emperor, *Lethrinus atkinsoni* showed a positive correlation during spermatogenesis. The percentage of cell components in the testis also was applied as the criterion of testicular development in Pacific steephead parrotfish, *Chlorurus microrhinos* (Ebisawa et al., 2016).

However, in present study the average values of spermatocyte and spermatozoa were chosen as one of cell identification to validate the well testicular maturation. This choice was based on the statistically significant differences observed and the distinct appearance in terms of size characteristics (Marques & Ferreira, 2011). Relatively the spermatocyte was most abundantly present at the developing phase while spermatozoa were at the spawning phase in the present study. This result was similar as demonstrated by Ebisawa et al., (1999). On the other hand, the absence of spermatozoa at IP regenerating, transition and TP developing is probably related to phagocytosis of the cell after spawning phase (Kahwa., 2013). Therefore, it was indicated that spermatocyte and spermatozoa were strongly important key indicators as one of the quantitative data to distinguish the different criteria or characteristic of gonad development and maturity of male parrotfish. However, more study is needed to determine the correlation of this character when dealing with different types of hermaphrodite fish and species.

The least number of spermatogenic cells such as Pgo, Psc and Psc which could only be observed in transition phase because these three cells were previous ovarian cells from female, yet later known as TP secondary male after complete sex change occurred (Munday et al., 2006). Other than that, the different number of Leydig cells present could be described as one of the important indicators to differentiate between IP and TP during histological observation as mentioned in the above subtopic before. This characteristic was confirmed by quantitative data in this section where the percentage of Leydig cells two times greater in TP than IP and dominant of the yellow-brown body at TP was observed. The numerous Leydig cells also had been found in TP parrot fish *S. ferrugineus* (Abdel-Aziz et al., 2006). Thus, the finding of this study suggests that, high number of Leydig cells present could act as mainly different characteristics of the terminal phase for parrotfish. Further study is necessary to examine more closely towards the functional of Leydig cells present at TP male of parrotfish during cell differentiation in the gonad.

Interpretation of total cell count pattern from developing to regressing according to the separate male adult phase (IP & TP) of parrotfish notified that the

highest dominant percentage of cell was homogenous to each other. From this result, it was assumed that the same mechanism of testicular differentiation in parrotfish gonad occurs during the gonad maturation at both IP and TP. As this study was the first attempt in which all gonad maturation phases were almost completely found in male parrotfish reproduction with differentiation criteria of adult phase and spermatogenic number, therefore no comparable could be done with the previous study.

5.4.3 Male Reproductive Hormone in Gonad Development Phase

The changes of gonad maturation phase were not only in line with the changes of spermatogenic cells but also were parallel with the changes of reproductive hormone 11-KT and E2. Meaning that, maturation of gonad development phase was concomitant with the increasing number of spermatogenic cells, accompanied with the changes of 11-KT and E2. There was elevation of spermatozoa from developing phase to spawning and simultaneously steady increase of 11-KT and drop of E2 from transition phase to regressing. The results observed in this current study mirror those of the previous studies that have demonstrated the changes of sex steroid hormone of sex change fish red porgy, *Pagrus pagrus* (Kokokiris et al., 2006), seabass, *Lates calcarifer* (Guiguen et al., 199l) and sobaity, *Sparidentex hasta* (Lone et al., 2001) was correlated with the change of gonad structure. There is a strong possibility that 11-KT and E2 have an important function during the transition of gonad in parrotfish.

According to the previous studies 11-KT could trigger the proliferation of germinal tissue in male (Godwin 2009), had significant role in testicular differentiation (Nakamura et al., 2003) and important for development of secondary sexual characteristics such as change in colour pattern of TP male (Godwin et al., 1996; Semsar and Godwin, 2003) while E2 responsible to maintain the ovarian tissue (Nakamura et al., 2015). In present study, IP male has significantly high number of spermatozoa and spermatocyte and slightly higher concentration of 11-KT than TP possibly due increase demand or high requirement for sperm production to adapt

sperm competition when a small IP male spawning in large groups individuals compare to large TP which usually observed with pair spawning behaviour (Godwin, 2009). This evidence may assume that high concentration of 11-KT may reflect more progressive of cell proliferation occuring in the male testis.

Meanwhile, sudden change of sex steroid hormone 11-KT and E2 pattern was also has been discussed as a sign or key of initiation sex change in hermaphrodite fish (Nakamura et al., 2015; Nakamura et al., 2007; Kokokiris et al., 2006; Bhandari et al., 2003; Nakamura et al., 2003). This may explain the sudden drop and slight decrease of E2 and 11-KT respectively from transition phase to developing phase in male parrotfish in the present study. Nakamura et al., 2007 found that low level of E2 occurred at female and transitional male grouper and steady increase of 11-KT from early transition to male phase. Similarly in Red Porgy fish, where the level of 11-KT reached to the highest value at male phase and precipitous drop of E2 occurred at early transitional phase concomitant with the proliferation of male spermatogenic cell and degeneration of perinucleolar oocytes (Kokokiris et al., 2006). In the meanwhile, Nakamura et al., (2003) had revealed that, sex inversion of three-spotted wrasse and saddleback wrasse occurred when the level of E2 reduce till below their threshold value which at same time had induced masculinization of germ and somatic cells. Also, it has been suggested that, a drop of E2 extremely to lowest level was the optimum condition which mediating the development of male germ cells in sex change fish (Nakamura et al., 2015; Nakamura et al., 2003; Bhandari et al., 2003).

Briefly, based on the evidence from previous study, it shows that, the significant and sudden change of 11-KT and E2 during gonad maturation in this present study might indicates that their important role in the tissue differentiation during spermatogenesis and most likely could be a factor of sex change in parrotfish. Therefore, it is reasonably to highlight that, there was close relation of gonad maturation phase with the proliferation of spermatogenic cell and reproductive hormone (11-KT & E2) in parrotfish at Pulau Bidong, Terengganu as presented in the present study.

5.4.4 Change of Reproductive Index in Gonad Development Phases of Male Parrotfish at Pulau Bidong

The lateral increase of GSI and HSI shown by the current study was another parameter to reflect the reproductive strategy in parrotfish. A similar pattern of GSI and HSI was shown in dusky parrotfish, *Scarus niger* (Yanti et al., 2019) and European hake, *Merluccius merluccius* (Dominguez-Petit et al., 2009). An increase of GSI from developing to spawning phase in the present result was due to the increase in gonad mass during spermatogenesis (Yanti et al., 2019). The insignificant difference of HSI in the gonad development phase might be due to independent energy reserve from the liver but dependent on lipids contained in muscle as suggested by Dominguez-Petit et al., 2009. Moreover, this might be the reason for a weak relationship found in the regression analysis described in the previous chapter (Table 4.3) in line with Dominguez-Petit et al., 2009. The evidence from this study suggested that there was an alternative source of energy involved in parrotfish or food that was continuously taken during reproduction.

The high demand of energy during parrotfish reproduction may not fully be allocated for gonad development but also for spawning behaviour and territory defense. Primary male parrotfish are mostly observed in the group spawning where more competition of sperm occurs and much sperm is released to ensure the breeding is successful. Meanwhile, Van Rooij et al., 1995 observed that, territory TP male experienced multiple spawning within female at territory areas where much energy was allocated for territory defence and mate acquisition rather than gonad development. Possibly, due to this slight difference in spawning behaviour, it might be also one of the reasons why GSI of IP was significantly higher than TP at spawning phase. Therefore, this territory TP male characterized to have slow growth efficiencies, increase size at sex maturation, spawning at a larger size and potentially achieving great reproductive success (Van Rooij et al.,1995).

Thus in order to support high energy demand, other sources of energy are required to fulfill this requirement (surplus energy). The alternative way is by using current energy resources means that food intake is necessary for individuals during

the reproductive period (Dominguez-Petit et al., 2010). Further study on the biochemical compound (lipid, glycogen, protein) and energy storage in various tissues (gonad, muscle, liver) of parrotfish during the spawning phase are necessary to strengthen the existing evidence and knowledge.

5.5 Conclusion

The compilation of male gonad maturation phase of parrotfish in the present study was the first attempt at the South China Sea water region. The gonad maturation phases of Scarus genus demonstrated in the present study begin with the transition phase, followed by the developing → spawning → regressing → regenerating phase of IP and then continues with TP adult phase which rotates the same gonad phase like IP from developing until regenerating phase. Based on microscopic observation, three parrotfish species of the Scarus genus have the same criteria of gonad characteristics. From histological observation, the gonads of IP and TP male differ in terms of the number of Leydig cells and yellow-brown body. The highest number of spermatozoa was observed at the spawning phase at both IP and TP male. Meanwhile, significant reduction of E2 and acceleration of 11-KT were probably related to the sex change event and proliferation of spermatogenic cells in male parrotfish during gonad development. Other than that, GSI and HSI of parrotfish pattern showed positively significant to the progression of the gonad phase in parrotfish where the highest GSI and HSI were observed during spawning phase. Taken together, the results in the present study have literally shown that, changes in spermatocyte and spermatozoa cells count, 11-KT and E2 concentration and GSI and HSI pattern were strongly associated with the progression of gonad maturation phase of male parrotfish of *Scarus* genus at Pulau Bidong, Terengganu.

CHAPTER 6

FATTY ACID PROFILE OF PARROTFISH SPECIES (*S. rivulatus, S. qouyi* and *S. ghobban)* IN DIFFERENT GONAD MATURATION PHASES

6.1 Introduction

In fish reproduction, the transfer of the nutrients along gonad development is a well-known process. Fatty acids are the main lipid derived after food assimilation, and play key roles in sexual maturation which is highly found in marine fish liver (S. Li et al., 2018). During male gonad maturation, the formation of spermatogonia to spermatozoa involved the utilization of energy for several processes such as spermatocytogenesis, spermatogenesis and spermiogenesis (Kahwa, 2013). Previous study showed that the fatty acid composition was found to vary at gonad developmental stages with no definite trends (Gonzalez-Felix et al., 2019). This is because near to the spawning phase, fish may utilize more energy derived from the liver for spermatogenesis illustrated through the reproduction index (HSI) of Limbough's damselfish (Gonzalez-Felix et al., 2019). The different patterns of fatty acid composition also may relate to the contribution of SAFA and MUFA in body metabolism during gonad maturation in fish. Therefore, this illustrates that energy demand has correlated with body metabolism which could be shown through the fatty acid profile.

Parrotfish are herbivore fish that coexist and inhabit coral reef areas. Parrotfish are grazing fish important for coral survival and preventing a surge of macroalgae population. Parrotfish are a common community found at Pulau Bidong (Arai, 2014) might be due to several factors such as food availability, less competition of space with other organisms and less exploitation, seen Pulau Bidong was a research area prohibited for any fishing activity. However, the study of fatty acid towards parrotfish reproduction is still limited yet scarcely been understood (Arai et al., 2015c). Up to date, there is no study regarding the fatty acid in

maturation phase of parrotfish has been done at Pulau Bidong although these species are highly abundant at the Malaysian South China Sea (Arai, 2014). The available data found only regarding the fatty acid on parrotfish towards feeding ecology (Arai et al., 2015a & Arai et al., 2015c) and habitat use (Arai et al., 2015b). Thus, this study was conducted on fatty acid profile in the gonad maturation phase which is associated with parrotfish reproduction.

Besides, fatty acid was also illustrating the feeding ecology of coral reef fishes (Arai et al., 2015a). Previous study by Arai et al., (2015c) revealed that the feeding ecology and habitat used among three parrotfish species from genus Scarus was the same at Pulau Bidong. The food source in their diet for Scaridae suggested was similar but might be changed according to growth factors and migration. This indicates that size of the individual may affect the amount of food source intake in parrotfish at Pulau Bidong but not due to the fish diet. There is another external factor which has a relation to different fatty acid profiles in fish life history such as physiological condition and sexual development (Arai et al., 2015b). Thus, how far the difference in the gonad maturation phase of parrotfish might result in the variations or difference of fatty acid profile in Pulau Bidong is still unclear. Moreover, whether the three parrotfish species within the Scarus genus in this study have similar or different fatty acid profiles is also unknown. Therefore, the objective of this chapter was to determine the fatty acid profile of parrotfish at different species and maturation phase access through fatty acid concentration at Pulau Bidong, South China Sea. There were two hypotheses in this chapter; (1) the coexisting three parrotfish species at Pulau Bidong have a similar fatty acid profile and (2) the fatty acid profile in parrotfish is associated with gonad maturation phases.

6.2 Materials and Methods

6.2.1 Sample collection

All three parrotfish species *S. rivulatus, S. qouyi* and *S. ghobban* were collected at Pulau Bidong of South China Sea. There were 46 individuals from *S. rivulatus*, 52 from *S. qouyi* and 36 from *S. ghobban*. The fish sampling method was according to the step which has been described at Chapter 3 section 3.3 paragraphs 2-3. The detail on fish identification was explained at Chapter 3 Section 3.4 and the different adult phases (initial & terminal) were shown in chapter 3 at Figure 3.3.

6.2.2 Sample Preparation

The liver sample of the parrotfish was taken after the fish was dissected. Then the weighted liver used for the HSI parameter was kept in a -20 freezer. Before the fatty acid analysis was done, liver samples were freeze-dried for 24 hours. To reduce the variation of fatty acids profile, the fatty acids were sorted according to species then followed by maturation phases.

6.2.3 One Step Method of Fatty Acid Analysis

Extraction and esterification of parrotfish liver were done according to the one-step method procedure following Abdulkadir and Tsuchiya (2008). There were three replicate samples for each sample. For the first step, about 200-300 mg of each sample for each replicate was mixed with 4 ml hexane and 1 ml of internal standard solution in a glass tube. Then the 14% BF_3 (boron trifluoride) in methanol was added for 2 ml and also a magnetic stirring bar was placed in the test tube. After that, the headspace of the glass test tube was flushed with nitrogen gas and rapidly closed with Teflon-line screw-cap tightly. Next, the glass test tube was placed into a glass

beaker of boiling water and heated on the hot plate at about 100 ºC for 120 minutes under continuous stirring.

After 120 minutes, the samples were kept cold at room temperature. Then the samples were added with 1 ml of hexane and 2 ml of distilled water. Next, the tube was then shaken vigorously for 1 minute and centrifuged for 3 minutes at 2500 rpm to obtain fatty acid methyl ester (FAME). The two layers formed after the sample was centrifuged and the upper layer was a hexane layer containing FAME. This FAME layer was transferred by using a Pasteur pipette into a clean sample vial to be injected into the GCFID (Gas chromatography with flame ionization detection) for FAME analysis. All the FAMEs which had been extracted were stored in a -20ºC freezer temporarily until gas chromatography (GC) analysis.

Fatty acid concentrations (CFA, mg/g of dry sample) were calculated by comparing the peak area of fatty acid in the sample with the peak area of internal standard as follows:

$$C_{FA} = A_S/A_{IS} \times C_{IS}/W_S$$

A_S = peak area of fatty acid in the sample in chromatogram
A_{IS} = peak area of internal standard in chromatogram
C_{IS} = concentration of internal standard (mg)
W_S = weight of sample (g)

6.2.4 Statistical Analysis

SIMPER analysis gives the percentage of similarity and dissimilarity of factors, between levels of factors and for specific levels of the factors. Percentage of contribution (Contrib %) will explain the similarity or dissimilarity. The data of fatty acid profile were analysed using PRIMER-E version 6 with PERMANOVA. The difference of fatty acid concentrations among parrotfish of *Scarus* genus at Pulau Bidong was tested using the PERMANOVA based on the Bray-Curtis dissimilarity. In order to avoid large variations of pooled data, the fatty acid profile was

differentiated according to species, maturation phases and seasons. Therefore, in PERMANOVA analysis species, maturation phases and seasons were used as a factor in the analysis. The null hypothesis of ‘no difference factors' was tested and significant levels with $p < 0.05$ indicated significant differences among those three factors (species, maturation phases & seasons) of the parrotfish genus at Pulau Bidong ($p < 0.05$). Any significant data between fatty acid groups such as SAFAs, MUFAs and PUFAs were further tested using PERMANOVA pair-wise test. The SIMPER was used to determine which components contribute to the dissimilarities and similarities in each factor (Clarke et al., 2014). The concentration of fatty acids was normalized using square root transformation.

6.3 Results

6.3.1 Fatty Acid Composition of Parrotfish Species

Overall, there were 27 fatty acids were identified in three parrotfish species *S. rivulatus*, *S. qouyi* and *S. ghobban* (Table 6.1). These compounds were classified into saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). In general, results of PERMANOVA had shown there was higher percentage similarity between or within the group of all fatty acid class (Table 6.6) of three parrotfish species at Pulau Bidong. Meanwhile, average dissimilarity between group species comparison for all fatty acid class was lower 27.63 % - 38.93 % (Table 6.5). Meaning that, fatty acid profiles between three parrotfish species had higher similarities than dissimilarities. However, PERMANOVA analysis revealed that the overall fatty acid composition of different fatty acid classes (SAFAs, MUFAs and PUFAs) were significantly different between three parrotfish species (Table 6.2) was shown at MUFAs ($p<0.05$). The pairwise test shows a comparison between groups (*S. rivulatus* vs *S. qouyi*, *S. rivulatus* vs *S. ghobban* and *S. qouyi* vs *S. ghobban*) where only MUFAs has significantly different ($p<0.01$) (Table 6.3). This was in line with SIMPER analysis of Bray Curtis dissimilarity within fatty acid class where MUFAs contribute to the highest dissimilarity percentage compared with SAFAs and PUFAs (Table 6.5).

Generally, the highest mean concentration of fatty acid in SAFAs (Figure 6.1) was at *S. ghobban* (11.603 ± 1.799 mg/g) followed by *S. qouyi* (5.486 ± 1.032 mg/g) and *S. rivulatus* (5.457 ± 0.896 mg/g). From PERMANOVA analysis fatty acid SAFAs showed no significant difference between the three parrotfish species with $p>0.05$ (Table 6.2). Also, through pair wise comparison SAFAs showed no significant different (Table 6.3). Besides, from SIMPER analysis SAFAs had contributed to lower dissimilarity about 24 % - 35 % (Table 6.5). There were five dominant of fatty acid in SAFAs (Figure 6.2) in three parrotfish species which the highest was C16:0 (palmitic acid), followed by C14:0 (myristic acid) and C18:0 (stearic acid). Fatty acid C16:0, C14:0 and C18:0 were highest at *S. ghobban* compared to *S. rivulatus* and *S. qouyi*. However, all these highest fatty acid (C16:0, C14:0 & C18:0) were not significantly different between group comparison (*S. rivulatus* vs *S. qouyi*, *S. rivulatus* vs *S. ghobban* and *S. qouyi* vs *S. ghobban*) range from 0.559 ± 0.255 mg/g to 6.053 ± 7.692 mg/g as shown through PERMANOVA analysis ($p>0.05$) (Table 6.3). This was associated with the lower average dissimilarity percentage of this three fatty acid based on the SIMPER analysis in the parrotfish species (Table 6.4). Meaning that, there was a higher similarity to the change of SAFAs at three parrotfish species at Pulau Bidong.

However, MUFAs showed significant different between parrotfish species (PERMANOVA, $p<0.05$) (Table 6.2). Results had showed that mean of MUFAs fatty acids (Table 6.1) at *S. ghobban* was highest (4.806 ± 0.746 mg/g) than *S. rivulatus* (2.128 ± 0.412 mg/g) and *S. qouyi* (2.443 ± 0.778 mg/g). The dominant fatty acid in MUFAs were C14:1 (myristoleic acid), C16:1 (palmitoleic acid), C18:1n9t (elaidic acid), C18:1n9c (oleic acid) and C20:1n9 (eicosenoic acid) was observed in three parrotfish species (Figure 6.2). Fatty acid C14:1 was significantly highest at *S. ghobban* (0.187 ± 0.237 mg/g) with *S. qouyi* (0.030 ± 0.024 mg/g), while C16:1 was highest in *S. qouyi* (1.980 ± 1.109 mg/g) compared to *S. rivulatus* (1.178 ± 2.604 mg/g) (Figure 6.2). In another hand, both fatty acids C18:1n9t and C18:1n9c was highest at *S. ghobban* compared to *S. rivulatus* and *S. qouyi*. From SIMPER analysis, comparisons between group species (*S. rivulatus* vs *S. qouyi*, *S. rivulatus* vs *S. ghobban*, *S.qouyi* vs *S. ghobban*) showed fatty acids MUFAs was

most contributes to highest the dissimilarity percentage about 36 % - 60 % within three class of fatty acid (Table 6.5).

Table 6.1 Fatty acid compositions (mg g^{-1} dry weight) three parrotfish species (*S. rivulatus*, *S. qouyi* and *S. ghobban)* collected in Pulau Bidong, Terengganu. Values are in means ± SD; n.d: not detected. Significant level denoted as *($p<0,05$) and ns (not significant).

Fatty acids	***S. rivulatus***	***S. qouyi***	***S. ghobban***
N	14	17	12
C6:0	0.057 ± 0.050	0.046 ± 0.034	0.040 ± 0.028
C10:0	0.063 ± 0.044	n.d	n.d
C12:0	0.048 ± 0.018	0.014 ± 0.012	0.015 ± 0.007
C13:0	n.d	n.d	0.250 ± 0.354
C14:0	1.443 ± 1.323	1.293 ± 0.962	1.681 ± 1.276
C15:0	0.202 ± 0.147	0.138 ± 0.114	0.462 ± 0.473
C16:0	2.761 ± 1.206	3.251 ± 0.273	6.053 ± 7.692
C17:0	0.114 ± 0.082	0.095 ± 0.096	0.603 ± 0.672
C18:0	0.652 ± 0.253	0.559 ± 0.255	2.107 ± 3.025
C20:0	n.d	0.017 ± 0.012	0.055 ± 0.067
C21:0	0.049 ± 0.023	0.034 ± 0.044	0.189 ± 0.322
C22:0	0.067 ± 0.048	0.038 ± 0.043	0.149 ± 0.209
Σ SAFAs^{ns}	**5.457 ± 0.896**	**5.486 ± 1.032**	**11.603 ± 1.799**
C14:1	0.041 ± 0.023	0.030 ± 0.024	0.187 ± 0.237
C15:1	0.020 ± 0.009	0.015 ± 0.004	0.099 ± 0.121
C16:1	1.178 ± 1.241	1.980 ± 1.109	1.785 ± 2.604
C17:1	0.117 ± 0.115	0.044 ± 0.034	0.184 ± 0.187
C18:1n9t	0.428 ± 0.594	0.012 ± 0.008	0.531 ± 1.334
C18:1n9c	0.274 ± 0.153	0.304 ± 0.140	1.736 ± 3.187
C20:1n9	0.071 ± 0.049	0.070 ± 0.067	0.285 ± 0.369
Σ MUFAs*	**2.128 ± 0.412**	**2.443 ± 0.778**	**4.806 ± 0.746**
C18:2	n.d	n.d	0.030 ± 0.036
C18:2n6c	0.091 ± 0.099	0.094 ± 0.122	0.630 ± 1.044
C18:3n6	0.037 ± 0.045	0.044 ± 0.122	0.221 ± 0.375
C20:2	0.053 ± 0.032	0.113 ± 0.150	0.199 ± 0.287
C20:3n6	0.343 ± 0.309	0.265 ± 0.187	0.332 ± 0.585
C20:3n3	0.728 ± 0.904	0.407 ± 0.474	1.073 ± 1.251
C22:2	0.068 ±0.003	0.049 ± 0.035	0.203 ± 0.144
C20:5(n3)	0.306 ± 0.213	0.128 ± 0.169	0.960 ± 1.053
Σ PUFAs^{ns}	**1.625 ± 0.317**	**1.102 ± 0.132**	**3.618 ± 0.374**

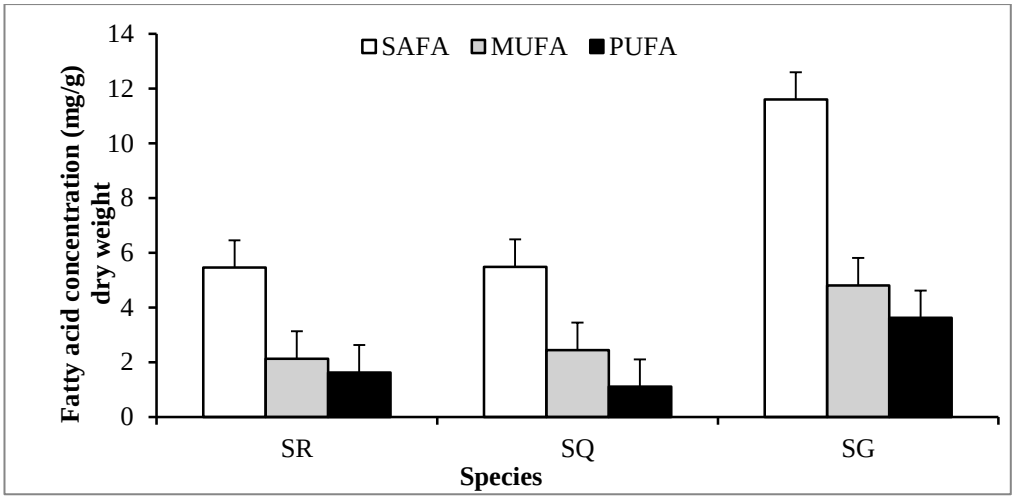

Figure 6.1 Fatty acid composition of total saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of three parrotfish of *Scarus* genus, SR = *S. rivulatus*, SQ = *S. qouyi* and SG = *S. ghobban* collected at Pulau Bidong, Terengganu. (mean±SD).

As same as SAFAs, PERMANOVA analysis revealed that, fatty acid PUFAs do not differ significantly between the three parrotfish species at Pulau Bidong ($p>0.05$) (Table 6.2). The mean concentration of PUFAs had shown the highest at *S. ghobban* (3.618 ± 0.374 mg/g) compared to *S. rivulatus* (1.625 ± 0.317 mg/g) and *S. qouyi* (1.102 ± 0.132 mg/g) (Table 6.1). In PUFAs five dominant fatty acids in three parrotfish species were C18:2n6c (linoleic acid), C18:3n6 (γ-linoleic acid), two types of eicosatrienoic acid (C20:3n6 & C20:3n3) and C20:5(n3) (eicosapentaenoic acid) (Figure 6.2). Fatty acid C20:3n3 and C20:5(n3) were highest in *S. ghobban* compared to *S. rivulatus* and *S. qouyi* (Figure 6.2). However, fatty acid C20:3n6 was highest at *S. rivulatus* (0.343 ± 0.309 mg/g) compared to *S. qouyi* (0.265 ± 0.187 mg/g) and *S. ghobban* 0.332 ± 0.585 mg/g) not differ significantly ($p>0.05$). Among all fatty acid classes, SIMPER analysis shows that, C20:3n3 and C20:5(n3) PUFAs were identified and contributed to the most dissimilarity between parrotfish species at Pulau Bidong with 18% until 26% (Table 6.4).

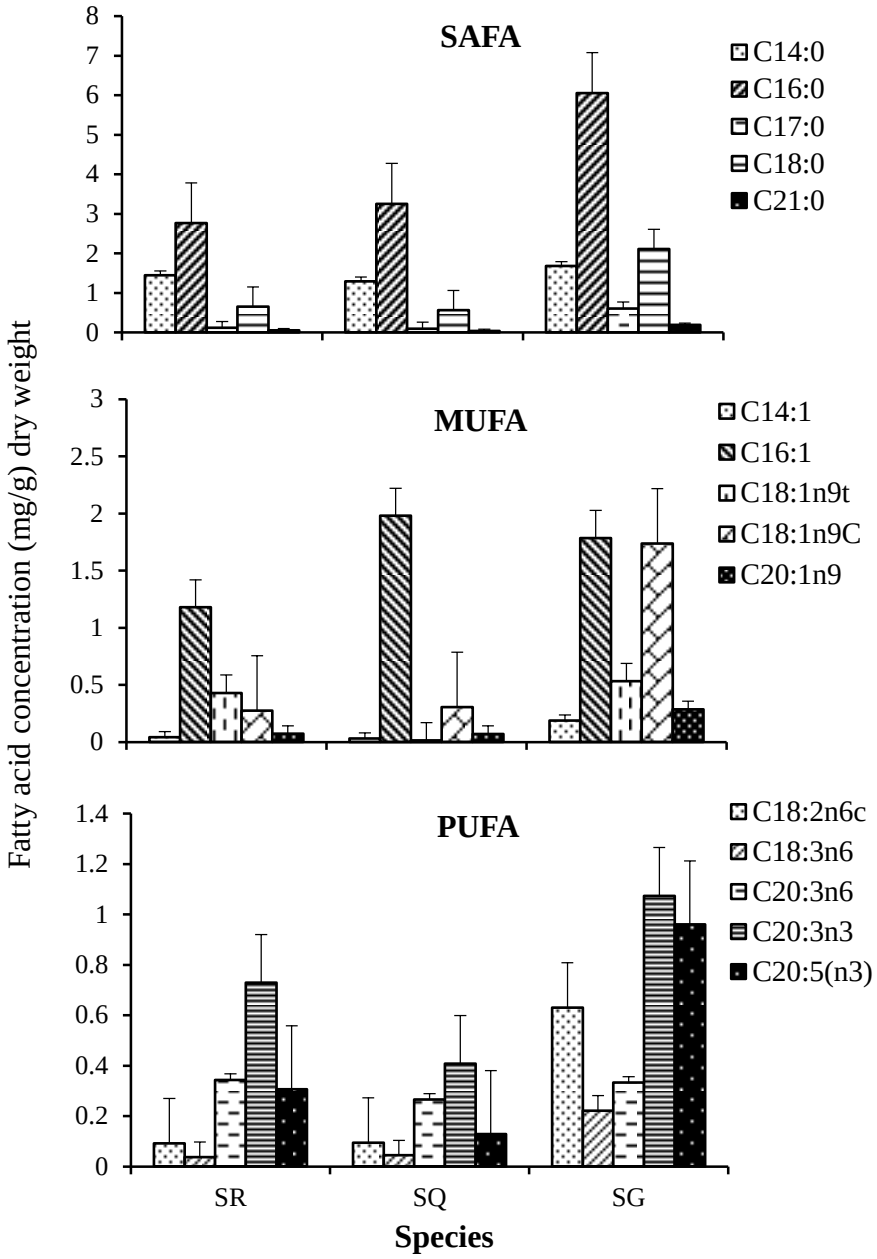

Figure 6.2 Concentrations of fatty acids group (mg/g) dry weight of three parrotfish species of *Scarus* genus SR = *S. rivulatus*, SQ = *S. qouyi* and SG = *S. ghobban* collected at Pulau Bidong. (mean±SD).

Table 6.2 Result of PERMANOVA on fatty acid of different parrotfish species. Significant level denoted as * (p<0.05), ** (p<0.01), *** (0.001).

Species	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Group	2	3348.3	1674.2	7.9152	0.001	999
Res	40	8460.6	211.51			
Total	42	11809				
SAFA	2	2644.5	1322.3	1.1569	0.318	999
Res	40	45717	1142.9			
Total	42	48361				
MUFA	2	5844.9	2922.4	2.8929	0.008	999
Res	40	40408	1010.2			
Total	42	46253				
PUFA	2	7056.6	3528.3	1.677	0.136	999
Res	35	73639	2104			
Total	37	80695				

Table 6.3 Result of pairwise test from PERMANOVA on fatty acid of different parrotfish species. Significant level denoted as * (p<0.05), ** (p<0.01), *** (0.001).

Group	t	P(perm)	Unique perms
S.rivulatus, S. qouyi	2.5676	0.002	999
S.rivulatus, S. ghobban	3.4111	0.002	998
S. qouyi, S. ghobban	2.2164	0.004	998
SAFA	**t**	**P(perm)**	**Unique perms**
S.rivulatus, S. qouyi	0.60505	0.585	999
S.rivulatus, S. ghobban	1.0591	0.293	999
S. qouyi, S. ghobban	1.4088	0.165	999
MUFA	**t**	**P(perm)**	**Unique perms**
S.rivulatus, S. qouyi	1.6726	0.045	998
S.rivulatus, S. ghobban	1.3664	0.093	998
S. qouyi, S. ghobban	2.0897	0.011	999
PUFA	**t**	**P(perm)**	**Unique perms**
S.rivulatus, S. qouyi	0.89144	0.465	995
S.rivulatus, S. ghobban	1.4888	0.085	998
S. qouyi, S. ghobban	1.453	0.115	998

Table 6.4 Summary Results of SIMPER analysis (Bray Curtis dissimilarity) on the average dissimilarity contribution between fatty acids components within each class.

Group	**Class**	**Fatty acid**	**Average dissimilarity %**
S. rivulatus, S. qouyi	SAFA	C16:0	16.95
		C14:0	10.3
		C18:0	5.4
		C17:0	3.82
	MUFA	C16:1	21.45
		C18:1n9c	9.1
		C20:1n9	3.77
		C14:1	3.24
	PUFA	C20:3n3	25.49
		C18:2n6c	21.82
		C20:5n3	12.36
		C18:3n6	6.77
S. rivulatus, S.ghobban	SAFA	C16:0	16.63
		C14:0	10.56
		C17:0	6.39
		C18:0	4.61
	MUFA	C16:1	15.92
		C18:1n9c	11.84
		C20:1n9	7.13
		C18:1n9t	6.91
		C14:1	6.57
	PUFA	C20:3n3	23.6
		C20:5n3	18.82
		C18:2n6c	15.43
		C18:3n6	6.59
S. qouyi, S. ghobban	SAFA	C16:0	16.91
		C14:0	9.4
		C17:0	7.63
		C18:0	5.42
	MUFA	C16:1	15.7
		C18:1n9c	10.84
		C20:1n9	6.5
		C14:1	5.89
		C18:1n9t	5.1
	PUFA	C20:3n3	22.05
		C20:5n3	18.04
		C18:2n6c	14.58
		C18:3n6	6.72

Table 6.5 Result of SIMPER analysis (Bray Curtis dissimilarity) on fatty acid between group comparison of different parrotfish species.

Group	Average Dissimilarity	Factor	Dissimilarity Contribution %	Similarity contribution %
S. rivulatus, S. qouyi	29.54	MUFA	59.05	40.95
		SAFA	24.29	75.71
		PUFA	16.67	83.33
S. rivulatus, S. ghobban	38.93	MUFA	51.68	48.32
		PUFA	24.39	75.61
		SAFA	23.93	76.07
S. qouyi, *S. ghobban*	27.63	MUFA	35.78	64.22
		SAFA	34.24	65.76
		PUFA	29.98	70.02

Table 6.6 Result of PERMANOVA on average similarity between/within group of all fatty acid with three parrotfish species.

Average Similarity between/within groups %			
	S. rivulatus	*S. qouyi*	*S. ghobban*
S. rivulatus	81.596		
S. qouyi	79.393	86.226	
S. ghobban	72.67	81.071	79.899

6.3.2 Fatty Acid Composition During Gonad Maturation Phases

A total of 27 fatty acids were identified at the gonad maturation phases of three parrotfish species (*S. rivulatus, S. qouyi* & *S.ghobban*) of *Scarus* genus at Pulau Bidong. These fatty acids were classified into SAFAs, MUFAs and PUFAs according to five maturation phases which were transition (Trans), developing (Dvp), spawning (Spw), regressing (Rgs) and regenerating (Rgn) phase. The fatty acid classification was also determined according to two adult phases of parrotfish of *Scarus* genus which were the initial phase (IP) and terminal phase (TP). Results of PERMANOVA analysis showed that the mean concentrations of total fatty acid did not differ significantly between the maturation phases ($p>0.05$) (Table 6.8). Meaning that, the changes in concentration of SAFAs, MUFAs and PUFAs during the gonad maturation phase were not significant in three parrotfish species of *Scarus* genus in the present study. This was associated with the highest average similarity between or

within a group of all fatty acids with maturation phase through PERMANOVA which was about 69 % - 89 % (Table 6.9). According to the fatty acid class, PUFA contributes to the highest average dissimilarities about 30 % - 54 % after MUFA and SAFA (Table 6.11). Meanwhile, between the pair wise comparison through SIMPER analysis, some group between IP-Spw vs IP-Rgs, IP-Spw vs TP-Dvp, IP-Rgs vs TP-Dvp, IP-Rgs vs TP-Spw and IP-Rgn vs TP-Dvp showed significantly differed (Table 6.10) with lower average dissimilarity 18 % - 27 % (Table 6.11). This result revealed that, the changes of fatty acid profile during the gonad maturation phase in parrotfish had higher similarities percentage than dissimilarity.

In SAFAs, fatty acid was highest at the Trans phase (19.08 ± 0.080 mg/g), followed by IP-Rgn (6.527 ± 0.473 mg/g), IP-Rgs (6.256 ± 1.108 mg/g) and TP-Rgs (5.577 ± 1.129 mg/g) (Figure 6.3). Fatty acid SAFAs were dominantly by C14:0 (myristic acid), C16:0 (palmitic acid), C18:0 (stearic acid) and C20:0 (arachidic acid) (Figure 6.4). The mean concentration of C14:0 was highest at the transition phase (2.072 ± 1.442 mg/g) and slightly higher at TP-Dvp (2.186 ± 1.427 mg/g) and IP-Rgs (1.889 ± 0.987 mg/g) compared to other maturation phases with no significant difference ($p>0.05$) between phases. Fatty acid C16:0 was highest at the transition phase (5.592 ± 3.957 mg/g), slightly higher at IP-Spw, IP-Rgs, IP-Rgn, TP-Spw and TP-Rgs (range 3.182 ± 0.370 mg/g to 3.333 ± 0.001 mg/g) and lowest at IP-Dvp (0.333 ± 1.721 mg/g) and TP-Dvp (1.428 mg/g) (Figure 6.4). Meanwhile, fatty acid C18:0 was highest at the transition phase but lower (range 0.158 ± 0.112 mg/g to 0.875 ± 0.430 mg/g) at other maturation phases in *Scarus* genus at Pulau Bidong. Based on the PERMANOVA, the overall change of fatty acid profile in SAFAs was not significant (Table 6.8) but three pair wise group comparison between IP-Spw vs IP-Rgs, IP-Spw vs TP-Dvp and IP-Rgs vs TP-Spw showed $p<0.05$ (Table 6.10) which contributed by C16:0 and C14:0 (Table 6.12). However, SIMPER analysis showed SAFAs contributes to lowest percentage dissimilarity among two other fatty acid class (Table 6.11).

Fatty acid MUFAs showed the highest concentration at Trans (7.306 ± 1.351 mg/g) and slightly higher at IP-Rgs (3.711 ± 0.729 mg/g) and TP-Rgs (2.158 ± 0.622 mg/g) compared to other maturation phases (Figure 6.3). The dominant fatty acid

observed was C16:1 (palmitoleic acid), C17:1 (heptadecanoic acid), C18:1n9c (oleic acid), C18:1n9t (elaidic acid) and C20:1n9 (eicosenoic acid) (Figure 6.4). Fatty acid C16:1 and C18:1n9c were highest at Trans phase (2.741 ± 3.535 mg/g and 3.273 ± 4.898 mg/g respectively), while C18:1n9t was highest at IP-Rgs (1.121 ± 1.916 mg/g). In general, changes of fatty acid MUFAs between the gonad maturation phases did not differ significantly (Table 6.8). From pairwise comparison, there were some significant differences in MUFAs between IP-Spw vs IP-Rgs and IP-Spw vs TP-Dvp (Table 6.10). However, the average percentage dissimilarity between this pairwise test was low about 18% - 19 % (Table 6.11).

The mean concentration of the PUFAs showed no significantly differ (PERMANOVA, $p>0.05$) between all gonad maturation phases of the parrotfish *Scarus* genus (Table 6.8). PUFAs were highest at Trans (4.304 ± 3.650 mg/g), followed by slightly higher at IP-Rgs (2.164 ± 0.229 mg/g) and TP-Rgs (2.14 ± 0.363 mg/g) compared to IP-Dvp, IP-Spw, IP-Rgn, TP-Dvp and TP-Spw (range 0.147 ± 0.025 mg/g to 1.982 ± 0.371 mg/g) (Figure 6.3). The dominant fatty acid PUFAs at gonad maturation phase were C18:2n6c (linoleic acid), C20:2 (eicosadienoic), two types of eicosatrienoic acid (C20:3n6 & C20:3n3) and C20:5(n3) (eicosapentaenoic acid) (Figure 6.4). Fatty acid C18:2n6c and C20:5(n3) were highest at the transition phase with 1.040 ± 1.656 mg/g and 1.640 ± 1.514 mg/g respectively, while C20:3n6 was highest at IP-Rgs (0.737 ± 0.667 mg/g) and TP-Rgs (0.561 ± 0.397 mg/g). The concentration of C20:3n3 was observed slightly higher at Trans (1.134 ± 1.689 mg/g), TP-Rgs (1.014 ± 1.282 mg/g), IP-Rgs (0.620 ± 0.512 mg/g) and IP-Rgn (0.512 ± 0.40 mg/g) (Figure 6.4). Although there was some different in fatty acid between the gonad phase at PUFAs of parrotfish of *Scarus* genus, but in overall these concentration differences contribute to low average dissimilarity (Table 6.11). PUFAs SIMPER analysis showed there were some significant different between IP-Spw vs IP-Dvp and IP-Rgs vs TP-Dvp (Table 6.12) which mostly percentage dissimilarity contributed by C20:3n3, C20:5(n3) and C18:2n6c (Table 6.12).

Table 6.7 Fatty acid compositions (mg g^{-1} dry wt.) of eight different maturation phases of parrot fish species (*S. rivulatus*, *S. qouyi* and *S. ghobban)* collected in Pulau Bidong, Terengganu. Values are in means ± SD; n.d: not detected . Significant level denoted as *(p<0,05) and ns (no significant).

FA	Trans	IP-Dvp	IP-Spw	IP-Rgs
N	6	6	6	9
C6:0	0.061 ± 0.065	0.038 ± 0.025	0.039 ± 0.035	0.056 ± 0.039
C10:0	0.009 ± 0.006	n.d	n.d	n.d
C12:0	0.008 ± 0.003	0.012 ± 0.009	0.015 ± 0.005	0.023 ± 0.016
C13:0	0.501 ± 0.354	n.d	n.d	n.d
C14:0	2.072 ± 1.442	1.045 ± 0.658	0.829 ± 0.714	1.889 ± 0.987
C15:0	0.592 ± 0.613	0.099 ± 0.024	0.133 ± 0.068	0.220 ± 0.144
C16:0	8.772 ± 10.880	0.333 ± 1.721	3.182 ± 0.370	3.333 ± 1.667
C17:0	0.747 ± 0.792	0.060 ± 0.025	0.112 ± 0.096	0.169 ± 0.120
C18:0	5.592 ± 3.954	0.495 ± 0.290	0.314 ± 0.222	0.435 ± 0.048
C20:0	0.073 ± 0.085	0.012 ± 0.0003	n.d	0.010 ± 0.007
C21:0	0.417 ± 0.482	0.016 ±0.011	0.037 ± 0.038	0.068 ± 0.050
C22:0	0.242 ± 0.326	0.014 ± 0.001	0.057 ± 0.054	0.053 ± 0.042
Σ SAFAs^{ns}	**19. 080 ± 3.125**	**5.121 ± 1.046**	**4.718 ± 1.029**	**6.256 ± 1.108**
C14:1	0.253 ± 0.336	0.024 ± 0.005	0.043 ± 0.029	0.044 ± 0.030
C15:1	0.140 ± 0.183	0.015 ± 0.001	0.037 ± 0.023	0.015 ± 0.005
C16:1	2.741 ± 3.535	2.073 ± 1.027	1.673 ± 0.881	1.926 ± 1.415
C17:1	0.275 ± 0.247	0.035 ± 0.020	0.054 ± 0.040	0.082 ± 0.069
C18:1n9t	0.278 ± 0.264	n.d	0.014 ± 0.003	1.121 ± 1.916
C18:1n9c	3.273 ± 4.898	0.252 ± 0.122	0.226 ± 0.108	0.405 ± 0.075
C20:1n9	0.345 ± 0.484	0.032 ± 0.019	0.136 ± 0.097	0.119 ± 0.065
Σ MUFAs^{ns}	**7.306 ± 1.351**	**2.432 ± 0.822**	**2.183 ± 0.337**	**3.711 ± 0.729**
C18:2n6c	1.040 ± 1.656	0.049 ± 0.034	0.209 ± 0.366	0.194 ± 0.177
C18:3n6	0.337 ± 0.538	0.023 ± 0.012	0.047 ± 0.043	0.084 ± 0.049
C18:2	0.046 ± 0.050	n.d	0.012 ± 0.003	0.013 ± 0.009
C20:2	0.020 ± 0.014	0.017 ± 0.012	0.044 ± 0.041	0.214 ± 0.219
C20:3n6	0.087 ± 0.062	Nd	0.012 ± 0.008	0.737 ± 0.667
C20:3n3	1.134 ± 1.689	0.326 ± 0.364	0.294 ± 0.385	0.620 ± 0.512
C22:2	nd	Nd	nd	0.126 ± 0.109
C20:5(n3)	1.640 ± 1.514	0.072 ± 0.054	1.022 ± 0.723	0.176 ± 0.220
Σ PUFAs^{ns}	**4.304 ± 0.650**	**0.487 ± 0.129**	**1.640 ± 0.276**	**2.164 ± 0.**

Table 6.7 continue

FA	IP-Rgn	TP-Dvp	TP-Spw	TP-Rgs
n	3	4	3	6
C6:0	0.036 ± 0.022	0.108 ± 0.072	0.023 ± 0.011	0.039 ± 0.015
C10:0	0.063 ± 0.441	n.d	n.d	n.d
C12:0	0.046 ± 0.033	0.050 ± 0.025	0.009 ± 0.006	Nd
C13:0	n.d	n.d	n.d	n.d
C14:0	1.477 ± 1.609	2.186 ± 1.427	0.456 ± 0.245[a]	1.098 ± 1.218
C15:0	0.296 ± 0.158	0.250 ± 0.167	0.161 ± 0.106	0.179 ± 0.129
C16:0	3.32 ± 0.008	1.428 ± 1.654	3.332 ± 0.002	3.333 ± 0.001
C17:0	0.251 ± 0.146	0.079 ± 0.036	0.173 ± 0.156	0.152 ± 0.101
C18:0	0.875 ± 0.430	n.d	0.158 ± 0.112	0.652 ± 0.253
C20:0	0.026 ± 0.013	n.d	0.017 ± 0.012	n.d
C21:0	0.039 ± 0.029	n.d	0.030 ± 0.026	0.050 ± 0.033
C22:0	0.085 ± 0.050	n.d	0.045 ± 0.014	0.074 ± 0.066
Σ SAFAs[ns]	**6.527 ± 0.473**	**4.101 ± 0.905**	**4.403 ± 1.025**	**5. 577 ± 1.129**
C14:1	0.046 ± 0.034	0.080 ± 0.057	0.104 ± 0.109	0.040 ± 0.018
C15:1	0.039 ± 0.027	n.d	0.052 ± 0.060	0.025 ± 0.001
C16:1	0.525 ± 0.384	0.996 ± 1.570	0.654 ± 0.211	1.605 ± 1.377
C17:1	0.148 ± 0.193	0.150 ± 0.088	0.055 ±0.030	0.073 ± 0.063
C18:1n9t	0.460 ± 0.549	n.d	0.018 ± 0.014	n.d
C18:1n9c	0.733 ± 0.446	0.119 ± 0.022	0.532 ± 0.450	0.349 ± 0.114
C20:1n9	0.047 ± 0.036	n.d	0.076 ± 0.086	0.066 ± 0.043
Σ MUFAs[ns]	**1.998 ± 0.220**	**1.345 ± 0.441**	**1.491 ± 0.063**	**2.158 ± 0.622**
C18:2n6c	0.108 ± 0.067	0.056 ± 0.055	0.091 ± 0.083	0.121 ± 0.144
C18:3n6	0.055 ± 0.039	n.d	0.059 ± 0.042	0.038 ± 0.022
C18:2	nd	n.d	n.d	n.d
C20:2	0.416 ± 0.297	n.d	0.068 ± 0.048	0.053 ± 0.032
C20:3n6	0.072 ± 0.074	0.0811 ± 0.01	nd	0.561 ± 0.397
C20:3n3	0.512 ± 0.400	n.d	0.249 ± 0.321	1.014 ± 1.282
C22:2	0.066 ± 0.047	n.d	nd	0.070 ± 0.050
C20:5(n3)	0.754 ± 1.061	n.d	0.278 ± 0.369	0.282 ± 0.223
Σ PUFAs[ns]	**1.982 ± 0.371**	**0.147 ± 0.025**	**0.745 ± 0.106**	**2.140 ± 0.363**

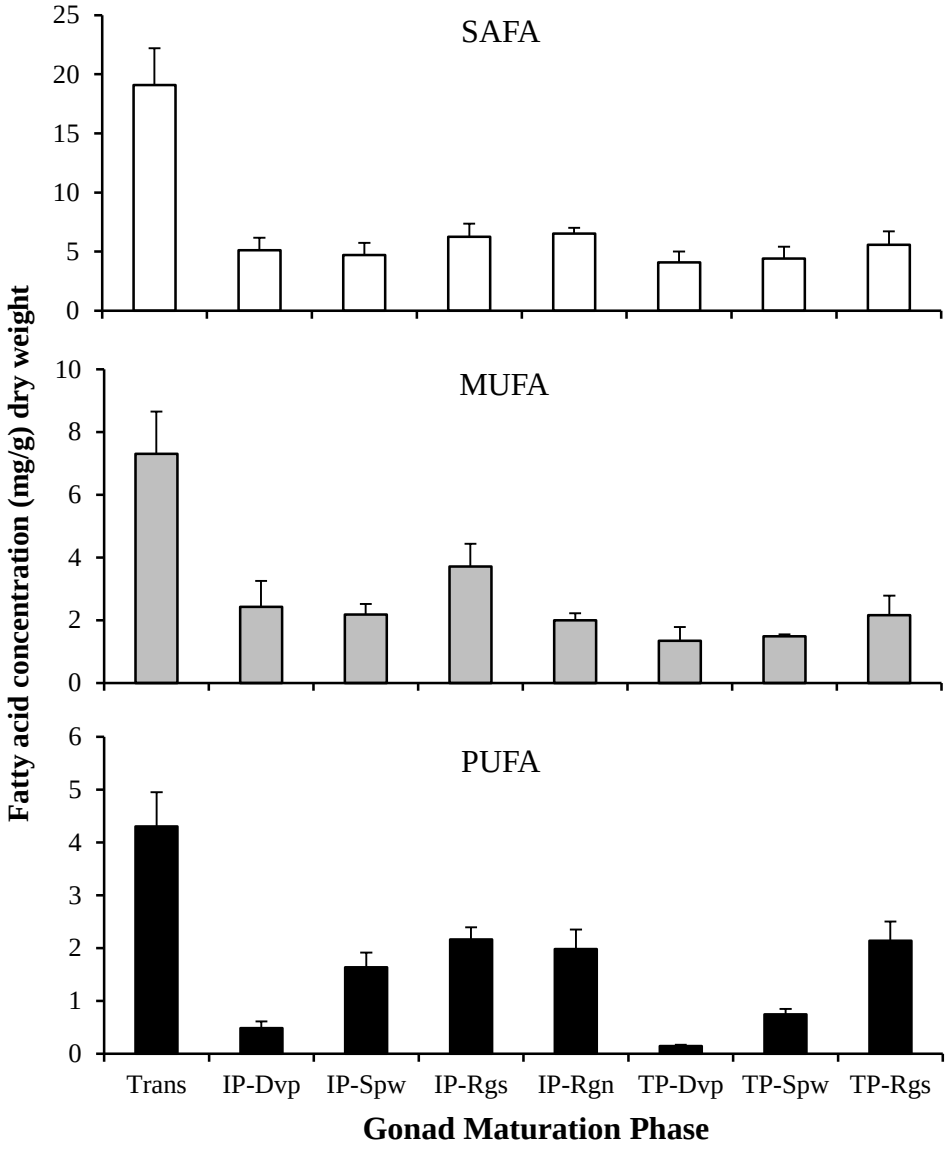

Figure 6.3 Total fatty acid (mg/g) dry weight according to of saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of adult phase (IP = initial phase and TP = terminal phase) of gonad maturation Trans = transition, Dvp = developing, Spw = spawning, Rgs = regressing and Rgn = regenerating of three parrotfish *Scarus* genus (*S. rivulatus*, *S. qouyi*, *S. ghobba*n) at Pulau Bidong. (mean±SD)

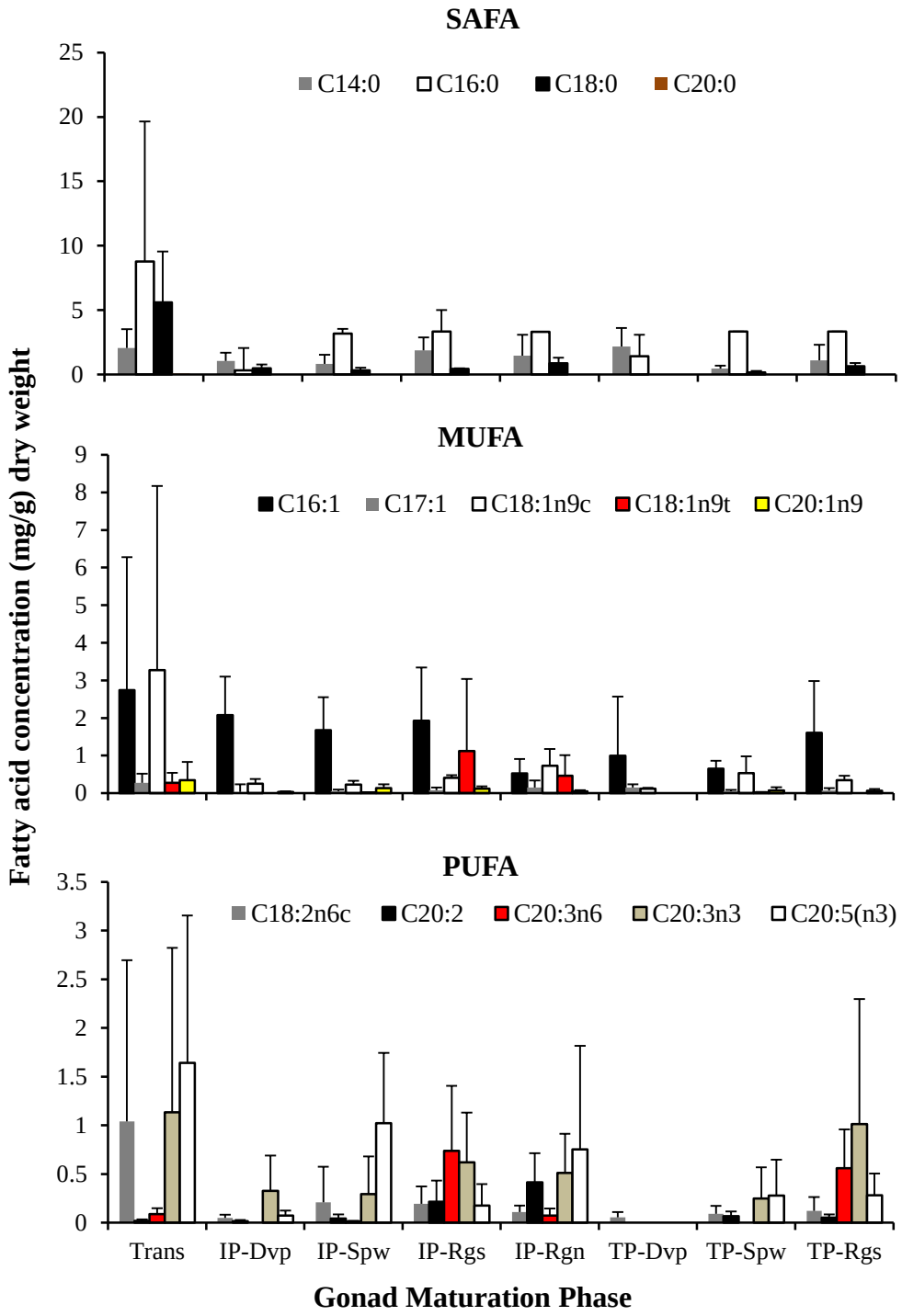

Figure 6.4 Composition of dominant fatty acid (mg/g) dry weight according to adult phase (IP = initial phase and TP = terminal phase) of gonad maturation Trans = transition, Dvp = developing, Spw = spawning, Rgs = regressing and Rgn = regenerating of three parrotfish *Scarus* genus (*S. rivulatus*, *S. qouyi*, *S. ghobba*n) according to group of saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) at Pulau Bidong. (mean±SD)

Table 6.8 Result of PERMANOVA on fatty acid of different gonad maturation phase. Significant level denoted as * (p<0.05), ** (p<0.01), *** (0.001).

Source	Df	SS	MS	Pseudo-F	P(perm)	unique	perms
Group	7	2479.3	354.19	1.3859	0.184		998
Res	35	8944.7	255.56				
Total	42	11424					
SAFA	7	9841.1	1405.9	1.3626	0.254		997
Res	35	36112	1031.8				
Total	42	45953					
MUFA	7	9841.1	1405.9	1.3626	0.243		996
Res	35	36112	1031.8				
Total	42	45953					
PUFA	7	18910	2701.4	1.3483	0.179		999
Res	29	58103	2003.5				
Total	36	77012					

Table 6.9 Result of PERMANOVA on average similarity between/within a group of all fatty acid with maturation phase of three parrotfish species.

Average Similarity between/within groups (%)								
	TRANS	IP-DVP	IP-SPW	IP-RGS	IP-RGN	TP-DVP	TP-SPW	TP-RGS
TRANS	68.33							
IP-DVP	76.237	84.155						
IP-SPW	78.092	86.679	88.09					
IP-RGS	74.908	81.844	81.285	80.557				
IP-RGN	77.449	81.631	83.605	82.265	86.307			
TP-DVP	70.477	78.956	81.533	73.75	73.984	80.035		
TP-SPW	77.158	84.633	87.333	80.884	84.612	82.119	82.893	
TP-RGS	75.635	82.863	84.806	80.25	82.783	79.123	84.382	79.571

Table 6.10 Results of pairwise test from PERMANOVA between different gonad maturation phases of three parrotfish species at Pulau Bidong. Significant level denoted as * $p<0.05$; ** $p<0.01$; ns = not significant.

Variable	**overall**		**SAFA**		**MUFA**		**PUFA**	
Groups	p (perms)							
TRANS, IP-DVP	0.295	ns	0.669	ns	0.666	ns	0.54	ns
TRANS, IP-SPW	0.236	ns	0.388	ns	0.356	ns	0.092	ns
TRANS, IP-RGS	0.525	ns	0.189	ns	0.192	ns	0.493	ns
TRANS, IP-RGN	0.8	ns	0.925	ns	0.906	ns	0.524	ns
TRANS, TP-DVP	0.113	ns	0.877	ns	0.866	ns	0.136	ns
TRANS, TP-SPW	0.601	ns	0.453	ns	0.432	ns	0.553	ns
TRANS, TP-RGS	0.667	ns	0.781	ns	0.782	ns	0.525	ns
IP-DVP, IP-SPW	0.53	ns	0.293	ns	0.322	ns	0.316	ns
IP-DVP, IP-RGS	0.301	ns	0.167	ns	0.183	ns	0.211	ns
IP-DVP, IP-RGN	0.104	ns	0.551	ns	0.509	ns	0.157	ns
IP-DVP, TP-DVP	0.078	ns	0.478	ns	0.488	ns	0.232	ns
IP-DVP, TP-SPW	0.375	ns	0.163	ns	0.17	ns	0.897	ns
IP-DVP, TP-RGS	0.631	ns	0.615	ns	0.624	ns	0.672	ns
IP-SPW, IP-RGS	0.084	ns	0.02	*	0.024	*	0.013	*
IP-SPW, IP-RGN	0.062	ns	0.284	ns	0.298	ns	0.087	ns
IP-SPW, TP-DVP	0.181	ns	0.022	*	0.029	*	0.953	ns
IP-SPW, TP-SPW	0.784	ns	0.48	ns	0.501	ns	0.785	ns
IP-RGS, IP-RGN	0.341	ns	0.213	ns	0.272	ns	0.539	ns
IP-RGS, TP-DVP	0.029	*	0.321	ns	0.301	ns	0.019	*
IP-RGS, TP-SPW	0.247	ns	0.021	*	0.026	ns	0.159	ns
IP-RGS, TP-RGS	0.354	ns	0.136	ns	0.129	ns	0.287	ns
IP-RGN, TP-DVP	0.032	*	0.504	ns	0.504	ns	0.103	ns
IP-RGN, TP-SPW	0.406	ns	0.518	ns	0.488	ns	0.308	ns
IP-RGN, TP-RGS	0.533	ns	0.57	ns	0.573	ns	0.409	ns
TP-DVP, TP-SPW	0.575	ns	0.092	ns	0.079	ns	0.693	ns
TP-DVP, TP-RGS	0.274	ns	0.472	ns	0.498	ns	0.614	ns

Table 6.11 Result dissimilarity of SIMPER analysis (Bray Curtis dissimilarity) of group comparison between maturation phases of parrotfish species.

Group	Average Dissimilarity	Factor	Dissimilarity Contribution %	Similarity contribution %
IP-SPW, IP-RGS	18.72	PUFA	47.54	52.46
		MUFA	30.85	69.15
		SAFA	21.61	78.39
IP-SPW, TP-DVP	18.47	MUFA	54.83	45.17
		PUFA	29.42	70.58
		SAFA	15.75	84.25
IP-RGS, TP-DVP	26.25	MUFA	42.69	57.31
		PUFA	40.29	59.71
		SAFA	17.02	82.98
IP-RGN, TP-DVP	26.02	PUFA	53.24	46.76
		MUFA	30.9	69.1
		SAFA	15.86	84.14
IP-RGS, TP-SPW	19.12	PUFA	39.83	60.17
		MUFA	39.36	60.64
		SAFA	20.82	79.18
TRANS, IP-RGS	25.09	PUFA	36.35	63.65
		MUFA	33.24	66.76
		SAFA	30.41	69.59
TRANS, TP-DVP	29.52	MUFA	40.79	59.21
		PUFA	35.06	64.94
		SAFA	24.15	75.85
TRANS, TP-RGS	24.37	PUFA	39.1	60.9
		MUFA	35.45	64.55
		SAFA	25.45	74.55

Table 6.12 Results of SIMPER analysis (Bray Curtis dissimilarity) on the average dissimilarity contribution within maturation phase group in parrotfish.

Group	Class	Fatty acid	Average dissimilarity %
IP-SPW, IP-RGS	SAFA	C16:0	28.32
		C14:0	12.46
	MUFA	C17:1	28.22
		C16:1	12.45
	PUFA	C20:3n3	27.69
		C20:5n3	15.51
		C18:2n6c	10.4
IP-SPW, TP-DVP	SAFA	C16:0	14.79
		C14:0	12.95
	MUFA	C17:1	14.77
		C16:1	12.94
	PUFA	C20:2	16.4
		C18:2n6c	15.65
		C20:3n3	7.87
IP-RGS, TP-DVP	SAFA	C16:0	22.24
		C14:0	10.91
	MUFA	C17:1	22.14
		C16:1	10.92
	PUFA	C20:3n3	34.14
		C20:5n3	15.23
		C18:2n6c	10.4
IP-RGS, TP-SPW	SAFA	C16:0	29.27
		C14:0	15.55
		C18:0	4.19
	MUFA	C17:1	29.25
		C16:1	15.54
		C18:1n9c	4.18
	PUFA	C20:3n3	22.66
		C20:5n3	12.57
		C18:2n6c	8.27
IP-RGN, TP-DVP	SAFA	C16:0	16.76
		C14:0	11.21
		C18:0	10.07
	MUFA	C17:1	16.75
		C16:1	11.22
		C18:1n9c	10.08
	PUFA	C20:3n3	25.49
		C20:5n3	24.86
		C20:2	13.72

6.4 Discussion

6.4.1 Diet Similarity in Parrotfish Species

Three parrotfish species of the *Scarus* genus at Pulau Bidong showed the similarities to most dominant contribution of the fatty acid group where composition of C16:0 (palmitic acid), C16:1 (palmitoleic acid) and C20:3n3 (eicosatrienoic acid) were highest at SAFAs, MUFAs and PUFAs respectively. This indicates that, the *S. rivulatus*, *S. qouyi* and *S. ghobban* were sharing the same food source. Previous study revealed that fatty acid C16:0 was higher in zooxanthellae and azooxanthellae of soft coral in Vietnam, South China Sea (Imbs et al 2010). There was also demonstrated that, higher fatty C16:0 and the present of other dominant fatty acid such as C14:0, C18:0, C18:3n6, C20:1n9 and C20:5n3 was identified in hard coral of Acroporidae family (Safuan, 2021) at East and West coast of Peninsular Malaysia as same as the present study at Scarus genus. Besides that, most of all dominant fatty acid at SAFAs, MUFAs, and PUFAs observed in the present study at three parrotfish of *Scarus* genus was the same as demonstrated in brown, green and red macroalgae (Berneira et al., 2020). Meanwhile, C18:3n6 PUFA was identified as markers of green macroalgae (Bachok et al., 2009). This fatty acid of PUFA (C18:3n6) also was within five dominant fatty acids in parrotfish of *Scarus* genus. This showed that three herbivorous parrotfish of the Scarus genus possibly feed on several types of hard coral, soft coral and macroalgae at Pulau Bidong.

Previous study on the feeding ecology of parrotfish *S. psittacus*, *S. rivulatus* and *S. qouyi* suggested, this three parrotfish at the South China Sea may ingest the same diet and have the same food source at Pulau Bidong (Arai et al., 2015). However, there was a significant difference in some fatty acid composition between three species in the *Scarus* genus, especially at *S. ghobban* in the present study. Although there was a similarity in food sources at the same area, the difference of fatty acid composition of the *Scarus* genus might be attributed to the growth (body size). For example, there was a significant relationship between the total SAFAs and MUFAs with the total length and body weight of moon wrasse *Thalssoma lunare* where the changes in fish size had affected the fatty acid composition (Amalina et al.,

2016). Another study of the fatty acid composition of three damselfishes *Abudefdaf* spp also found that there was a significant relation between diets to body size (Arai et al., 2015). Thus, the different adult phases in parrotfish which are IP and TP could potentially influence the variation in body weight and total length, as seen in this study. Feeding intensity has also been demonstrated to effect the accumulation of PUFAs in the muscle of wild cobia at Dungun coast of Malaysia (Barbatunde et al., 2017). It was mentioned that, at monsoon season, the increased of several fatty acids (C15:0, C16:1, C17:1, C18:2n6 & C18:3n3) in wild cobia was possibly due to an increase of feeding activities (Barbatunde et al., 2017). This previous study showed that different feeding activities of parrotfish individuals of *Scarus* genus at Pulau Bidong might also affect the fatty acid composition between species. Thus, from this evidence, it was strongly supported that diet or differences in the fatty acid composition of coral reef fishes possibly be affected by growth factor (Arai et al., 2015) and the feeding intensity of fish (Barbatunde et al., 2017).

Besides that, composition of fatty acid at the fish species can be varied due to state of reproductive cycle or gonad maturation. Study in Mesopotamia catfish (*Silurus triostegus*) showed that total PUFA in male fish was decreased during spawning and at highest level after post spawning (Kacar et al., 2016). In Atlantic salmon (*Salmo salar*) increased of long chain PUFA such as arachidonic acid had positively impacted the steroidogenesis and sexual maturation in male fish (Bogevik et al., 2020). There was also significant different of fatty acid composition such as SAFA (C16:0) and MUFA (C18:1n9) was observed in mature Albacore tuna (*Thunnus alalunga*) at different maturation stages (Dhurmeea et al., 2018). In the present study, the male parrotfish species had various states of gonad maturation which also will possibly affect the composition of fatty acid between each species. Furthermore, study in male gonad maturation in Atlantic salmon had suggested where change of lipid or fatty acid composition was positively related toward their importance in spermatogenesis and serve as structural components during testicular growth and maturation phase (Bogevik et al., 2020).

The composition of MUFAs and PUFAs showed significant differences in parrotfish of the Scarus genus. In spiny eel (*Mastacembelus mastacembelus*),

C18:1n9 MUFA was the prominent fatty acid in liver and gonads (Kacar and Bashan, 2017) as was observed among parrotfish species in the present study. MUFAs are important as rapid energy production (Tocher, 2003), are components in fatty acid biosynthetic pathways (Rocker et al., 2019) and closely related to fish reproduction (Kacar et al., 2016). It was reported that fatty acid MUFA such as C18:1n9 was released during the catabolic period which is during the breakdown of larger molecules to yield energy for gonad development (Kacar and Bashan, 2017). Meanwhile, PUFAs were related to gametogenesis (Gonzalex-Felez et al., 2019) and maintain the fluidity of cell membrane in teleost fish (Tocher, 2003). Therefore, this indicates that the difference in PUFAs between the parrotfish of *Scarus* genus might be attributed to the changes during reproductive status such as change of maturation phase in parrotfish at Pulau Bidong.

The fatty acid content of three species of parrotfish belonging to Scarus genus exhibited a consistent pattern, with SAFAs being the predominant group compared to MUFAs and PUFAs across all species. Besides, fatty acids C16:0, C14:0 and C18:0 were most contributed to the highest composition of SAFAs among three parrotfish *S. rivulatus*, *S. qouyi* and *S. ghobban* at Pulau Bidong. This high concentration of SAFAs among the two other fatty acid groups (MUFAs and PUFAs) also was observed in several marine fish at the South China Sea such as damselfish *Abudefduf spp*. (Arai et al., 2015a), moon wrasse *Thalassoma lunare* (Amalina et al., 2016), bigeye snapper *Lutjanus lutjanus* (Arai et al., 2015b) and most of the commercial fish at Terengganu (Tramice et al., 2021). SAFAs have an important role in metabolism as a major source of energy and growth (Sargent et al., 2002). Previous study in Atlantic sturgeon (*Acipencer oxyrhynchus*) revealed that C16:0 SAFA was a key in fish metabolism and the composition not influenced by diet (Ackman et al., 1975). A study showed that the high level of palmitic acid (C16:0) of SAFAs at warm water rather than cool water indicates the difference in metabolic rate in fish and is not also associated with the fish diet (Huynh and Kitts, 2009). Again, study in fatty acid profile of catfish (*Silurus triostegus*) during spawning season mentioned that SAFAs and MUFAs were important for source of energy while PUFAs was involved toward structural function (Kacar et al., 2016).

6.4.2 Important of Fatty Acid to Parrotfish Reproduction and Physiological Condition

The nutrient allocation for gonad maturation is a well-known process in fish. Generally, total SAFAs, MUFAs, PUFAs and all dominant fatty acids were highest in the Trans phase. A study shows that concentrations of lipids in fish muscle were higher in the non-reproductive period (Kacar et al., 2016). Parrotfish are hermaphrodite fish that change their sex from female to male (Fennessy & Sodavy, 2002; Allops & West, 2003). The transition phase is the temporary phase where the gonads of hermaphrodite fish change their sex. The male spermatogenic cell is not well developed at transition phase and the proliferation of cells has not actively occurred (El-Sayedah et al., 2012; Hamilton et al., 2008). With regard to this condition, meaning that parrotfish at the Trans phase were assumed as a non-reproductive stage which conforms with their significantly lowest GSI value shown in Figure 5.6 of the previous chapter (Chapter 5) and the absent of both dominant spermatogenic cells (spermatocyte & spermatozoa) showed in Figure 5.4 (Chapter 5).

Besides, at the Trans phase the sex changes possibly require high energy demand for this transformation. Although the composition of C14:0, C16:0 and C18:0 were shown to be higher throughout reproductive cycle or gonads of fish but their specific functions in fish remain largely unclear (Sardenne et al., 2022; Sulamo & Ogata, 2012). However, a study on genetic analysis confirmed that the C14:0 SAFA was involved during the sex-determination or transform in hermaphrodite worms *Caenorhabditis elegans* (Tang & Han, 2017). Based on this evidence, there might be one of the possible reasons for the high composition of C14:0 at the Trans phase in parrotfish where their sex starts to be transformed. Other than that, the highest SAFAs (C14:0, C16:0, C18:0) and MUFAs (C18:1n9c, C20:1n9, C16:0) level at Trans phase in parrotfish species may also correlated with the main function of SAFAs for energy supply in fish metabolism (Anido et al., 2016; Sargent et al., 2002; Tocher, 2003) during sex transformation.

To date there was very little information has been available towards the main function of predominant SAFAs (C14:0, C16:0, C18:0) and MUFAs (C18:1n9c,

C20:1n9, C16:0) during gonad maturation even though several studies on the change of fatty acid profile towards gonad development (Sharma et al., 2022; Gonzalex-Felex et al., 2019; Li et al., 2018; Anido et al., 2015) had been investigated. However, some studies had mentioned relation between SAFAs and MUFAs in reproduction generally (Sardenne et al., 2022; Dhurmeea et al., 2018; Kacar et al., 2016; Esmaeili et al., 2015). In European eels (*Anguilla anguilla*), de novo synthesis of C16:0 SAFAs were observed in the male liver during sperm cell production or called spermatogenesis (Baeza et al., 2014). The increased production of C16:0 with GSI in Atlantic salmon (*Salmon salar*) in sexual maturation was proposed as their functional for growth and structural purposes during testicular maturation (Bogevik et al., 2020). Meanwhile, C18:1n9c MUFAs were recognized as basic fatty acids before and after reproduction period in spiny eel, *Mastacembelus mastacembelus* (Kacar and Bashan, 2017). Besides that, C18:1n9 appears to be a major fatty acid in spermatozoa of mammalian biological system (Esmaelli et al., 2015). It was reported that the high composition of C18:1n9c MUFAs with C16:0 SAFAs during reproduction were due to energy requirements for the course of gonad development (Kacar and Bashan, 2017). Thus, from these previous studies, plausibly the high composition of SAFAs and MUFAs such as C16:0 and C18:1n9c were closely related to reproduction, also equally important in gonad maturation of fish.

Other than that, gonad fatty acid composition showed some pattern along the maturation phases of parrotfish, especially for PUFAs. The composition of PUFAs slowly increased from Dvp and reached peak value at Rgs in both IP and TP. Among all PUFAs, groups of n-3 and n-6 such as C20:5(n3) known as eicosapentaenoic acid (EPA), C20:3n3 and C20:3n6 were highest during spawning and regressing. Similarly, in male wild devil stinger, *Inimicus japonicas* where the total PUFAs, n-3, n-6 and high unsaturated fatty acid (HUFA) such as EPA was elevated during gonad development, which was lower at phase III then significantly increased during phase IV (Li et al., 2019). Meanwhile, there was a significant increase of EPA, C20:3n3, C20:3n6 at stage IV compared to stage I of female jundia catfish, *Rhamdia quelen* in the sub-tropical region (Southern Brazil) at gonad development phases (Anido et al., 2015). Another significant increase of n-3 and long chain PUFA including C20:3n3, C20:3n6 and EPA were also identified in male damselfish, *Chromis limbaughi* at

temperate region (Gulf of Califonia) from developing to spawning capable (Gonzalex-Felez et al., 2019). This highlighted that EPA and some other fatty acids were important during gonad maturation and fertilization (Li et al., 2019). The evident was supported by the significantly increased of male GSI and HSI from stage III to V of wild devil stinger, *Inimicus japonicas* (Li et al., 2019) which were similarly identified at parrotfish of *Scarus* genus in the present study where GSI and HSI increased significantly from developing to spawning phase (Figure 5.6, Chapter 5).

In the physiological aspect, PUFAs composition was also related to the secretion of sex steroid hormone and spermatogenesis in fish (Ferosekhan et al., 2021; D Crespo et al., 2020; Wade et al., 1994). This was shown in the parrotfish of the *Scarus* genus where the high concentration of PUFAs at IP-Rgs and TP-Rgs was associated with the significantly high level of 11KT hormone at the same maturation phase (regressing) and low E2 shown in the present study at Figure 5.5 of the previous chapter (Chapter 5). The number of spermatozoa was also significantly lowest at the regressing phase in both IP and TP of parrotfish. High PUFAs content was needed by spermatozoa during fertilization for their plasma membrane fluidity (Wathes et al., 2007).

The 11KT was the sex steroid hormone which is important in controlling spermatogenesis (Borg, 1994; Hashida & Kawamoto 1970), principally synthesised from testosterone (Tenugu et al., 2021). 11KT was strongly correlated with testosterone (Ferosekhan et al., 2021). Testosterone is involved in the proliferation of spermatogenic cells from spermatogonia to spermatocytes in fish (Billard et al., 1974). Ferosekhan et al., (2021) revealed that fish fed with a high PUFA diet such as C20 and C22 of n-3 with n-6 after three months showed a significant difference in 11KT and testosterone concentration. Study in tongue fish (*Cynoglossus semilaevis*) had confirmed the functional of longchain PUFA C20:5(n3) toward the regulation of sex steroid hormone production through the accumulation of this fatty acid in fish gonad and response of gene expressions (Xu et al., 2017). Besides, a study of steroidogenic action in testis of goldfish demonstrated the increase of fatty acids C20:2n4, C20:3n6 and EPA had stimulated a higher increase of testosterone

production (Wade et al., 1994). The increase of 11KT and testosterone was also strongly correlated to the increase in sperm concentration (Foresekhan et al., 2021). Meanwhile, sperm concentration has strong relationship with sperm mortality and sperm viability in male gilthead seabream, *Sparus aurata* (Foresekhan et al., 2021).

Another study showed the high of n-3 fatty acid composition resulted in a significant increase of sperm density and lower sperm deformity in boars (Lin et al., 2016). Also, a review study on broodstock nutrition recognized that n-3 PUFA such as C20:5(n3) were important for gonad gonad development, fertilization and spawning in fish (Izquierdo et al., 2001). Multiple studies have provided evidence indicating that the fatty acid compositions in fish are linked to the process of steroidogenesis (synthesis and production of hormone), sperm production and gonad development. Therefore, the result in this present study probably support the previous evidence indicating that fatty acid from fish diet especially PUFAs has a close relationship with releasing of steroid hormones concentration (11KT & E2), reproductive index (GSI & HSI) and spermatogenic cells numbers (spermatozoa & spermatocyte) in parrotfish of *Scarus* genus at Pulau Bidong.

6.5 Conclusion

There were same 27 fatty acids composition was identified in three parrotfish species *S. rivulatus*, *S. qouyi* and *S. ghobban*. The significant change of MUFAs among three parrotfish species was assumed to be related to the different energy demand either for growth or gonad development purpose. Meanwhile, according to gonad maturation of parrotfish, fatty acid of C16:0 SAFAs, C16:1 and C18:1n9c MUFAs and C20:3n3 and C20:5(n3) PUFAs might have the most important role due to highest dissimilarity contribution. It was suggested that, from previous study where C16:0 SAFAs together with C18:1n9c MUFAs may provide the energy for gonad development (Kacar & Basham, 2017) and C20:5(n3) PUFAs play a significant role towards the production of sex steroid hormone during fish reproduction (Xu et al., 2017) .

CHAPTER 7

GENERAL CONCLUSION AND RECOMMENDATION

7.1 General Conclusion

Parrotfish species in this study may consider as among important coral reef fish at Pulau Bidong, Terengganu, as well as in other parts of the reef ecosystem in Malaysia. From family Scaridae, they are known as an important functional group within herbivorous fish and most of them are diandric protogynous hermaphrodite, which has complex initial and terminal adults phase. This study is the first attempt that classifies the parrotfish gonad developments phases from East Coast of Malaysia specifically at Pulau Bidong, a case study in three parrotfish species; Surf parrotfish (*Scarus rivulatus)*, Quoy's parrotfish (*S. qouyi)* and Yellowscale parrotfish (*S. ghobban).* From microcharacteristic criteria of histological examination had confirmed that after transition phase, gonad development at initial phase of male individuals possess four maturation phases, developing → spawning → regressing → regenerating, while for terminal phase possess developing → spawning → regressing.

At the spawning phase of gonad maturation, significantly high mean values of spermatogenic cells which were spermatocyte and spermatozoa correlated with maximum GSI to HSI value and reproductive hormone was observed indicating that spawning may occur in parrotfish species. Toward gonad maturation phase, male sex steroid hormone such as 11-ketotestosterone was released at a constantly higher level compared to estradiol. The changed pattern of sex steroid hormone profile according to gonad development phase likely showed their important role during fish maturation.

Transitioning phase required the highest energy demand in individual parrotfish in the population due to sex transformation. These had been approved by higher values of the energy reserve indicator, the SAFAs in parrotfish during the transition phase. Possibly further advancement of gonad maturation phases may influence food intake for essential nutrients. Consequently, there were increases of SAFAs, MUFAs and n3 and n6 PUFAs concentration during the initial and terminal phases after the parrotfish passage to the male adult phase. The mean concentration increases from the developing to regressing phase, which indicates that the SAFAs, MUFAs and PUFAs are essential for male parrotfish reproductive performance.

. There are several factors that correlate with gonad maturation in parrotfish, which are length-weight relationship, secretion of reproductive hormone and nutrition from food intake. Returning to the hypothesis stated at the beginning of this study, based on the present results, it is possible to state that, all the null hypothesis was accepted which exclusively conclude; 1) There was significant relation between the length-weight relationship with reproductive index (GSI, HSI, CF), 2) The gonad maturation phases of all three species of parrotfish had shown a similar micro-characteristic pattern, also the gonad maturation phases was significantly influenced the reproductive hormone (E2 & 11KT) profile, spermatogenic cell numbers and reproductive index values (GSI & HSI) and 3) the pattern of fatty acid profiles was associated towards gonad maturation phases in male parrotfish species at Pulau Bidong.

7.2 Limitation and Recommendation

Although the present study was able to completely achieve the null hypothesis, it has certain limitations such as in terms of sample size. The sample size in this study has less variation because through the random sampling technique it was very hard to specify the sample size at reef ecosystems. The only availability of size sample present was taken during the sampling. Thus, for *S. rivulatus* there was an absence of IP while *S. qouyi* was absent of TP. Only for *S. ghobban* both IP and TP were found but only three individuals for TP males.

Possibly, in the first objective, if more number of samples were present the better variation of size class sample may be observed. Study on the relation of length with GSI, HSI and CF in other male parrotfish species in Pulau Bidong is recommended to compare with the present results. Meanwhile, studies on the second objective of male gonad maturation were unable to complete the TP-Rgn phase. It is suggested that, larger sample size is required in order to complete the male gonad phases of parrotfish species in the Pulau Bidong. Besides, the study of sex steroid hormone profiles in relation to the maturation phase in other diandric male hermaphrodite fish species or other parrotfish genera in Pulau Bidong would be very interesting and valuable for the comparisons proposed.

Other than that, further study on size at first maturity in this parrotfish can provide additional evidence on the different growth pattern and reproductive index pattern at parrotfish species at Pulau Bidong. The change of nutrient requirement during gonad development is important in fish reproduction. This present study observed the changes of fatty acid profile SAFAs, MUFAs and PUFAs during gonad maturation phase in terms of lipid form after food assimilation had occurred. Thus, in order to understand whether this food source comes from current food intake or reserve energy in fish, additional study on the stomach content during gonad maturation phase is suggested as a good approach.

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APPENDICES

Appendix 1-(Chapter 4)

a)One-Way ANNOVA and Post-hoc Analysis of the reproductive index (GSI, HSI,CF) between species

ANOVA

GSI	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.856	2	.428	4.382	.014
Within Groups	12.408	127	.098		
Total	13.265	129			

GSI

Post-hocv test	species	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a,b]	S. ghobban	35	.0848	
	S.rivulatus	43	.1976	.1976
	S. qouyi	52		.2869
	Sig.		.225	.391

ANOVA

HSI	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.869	3	5.956	1.134	.338
Within Groups	688.004	131	5.252		
Total	705.873	134			

ANOVA

CF	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.599	2	.799	7.815	.001
Within Groups	13.297	130	.102		

Total	14.896	132			

CF- Post-Hoc	species	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a,b]	S. ghobban	37	1.5920	
	S. qouyi	50		1.7730
	S. rivulatus	46		1.8692
	Sig.		1.000	.341

Appendix 2 – Chapter 5

a) One-Way ANNOVA and Posthoc Analysis of the reproductive index (GSI & HSI) with gonad maturation phases in *S. rivulatus*.

ANOVA

GSI SR	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.269	2	.634	2.307	.113
Within Groups	10.724	39	.275		
Total	11.993	41			

GSI SR	stage	N	Subset for alpha = 0.05	
Posthoc test			1	2
Duncan[a,b]	TP-DVP	20	-1.1386	
	TP-RGS	15	-1.0575	-1.0575
	TP-SPW	7		-.6479
	Sig.		.712	.068

ANOVA

HSI SR	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.945	2	2.973	.515	.601
Within Groups	225.100	39	5.772		
Total	231.046	41			

SR_HSI

HSI SR	stage	N	Subset for alpha = 0.05
Posthoc			1
Duncan[a,b]	TP-RGS	15	3.6627
	TP-DVP	20	4.4075
	TP-SPW	7	4.5400
	Sig.		.415

b)One-Way ANNOVA and Posthoc Analysis of the reproductive index (GSI & HSI) with gonad maturation phases in *S. qouyi*

ANOVA

GSI SQ	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.450	3	.483	2.323	.088
Within Groups	9.358	45	.208		
Total	10.808	48			

GSI SQ	stage	N	Subset for alpha = 0.05
Posthoc			1
Duncan[a,b]	IP-DVP	17	-1.0015
	IP-SPW	17	-.9916
	IP-RGN	4	-.7368
	IP-RGS	11	-.5930
	Sig.		.093

ANOVA

HSI SQ	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.237	3	.079	.475	.701
Within Groups	7.480	45	.166		
Total	7.717	48			

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HSI SQ	stage	N	Subset for alpha = 0.05
Posthoc			1
Duncan[a,b]	IP-RGS	11	.4185
	IP-DVP	17	.4445
	IP-RGN	4	.5067
	IP-SPW	17	.5822
	Sig.		.452

c)One-Way ANNOVA and Posthoc Analysis of the reproductive index (GSI & HSI) with gonad maturation phases in *S. ghobban*

ANOVA

GSI SG	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.827	5	.965	4.035	.007
Within Groups	6.699	28	.239		
Total	11.525	33			

GSI SG	STAGE	N	Subset for alpha = 0.05		
Posthoc test			1	2	3
Duncan[a,b]	TRANS	11	-1.5962		
	IP-DVP	6	-1.4944	-1.4944	
	IP-RGN	4	-1.0822	-1.0822	-1.0822
	TP-SPW	3		-.8339	-.8339
	IP-SPW	3		-.8045	-.8045
	IP-RGS	7			-.7141
	Sig.		.145	.060	.310

ANOVA

HSI SG	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	40.717	5	8.143	2.641	.044
Within Groups	89.431	29	3.084		
Total	130.148	34			

HSI SG Posthoc	STAGE	N	Subset for alpha = 0.05	
			1	2
Duncan[a,b]	IP-RGS	7	1.8171	
	IP-RGN	4	1.8600	
	TRANS	12	3.1083	3.1083
	TP-SPW	3	4.0433	4.0433
	IP-DVP	6	4.2433	4.2433
	IP-SPW	3		5.1533
	Sig.		.070	.117

d)One-Way ANNOVA and Posthoc Analysis of the reproductive index (GSI & HSI) with gonad maturation phases in all species

ANOVA

All GSI species	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.244	7	1.321	5.519	.000
Within Groups	28.476	119	.239		
Total	37.720	126			

All species GSI Posthoc	STAGE_TTL_SAMPLE	N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a,b]	TRANS	13	-1.6352			
	TP-DVP	20		-1.1386		
	IP-DVP	23		-1.1301		
	TP-RGS	15		-1.0575	-1.0575	
	IP-SPW	20		-.9635	-.9635	-.9635
	IP-RGN	8		-.9095	-.9095	-.9095
	TP-SPW	10			-.7037	-.7037
	IP-RGS	18				-.6401
	Sig.		1.000	.277	.082	.112

ANOVA

All species HSI	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.672	7	.239	1.204	.306
Within Groups	23.801	120	.198		
Total	25.473	127			

All HSI	STAGE_TTL_SAMPLE	N	Subset for alpha = 0.05
Posthoc			1
Duncan[a,b]	IP-RGS	18	.2784
	TP-RGS	15	.3259
	IP-RGN	8	.3706
	TRANS	14	.3931
	IP-DVP	23	.4726
	TP-DVP	20	.5401
	TP-SPW	10	.5866
	IP-SPW	20	.6008
	Sig.		.103

BIODATA OF AUTHOR

Nor Hazirah Mohd Zuki was born in Bukit Mertajam, Pulau Pinang on 2nd March 1990 early in the morning. She attended two primary schools that was Sekolah Kebangsaan Jejawi (1997 – 2001) and Sekolah Kebangsaan Bandar Baru Darulaman (2001 – 2002). After finished her primary school, she had enter the secondary school at Sekolah Menengah Kebangsaan Sultanah Asma (2003 – 2007). Thereafter, she joined the Pulau Pinang Matriculation College (2008 – 2009). Her first degree is Fisheries of Science (2009 – 2012) and continues her Master in Science of Aquaculture (2012 – 2013) in UMT. She has gained some experience as Research Assistant (RA) for six month. Hazirah had deep interest in biological in relation with physiological study. Therefore, she furthers her PhD in fish biology connected with physiology specifically in gonad maturation and fish hormone of coral reef fish. She had ability in blood drawing for several fish species and conducted the histological examination. Her Phd was under the supervision of Prof. Zainudin Bachok, Dr. Siti Ariza Aripin, Prof Takaomi Arai and late Dr. Safiah Jasmani. As a researcher she had plan to publish several papers with her committees about fish reproductive biology and physiology in parrotfish to share with other researchers especially about the diandric hermaphrodite fish at coral reef ecosystem in the South China Sea water region.