INFLUENCE OF CHARACTERISTICS OF MARINE MACROALGAE ON FEEDING PREFERENCES OF THE MILKFISH (*Chanos chanos*) IN PENINSULAR MALAYSIA

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INFLUENCE OF CHARACTERISTICS OF MARINE MACROALGAE ON FEEDING PREFERENCES OF THE MILKFISH (*Chanos chanos*) IN PENINSULAR MALAYSIA

ABSTRACT

Herbivores can alter several ecological mechanisms including interactions between native and non-native autotrophs that may determine invasion success of the non-native autotrophs. This study looked into the autotroph-herbivore interactions in coastal marine ecosystems of Peninsular Malaysia, mainly to determine if autotroph nutritional components and geographical differences significantly influence herbivore feeding behaviours. The relative consumption rate (RCR) of milkfish (*Chanos chanos* Forsskål), a generalist herbivore obtained from the Straits of Malacca was documented through feeding trials using 12 macroalgal species collected from different coastal areas of the Straits of Malacca, the Straits of Johor, and the South China Sea. The herbivore was observed to feed selectively ($P<0.05$) on the tested macroalgal species, with *Valoniopsis pachynema* (G.Martens) Børgesen and *Cladophora prolifera* (Roth) Kützing from the coastal areas of the South China Sea has the highest RCR (94.04 mg g^{-1} day^{-1} and 78.20 mg g^{-1} day^{-1}, respectively). Contrarily, *Padina gymnosphora* (Kützing) Sonder collected from the Straits of Johor was the least preferred species with the lowest RCR (1.74 mg g^{-1} day^{-1}). Based on the nutritional analysis, the most consumed species *V. pachynema* and the least consumed species *P. gymnosphora* had the lowest and highest total nitrogen content, respectively. A significant inverse correlation was found between the RCR and total nitrogen content of macroalgae, implying the possibility of nutrient-specific compensatory feeding by the herbivore. Besides total nitrogen content, the RCR was also negatively correlated with total phenolic content, where the least consumed species *P.*

gymnosphora recorded the highest total phenolic content. This study also found that the morpho-functional groups of macroalgae may exert influence over herbivore feeding preferences, whereby two tested filamentous macroalgae were consumed in greater amounts than species from other functional groups such as corticated foliose macroalgae. The overall findings demonstrate that the feeding behaviours of a generalist herbivore may depend directly or indirectly on multifaceted factors, including the nutritional quality, novelty, and morphology of the autotrophs.

Keywords: Autotroph-herbivore interaction, feeding preference, nutritional quality, novelty, morphology

KESAN CIRI MAKROALGA MARIN KE ATAS PILIHAN MAKANAN IKAN SUSU (*Chanos chanos*) DI SEMENANJUNG MALAYSIA

ABSTRAK

Herbivor mengubah sebilangan mekanisme ekologi termasuk interaksi antara autotrof tempatan dan bukan tempatan yang berkemungkinan menentukan kejayaan pencerobohan autotrof bukan tempatan. Projek ini mengkaji interaksi autotrof-herbivor dalam ekosistem marin pantai di Semenanjung Malaysia, terutamanya untuk menentukan sama ada ciri kualiti makanan autotrof dan perbezaan geografi mempengaruhi pemilihan makanan herbivor secara ketara. Kadar pemakanan relatif (RCR) ikan susu (*Chanos chanos Forsskål*), iaitu satu herbivor generalis yang diperoleh dari Selat Melaka didokumentasikan melalui ujian pemakanan menggunakan 12 spesies makroalga yang dikumpulkan dari kawasan pantai di Selat Melaka, Selat Johor, dan Laut China Selatan. Herbivor diperhatikan memakan secara selektif ($P<0.05$) pada makroalga yang diuji, dengan *Valoniopsis pachynema* (G. Martens) Børgesen dan *Cladophora prolifera* (Roth) Kützing dari kawasan pesisiran Laut China Selatan mencatatkan RCR yang tertinggi (94.04 mg g^{-1} $hari^{-1}$ dan 78.20 mg g^{-1} $hari^{-1}$, masing-masing). Sebaliknya, *Padina gymnosphora* (Kützing) Sonder yang didapati dari Selat Melaka adalah spesies yang paling kurang dimakan dengan RCR yang paling rendah (1.74 mg g^{-1} $hari^{-1}$). Berdasarkan analisis pemakanan, spesies yang paling banyak dimakan *V. pachynema* dan spesies yang paling kurang dimakan *P. gymnosphora* masing-masing mempunyai jumlah kandungan nitrogen yang terendah dan tertinggi. Satu korelasi songsang yang signifikan didapati antara RCR dan jumlah kandungan nitrogen makroalga, menunjukkan kemungkinan berlakunya pemberian pampasan spesifik nutrien oleh herbivor. Selain kandungan nitrogen, RCR juga berkorelasi negatif dengan kandungan jumlah phenolic, di mana spesies yang paling kurang dimakan *P. gymnosphora* mancatatkan jumlah kandungan phenolic yang tertinggi. Kajian ini juga mendapati bahawa kumpulan morfologi berfungsi makroalga boleh memberi pengaruh terhadap pemilihan makanan herbivor, di mana dua makroalga jenis filamen yang diuji dimakan dengan jumlah yang lebih besar daripada spesis dari kumpulan fungsi yang lain seperti makroalga jenis *corticated foliose*. Penemuan keseluruhan menunjukkan bahawa pemilihan makanan herbivor generalis mungkin bergantung secara langsung atau tidak langsung kepada pelbagai faktor, termasuk kualiti makanan, novelti, dan morfologi autotrof.

Kata kunci: Interaksi autotrof-herbivor, pemilihanan makanan, ciri nutrisi, novelti, morfologi

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

°C : Degree Celsius

g : Gram

L : Liter

mg : Milligram

mL : Milliliter

nm : Nanometer

µg : Microgram

% : Percentage

ANOVA : Analysis of variance

BRH : Biotic resistance hypothesis

$DW_{algae:final}$: Final dry mass of macroalgae

$DW_{control}$: Final dry mass of control macroalgae

$DW_{algae:initial}$: Initial dry mass of macroalgae

DW_{fish} : Dry body mass of fish

GAE : Gallic acid equivalents

$FW_{algae:initial}$: Initial fresh mass of macroalgae

$FW_{control}$: Initial fresh mass of control macroalgae

RCR : Relative consumption rate

SD : Standard deviation

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Research Background

Mounting evidence indicates that biological invasions by non-native species are a growing threat to global biodiversity, and is exacerbated by globalization and climate change (Meyerson et al., 2019; Seebens et al., 2017). In the marine system, non-native macroalgae have become widespread and, despite increasing awareness and mitigations to minimize the phenomenon, the number of macroalgal species across the invasive range in coastal waters has substantially increased over the last 20 years (Low et al., 2015). The anthropogenically introduced macroalgae can adversely influence both ecosystem function and structure, by changing community composition and diversity, reducing the abundance of native species, and altering food web and energy flow (Schaffelke et al., 2006). These environmental and economic costs of invasions by non-native macroalgae therefore have prompted intense studies in the mechanisms that contribute to invasion success (Fleming & Dibble, 2015).

The response of marine herbivores to the entry of non-native macroalgae to the community has important implications for invasion success. In the new community, marine native herbivore may able to consume the non-native macroalgae or, alternatively, the non-native macroalgae may well adapt to defend against the new herbivore (Maron & Vila, 2001). Evolutionary novelty is thus a key aspect of these interactions, potentially contribute to both the successful and unsuccessful of autotroph invasions (Verhoeven et al., 2009). To resolve the inconsistent results of novelty, the novelty-based hypothesis may need to be extended by including mechanisms underlying autotroph characteristics that affect herbivore feeding. In the case of marine ecosystems, several studies have demonstrated that the characteristics of marine macroalgae largely influence the feeding preference and consumption patterns of marine herbivores (Bakker et al., 2016; Kelly et al., 2017; Van Alstyne et al., 2009). One of the most-studied intrinsic characteristics of macroalgae that regulate feeding decisions by herbivores is the nutritional quality (Cebrian et al., 2009). Herbivore is often limited by the low nutritional value of their foods, which should, therefore, focus their foraging effort on autotroph with higher nutritional value. Indeed, nitrogen and phosphorus in macroalgae have been long acknowledged as important determinants of marine herbivore feeding selectivity (Bakker et al., 2009; Barile et al., 2004). For example, Shantz et al. (2017) found that several types of herbivorous reef fishes increased their foraging effort on brown alga Sargassum sp. with higher nitrogen and phosphorus values. Likewise, in freshwater systems, decreasing of the phenolic compounds from emergent to floating to submerged autotrophs, suggesting that not only higher nutritional quality but less defended tissues, resulting in relatively higher consumption of herbivores (Bakker et al., 2016). Nonetheless, the effects of macroalgal nutritional quality and novelty on the marine community, particularly herbivores capable of consuming non-native macroalgae, still remain largely unexplored in marine studies.

Interactions between herbivores and autotrophs have far displayed a dynamic evolutionary history, whereby the autotrophs have evolved both morphological and chemical defences that reduce the impact of herbivory (Sanchez & Trexler, 2016). Many marine macroalgae produce secondary metabolites to deter herbivore feeding. Secondary metabolites such as phenolics, terpenoids, and nitrogenated compounds can be efficient deterrents of aquatic herbivores and it is assumed these compounds have evolved countermeasures subject to natural selection by herbivores. Further, secondary metabolites are more abundant and diverse in tropical than temperate macroalgae which result from high selective pressure through grazing in tropical ecosystems (Amsler et al., 2005; Fong & Paul, 2011). Morphological defences such as calcification and toughness are common in certain macroalgal species, which have been studied extensively, include a high proportion of carbon-rich structural compounds that strengthen cell walls and thus increase resistance to herbivores. In nature, most of the herbivores, especially fish species, were observed to preferentially consume fleshy macroalgae, particularly those with simple thalli such as filamentous macroalgae, rather than heavily calcified macroalgae (Mendes et al., 2015; Suskiewicz & Johnson, 2017). As such, the effects of herbivore preferences are likely to be most evident when macroalgal taxa classified according to their morpho-functional groups, for example crustose, corticated, articulated calcareous, and filamentous (Griffin et al., 2010).

Many marine ecosystems are typified by a taxonomically rich suite of primary producers and their grazers. The grazers often include marine herbivorous fishes, such as milkfish (*Chanos chanos*) which are abundant and known to have a profound impact on the composition, abundance, and evolution of macroalgae (Puk et al., 2016; Sala et al., 2011). On reef systems, grazing by native herbivorous fishes can prevent native blooms of tuft and fleshy macroalgae that frequently reduce the resilience of coral reefs (Cheal et al., 2013; Hughes et al., 2007). The high mobility, broader diets and varied feeding apparatus, and visual acuity of many generalist herbivorous fishes have relatively larger impacts than another type of herbivores, on survivorship of macroalgal species (Floeter et al., 2005; Goatley & Bellwood, 2009). Given these strong impacts on populations and communities of native macroalgae, native herbivorous fishes apparently have great potential to influence invasion dynamics as well. According to the biotic resistance hypothesis, the failure of non-native autotroph species to establish in the invasive range is commonly attributed to healthy populations of native herbivores that can provide biotic resistance to autotroph invasions (Parker et al., 2006). In support of this hypothesis, several studies on aquatic ecosystems showed that native herbivores can potentially restrict the growth of non-native autotrophs and allow the growth of other autotroph species as the non-native autotrophs are favored food items (Morrison & Hay, 2011; Noè et al., 2018; Petruzzella et al., 2017). When testing the biotic resistance hypothesis, it is critical to incorporate the fundamental difference between specialist and generalist herbivores. In a new range of communities, non-native autotrophs are typically more completely suppressed by generalist than specialist herbivores as they encounter a new suite of generalist herbivores with which they have not coevolved with (Schaffner et al., 2011; Verhoeven et al., 2009). Accordingly, in comparison to specialist herbivores, generalist herbivores more likely to confer biotic resistance to non-native autotrophs as they can potentially consume a diverse and broadly array of autotroph species (Noè et al., 2018).

Although the dynamics of autotroph-herbivore interactions have been previously discussed for both terrestrial and aquatic environment in different parts of the world, many studies have reported contradictory findings and conclusions on the effects of autotroph characteristics and feeding patterns of generalist herbivore (Cronin et al., 2002; Grutters et al., 2017; Raubenheimer et al., 2009; Wong et al., 2010). To the best of our knowledge, the critical characteristics in marine macroalgae such as their nutritional qualities and geography (especially in terms of novelty) that attract or deter herbivores have not been well-addressed in Malaysia. Hence, a greater understanding of how macroalgal characteristics influence herbivore feeding is necessary to predict the potential role of native generalist herbivores in controlling the populations of non-native macroalgae in a changing climate. In the present study, the effects of macroalgal characteristics (nutritional quality, novelty, and morphology) and their relationships on the feeding preferences of generalist herbivorous milkfish (*Chanos chanos* Forsskål) were considered. Milkfish *C. chanos* is well-suited to this study purpose, as it appears to be generalist in their foraging behaviour, which consuming a wide range of macroalgae types in nature. By using milkfish *C. chanos* whose distribution is primarily native to the northwest coast of Peninsular Malaysia, the novelty of macroalgal species based on the presence and absence in the milkfish native range was assessed.

1.2 Problem Statement

Presently, the available literature on the interactions between marine macroalgae and herbivores based on macroalgal nutritional quality, novelty and morphology, is still scattered and fragmentary. It is also important to note that the nutritional characteristics in marine macroalgae that attract or deter their grazers have not been well-addressed. With regard to improving success rate of biological control of macroalgal invasions, it is essential to enhance the understanding of the marine ecosystem dynamics, which are primarily driven by the co-evolutionary interaction between marine macroalgae and herbivores. The current study delved into the value of nutritional characteristics of marine macroalgae and also other possible factors such as their novelty and morphology to predict the feeding preferences of a generalist herbivore. This study also aimed to determine which macroalgal characteristics that have significant influence over herbivore's feeding preferences.

1.3 Research Objective and Hypothesis

1.3.1 Objectives

i. To investigate the effects of macroalgal nutritional characteristics on the relative consumption rate (RCR) of a generalist herbivore.

ii. To determine which macroalgal nutritional characteristics pose the most significant influence on herbivore feeding preferences.

iii. To study the relationships between macroalgal nutritional characteristics and other factors (novelty and morphology) in determining herbivore feeding preferences.

1.3.2 Hypotheses

i. There is a causal relationship between herbivore feeding preferences and nutritional characteristics of marine macroalgae.
ii. Total nitrogen and total phenolic contents have the most influence on herbivore feeding preferences.
iii. The feeding preference of herbivore is affected by multiple factors, including nutritional quality, novelty and morphology of the marine macroalgae.

CHAPTER 2: LITERATURE REVIEW

2.1 Species Invasions: A Growing Threat to Marine Ecosystems

Biological invasions are considered one of the most severe threats to global biodiversity. Over the last 20 years, the observed rates and effects of invasions have increased dramatically in marine systems, and in most cases, macroalgal species responsible for a large percentage of invaders due to their ability to alter ecosystem structure by dominating primary space (Krueger-Hadfield, 2020; Low et al., 2015). Such increases are often attributed to human activities. Globalization, the transformation of technological regimes, and expansions of transportation networks that modify the marine habitats are some of the recognized drivers behind the rapid shifting of non-native macroalgal species across a broad geographical range (Pyšek et al., 2012; Richardson et al., 2007). As compared to terrestrial systems, the community structure of marine systems is much vulnerable to the impacts of macroalgal invasions due to its openness and greater primary producer turnover (Kimbro et al., 2013). Human activities such as shipping, aquaculture, construction of a canal, and climate change provide increasing opportunities for non-native macroalgal species to be transferred, introduced, and finally established in new environments (Copp et al., 2016; Rahel & Olden, 2008; Seebens et al., 2013). Future threats caused by macroalgal invasions would be greatest in emerging economics due to these factors (Seebens et al., 2015).

The invasion of non-native species is an important factor affecting biodiversity on a global scale (Castells & Berenbaum, 2008). Equally well-known is that Southeast Asia considered the important hotspot harbors a large proportion of biological diversity globally. Such rich reservoirs of biodiversity are spread across this biogeographical region that comprises of Burma, Cambodia, Laos, Malaysia, Thailand, Vietnam as well as insular countries such as Brunei, Indonesia, Singapore, East Malaysia, and the

Philippines (Peh, 2010). However, the number of species threatened with extinction in Southeast Asian tropical ecosystems far outstrips available conservation resources (Kier et al., 2009; Myers et al., 2000). At present, there are several examples of species invasions in tropical Southeast Asia, and evidence suggests that species invasions are a significant component of native biota displacements in Southeast Asia. For example, the introduction of golden apple snail *Pomacea canaliculata* has resulted in an almost complete collapse of the aquatic autotroph community in the wetlands of Southeast Asia (Scheffer et al., 1993). In Thailand, the occurrence of non-native snail has decreased the richness of native autotrophs in the wetlands due to the absence of native predators (Carlsson et al., 2004). Apart from the high cost of such species expulsions, invasion of non-native species has also been proven to initiate and accelerate the process of ecosystem modification. Both losses of native species and addition of non-native species may have dramatic effects on ecosystem functioning by affecting assemblages of ecological traits between species, and thereby, alter the ecosystem processes (Carlsson et al., 2004). Probably, the best-known biological invasion that can potentially modify the marine ecosystem was observed in a study of near in Peninsular Malaysia, where the invasion by non-native fish species was observed to alter the process of marine regeneration through changing the structure of native ichthyo-fauna groups in the natural aquatic habitats (Rahim et al., 2013).

Species invasions can be hard to predict, given that the likelihood of a particular non-native species successfully spreading and persisting in an invaded environment depends on the interactions of both abiotic and biotic factors (Kinney et al., 2019; Mitchell et al., 2006; Pearson et al., 2018). For example, the non-native species are expected to persist and increase impact when the climate of the incumbent ecosystem matches the climate of the native range (Capinha et al., 2013; Iacarella et al., 2015). This highlighted that climate-mediated changes in biotic interactions may play a central role in altering the ecological impacts of non-native species. Moreover, in a new range of environments, the establishment and spread of non-native populations have been predicted by the interactions with native community assemblage, which either facilitate or prevent via biotic resistance (DeRivera et al., 2005). Biotic resistance of the native community towards invasions can be exhibited in both consumptive and competition manners, but, in most cases, the strength of consumptive resistance is significantly stronger than competitive resistance (Alofs & Jackson, 2014; Cebrian & Lartigue, 2004; Stanley, 2008). This difference matches well with the observation that there is more predation than species competition in addressing the marine environments (Alofs & Jackson, 2014). When compared to aquatic or terrestrial environments, benthic marine systems show a higher occurrence of herbivore feeding leading to a lack of intraspecific and interspecific competition (South et al., 2020). Despite this, most studies of invasions provide evidence that the synergies between marine herbivores and various autotrophs could reduce or prevent the detrimental impacts of species invasions (Grutters et al., 2017; Parker et al., 2006). The establishment of the non-native green alga *Codium fragile*, for instance, was enhanced by sea urchin food preference for kelps under increased water temperature and wave action, leading to increased herbivore pressure on local kelp stands (Lyons & Scheibling, 2008). Moreover, generalist marine herbivores such as sea urchins and fishes that feed on autotrophs are common biological control agents that suppress the establishment and abundance of non-native species in the recipient communities (Hay, 1991; Hokkanen & Pimentel, 1989; Seastedt, 2015; Tomas et al., 2011). Thus, it is essential to incorporate mechanisms of biotic resistance by herbivory into predictions of ecological impact conferred by non-native-species.

2.2 Interactions Between Marine Autotrophs and Herbivores

Marine herbivore constantly depends on macroalgae for food and habitats, suggesting that persistent interactions between marine herbivores and their prey (Molis et al., 2008).

In both terrestrial and aquatic ecosystems, the autotroph-herbivore interactions have thus far displayed a dynamic evolutionary history. When introduced non-native autotrophs encounter a group of non-coevolved herbivores in their invasive range, the evolutionary novelty would be a key aspect of these interactions. In marine systems, the interaction between marine autotrophs and herbivores plays a dominant role in structuring marine ecosystems, promoting coral recovery, cycling nutrients, and materials, as well as determining the success of invasions (Ledlie et al., 2007; Traill et al., 2010; Vergés et al., 2014). With the entry of non-native species, a mismatch between autotroph defence traits and herbivore virulence traits is likely to occur. While many non-native autotrophs have been reported to have better intricate defence mechanisms to reduce the impact of herbivory, many native herbivores have evolved countermeasures to outwit these mechanisms (Burkepile & Parker, 2017; de Vries et al., 2017). Many studies have found that the preference of native herbivores was observed, whether to consume native or non-native autotrophs, may lie beyond the evolutionary novelty theory which consists of two dominant hypotheses: (1) enemy release hypothesis (ERH) which posits that herbivores are maladapted to consume non-native autotrophs; and (2) biotic resistance hypothesis (BRH) which posits that non-native autotrophs are maladapted to fend off herbivores (Grutters et al., 2017; Maron & Vila, 2001). Because non-native autotrophs do not share an evolutionary history with the native herbivore, this suggests that the feeding preference of herbivores can be hard to predict (Cogni, 2010). Nonetheless, a recent study conducted by Grutters et al. (2017) explained that mechanisms underlying autotroph palatability could be used to explain and resolve the inconsistent results of novelty.

The response of an herbivore to a non-native autotroph depends on features including behaviour (preference for host autotrophs), phenology, and physiological adaptation related to autotroph characteristics that affect the herbivore’s performances such as growth, survival, and reproduction (Duarte et al., 2010; Johnson et al., 2017). However, the preference-performance correlation has not been observed consistently in some marine herbivores, notably mesograzers (Taylor & Brown, 2006; Williamson & Steinberg, 2012). For example, using agal foods varying in nutritional content, Machado et al. (2018) showed that the generalist amphipod *Ampithoe valida* did not have better performance on preferred macroalgae (*Ulva* sp.) than other less preferred macroalgae (*Padina* sp. and *Egregia* sp.). On the other hand, the response to a host autotroph is likely to differ between generalist and specialist herbivores as the autotrophs varying their defence strategy according to variation in herbivory risk (Joshi & Vrieling, 2005). For example, during invasions, non-native autotrophs are likely to be escaped suppression from co-evolved specialist herbivores and at the same encounter strong biotic resistance from generalist herbivores in their native ranges (Maron & Vila, 2001). This may lead to the evolution of resistance against herbivore in non-native autotrophs, where the non-native autotrophs decreased their defence against specialist herbivore and increased defence against generalist herbivore (Z. Zhang et al., 2018).

The enemy release hypothesis (ERH) is widely-cited explanation for invasion success, stated that the success of non-native species is caused by reduced enemy pressure in their invasive range (Prior et al., 2015). Based on ERH theory, native herbivores will be better adapted to consume native than non-native autotrophs due to co-evolutionary history and thus lead to an increase in distribution and abundance of the non-native autotrophs in their invasive range (Keane & Crawley, 2002; Lankau et al., 2004). However, this assumption ignores the possibility that non-native autotrophs may lack effective defences to deter native herbivores as they share no evolutionary history with these natural consumers in their new invaded range (Colautti et al., 2004; Parker & Hay, 2005). There are two reasons why non-native autotrophs are more susceptible than native autotrophs to the impacts of native herbivores. First, the genetic diversity of non-native species could reduce by probable invasion bottlenecks through an invading subsample of the original population, followed by a rapid range expansion (Colautti et al., 2004; Golani et al., 2007; Sax et al., 2005). Correspondingly, native enemies will have disproportionate impacts on populations of non-native species as compared to more genetically diverse native groups (Colautti et al., 2004). Second and more importantly, non-native species represent native targeted hosts to native enemies, which can result in novel and often serious instances of attack (e.g. the new association or increased susceptibility principles) (Hokkanen & Pimentel, 1989). If these two possibilities are true, native herbivore has great potential to control invasion since non-native autotrophs are subjected to greater herbivory effects than native autotrophs, as proposed by the biotic resistance hypothesis (Maron & Vila, 2001).

Paradoxically, evolutionary novelty can also be argued to suppress species invasions (Lind & Parker, 2010). From both theoretical and applied standpoint, the role of biotic resistance in controlling the invasion success of non-native species is interesting and it has received considerable attention in invasion literature (Levine et al., 2004). Invasions empirically test community assembly and coexistence theory (Godoy, 2019; Pearson et al., 2018), and communities containing a great number of native species that are interested in community restoration will be the most resistant to invasions (Funk et al., 2008). As suggested by the biotic resistance hypothesis (Elton, 2020), species-rich communities, which are more likely to harbor strong native competitors and predators, would be more resistant to invasion than species-poor communities (Michelan et al., 2013; Ricciardi, 2001). Thus, evidence in support of this hypothesis would suggest that promoting the abundance of native species is an effective strategy to limit the non-native establishment and thereby contributing to decreasing in the number of invasions (Beaury et al., 2020). However, biotic resistance from generalist herbivore in controlling autotroph invasions has received relatively less attention, perhaps in part because the responses of generalist herbivore to non-native species are comparable in both the native and invasive ranges (Schaffner et al., 2011).

Biotic resistance from generalist herbivore to autotroph invasions has the potential to be substantial, especially in marine systems. This is because marine communities harbor a high proportion of generalist herbivores that could serve as a source of biotic resistance against autotroph invasions (Anton et al., 2019; Cebrian et al., 2011). Although generalist herbivores feeding on a mixture of autotroph species in their diets, they also exert intense grazing pressure that can substantially transform the composition and diversity of native autotroph communities (Strauss et al., 2009; Wikström et al., 2006). Given this, it is reasonable to predict that native generalist herbivore may similarly affect the invasion success of non-native. Many studies indicated that the effect of generalist herbivores on non-native species has differed from that of specialist herbivores. As relative to specialist, generalist herbivores are more likely to consume non-native autotrophs in their native range and thereby potentially contributing to outcomes of invasions (Parker & Hay, 2005). Previous studies have shown that native generalist herbivore can provide direct biotic resistance across various ecosystem types by feeding on non-native autotroph species, such as in grasslands (Slate et al., 2019), salt marsh (Ning et al., 2019), wetlands (Zhang et al., 2019), coastal dunes (Cushman et al., 2011), freshwater (Petruzzella et al., 2020) and also marine ecosystems (Cebrian et al., 2011). However, the ability of a generalist herbivore to switch from a native to non-native host autotroph depends on the geographical distribution of autotroph, as well as the structural and chemical similarities between its native and non-native host autotrophs (Castells & Berenbaum, 2008).

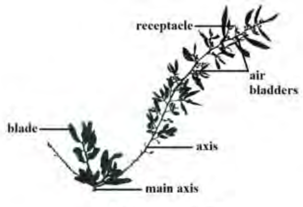
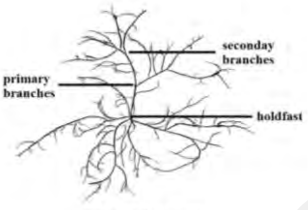
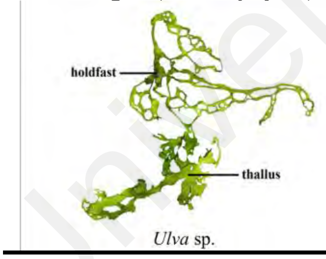
2.3 Marine Macroalgae

The feeding preference of marine herbivores depends primarily on several macroalgal characteristics and their interactions (Pennings et al., 2001). Characteristics including chemical repellents, morphological defences, and nutritional constituents, such as nitrogen content and phosphorus content, are reported to influence preference and avoidance of certain marine macroalgae by marine herbivores (Elger & Willby, 2003; Jiménez-Ramos et al., 2018). Unlike terrestrial autotrophs, most submerged aquatic autotrophs have a higher internal concentration of nutrient-rich compounds and lower concentrations of lignin and other hardy structural compounds. On this basis, aquatic autotrophs would promote herbivore metabolism and growth, and thereby leads to higher herbivory rates and larger autotroph biomass consumed by herbivores (Cebrian et al., 2009). In marine systems, there is a wide variety of species in the submerged autotrophs, predominantly macroalgae (or seaweeds). Marine macroalgae are classified into three main groups, which include Rhodophytes (red algae), Chlorophytes (green algae), and Phaeophytes (brown algae). They are generally found from intertidal to shallow subtidal zones and can be many different shapes, sizes, colors and composition (Makkar et al., 2016). A set of specific characteristics are used to classify marine macroalgae, including chloroplast pigmentation, the nature of photosynthesis products, the organization of cell membranes, and other morphological and nutritional features (Bocanegra et al., 2009) (Table 2.1).

2.3.1 Feeding Preferences Related to the Biochemical Composition of Marine Macroalgae

Marine macroalgae differ greatly from terrestrial vascular autotrophs in that the biochemical composition of macroalgae can be highly ranged between divisions, genus and species (Wan et al., 2019), with their habitat, season and age (Marinho-Soriano et al., 2006; Serviere-Zaragoza et al., 2002). Generally, brown algae are of lesser nutritional value than red and green algae, due to their low protein content and a higher content of low digestible refractory components, such as phlorotannins (Harnedy & FitzGerald, 2011; Makkar et al., 2016). In contrast, the red and green algae that appeared to have relatively high protein content are potentially more readily consumed by herbivores (Adin & Riera, 2003). Given the macroalgae can differ greatly in their nutritional quality, some studies suggested that marine herbivores feed selectively on macroalgae and macroalgal parts of high nutritional value to fulfil their nutritional requirements.

Table 2.1: The classification of three major groups of marine macroalgae, which includes their unique physical and chemical autotroph traits, as well as their ecological functional role in marine ecosystems

Marine macroalgal group	Morphology	Photosynthetic pigments	Cell wall components	Ecological role in the marine environment
Red algae (Rhodophytes)  *Sargassum* spp.	Eukaryotic; multicellular	Chlorophyll *a*, *b*, carotenoids, and phycobilins (phycocyanin & phycoerythrin)	Agar, carrageenan, cellulose, and calcium carbonate in coralline algae	Primary producers; as an important source of calcareous deposits in coral reefs
Brown algae (Phaeophytes)  *Gracilaria* sp.	Eukaryotic; multicellular	Chlorophyll a, c, carotenoids, and xanthophylls	Cellulose and alginates	Primary producers; dominant components of kelp forests
Green algae (Chlorophytes)  *Ulva* sp.	Eukaryotic; unicellular	Chlorophyll a, b, and carotenoids	Cellulose, and calcium carbonate in calcareous algae	Primary producers; as important sources of calcareous deposits in coral reefs

In the marine chemical literature, nitrogen is traditionally regarded as nutritional essential not only for macroalgal production, but also for herbivores to meet requirements for maintenance, growth, development, and health (Hou et al., 2015; Mattson, 1980). One approach for a marine herbivore to increase its nitrogen intake is to preferentially consume macroalgae and macroalgal parts with higher nitrogen value. According to this, recent evidence suggests that marine herbivores select for macroalgae with elevated nitrogen content (Barile et al., 2004; Chan et al., 2012; Cruz-Rivera & Hay, 2003). For example, the findings of Van Alstyne et al. (2009) provide evidence that marine littorinid snail, *Littorina sitkana*, preferentially selects nitrogen-rich macroalgae, although these foods contain chemical defences. More recently, Shantz et al. (2017) studying the feeding behaviour of herbivorous coral reef fishes found that, in both mechanistic experiments and observational field studies, macroalgae with higher nitrogen and phosphorus were significantly preferred by all four species of herbivorous fishes, including three parrotfish species (*Sparisoma aurofrenatum*, *Sparisoma rubripinne*, and *Sparisoma chrysopterum*) and one surgeonfish species (*Acanthurus coeruleus*). However, overconsumption of nitrogen-rich macroalgae can impose additional energetic cost, both in terms of energy and time spent foraging (Catano et al., 2016), as well as the physiological costs of excreting excess nutrient to maintain homeostatic balance (Boersma & Elser, 2006). Therefore, marine herbivores should be able to select their autotroph foods which most closely match their nutritional needs (Meunier et al., 2016; Sterner & Elser, 2002).

The nutritional characteristics, other than total nitrogen, that determine the quality of an animal diet include organic matter, total carbon, and total phosphorus contents. Several studies measured the autotroph nutritional quality in the form of total nitrogen, total carbon, and total phosphorus as they are the most important structural and functional elements in organisms (He et al., 2006; Hillebrand et al., 2009; Lapointe et al., 2004; Sterner & Elser, 2002; Zhang et al., 2011). Providing phosphorus to herbivore is critical for their growth and bone mineralization (Sterner & Elser, 2002), as phosphorus is a determinant component of nucleic acids, membrane phospholipids, and many intermediary metabolites (Herrera-Estrella & López-Arredondo, 2016). Because of its important roles in several energy-dependent metabolic processes, herbivores often adjust their foraging effort to resources rich in phosphorus. For example, Boyer et al. (2004) found that elevated tissue nitrogen and phosphorus contents of macroalgae *Acanthophora* *spicifera* led to significantly increased fish grazing across multiple habitats, especially in the mangrove. Similar preferences for marine autotrophs with high nitrogen and phosphorus contents have been suggested for other marine herbivores, include green turtles (*Chelonia mydas*), dugongs (*Dugong dugon*), and teleosts (*Pelates octolineatus* and *Pelates sexlineatus*) (Burkholder et al., 2012). Moreover, some studies have demonstrated how the growth of marine herbivores, notably phosphorus-demanding species, for example, maybe constrained with the diets with low phosphorus content (Capps & Flecker, 2013). Phosphorus limitation of growth may be common for herbivorous fishes because of the body phosphorus concentration higher than all but the most phosphorus-rich macroalgae (Hood et al., 2005; McIntyre & Flecker, 2010).

Apart from that, dry matter content (DMC) could be another nutritional quality that influences the consumption rate of marine herbivores. For example, Cruz-Rivera and Hay (2001) found that the DMC of marine macroalgae was negatively correlated with marine amphipod *Ampithoe longimana* consumption rate, and Elger and Willby (2003) reported similar results. As an increase in herbivore consumption of macroalgae with low organic matter, they attributed this pattern to compensatory feeding, suggest that palatability differences in macroalgae may drive herbivores to compensate for the low nutritional quality of macroalgae. The ability of DMC to explain variations in macroalgae palatability was also supported by a recent study conducted by Paz et al. (2019), who found that there was a highly significant inverse correlation between the consumption rate of aquatic apple snail *Pomacea canaliculate* and DMC of three macroalgal species. In most cases, there was a strong positive correlation between DMC and thallus toughness for tropical macroalgal species, probably because of the thallus constituents such as lignin, fiber, and silica contents, which contribute to thallus toughness, have a transparent link to DMC (Elger & Willby, 2003; Kergunteuil et al., 2018). Since thallus toughness is an important determinant of macroalgal palatability to marine herbivores (Taylor et al., 2002), macroalgae with higher DMC may therefore lead to a decrease in herbivore consumption rate (Elger & Lemoine, 2005; Wong et al., 2010).

Nonetheless, the body stoichiometry for every consumer is somewhat different and chemical composition can differ greatly between closely-related species, suggesting potentially strong interspecific variation in foraging based on stoichiometry (Shantz et al., 2017; Vanni et al., 2002). As individuals age, variation in growth and metabolic rates, which require different ratios of nutrients and energy, likewise leads to selective feeding to encounter varying nutrient intake targets (Moody et al., 2018). For example, more rapidly growing fishes, mostly those in juvenile life-stages, often exhibit elevated phosphorus of mass gain due to the abundance of ribosomal RNA needed to support rapid growth rates, but the proportion of phosphorus drops as growth rates declines (Elser et al., 2003). In a similar vein, Shantz et al. (2017) found that the influence of algal nutrient content on foraging efforts declined with increasing fish size, such that full-grown fishes did not respond to assay nutrient content. Because fish have the highest inter-taxonomic differences in body stoichiometry among vertebrates, different species are likely to forage nutrients in different proportions.

2.3.2 Evolutionary Strategies of Anti-Herbivore Defence in Marine Macroalgae

Given the strong top-down pressure of marine communities (Burkepile & Hay, 2006; Shurin et al., 2005) and the high intensity of predation, it is not surprising that marine benthic autotrophs have evolved a wide array of defence strategies to minimize damage by herbivores (Van Donk et al., 2011). Defences of macroalgae against marine herbivores come in many forms, including morphological and chemical features that either directly deter, hinder or intoxicate the herbivores. In tropical waters where the herbivore diversity is higher, macroalgal species with lower susceptibility to herbivores often use both morphological and chemical defences against herbivores (Rodríguez et al., 2017;

Sakanishi et al., 2020; Van Alstyne et al., 2001). At first, macroalgal defences were initially presumed to be constitutive, which is always expressed in the plant. However, this prediction has evolved based on evidence that many defences are only expressed in response to herbivore attack (Van Donk et al., 2011). Similar to those in terrestrial autotrophs, induction of specific chemical defences against herbivory have also been found in marine macroalgae (Amsler, 2001). Thus far, since 2001 more studies on macroalgal metabolites potentially responsible for induced defence have concentrated on brown and red macroalgae, upon which herbivores has been shown to trigger the defence (production of secondary metabolites like phlorotannins) by damaging the macroalgae (Haavisto et al., 2010; Jormalainen & Ramsay, 2009; Lüder & Clayton, 2004; Molis et al., 2006; Toth et al., 2005).

Many tropical macroalgae produce a variety of chemical compounds, traditionally in the form of secondary metabolites, that facilitate the vital role of chemical defence against herbivore and pathogens (Nylund et al., 2013). These chemical compounds, including terpenes, phenolics, acerogenins, amino acid-derived, and halogenated compounds, were broadly assumed to act as chemical defences due to the negative relationship between the preference by herbivores and the presence of secondary metabolites recorded in the chemical literature (Pereira & da Gama, 2008). Yet, the concentration and composition of secondary metabolites can be differed greatly among spatial and temporal scales, including among types of tissues in an individual (Erickson et al., 2006; Hartmann, 2007; Theis & Lerdau, 2003). For example, fucoids have higher polyphenolic content than laminarians and the content of polyphenol is higher in meristems than in non-meristematic vegetative tissues for both fucoids and laminarians (Van Alstyne et al., 2001). Moreover, variation in polyphenol contents may also occur at temporal scales from few days to weeks in response to various cues, such as grazing pressure or different environmental factors (Cacabelos et al., 2010; Rohde et al., 2004). For most tropical macroalgae, the majority of deterrent metabolites are terpenoids, followed by acerogenins (acetate-derived compounds) and mixed biosynthetic origin (meroterpenoids) (Pereira & da Gama, 2008; Soares et al., 2015). However, it is important to emphasize that many chemical defences are only effective to deter certain marine herbivores, suggesting some herbivores may have physiological mechanisms that allow them to cope with noxious (e.g. unpalatable or toxic) compounds in their diets. For example, previous studies have shown that small marine herbivores (termed mesograzers), which utilize individual macroalgae as both habitat and foods, can inhabit and consume macroalgae that are chemically defended against larger grazers, such as sea urchins and fishes (Taylor & Steinberg, 2005; Van Alstyne et al., 2009). Since mesograzers are less mobile than larger grazers and depend on macroalgae for shelter as well as foods, they may evolve tolerance to chemically defended macroalgae that deter larger grazers, to obtain "enemy-free space" (Lasley-Rasher et al., 2011).

In some cases, palatability among macroalgae seems to be related to morphological defences but not to their nutritional quality. For example, in a recent study of ontogenetic changes in macroalgal resistance to herbivory, the nutritional quality of algae species was much less effective than the morphological defence in determining the feeding behaviour of sea urchins *Diadema africanum* and *Paracentrotus lividus* (Rodríguez et al., 2017). Morphological defences such as calcification and toughness are common in certain macroalgal species, which have been studied extensively, include a high proportion of carbon-rich structural compounds that strengthen cell walls, and thus provide a significant barrier to herbivores (Sakanishi et al., 2020). In tropical waters, marine macroalgae come in a great gradient of morphological forms, ranging from small filamentous, encrusting coralline species, to large canopy-forming species that adhering to the substratum (Marques et al., 2006). These different types of macroalgae often differ significantly in susceptibility to herbivory, where most herbivorous fishes prefer filamentous and turf fleshy macroalgae over calcareous coralline and encrusting species (Tolentino-Pablico et al., 2007).

2.4 Marine Herbivores

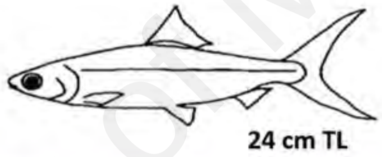
Efforts have been undertaken since the 1980s to understand the feeding behaviour of marine herbivores, especially the generalists (Behmer et al., 2002; Martinez et al., 2016; Senft et al., 1987; Wahl & Hay, 1995). In many instances, generalist herbivores tend to make their feeding decision based on autotroph palatability that depends largely on some unique attributes such as secondary metabolites, morphology, and physical stress (Chavanich & Harris, 2002; Jormalainen et al., 2001; Molis et al., 2015; Pennings et al., 2001; Rosenthal & Berenbaum, 2012; Sudatti et al., 2018). Although significant research has been done to elucidate the role of secondary metabolites in the survival and adaptation of autotrophs, little attention has been paid to understand the value and significance of their other attributes that may also influence the preferences of herbivore (Grutters et al., 2017; Ianora et al., 2006; Maschek & Baker, 2008; Wink, 2003). In several feeding assays, nutritional quality (measured as protein, nitrogen, and organic carbon content) of macroalgae has been shown to influence herbivore feeding preference, growth, and performance (Aquilino et al., 2012; Barile et al., 2004; Cruz-Rivera & Hay, 2003; Duarte et al., 2010; Taylor & Brown, 2006). Selective feeding, compensatory feeding, and diet mixing were thus reported as feeding strategies by herbivores aim to optimize their intake of energy, or limiting nutrients, to fulfill their fitness (Cruz-Rivera & Hay, 2003; Duarte et al., 2014; Lyons & Scheibling, 2007; Machado et al., 2018). For example, some marine herbivores appeared to successfully compensate for nutritional quality by intaking more of the low-quality algal foods without suffering a decrease in survivorship and growth (Cruz-Rivera & Hay, 2003). By selectively removing some macroalgae or avoiding others, herbivores, therefore, exert a profound impact on the biological structure of many marine ecosystems (Bakker et al., 2006; Fields et al., 2003; Schmitz et al., 2008; Taylor & Schiel, 2010).

Herbivorous fishes are the major consumers of tropical marine macroalgae, particularly in the undisturbed coral reef systems (Hughes et al., 2007; Mumby, 2006). As consumers of macroalgae, herbivorous fishes directly affect the structure and function of marine benthic communities (Burkepile & Hay, 2008). On coral reefs, the removal of herbivorous fishes through overfishing and disease, and the subsequent degradation of those reef has emphasized the role of this group plays in benthic communities (Bellwood et al., 2004; Edwards et al., 2014; Thacker et al., 2001). Indeed, many observers have reported a reduction in herbivorous fish biomass and a consistent increase in the number of macroalgae, suggesting that intense feeding by herbivorous fishes can profoundly exert top-down control on macroalgal abundance, but the magnitude of these impacts may vary across different geographical regions (Roff & Mumby, 2012). Thus far, herbivorous fishes regarded as the single most important functional group of reef fishes, bolstering the resilience of marine ecosystem by removing upright macroalgae that negatively affect the recruitment, growth, and survivorship of corals (Diaz-Pulido & McCook, 2003; Pratchett et al., 2011; Vieira et al., 2016). Compared to other herbivore groups, herbivorous fishes do not constitute an ecologically uniform group but can be categorized into grazer and browser functional groups, based on their feeding behaviour, jaw morphology, and feeding preferences (Bellwood et al., 2004; Hoey & Bellwood, 2010; Stamoulis et al., 2017). The fishes in the grazer functional group generally feed on turfing, filamentous, and smaller thallate macroalgae, while the browser functional group feeds almost exclusively on macroalgae and associated epiphytic material (Bellwood et al., 2006; Edwards et al., 2014; Fox & Bellwood, 2008). Tada et al. (2017) discovered notable morphological differences between grazers and browsers in terms of their jaw and teeth. In grazers, jaws and opening direction of mouth were the most significantly diverse morphology, advocating that these species have adapted to consume on the various substrate.

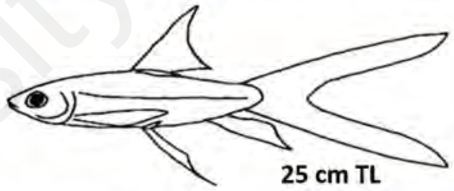
2.4.1 Milkfish

The milkfish, (*Chanos chanos* Forskål, 1775) is the only living species of the family *Chanidae* which belongs to the *Gonorynchiformes* order. The milkfish *C. chanos* is widely distributed in the tropical and subtropical Indo-Pacific Oceans, from 40° E to about 100° W and 30-40° N to 30-40° S (Bagarinao, 1994; Schuster, 1960). The first record of *C. chanos* was in the Red Sea, initially described as *Mugil chanos* by Petrus Forskål in the year 1775 (Bagarinao, 1991; Khalaf, 2005). It has spread from the Indian Ocean to other coastal areas such as those in Africa, the Pacific Ocean of Central America, Mexico, Hawaii, and southern Australia (Nelson et al., 2016). Adult *C. chanos* is characterized by a few observed external features. They have an elongated and compressed streamlined body with a large and powerful forked tail, allowing them to swim fast in open pelagic water (Bagarinao, 1994). These fishes are generally olive green in color for the dorsal, have silver flanks and dark margins around the unpaired fins. There are various morphological forms of *C. chanos* which occasionally being discovered from different regions, such as 'goldfish' type from Iloilo, Philippines and 'shad' type from Hawaii, Indonesia and Australia (Figure 2.1) (Bagarinao, 1994). Nothing is known about these variant forms of *C. chanos* since they rarely occur (Bagarinao, 1999a). In most cases, a matured *C. chanos* can have a length of up to 1.5 m, weigh up to 15 kg, and live up to 15 years (Bagarinao, 1994).

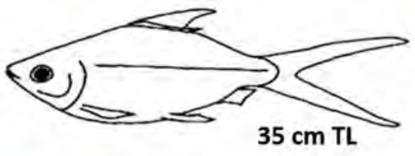
In the life cycle of the *C. chanos*, they live in different places based on their ecological requirements. Throughout the cycle, *C. chanos* spawns in the ocean and later on moves to mangrove swamps, coral lagoons, estuaries, and lakes when they are larvae and juveniles. Those habitats share the common characteristics of a sustainable supply of foods and sufficient places to find shelter (Bagarinao, 1999b). When approaching the sub-adult and adult stages, most of the *C. chanos* will return to the ocean where they feed on a diet of plankton. In terms of feeding habits, *C. chanos* are considered herbivorous with generalist tendencies (polyphagous) due to their role as substratum-feeders in shallow-water surroundings. Adult and juvenile *C. chanos* in the wild generally feed on an array of marine macroalgal species in their natural surroundings (Bagarinao, 1994; Martinez et al., 2006). Based on their jaw morphology and feeding strategy, *C. chanos* have generally been assigned to the grazer functional group which primarily comb multicellular algae from epilithic assemblages using multiple rows of similar-sized slender teeth with fork-like tricuspid tips (Hata et al., 2015).



(a) Juvenile milkfish (similar to adult)



(b) 'Goldfish' type from Philippines



(c) 'Shad' type from Indonesia

Figure 2.1: Outlines of the morphology of *C. chanos*

Fast growth, efficient use of natural foods, and high tolerance to environmental conditions make *C. chanos* the most important cultured finfish species in East and Southeast Asian countries, including Malaysia, Taiwan, Philippines, and Indonesia (Lee

& Banno, 1988; Liu & Stiling, 2006). According to the Philippine Fisheries Profile (2018), almost 303,402 metric tons (a quarter of the total aquaculture production) of *C. chanos* were harvested which were valued close to 5279 million US dollars (Borlongan & Satoh, 2001). Traditionally, *C. chanos* was cultured in brackish water-pond and freshwater pen culture (Lin et al., 2003). However, over the last 10 years in practice and research, the *C. chanos* farming has been switched to being cultured intensively in brackish water pond and marine cages (Borlongan & Satoh, 2001; Sumagaysay-Chavoso, 2003). During farming, basic knowledge on the nutrient requirement of *C. chanos* is essential to the formulation of nutritionally balanced and economical feeds that improve growth, survival, and total production of *C. chanos* (Borlongan & Satoh, 2001; Webster & Lim, 2002). Previous studies found that the minimum requirement of dietary protein for *C. chanos* growth is around 40.0 %, whereas requirements of total lipid and fatty acid are not less than 7.0 % and 1.0 % respectively in their diet intakes (Borlongan & Coloso, 1993). Like other fish, *C. chanos* require a balanced mixture of essential and non-essential amino acids. The ten essential amino acids are arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine (Borlongan & Coloso, 1993). For juvenile *C. chanos*, the limiting essential amino acids are arginine, leucine, and lysine because their concentrations in the protein's amino acid pattern are typically higher in the juvenile body as compared to adult *C. chanos* (Coloso et al., 1988).

CHAPTER 3: MATERIALS AND METHODS

3.1 Sample Collection and Experimental Design

3.1.1 Generalist Herbivore

In this study, generalist was chosen rather than specialist herbivores because generalists are more likely than specialists to feed on and therefore have stronger impacts on the autotroph community structure. Milkfish (*Chanos chanos* Forsskål), which is relatively abundant in the tropical waters of the Indo-Pacific Ocean, was used in this study because it is an herbivore with generalist tendencies (Bagarinao, 1999b). Their specialized teeth and pharyngeal mill enable them to graze efficiently on a wide variety of phytoplankton and macroalgae, including calcareous macroalgae (Olsen, 2011; Tulkani, 2017). Juvenile *C. chanos* were used in this study because of their higher rates of metabolism than adults, giving them the ability to access high nutritional quality food patches (Gotceitas & Godin, 1992; Millidine et al., 2009). The juvenile *C. chanos* individuals were collected from a commercial fish hatchery located on the northwest coast of Peninsular Malaysia (06º09'20"N, 100º34'11"E). They were transferred and kept in rectangular fiberglass tanks (i.e., artificial seawater systems) in an indoor greenhouse in Rimba Ilmu, University of Malaya (UM). During an acclimation period of two weeks, the juvenile *C. chanos* were only fed with Malaysian sea lettuce (*Ulva reticulata*) once a day and fish food pellets (Cargill, Malaysia) once a week to provide other nutrients. Previous pilots showed that there was no effect on feeding results when using lettuce and food pellets as control foods, as long as the herbivores were starved for at least 48 hours prior to the start of the feeding experiment (Duarte et al., 2011; Grutters et al., 2017; P. Zhang et al., 2018). After the acclimation period, only those with 1.4 ± 0.5 g weight and 11.0 ± 3.5 cm total length of juvenile milkfish were selected for feeding trials.

3.1.2 Marine Macroalgae

The 12 marine macroalgal species used in this study consist of those commonly found in Peninsular Malaysia: *Acanthophora muscoides*, *Amphiroa fragilissima*, *Ceratodictyon spongiosum*, *Chaetomorpha antennina*, *Cladophora prolifera*, *Cladophora sericea*, *Dictyota ciliolata*, *Gracilaria firma*, *Gracilaria salicornia*, *Padina gymnospora*, *Ulva reticulata*, and *Valoniopsis pachynema*. These species were chosen because they are relatively abundant throughout the study area, encompass all three major macroalgal groups (Rhodophytes, Phaeophytes, and Chlorophytes), and possess different morphologies. To cover a broad range of geographical regions, macroalgae were collected from six sampling sites across three coastal areas in Peninsular Malaysia (Blue Lagoon and Pantai Morib from Straits of Malacca; Merambong Shoal and Tanjung Balau from Straits of Johore; Tanjung Bukit Keluang and Pantai Kemasik from the South China Sea) to be used for feeding trials (Figure 3.1; Table 3.1).

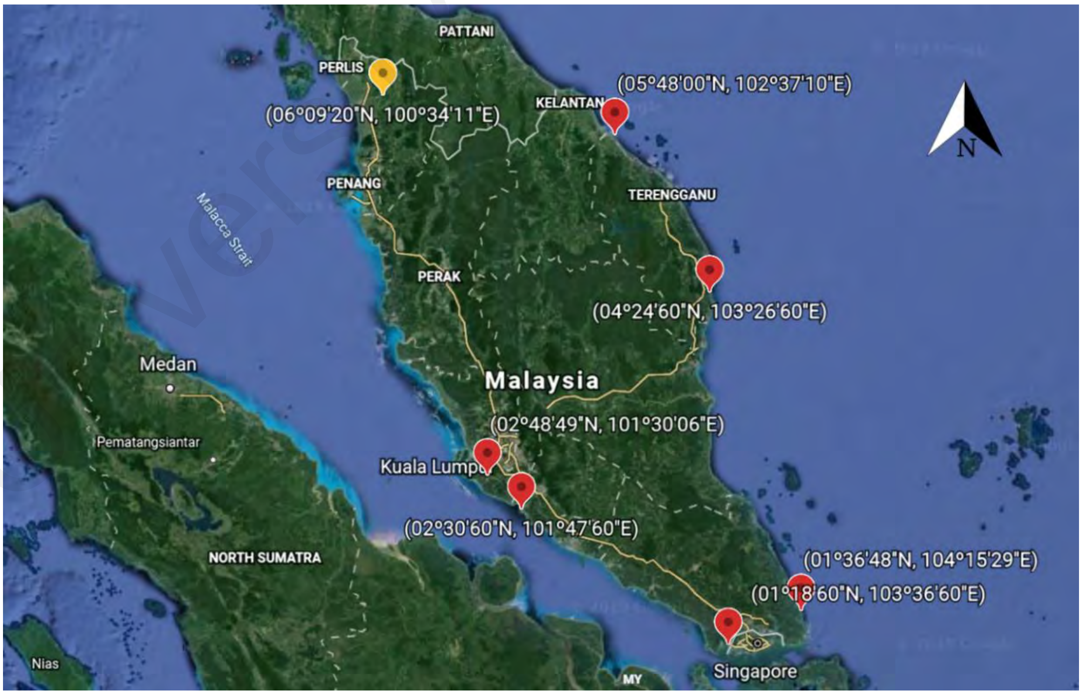


Figure 3.1: Red colour placemarks represent macroalgae's sampling sites (n=6) along the coastline of Peninsular Malaysia, whereas yellow colour placemark represent juvenile *C. chanos*'s sampling site

Table 3.1: Coastal areas and sampling sites of 12 species of macroalgae

Macroalgal species	Coastal area	Sampling site
Acanthophora muscoides (Linnaeus) Bory de Saint-Vincent	South China Sea	Tanjung Bukit Keluang, Terengganu
Amphiroa fragilissima (Linnaeus) Lamouroux	Straits of Johor	Merambong Shoal, Johore
Ceratodictyon spongiosum Zanardini	Straits of Johor	Merambong Shoal, Johore
Chaetomorpha antennina (Bory de Saint-Vincent) Kutzing	South China Sea	Tanjung Bukit Keluang, Terengganu
Cladophora prolifera (Roth) Kutzing	South China Sea	Pantai Kemasik, Terengganu
Cladophora sericea (Hudson) Kutzing	Straits of Johor	Merambong Shoal, Johore
Dictyota ciliolata Kutzing	Straits of Malacca	Blue Lagoon, Negeri Sembilan
Gracilaria firma Chang et Xia	Straits of Malacca	Pantai Morib, Selangor
Gracilaria salicornia (C. Agardh) Dawson	Straits of Malacca	Pantai Morib, Selangor
Padina gymnospora (Kutzing) Sonder	Straits of Johor	Tanjung Balau, Johore
Ulva reticulata Forsskaal	Straits of Johor	Merambong Shoal, Johore
Valoniopsis pachynema (G. Martens) Børgesen	South China Sea	Pantai Kemasik, Terengganu

Source of references: (Asmida et al., 2017; Lee et al., 2014; Muta Harah et al., 2014; Phang et al., 2010a; Phang et al., 2010b).

The collected macroalgae have represented a variety of morpho-functional groups to herbivore when employed in the feeding trials. Based on their morphology and anatomy, they can be subdivided by the following-form groups, which are filamentous macroalgae, foliose macroalgae, corticated macroalgae, corticated foliose macroalgae, corticated terete macroalgae, encrusting non-calcified macroalgae, and articulated calcareous macroalgae (Balata et al., 2011; Steneck & Dethier, 1994).

Samplings were done in intertidal and subtidal zones at low tide between September 2017 to April 2019. During sampling, most of the macroalgae were collected intertidally by hand. Only healthy-looking macroalgae with minimal epiphytes were selected for feeding experiments. The macroalgal samples were transferred to the Rimba Ilmu, UM after thorough cleansing with seawater to remove sediments. The macroalgal samples were then allocated in recirculating low nutrient artificial seawater holding tanks, kept at constant conditions of temperature (24-28°C), and light intensity (92-350 $\mu mol/m^2s$) for not more than two weeks until the feeding trials were conducted (Figure 3.2). Regarding the macroalgae, macroalgal cultivation in the open seawater creates a lot of diversity changes and may affect macroalgal species composition (Sudhakar et al., 2019). Hence, the development of closed tank system for culturing macroalgae can be essential in feeding experiments involving determination of nutritional composition of macroalgae (Mendes et al., 2012).

Figure 3.2: Macroalgae were kept separately in the artificial seawater tanks according to their species after a process of sorting

3.2 Experimental Design

Artificial seawater tanks were set up in Rimba Ilmu, UM to store both juvenile *C. chanos* and macroalgal samples in preparation for feeding trials. Figure 3.3 shows the experimental design of artificial seawater tanks (100 L) and each tank consisted of six rectangular separated containers (7 L). The use of separate tanks had the benefit of restricting potential cross-contamination between different treatments. These tanks were equipped with a mechanical filtration system with appropriate water quality levels of 30 ppt salinity, 7-8 pH, and 24-28°C temperature, monitored with a multiparameter YSI-Pro Plus (YSI, USA) (Ask & Azanza, 2002; Bhatnagar & Devi, 2013). These water quality levels were adjusted according to natural seawater conditions at the macroalgal sampling sites to avoid salinity and temperature stress (Joly et al., 2007). Every two days, total ammonia, nitrates, and nitrites were measured with a colorimetric API saltwater test kit. A protein skimmer was added in the tanks to remove the excess organic nutrients and wastes from water. The containers within the tanks were covered with individual mesh to avoid the fish jumping out from tanks (Figure 3.4).

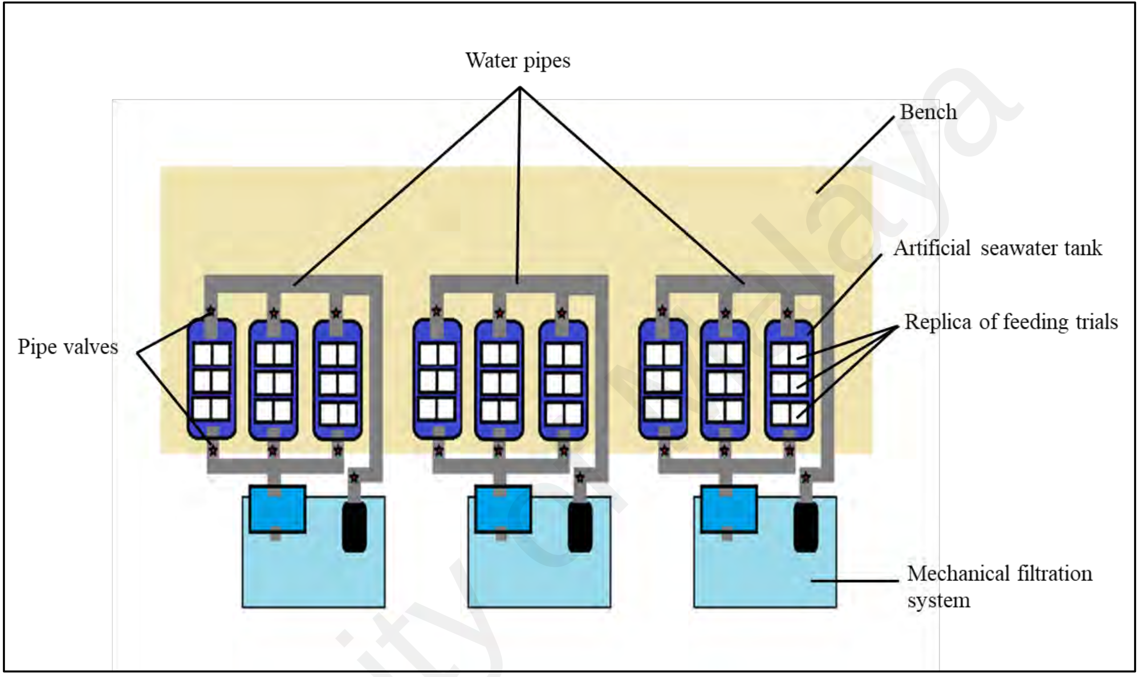


Figure 3.3: Experimental design of tanks (with labels) for replicate feeding trials

Figure 3.4: The setup of artificial seawater tanks in Rimba Ilmu, the University of Malaya in preparation for feeding trials

3.3 Feeding Trials

A no-choice feeding trial was employed in this study because this approach provides the flexibility to determine compensatory feeding in juvenile *C. chanos* (Duarte et al., 2010; Elger & Barrat-Segretain, 2002; Grutters et al., 2017). Based on established protocols, 48 hours of no-choice feeding trials were conducted for all macroalgal species in triplicate (Burlakova et al., 2009; Elger & Barrat-Segretain, 2002). All the juvenile *C. chanos* were starved for 48 hours before the trials. Three fish with almost same body weight (1.4 ± 0.5 g) were grouped and placed into each experimental unit and randomly fed with each macroalgal species. Fresh fragments from the tested macroalgal species were blotted dry, weighed ($FW_{algae:initial}$), and offered to starved *C. chanos*. The amount of macroalgal fragments that were offered to juvenile *C. chanos* was standardized based on an equal volume of macroalgae, ranged from 0.6 g *Padina gymnospora* to 10.0 g *Valoniopsis pachynema*. After 48 hours, juvenile *C. chanos* from each treatment were removed from their experimental units and dried to obtain their body dry mass, which was used to express the consumption standardized for fish mass (DW_{fish}). The remaining macroalgal fragments from each treatment were then dried in an oven at 60°C for at least 72 hours before reweighing ($DW_{algae:final}$). Six tanks were remained as control treatments with herbivore-free during the feeding trial to determine initial fresh mass ($FW_{control}$) and final dry mass ($DW_{control}$) for each macroalgal species.

To calculate the relative consumption rate (RCR), the mean of initial fresh-to-final dry mass ratio of controls was used to calculate $DW_{algae:initial}$ from $FW_{algae:initial}$ (Elger & Barrat-Segretain, 2004; Grutters et al., 2017). For each macroalgal species, the linear relationship was assessed separately to calculate $DW_{algae:initial} = a \times FW_{algae:initial} + b$, where $FW_{algae:initial} = FW_{control}$. Following Elger and Barrat-Segretain (2004), the relative consumption rate (RCR) (mg g^{-1} day^{-1}) was calculated as follows: -

$$RCR = DW_{algae:initial} - DW_{algae:final} / DW_{fish} / time_{day}$$

3.4 Macroalgal Nutritional Analyses

Previous studies reported the correlation between macroalgal nutritional quality to herbivore consumption rates and it is hypothesized that nutritional characteristics such as nitrogen and phosphorus contents can affect the palatability of macroalgae to herbivores (Cronin et al., 2002; Dorenbosch & Bakker, 2011). Hence, five critical nutritional characteristics of macroalgae, including total dry matter, total nitrogen, total phosphorus, total carbon, and total phenolic contents, were evaluated in this study. Each analysis was performed in triplicate, with the average value used as the datum for each variable.

3.4.1 Total Dry Matter Content

The total dry matter content of each macroalgal species was assessed by subjecting the macroalgal samples through a series of drying procedures and ultimately a constant weight is achieved to obtain the percentage of total dry matter (%). For each macroalgal species, a minimum of five replicates of 4.0-10.0 g of fresh algae fragments was dried for at least 48 hours at 60 ℃ and then reweighted. The dried material samples were finely ground and was used to determine the total nitrogen, phosphorus, carbon, and phenolic contents.

3.4.2 Total Carbon Content

Analysis of total carbon content was performed with the CHN628 Series Elemental Determinator according to the manufacturer's protocol (LECO, Michigan, USA). The instrument automatically determined carbon content by oxidizing 1.5 mg of dried sample. After the oxidation process, the combination gases (combustion products) were then separated and determined via a thermal conductivity detector. In the combustion chamber, the oxidation process was carried out in the presence of catalyst at the nominal temperature of 950º C in a stream of pure oxygen (99.995%).

3.4.3 Total Nitrogen Content

A modified Nessler's reagent spectrophotometry method was used to analyze the total nitrogen content (Makino & Osmond, 1991). Ammonium sulphate was used as the standard compound. The Nessler's reagent was prepared by mixing 10.0 g of mercury(ll) iodine, 7.0 g of potassium iodine, and 50 ml of 20% sodium hydroxide. Concentrated sulfuric acid and saturated potassium were added separately to digest 0.1 g of sample. After digestion, 0.5 ml aliquot of the digested solution was poured into a 10.0 ml graduated test tube, followed by additions of 7.0 ml of Nessler's reagent, 0.1 ml of 50% concentrated sulfuric acid, 0.1 ml of saturated potassium persulphate, and 2.3 ml of distilled water. The reagent blank was prepared in the same way except the sample solution. The absorbance of the sample was then measured at 480 nm using a UV Mini-1240 spectrophotometer (Shimadzu, Kyoto, Japan) and the total nitrogen content was determined by comparing the absorbance value of ammonium sulphate standard to sample (see Appendix A, Figure A1). The total nitrogen content was expressed as % dry weight.

3.4.4 Total Phosphorus Content

Total phosphorus content was determined using the vanado-molybdate method with minor modifications (Legiret et al., 2013). For the assay, 0.5 g of dried sample was digested with diacid mixture (3: 1: nitric acid: perchloric acid) and the volume was made up to 50 ml with distilled water. Then, 10.0 ml of digested solution were mixed with 10.0 ml of ammonium molybdate solution. The mixed solution was transferred into a 50.0 ml volumetric flask and topped up the volume with distilled water. The absorbance was measured at 440 nm using a UV Mini-1240 spectrophotometer (Shimadzu, Kyoto, Japan). A calibration curve of phosphorus standard solution was plotted (see Appendix A, Figure A2) and used to determine the phosphorus concentration of the sample. The total phosphorus content was expressed as % dry weight.

3.4.5 Total Phenolic Content

The total phenolic content of the macroalgal samples was estimated as gallic acid equivalents (GAE), according to the Folin-Ciocalteu reagent method as described by Blainski et al. (2013), with slight modification. A 0.25 ml aliquot of sample extract (1.0 mg/ml of ethanol) was mixed with 1.25 ml of Folin-Ciocalteu reagent. After 5 minutes of dark incubation at room temperature, 1.0 ml of 7.5% sodium carbonate solution was added, and the mixture was mixed thoroughly. A control was prepared under the same condition, without adding any sample extracts. The sample was incubated at room temperature for 2 hours before obtaining the absorbance value at 760 nm using a UV Mini-1240 spectrophotometer (Shimadzu, Kyoto, Japan). The results were compared to a gallic acid calibration curve and given in µg of gallic acid equivalents (GAE) per gram dry weight.

3.5 Statistical Analysis

All data sets obtained were subjected to descriptive statistics. The normality of the distributions was tested using the Shapiro-Wilk Test. One-way analysis of variance (ANOVA) was carried out to compare means between two or more variables, followed by the Tukey’s honestly significant difference (HSD) post hoc test. The linear regression was used to evaluate the relationships among studied variables. All analyses were performed using SPSS Statistics Software Version 25 (SPSS Inc., Chicago, USA).

CHAPTER 4: RESULTS

4.1 Analysis of Consumption Rates of Juvenile *C. chanos*.

The results from the no-choice feeding trials showed that the RCR of juvenile *C. chanos* were significantly different ($P < 0.05$) when fed with different species of macroalgae (n=12) (Table 4.1; Figure 4.1).

Table 4.1: The relative consumption rates (RCR) of juvenile milkfish *C. chanos* for 12 species of tested macroalgae

Macroalgal species	RCR (mg $g^{-1}day^{-1}$)[1]
Acanthophora muscoides	37.34 ± 2.76^{bc}
Amphiroa fragilissima	29.32 ± 3.14^{bc}
Ceratodictyon spongiosum	39.80 ± 0.65^{bc}
Chaetomorpha antennina	46.85 ± 4.39^{bc}
Cladophora prolifera	78.20 ± 7.31^{a}
Cladophora sericea	50.02 ± 8.72^{b}
Dictyota ciliolata	27.49 ± 2.11^{c}
Gracilaria firma	34.67 ± 3.48^{bc}
Gracilaria salicornia	39.13 ± 0.05^{bc}
Padina gymnospora	1.74 ± 0.81^{d}
Ulva reticulata	29.37 ± 3.61^{bc}
Valoniopsis pachynema	94.04 ± 6.04^{a}

[1] Data are given as means (± SD). Means followed by the different superscript within columns do differ significantly between treatments ($P < 0.05$).

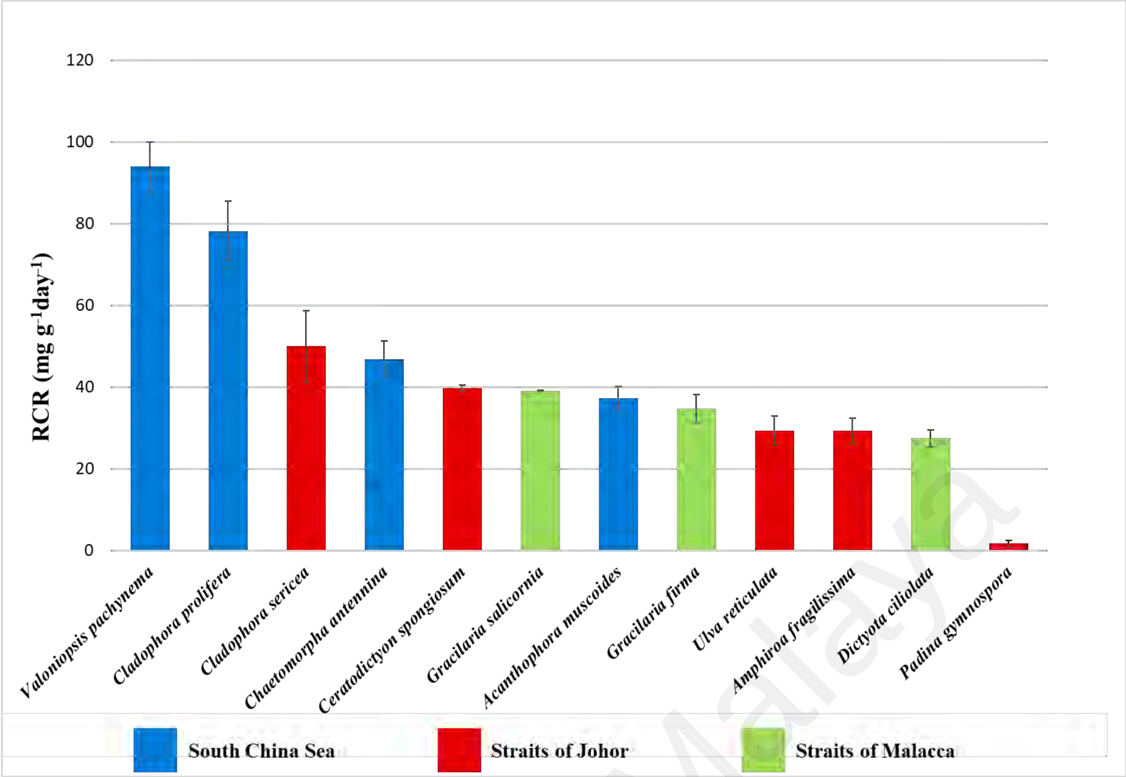


Figure 4.1: Relative consumption rate (RCR; mg $g^{-1}day^{-1}$) of juvenile milkfish *C. chanos* on tested macroalgae from three territorial waters of Malaysia. Error bars represent standard deviations.

Additionally, juvenile *C. chanos* displayed variation in RCR across the macroalgal geographical origin. *Valoniopsis pachynema* and *Cladophora prolifera*, which was collected from the coastal areas of South China Sea, were the two most consumed species, having significantly higher of RCR (94.04 mg g^{-1} day^{-1} and 78.20 mg g^{-1} day^{-1}, respectively) than other tested species (Table 4.1). On the contrary, the RCR of *Padina gymnospora* showed that it was the least consumed species, with only 1.74 mg g^{-1} day^{-1} (Table 4.1). This species was collected from coastal areas of Straits of Johor.

4.2 Analysis of Nutritional Qualities of Macroalgae

All the five nutritional values showed significant differences ($P < 0.05$) among all tested macroalgal species (Table 4.2). The content of total dry matter was highest in *Amphiroa fragilissima* (59.21%) and the lowest level was in *Ceratodictyon spongiosum* (7.82%). Total carbon and total phosphorus contents of the macroalgae ranged from

5.90% to 26.05% and 0.04% to 0.23%, respectively. The most consumed species, *V. pachynema*, recorded the lowest mean for both total carbon and total phosphorus contents. Similarly, the lowest total nitrogen content was also found in the same species, *V. pachynema* (0.69%), followed by *Acanthophora muscoides* (0.86%) as the second lowest. Going further, the least consumed species, *P. gymnospora,* recorded the highest mean for both total nitrogen content (7.55%) and total phenolic content (1261.80 µg/g). The total phenolic content for *P. gymnospora* was almost two times higher than the *C. spongiosum,* species with the second highest of total phenolic content. The total phenolic content for six species was not detected in the present study (Table 4.2), indicating that these species had total phenolic content lower than 5 µg/g and this is not uncommon in phycological research (Figueroa et al., 2014; Jerez-Martel et al., 2017; Machu et al., 2015).

Table 4.2: Nutritional value of tested macroalgae in dry mass basis (% or µg/g)[1]

Macroalgal species	Total dry matter content (%)	Total carbon content (%)	Total nitrogen content (%)	Total phosphorus content (%)	Total phenolic content (µg/g)
Acanthophora muscoides	29.74 ± 0.48^{e}	6.29 ± 0.00^{j}	0.86 ± 0.10^{g}	0.04 ± 0.00^{g}	ND[2]
Amphiroa fragilissima	59.21 ± 0.81^{a}	14.30 ± 0.00^{f}	2.36 ± 0.04^{e}	0.12 ± 0.00^{d}	ND
Ceratodictyon spongiosum	7.82 ± 0.07^{i}	11.20 ± 0.00^{h}	6.66 ± 0.04^{b}	0.06 ± 0.00^{f}	691.80 ± 0.85^{b}
Chaetomorpha antennina	19.33 ± 0.90^{fg}	19.25 ± 0.00^{c}	1.91 ± 0.05^{ef}	0.18 ± 0.00^{c}	231.90 ± 0.99^{c}
Cladophora prolifera	47.89 ± 1.87^{b}	12.40 ± 0.01^{g}	2.44 ± 0.19^{de}	0.10 ± 0.01^{e}	ND
Cladophora sericea	16.25 ± 0.58^{gh}	17.10 ± 0.00^{e}	1.63 ± 0.03^{f}	0.19 ± 0.00^{bc}	191.60 ± 1.13^{d}
Dictyota ciliolata	21.92 ± 0.79^{f}	26.05 ± 0.00^{a}	4.62 ± 0.22^{c}	0.09 ± 0.00^{e}	9.70 ± 0.14^{f}
Gracilaria firma	16.91 ± 0.03^{g}	25.31 ± 0.00^{b}	2.99 ± 0.05^{d}	0.23 ± 0.00^{a}	ND
Gracilaria salicornia	12.53 ± 0.11^{h}	19.22 ± 0.00^{d}	2.94 ± 0.11^{d}	0.19 ± 0.00^{b}	ND
Padina gymnospora	31.53 ± 0.55^{de}	9.40 ± 0.00^{i}	7.55 ± 0.09^{a}	0.13 ± 0.00^{d}	1261.80 ± 0.06^{a}
Ulva reticulata	40.98 ± 0.06^{c}	25.30 ± 0.00^{b}	6.60 ± 0.12^{b}	0.13 ± 0.00^{d}	ND
Valoniopsis pachynema	34.62 ± 0.76^{d}	5.90 ± 0.00^{k}	0.69 ± 0.04^{g}	0.04 ± 0.00^{g}	17.40 ± 0.05^{e}

[1] Data are given as means (± SD). Means followed by the different superscript within columns do differ significantly between treatments ($P < 0.05$).

[2] ND = not detected (< 5 µg/g).

A linear model was constructed to identify the parameters that best explained the RCR of the juvenile *C. chanos*. All five nutritional parameters were included in the linear model, which accounted for 50.1% of the variance in consumption rate ($R^2 = 0.501$) (Table 4.3). Among all tested parameters, total nitrogen and total phenolic contents were significant and best correlated with RCR of juvenile *C. chanos* (total nitrogen content: $F = 18.0$, $P = 0.0002$; total phenolic content: $F = 18.3$, $P = 0.0001$) (Table 4.3). In linear regression analysis, total nitrogen content showed high variance in RCR ($R^2 = 0.378$) (Figure 4.2A), followed by total phenolic content ($R^2 = 0.216$) (Figure 4.2B). Both total nitrogen and total phenolic contents were negatively, marginally significant, correlated with RCR ($P < 0.05$). This was observed when the highest total nitrogen and total phenolic contents were found in the least consumed macroalgal species (*P. gymnospora*), whereas the most consumed macroalgal species (*V. pachynema*) recorded the lowest total nitrogen content (Table 4.1; Table 4.2).

Table 4.3: Results of linear model for testing five nutritional parameters best related to juvenile milkfish *C. chanos* consumption rates[1]

Fixed effects	***F***	**Numerator d.f.**	***P* value**
Total dry matter content	0.6	1	0.4620
Total carbon content	3.1	1	0.0852
Total nitrogen content	18.0	1	**0.0002**
Total phosphorus content	2.7	1	0.1107
Total phenolic content	18.3	1	**0.0001**
		Denominator d.f.	R^2-adjusted
		30	0.501

[1] Bold value indicate statistical significance ($P < 0.05$).

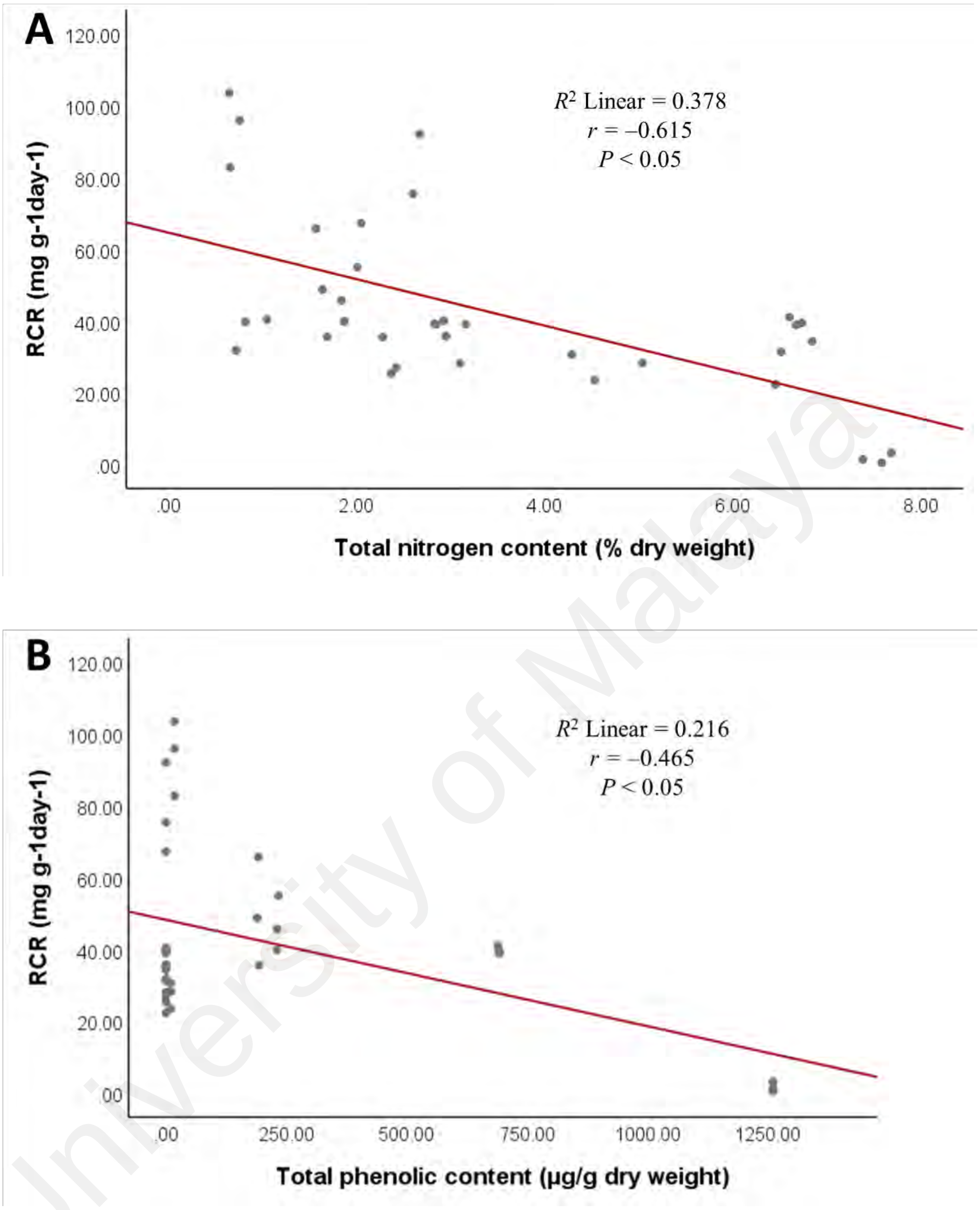


Figure 4.2: Bivariate scatter plots showing the relationships between relative consumption rate (RCR; mg $g^{-1}day^{-1}$) of the juvenile milkfish *C. chanos* with (A) total nitrogen content and (B) total phenolic content of the tested macroalgae

4.3 Relationship Analysis

4.3.1 Evolutionary novelty

It was observed the juvenile *C. chanos* less preferred to consume the macroalgal species collected from the Straits of Malacca, where the juvenile *C. chanos* was obtained. *V. pachynema* and *C. prolifera*, which collected from the South China Sea, were the two most consumed species in the present study (Figure 4.1). On the contrary, the least consumed species, *P. gymnospora* was collected from Tanjung Balau beach near the Straits of Johor, which is situated north of Singapore where *C. chanos* were reported not to be found (Bagarinao, 1994) (Figure 4.1; Figure 4.3).

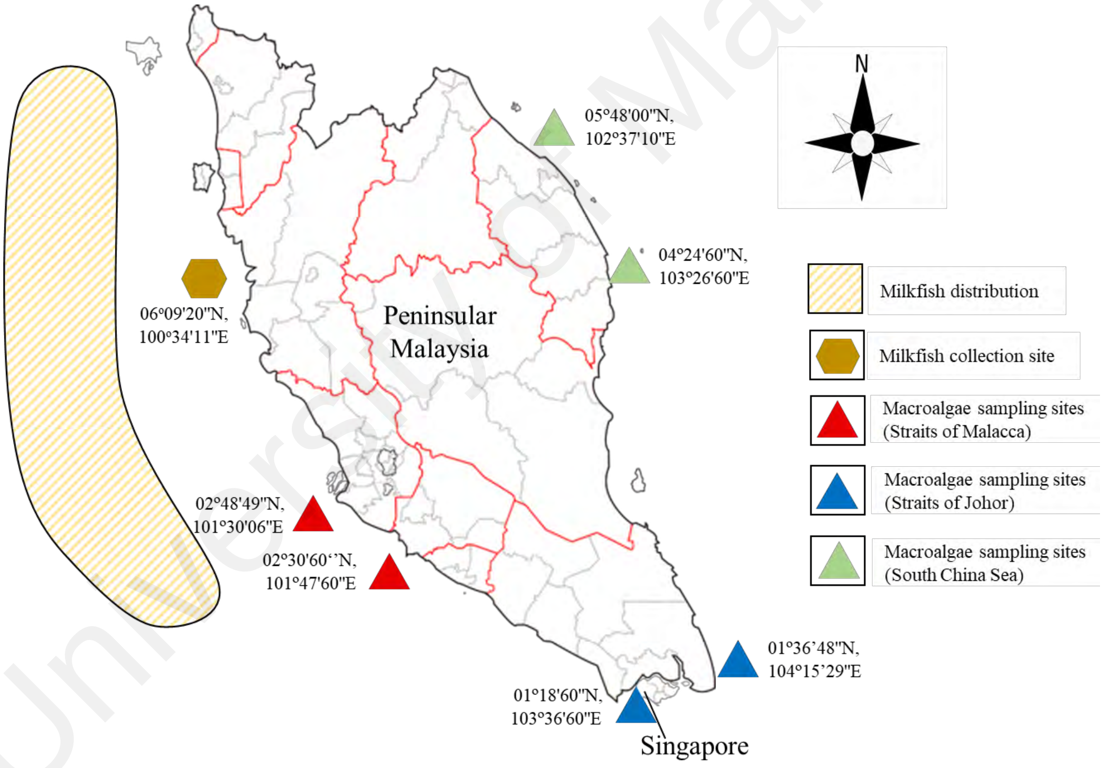


Figure 4.3: Distribution and collection site of *C. chanos* and sampling sites of macroalgae in Malaysia

4.3.2 Morphological Traits

Based on the results of RCR, all the filamentous macroalgae were consumed in greater amounts than other functional form groups of macroalgae. Two of the filamentous species

(*V. pachynema* and *C. prolifera*) were among the most consumed ($P < 0.05$) species by juvenile *C. chanos*, with a preference order of: *V. pachynema* > *C. prolifera* > *Cladophora sericea* > *Chaetomorpha antennina*. Conversely, the RCR of both corticated foliose macroalgae (*P. gymnospora* and *Dictyota ciliolata*) showed a similar magnitude and pattern that they were consumed in lesser amounts than those of other functional form groups, where *P. gymnospora* showed the lowest RCR ($P < 0.05$). Surprisingly, the most heavily calcified macroalgae (*Amphiroa fragilissima*), which expected to have the least RCR due to calcification in their thalli, was consumed within the intermediate range by the juvenile *C. chanos*. Table 4.4 shows the morpho-functional groups of all the 12 macroalgal species in this study.

Table 4.4: Morpho-functional groups of tested macroalgae, ranked by decreasing relative consumption rate (RCR) of juvenile milkfish *C. chanos*

Macroalgal species	Functional form group	References
Valoniopsis pachynema	Filamentous macroalgae	Sidik et al. (2001)
Cladophora prolifera	Filamentous macroalgae	Balata et al. (2011); Benvenuto et al. (2003)
Cladophora sericea	Filamentous macroalgae	Benvenuto et al. (2003)
Chaetomorpha antennina	Filamentous macroalgae	Vinueza et al. (2006)
Ceratodictyon spongiosum	Encrusting non-calcified macroalgae	Balata et al. (2011); Titlyanov et al. (2015)
Gracilaria salicornia	Corticated terete macroalgae	Sousa-Dias and Melo (2008)
Acanthophora muscoides	Corticated macroalgae	Lambrinidis et al. (1997)
Gracilaria firma	Corticated terete macroalgae	Beach et al. (2006)
Ulva reticulata	Foliose macroalgae	Sidik et al. (2001); Wolf et al. (2012)
Amphiroa fragilissima	Articulated calcareous macroalgae	Dolan (2001); Williams et al. (2008)
Dictyota ciliolata	Corticated foliose macroalgae	Benvenuto et al. (2003)
Padina gymnospora	Corticated foliose macroalgae	Caldeira et al. (2017); Fleury et al. (2011)

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CHAPTER 5: DISCUSSION

The synergies between autotroph and herbivore have been previously discussed for both terrestrial and aquatic environments; however, many studies have reported conflicting findings and conclusions on the effects of autotroph nutritional quality and novelty on herbivore feeding patterns (Cronin et al., 2002; Grutters et al., 2017; Raubenheimer et al., 2009). With regard to improving success rate of biological control of macroalgal invasions, it is essential to enhance the understanding of the marine ecosystem dynamics, which are primarily driven by the co-evolutionary interaction between marine macroalgae and herbivores. In Malaysia, anthropogenic activities near coastal areas such as fishing and trade have been reported as one of the major threats to marine ecosystems (Islam & Tanaka, 2004). It is evident that the coastal ecosystems are getting more vulnerable to both point source and non-point source pollution, which may lead to environmental degradation (Bakker et al., 2013; Islam & Tanaka, 2004). As environmental damage increases, numbers of macroalgal species have been observed to cross biogeographical barriers, thus establishing a series of unpredictable consequences for ecosystems functioning and evolutionary pathways (Sax et al., 2005; Simberloff, 2011). As proposed by the biotic resistance hypothesis, the success of autotroph invasions is typically hindered by native herbivores (herbivory). Nonetheless, the important characteristics of marine macroalgae that attract or deter herbivores in the coastal areas have not been well-addressed. It is, therefore, important to the interactions between the current study was carried out to predict the feeding preferences of a generalist herbivore based mainly on the nutritional characteristics of macroalgae collected from some coastal areas in Peninsular Malaysia, and to deduce if other factors such as the novelty and morphology of macroalgae also play a role in determining the herbivore feeding behaviours.

5.1 Effects of Major Characteristics of Macroalgae on the Feeding Preferences of Generalist Herbivore

The characteristics of the tested macroalgae in this study, especially their total nitrogen content, were found to influence the RCR of the juvenile *C. chanos* (Table 4.3). The highest and lowest total nitrogen content was found in the least (*P. gymnospora*) and most (*V. pachynema*) consumed macroalgal species, respectively. This is further supported by regression analysis, which revealed a strong negative correlation between the RCR and the total nitrogen content of macroalgae (Figure 4.2A). Interestingly, *V. pachynema* also recorded the lowest total phosphorus and total carbon contents (Table 4.2). These results may be explained by compensatory feeding, which is regarded as an adaptive strategy that allows herbivores to enhance nutrient intake from low-quality autotrophs (Cruz-Rivera & Hay, 2001). This strategy has been reported in several studies, where herbivores were observed to graze more on autotrophs with lower nutritional quality to maintain their growth and development (Cruz-Rivera & Hay, 2001; Duarte et al., 2010). It is important to note that compensatory feeding is necessary for generalist herbivores to maintain their populations during seasonal changes or periods when high-quality foods are unavailable, especially when generalists are less constrained that specialists in their selection of foods that have noticeable nutritional disparity (Cruz-Rivera & Hay, 2000).

Nitrogen, and more specifically protein content, has been traditionally considered to be a primary limiting nutrient to herbivore growth due to its essential role in all biological processes, such as cellular structure building and genetic coding (Angell et al., 2012; Lemoine et al., 2014; Wu et al., 2014). Furthermore, total nitrogen is much crucial for juvenile than adult herbivores in nature as they are required to fulfill an elevated total nitrogen demand, to maintain a rapid growth rate (Day et al., 2011). For example, You et al. (2014) proposed that the preferences of juvenile rabbitfish (S*iganus canaliculatus*) for macroalgae were closely related to the content of protein and soluble sugar, and such a preference may because of the high energy costs and nutrient requirements of juvenile fishes. Therefore, juvenile herbivorous are often forced to consume a protein-rich diet until their high total nitrogen requirement subsides (White, 1985). The nutritional characteristics, other than total nitrogen, that determine the quality of an animal diet include organic matter, total carbon, and total phosphorus contents. Several studies measured the autotroph nutritional quality in the form of total nitrogen, total carbon, and total phosphorus as they are the most important structural and functional elements in organisms (He et al., 2006; Hillebrand et al., 2009; Lapointe et al., 2004; Sterner & Elser, 2002; Zhang et al., 2011). As Nordhaus et al. (2011) discuss, however, the nutritional quality of preferred food is usually explained by its carbon: nitrogen ratio, assuming that marine herbivores prefer to consume autotrophs with low carbon: nitrogen ratio because of the high contributions of easily digestible energy-rich nitrogenous organic compounds.

From the regression analysis, the RCR of the herbivore was also found to be negatively correlated with the total phenolic content of macroalgae (Figure 4.2B), indicating that the juvenile *C. chanos* preferred to consume macroalgae with low phenolic compounds. Previous studies have demonstrated that the presence of substantial amounts of phenolic compounds in marine autotrophs may play a role in anti-herbivore defence (Plouguerné et al., 2006; Qiu & Kwong, 2009; Vergés et al., 2007). In general, marine autotrophs have a lower total phenolic content than most terrestrial autotrophs, although it is considered an important characteristic that may involve in defending aquatic autotrophs against pathogens and herbivores (Smolders et al., 2000). Total phenolics have been shown to be involved in both passive and active forms of autotroph defence (Vermerris & Nicholson, 2008) and their accumulation in both compatible and incompatible interactions leads to the expression of resistance of marine autotrophs to pathogens and herbivores (Latha et al., 2007; Nicholson & Hammerschmidt, 1992). They are suggested to be highly susceptible to oxidation and resulted in forming oxidized substances toxic to herbivores

(Tuominen, 2013). The defensive role of total phenolics in macroalgae can be supported by several observations conducted previously on some generalist herbivores, including freshwater fish *Scardinius erythrophthalmus* (Linnaeus) and *Ctenopharyngodon idella* (Cuvier and Valenciennes) (Dorenbosch & Bakker, 2011), snail species *Pomacea canaliculate* (Lamarck) and *Lymnaea stagnalis* (Linnaeus) (Grutters et al., 2017; Qiu & Kwong, 2009) and sea urchins *Diadema antillarum* (Phillipi) and *Strongylocentrotus droebachiensis* (Echinoidea) (Pelletreau & Muller-Parker, 2002; Tuya et al., 2001). According to Taylor et al. (2003), defensive roles of phenolic compounds were regarded as non-specific and their activity often concentration-dependent. For example, Van Alstyne and Paul (1990) showed that the total phenolic contents with concentrations of more than 2% dry weight were consistently deterred feeding by herbivorous fishes, whereas concentrations of less than 2% dry weight did not. Similarly, in the study of Pavia and Toth (2000), the increased phlorotannin content in the brown algae *Ascophyllum nodosum* was strongly correlated with increased resistance to grazing by marine gastropod *Littorina obtusata*. In the present study, the high amounts of total phenolic contents in *P. gymnospora* were comparable to those previously reported in East Malaysia (Chye et al., 2015) and therefore, the large amount of total phenolic could be broken down into phlorotannins and other secondary substances to deter herbivore feeding.

On the contrary, the total dry matter, total carbon, and total phosphorus contents did not exhibit a significant correlation with RCR in the present study. Similar results were obtained in some earlier studies, which highlighted that nutritional characteristics of autotrophs alone could merely provide an accurate prediction on herbivore preference (Paz et al., 2019; Pillans et al., 2004; Tomas et al., 2011). For example, a study conducted by Van Alstyne et al. (2001) demonstrated that the feeding preferences of marine herbivores are likely to be affected by a combined effect of physical and chemical factors. These factors would able to combine in multiple ways to affect the interactions between marine autotrophs and herbivores, not only on grazing rate but also on autotroph survival and the benefit of consumption to herbivore (Fairhead et al., 2005; Taylor et al., 2002; Wakefield & Murray, 1998). Overall, 50.1% of the variation in the strength of the herbivore consumption rate could be explained by the five nutritional parameters tested in this study. This suggests the prospects are good for a nutritional approach of marine macroalgal palatability and herbivore feeding preferences that are based on a mechanistic understanding of autotroph nutritional quality and the factors underlying herbivore consumption.

5.2 Relationships of Macroalgal Nutritional Characteristics with Other Possible Factors in Determining Herbivore Feeding Preferences

Apart from macroalgal nutritional quality, current results indicated that the morphology of the macroalgae might play an indirect role in determining the herbivore feeding preferences. Juvenile *C. chanos* consumed structurally diverse range of macroalgal species in no-choice feeding trials. The two most consumed species (*V. pachynema* and *C. prolifera*) are both filamentous macroalgae, and each of the other three tested filamentous macroalgae (*C. spongiosum, C. antennia*, and *C. sericea*) recorded an RCR of at least 43 mg $g^{-1}day^{-1}$ (Table 4.1; Table 4.4). The similar and high consumption rates shown for the different filamentous macroalgae by juvenile *C. chanos* are likely to reflect generally high feeding rates on filamentous macroalgae by herbivores in marine communities. Alternatively, these findings were in line with studies highlighting that filamentous macroalgae are generally preferred by marine herbivores over other macroalgal morphologies (Tolentino-Pablico et al., 2007; Vermeij et al., 2013). In contrast to other functional form groups of macroalgae, filamentous macroalgae can tolerate the high intensity of herbivory by investing energy in continuous rapid growth rather than costly structural and/or chemical defence (Duffy & Hay, 1990). For this reason, a trade-off relationship between productivity and thallus toughness was confirmed in filamentous macroalgae, suggesting that the high productivity of filamentous macroalgae often exhibits lower thallus toughness which could reduce the difficulty of herbivore ingestion (Sakanishi et al., 2019; Sakanishi et al., 2017). Thallus toughness has been reported as one of the primary morphological properties that could affect the feeding rates of herbivores marine invertebrate grazing herbivores, such as abalone, sea snails, and amphipods (Goecker & Kåll, 2003; McShane et al., 1994; Pennings et al., 2000). Nevertheless, the articulated calcareous species *A. fragilissima* with tough thallus was not the least consumed macroalgae in this study. This result is in line with some previous studies where some marine herbivorous fish species were relatively insensitive to macroalgal calcification (Mantyka & Bellwood, 2007). According to Dromard et al. (2015), calcified macroalgae contributed principally to the diet of several herbivorous reef fishes, mainly surgeonfish (*Acanthuridae*), probably due to their muscular stomachs which enable them to digest calcium carbonate deposits from calcified macroalgae, in contrast to other herbivores.

In terms of novelty, it was observed that the juvenile *C. chanos* in this study generally had a higher preference for macroalgae collected from the South China Sea, including the two most consumed species, *V. pachynema* and *C. prolifera* (Table 4.1; Figure 4.3). One the other hand, the least consumed species *P. gymnospora* was collected from the Straits of Johor, which is located close to Ponggol estuary in Singapore where *C. chanos* was reported not to be found (Bagarinao, 1994) (Figure 4.3). Although *C. chanos* has a wide geographical distribution, it has not been observed in several tropical estuaries in Singapore and North Borneo, which are close to the Straits of Johor and the South China Sea, respectively (Bagarinao, 1994). In contrast, *C. chanos* has been found to occur around the northwest coast of Peninsular Malaysia and also along the coast of Thailand (Bagarinao, 1994). Presuming that macroalgae collected from the Straits of Malacca were native to the herbivore and those collected from the Straits of Johor and the South China

Sea were non-native, results of this study are in line with some earlier reports, whereby certain herbivores preferentially consumed non-native autotrophs although both native and non-native autotrophs contained similar nutritional values (Lyons & Scheibling, 2007; Parker et al., 2012; Parker & Hay, 2005; Strong et al., 2009).

When a native herbivore has a preference for non-native autotrophs, it can perhaps be explained by the biotic resistance hypothesis. The hypothesis posits that non-native autotrophs fail to either establish or spread in new communities because of the defence mechanisms of the native species (Alpert, 2006; Levine et al., 2004; Maron & Vila, 2001). Likewise, some herbivores have been reported to confer biotic resistance to autotroph invasions (Morrison & Hay, 2011; Ricciardi & Ward, 2006). For example, a meta-analysis on plant-herbivore interactions using more than 100 plant species demonstrated that generalist herbivores often incorporate non-native species into their diets (Parker et al., 2006). Given that generalist herbivores often play a critical role in maintaining a healthy balance in marine ecosystems, particularly in controlling the spread of non-native autotrophs, the results of this study may contribute to the success of the invasion control in Peninsular Malaysia, or possibly beyond. Controlling the occurrence and abundance of widespread non-native species is a means to maintain biodiversity, which is one of the critical elements for habitat management. Table 5.1 lists examples of studies that test assumptions of the biotic resistance hypothesis (BRH).

In light of the growing threat of biological invasions to biodiversity worldwide, where the rate of invasions is expected to increase due to the effects of climate change, addressing the knowledge gaps on the potential synergies between autotrophs and herbivores has become urgent. Based on the results of this study, feeding preferences of the generalist herbivore are largely determined by the characteristics of macroalgae (Table 4.3), and their morphology and novelty. They are consistent with a recent study conducted by Grutters et al. (2017), which utilized freshwater species widely spread in Europe. The findings of this study lead to further insights into the dynamics that occur between marine autotrophs and herbivores based on important autotroph characteristics, and reconciling some confusion surrounding the conflicting evidence as to whether species interactions vary geographically. Intensive research should be carried out to test the major hypotheses in invasion biology which are biotic resistance and enemy release hypotheses to ensure that the current ecosystems continue to have the ability to resist disturbances to their dynamics. Incorporation of geographic variation should be recommended in future studies that aim to develop theories of ecology.

Table 5.1: Examples of studies underpin the biotic resistance hypothesis (BRH) in aquatic invasion biology

Studies	**Support for BRH**^{1}	**Generalist herbivores**	**Number of autotroph species tested**
Castilla et al. (2004)	+	Juveniles marine snails (*Thais haemastoma*), muricid gastropods (*Concholepas concholepas*) and starfishes	One non-native species
Cebrian et al. (2011)	+	Sea urchins (*Paracentrotus lividus*)	Seven native and two non-native species
Dumont et al. (2011)	+	Crustaceans (*Rhyncocinetes typus*, *Pagurus edwardsii*, *Paraxanthus barbiger*, *Cancer setosus*), echinoderms (*Tetrapygus niger* and *Heliaster helianthus*), and blennid fishes (*Scartichthys viridis* and *Hypsoblennius sordidus*)	One pairs of native and non-native species
Gollan and Wright (2006)	+	Herbivorous fishes (*Girella tricuspidata*), and sea-hare (*Aplysia dactylomela*), mesograzers (*Cymadusa setosa* and *Platynereis dumerilii antipoda*)	Seven native and one non-native species
Grutters et al. (2017)	–	American freshwater snails (*Pomacea canaliculata*) and Eurasian freshwater snails (*Lymnaea stagnalis*)	20 pairs of native and non-native species

Table 5.1: Continued

Studies	**Support for BRH[1]**	**Generalist herbivores**	**Number of autotroph species tested**
Lyons and Scheibling (2007)	+	Green sea urchins (*Strongylocentrotus droebachiensis*)	One pair of native and non-native species
Monteiro et al. (2009)	–	Gastropods (*Gibbula umbilicalis*), sea-hare (*Aplysia punctata*), sea urchins (*Paracentrotus lividus*), amphipods (*Gammarus insensibilis*), and gastropods (*Stypocaulon scoparium*)	13 native and one non-native species
Morrison and Hay (2011)	+	South American apple snails (*Pomacea* sp.) and North American crayfish (*Procambarus spiculifer*)	Nine pairs of native and non-native species
Noè et al. (2018)	+	Sea urchins *Paracentrotus lividus*)	Two pairs of native and non-native species
Parker and Hay (2005)	+	American crayfishes (*Procambarus spiculifer*)	57 native and 15 non-native species
Petruzzella et al. (2020)	+	Eurasian freshwater snails (*Lymnaea stagnalis*)	Three native and one non-native species
Scheibling et al. (2008)	+	Periwinkle (*Littorina littorea*)	One non-native species
Strong et al. (2009)	+	Amphipods (*Dexamine spinosa*)	Three native and one non-native species
Suárez-Jiménez et al. (2017)	+	Talitrid amphipod (*Bellorchestia quoyana*)	Three native and one non-native species
Tomas et al. (2011)	+	Sea urchins (*Paracentrotus lividus*)	Four non-native species
Wikström et al. (2006)	–	Isopods (*Idotea granulosa*) and littorinid gastropods (*Littorina obtusata* and *Littorina littorea*)	Three native and one non-native species
Xiong et al. (2008)	–	Pond snails (*Radix swinhoei*)	20 native and seven non-native species

[1] Studies that support the BRH are indicated by '+', whereas those findings opposite or no support to BRH predictions are indicated by '–'.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The overall outcome of this study demonstrated that the feeding preferences of the generalist herbivore may be largely determined by the nutritional characteristics of macroalgae, and their novelty and morphology. The feeding preferences of the selected generalist herbivore (*C. chanos*) in this study could be best explained by the level of nitrogen content in the macroalgae. Results from the feeding trials indicated that the generalist herbivore preferred macroalgal species with lower total nitrogen content, whereby the relative consumption rates and total nitrogen content were found to be inversely correlated. The increased consumption of nitrogen-deficient macroalgae may be driven by compensatory feeding of the generalist herbivore, where it fed more to overcome the negative effects of low-quality foods. The present study also found that the generalist herbivore, which collected from the Straits of Malacca, showed significantly higher preference for macroalgal species obtained from the coastal areas of the South China Sea where the generalist herbivore was reported not to be found. This supports the biotic resistance hypothesis which suggests that generalist herbivores could inhibit the spread of non-native autotroph species by consuming them more than the native species. It was also observed that two tested filamentous macroalgae in this study were consumed in greater amounts than species from other functional groups, indicating that the morphology of the macroalgae may also play a role in determining herbivore feeding preferences. The three research objectives designed at the beginning of the study were achieved, as the findings of this study have led to further insights into the dynamics that occur between marine autotrophs and herbivores based on several important factors, including the nutritional quality, novelty, and morphology of the marine autotrophs.

6.2 Future Research Recommendations

The present study examined the causal relationship between herbivore feeding preferences and characteristics of marine macroalgae based on the samples collected from a few coastal areas in Peninsular Malaysia. Larger scale studies in both laboratory and field can be considered in future research, which should involve a larger number of autotrophs and herbivores from different geographical regions beyond Peninsular Malaysia.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

PUBLICATIONS:

1. **Lim, W. Y.**, Lim, P. E., Amri, A. Y., Song, S. L., & Cheng, A. (2020). Exploring the role of macroalgal traits on the feeding behaviour of a generalist herbivore in Malaysian waters. *Botanica Marina*, *1*. https://doi.org/10.1515/bot-2020-0029 (see Appendix B)

PAPERS PRESENTED:

1. **Lim, W. Y.**, Lim, P. E., Amri, A. Y., & Cheng, A. (2018). The role of plant chemical traits in marine plant-herbivore interactions. Paper presented at the 23rd Biological Sciences Graduate Congress, 18th–20th December 2018, Chulalongkorn University, Bangkok, Thailand.
2. **Lim, W. Y.**, Lim, P. E., Amri, A. Y., Lim, S. C., Zulkarnai, L. S. & Cheng, A. (2019). Marine autotroph-herbivore synergies: characteristics of macroalgae that predict feeding preferences of generalist herbivores. Paper presented at the 3rd South China Sea Conference, 24th–28th June 2019, Kuala Lumpur, Malaysia.