GROWTH AND MORTALITY OF GREEN MUSSEL, *Perna viridis* IN THE HIGH SPAT-FALL AREAS OF MARUDU BAY AND AMBONG BAY, SABAH, MALAYSIA

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ABSTRACT

Present study was conducted with the aim to understand the growth performance of green mussel, *Perna viridis* in two high spat-fall areas on the west coast of Sabah, Malaysia: Marudu Bay and Ambong Bay, by comparing environmental parameters data with growth data. The three selected study sites, which included river mouth station (Marudu Bay), coastal and open sea stations (Ambong Bay) could help to understand the favourable and suitable environmental conditions for better growth performance of green mussels. Samplings were carried out once a month for 12 months period (October 2015 - September 2016). The results showed the growth parameters of green mussel in coastal station have the highest increment, with 3.15cm in shell length, 11.23g in live weight, 2.37g in wet meat weight, 0.23g in dry meat weight, and 0.16g in ash free dry meat weight. The growth increments were significantly higher ($p<0.05$) in coastal than river mouth and open sea stations. The mussels cultured in the river mouth station attained highest mean meat yield (28.67%) but also recorded the highest cumulative mortality with 6.48%. In contrast, mussels in coastal and open sea stations recorded 1.48% and 2.96% respectively. Cumulative mortality of mussels in all stations showed significant different ($p<0.05$). Phytoplankton and zooplankton concentrations in the three stations showed no significant difference ($p>0.05$). However, coastal and open sea stations were found to have dominated by potential harmful algae (*Chaetoceros* spp. and *Prorocentrum* spp.). Overall, growth parameters in all stations showed negative correlation with salinity, temperature, DO and chlorophyll-*a*. Meat yield and condition index were positively correlated with chlorophyll-*a*, TPM and PIM in all stations. Water current speed in coastal and open sea stations were positively correlated with the growth parameters, but negatively correlated in river mouth station. pH showed to have positive correlation with the growth parameters in open sea station but only positive correlation with dry meat weight and ash free dry meat weight in coastal station. Phytoplankton cell density was found negatively correlated with meat yield in river mouth and open sea station but positively correlated in coastal station. On the other hand, zooplankton density was positively correlated with meat yield in river mouth and coastal station but negatively correlated in open sea station. In conclusion, green mussels in Ambong Bay (coastal station) exhibited better growth performance in term of shell length and live weight but lower meat yield, which are unfavourable to consumers. Green mussels in Marudu Bay (river mouth station), on the other hand, exhibited higher meat content but also experienced higher mortality rate, which is unfavourable by farmer. The higher mortality of mussels in Marudu Bay warrant further investigation on factors other than food availability and environmental conditions, such as predation and disease.

ABSTRAK

PERTUMBUHAN DAN KEMATIAN KUPANG, Perna viridis DI KAWASAN TINGGI BENIH DI TELUK MARUDU DAN TELUK AMBONG, SABAH, MALAYSIA

Kajian telah dijalankan untuk memahami prestasi pertumbuhan kupang, Perna viridis di Teluk Marudu dan Teluk Ambong, melalui perbandingan data parameter sekitaran dengan data pertumbuhan kupang. Ketiga-tiga kawasan pensampelan yang dipilih, termasuklah muara sungai (Teluk Marudu), pesisir pantai dan laut terbuka (Teluk Ambong) dijangka dapat membantu memahami keadaan persekitaran yang baik dan sesuai untuk prestasi pertumbuhan kupang yang lebih baik. Pensampelan dijalankan sekali sebulan selama 12 bulan (Oktober 2015 - September 2016). Hasil kajian menunjukkan bahawa pertumbuhan kupang yang diternak di stesen persisir pantai mencapai peningkatan paling tinggi berbanding stesen-stesen lain, iaitu 3.15cm dalam panjang cengkerang, 11.23g dalam berat keseluruhan, 2.37g dalam berat daging basah, 0.23g dalam berat daging kering, dan 0.16g dalam berat daging bebas abu. Peningkatan pertumbuhan adalah signifikan ($p<0.05$) di antara stesen. Kupang diternak di stesen muara sungai mencapai purata hasil daging tertinggi (28.67%) tetapi juga mencatatkan purata kematian kumulatif tertinggi iaitu 6.48%. Sebaliknya, kupang di stesen pesisir pantai dan laut terbuka masing-masing mencatatkan purata kematian kumulatif sebanyak 1.48% dan 2.96%. Purata kematian kumulatif di semua stesen menunjukkan perbezaan yang signifikan ($p>0.05$). Kepekatan fitoplankton dan zooplankton di semua stesen tidak menunjukkan perbezaan yang signifikan ($p>0.05$). Walau bagaimanapun, stesen pesisir pantai dan laut terbuka didominasi oleh alga berpotensi bahaya (Chaetoceros spp. dan Prorocentrum spp.). Keseluruhannya, parameter pertumbuhan di semua stesen berkorelasi negatif dengan kemasinan, suhu, DO dan klorofil-a. Indeks daging dan hasil daging berkolerasi positif dengan klorofil-a, TPM dan PIM di semua stesen. Kelajuan arus air di stesen pesisir pantai dan laut terbuka berkorelasi positif dengan parameter pertumbuhan, tetapi berkorelasi negatif di stesen muara sungai. pH menunjukkan korelasi positif dengan parameter pertumbuhan di stesen laut terbuka tetapi hanya berkorelasi positif dengan berat daging kering dan berat daging bebas abu di stesen pesisir pantai. Kepekatan fitoplankton didapati berkorelasi negatif dengan hasil daging di stesen muara sungai dan laut terbuka tetapi berkorelasi positif di stesen pesisir pantai. Sebaliknya, ketumpatan zooplankton berkorelasi positif dengan hasil daging di muara sungai dan stesen pesisir pantai tetapi berkorelasi negatif di stesen laut terbuka. Kesimpulannya, kupang di Teluk Ambong (stesen pesisir pantai) menunjukkan prestasi pertumbuhan yang lebih baik khususnya panjang cengkerang dan berat hidup tetapi hasil daging yang lebih rendah, yang mana tidak digemari oleh pengguna. Manakala kupang yang diternak di Teluk Marudu (stesen muara sungai) mempamerkan kandungan daging yang lebih tinggi tetapi mengalami kadar kematian yang tinggi, yang mana tidak baik untuk penternak. Kadar kematian yang tinggi bagi kupang yang diternak di Teluk Marudu memerlukan kajian lanjutan terutama faktor-faktor selain daripada ketersediaan makanan dan keadaan persekitaran, khususnya pemangsa dan penyakit.

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°C	Degree Celsius
%	Percentage
cell/ml	Cell per milliliter
L	Liter
m	Meter
m^2	Meter square
m^3	Meter cube
mg/l	Milligram per liter
ml	Milliliter
m/s	Meter per second
nm	Nanometer
µg/l	Microgram per liter
µm	Micrometer
psu	Practical salinity unit
ppm	Part per million
rpm	Revolution per minute
spp.	Species
TPM	Total particulate matter
PIM	Particulate inorganic matter
POM	Particulate organic matter
OM	Organic matter
SL	Shell length
WMW	Wet meat weight
DMW	Dry meat weight
AFDMW	Ash free dry meat weight

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CHAPTER 1

INTRODUCTION

1.1 Green mussel, *Perna viridis*

Green mussel (*Perna viridis*) provided cheap source of protein for human consumption and has been cultured with great success using various methods at different places of both hemispheres (Srinibhadh, 1976). According to Choo and Ng (1990), there was about 60% of protein in every 100 g of mussel soft tissues (dry meat weight). The harvesting of *Perna viridis* can have economic, ecological, and human health impacts (Azman *et al.*, 2012). They are harvested commercially as a human food resource due to their dense and fast growth. Moreover, they are also used as a biomonitoring agent for heavy metals because they are commercially important seafood species worldwide (Farrington *et al.*, 1987), sedentary organisms, long-lived, easily identified and sampled, reasonably abundant and available throughout the year, tolerant to natural environmental fluctuation and pollution, and have good net accumulation capacities (Yap *et al.*, 2004).

In the natural habitat, *Perna viridis* are mostly found in the littoral zone, attached in clusters on various substrates, including vessels, wharves, aquaculture equipments, buoys and other hard substrates (Sallih, 2005). The native range of *Perna viridis* is along the Indian coast and throughout the coastal areas of the Indo-Pacific (Vakily, 1989; Siddall, 1980). It ranges from the north as far as Hong Kong, the southern provinces of Guangdong and Fujian in China and southern Japan to the east-west from the Persian Gulf to the west of New Guinea (Al-Barwani *et al.*, 2007; Vakily, 1989; Siddall, 1980). In the Southeast Asia region, *Perna viridis* is mainly cultivated in India, Indonesia, Philippines, Singapore, Thailand and Malaysia (Spencer, 2002).

Over the past several decades, *Perna viridis* has been distributed to many islands outside its native range in the South Pacific. It was first found outside their native range in waters surrounding the Carribbean island of Trinidad in 1990 (Benson, 2009; Agard *et al.*, 1992) and later along the nearby coast of Venezuela in 1993 (Benson, 2009; Rylander *et al.*, 1996). Since then, this species has continued to distribute to other countries such as Western Australia (McDonald, 2012) and South America (Benson *et al.*, 2001) through ship ballast, hull fouling, experimental farming and intentional release (Vakily, 1989).

In Malaysia, *Perna viridis* occurs widely in coastal waters along the west coast of Peninsular Malaysia (Al-Barwani *et al.*, 2007; Yap *et al.*, 2002). The culture activities of *Perna viridis* in Malaysia were started in the Johor strait due to the availability of natural seeds. It was then spread to Melaka where natural spats are available and Perak by obtaining seeds for transplantation (Sallih, 2005). At present, culturing of *Perna viridis* in Peninsular Malaysia has extended from Kedah in the north and east coast using seeds transplantation (Al-Barwani *et al.*, 2007). The production of *Perna viridis* in Malaysia reached its highest peak in 2010, with total production of 10,529 metrics tonnes (DOF, 2010). However, the production has declined and reduced for the following years due to the massive mortality event in *Perna viridis* farm across the country (Taib and Ransangan, 2016).

In Sabah, *Perna viridis* was first introduced and cultured in Tawau using broodstock originated from Johor strait (Tan and Ransangan, 2016a; DOF 2008). It was then extended to Kota Marudu, Tuaran and Kuala Penyu (Tan and Ransangan, 2016a). Since then, this industry has contributes local protein supply and economic benefits to the livelihood of local community. Production of *Perna viridis* in Sabah was significantly low when compared to Peninsular Malaysia, where only about two metrics tonnes was contributed from the total production of 1,600 metrics tonnes in 2015 (DOF, 2015). However, culture of *Perna viridis* in Sabah still holds considerable potential due to availability of natural spats.

In late 2009, a mass mortality event has wipe out the entire stock of *Perna viridis* in Marudu Bay and it is still struggling to restore even after five years. The main causative factors is still remain uncertain. Same incident also happen in

Tawau. Such incident has affected the livelihood of local farmers. On the other hand, culture of green mussel in Tuaran and Kuala Penyu also facing problem of the recurrent of red tides in the areas, where blooms of *Pyrodinium bahamense* var. *Compressum* have occur annually in the west coast of Sabah (Mohammad-Noor *et al.*, 2012; Adam *et al.*, 2011)

According to Barkai and Branch (1989) and Petraitis (1995), growth and mortality in mussels are good indicators of competitive ability. Greater growth rates can cause mortality or reduced fitness over poorer growing individuals either through overgrowing, undercutting and crushing, or exploitation of important resources such as food and space (Bownes and McQuaid, 2010; Wootton, 1993; Harger, 1972). Different in growth and mortality rates between species can reflect physiological tolerances to environmental factors and biological pressure (Bownes and McQuaid, 2010).

The growth rate of *Perna viridis* is high compared to other species of mussel (Sallih, 2005; Shafee. 1979) due to highly adaptable and tolerant to a wide range of environmental conditions. However, their growth rates are also highly influenced by the fluctuation in environmental conditions (Gosling, 2003). This suggests that growth of *Perna viridis* is the result of the combined effects of a number of environmental factors such as temperature, salinity, food availability, current speed, particulate matter, and nutrient supply (Vakily, 1989; Wilbur and Owen, 1964). Among these factors, food availability is considered to be most important since it promotes sustainable growth (Seed and Suchanek, 1992).

1.2 Significance of Study

In order to understand the possible reasons of *Perna virids* still struggling to recover after the mass mortality event in Marudu Bay, it is important to collect and examine the environment conditions in the bay and perhaps comparing it with a bay that reported less affected by the mortality. Throughout the past two years, few studies have been carried out in Marudu Bay (e.g. Tan and Ransangan, 2015; Tan and Ransangan, 2016a; Tan and Ransangan, 2016b; Taib and Ransangan, 2016) with the aims to determine the cause of the mortality and to suggest means to restore the *Perna viridis* population in the area. Previous study on food availability in Marudu Bay (Tan and Ransangan, 2016b) showed that the selective ingestion behaviour of *Perna viridis* in Marudu Bay is mainly influenced by seston concentration, phytoplankton cell density and composition. This indicates that environmental parameters play a crucial role in survival of *Perna viridis*.

In this study, focus was on the growth rate of *Perna viridis* in two different areas: Marudu Bay and Ambong Bay, with the aim to determine the reason of poor growth rate of *Perna viridis* in Marudu Bay by comparing the environmental and growth data in Ambong Bay. The three selected study sites, which included river mouth, coastal and open sea could help to understand the favourable and suitable environmental conditions for better growth rate of *Perna viridis*. Study also carried out to determine the potential farming of *Perna viridis* in Ambong Bay.

1.3 Objectives

The objectives of this study are:

I. To determine the physico-chemical parameters (environmental water parameters, seston quantity and quality, and water inorganic nutrients) in the study areas which could affect the growth of green mussel;

II. To determine the composition and abundance of phytoplankton and zooplankton in the study areas which contribute to the growth performance of green mussel;

III. To determine the growth rate and mortality of green mussels in the study areas.

CHAPTER 2

LITERATURE REVIEW

2.1 Species Description

Green mussel, also known as green-lipped mussel and Philippines green mussel belongs to the family Mytilidae and species *Perna viridis*. It is a comparatively large mussel, with average size 80-100 mm in length and has been reported occasionally to achieve a length of 150 mm (Sallih, 2005). It has two identical shell valves, pear shaped and smooth exterior surface characterized by concentric growth rings and a ventral margin that is distinctly concave on one side (Sallih, 2005). The shell surface of *Perna viridis* is covered by a smooth and firm periostrocum, which is bright and vivid green in juveniles but olive or brown with green margins in adult (Gosling, 2003). The inner surface of the valves is smooth and iridescent blue to bluish green in colour. A prominent, kidney shaped retractor muscle scar is present but the species lacks anterior adductor muscles. Close examination of the beak reveals a pair of hinge teeth on the left valve that interlock with a single hinge tooth on the right valve (Rajagopal *et al.*, 2006).

As a filter feeder, *Perna viridis* mainly feeds on a wide range of phytoplankton, while small zooplankton and suspended fine organic materials may also supplement their diets (Sallih, 2005). They feed by pumping water through a set of gill filaments and discharged through the excurrent siphon. Only suitable size food particles are retained and passed into the stomach where they are digested (Gosling, 2003; Tan and Ransangan, 2014).

The life span of *Perna viridis* is approximately three years (Power *et al.*, 2004). Reproduction is through sexual, where sexes are separated, and fertilization occurs externally. However, males and females are not distinguishable by external morphology (Rajagopal *et al.*, 2006). The sexes of truly mature species can be determined by the colour of the gonads: milky to creamy white in males, orange to red orange for females (Yap *et al.*, 1979). *Perna viridis* is sexually matured at 20-30 mm in length which is attained after two to three months.

Spawning of *Perna viridis* can be initiated by the presence of individuals releasing streams of gametes into the water (Stephen and Shetty, 1981; Rajagopal *et al.*, 2006). Spawning can also be triggered by a change in environment conditions, such as rising or dropping in water temperature (Rajagopal *et al.*, 1998) and salinity (Stephen and Shetty, 1981; Rajapogal *et al.*, 2006). In tropical countries, including Malaysia, *Perna viridis* spawns all year-round, with peak spawning period occurs during monsoon seasons, especially March to April, and October to November (Sivalingam, 1977; Tan and Ransangan, 2014). If gonads are ripen, mussels can easily be stimulated to release eggs and sperms, which could make them suitable for use as transplants to increase natural spat production (Yap *et al.*, 1979).

2.2 Growth

Growth is a parameter that is often quantitatively measured in order to understand the suitability of the environment to marine organisms (Berge *et al.*, 2006). Growth is also one of the main aspects that mussel farmers considered when assessing their culture system in order to meet the market demand (Vakily, 1989). Thus, farmers are usually interested in growing their mussels to reach the marketable size in the shortest period of time possible.

Perna viridis is a fast growing bivalve, with growth rate of 6-10 mm per month (Power *et al.*, 2004). Their growth can be distinguished into shell and body growth. In mussel, growth is most commonly measured as the increase in length, which is the maximum distance between the anterior and posterior axis of the shell (Vakily, 1989; Gosling, 2003). The shell length does not necessarily reflect the meat content. During spawning or food shortage, internal energy reserves are consumed while the shell may continue to grow (Power *et al.*, 2004, Tan and Ransangan, 2014).

2.3 Factors Affecting Growth

There are many factors which influence growth of *Perna viridis*. Food availability is considered to be most important because this provides energy for continuous growth (Seed and Suchanek, 1992; Gosling, 2003). However, the availability of food is influenced by environmental conditions such as temperature, salinity, and water nutrients. The growth rate of mussel is also influenced by age, where older mussels have poorer growth rate because of reduced metabolic activity (Cheung, 1993), declined in filtration rate (Seed and Suchanek, 1992) and increased in gamete production (Hilbish, 1986; Tan and Ransangan, 2014).

Other factors which have been shown to decrease mussel growth are turbidity, current speed, dissolved oxygen, depth, fouling organisms, pollutants, and predation. Because of the combined interaction and fluctuation of these environmental factors, it appears difficult to determine the relationship of growth and environmental factors in natural population of mussel (Wilber and Owe, 1964; Vakily, 1989). Such difficulty is also occurring in bivalve other than green mussel.

2.3.1 Food Availability and Quality

Studies have shown that filter-feeding bivalve molluscs can alter their filtration, ingestion and absorption efficiencies and rates depending on the quantities of food in the water (Winter, 1978; Bayne and Newell, 1983; Bayne *et al.*, 1987). Higher concentrations of phytoplankton and organic matter in the water will require lower filtration rates to intake the maximum volume of food and thus generate faster growth rate (Iglesias *et al.*, 1992; Hawkins *et al.*, 1997 and 1998).

According to Cheong (1982), suitable phytoplankton biomass for *Perna viridis* culture was 17 to 40 µg/l. Rajagopal *et al.* (1998) suggested that chlorophyll-a concentration ranged from 0.7 to 17 µg/l favour the *Perna viridis* culture. On the other hand, studied by Sivalingam (1977) reported that even 5.2 to 3.5 µg/l of chlorophyll-a was considered sufficient for *Perna viridis* culture in Malaysia. However, poor *Perna viridis* growth in moderate levels of chlorophyll-a has been reported by Ren and Ross (2002). Recent study by Tan and Ransangan (2016b) on *Perna viridis* in Marudu Bay farm also demonstrated poor growth and high mortality even though the phytoplankton concentration was within the recommended value. These suggest that feeding behaviour of mussels is not only affected by the quantity of food but quality also plays important role.

The size of the individual particles and the balance in the nutritional values are the two important features of food quality (Bayne *et al.*, 1987). Some of the phytoplankton species may be relatively poor in nutrition (of the wrong size or poorly digestible). For example, despite of high concentration of phytoplankton in water during algal blooms, the closure of the shells has been observed and reduction of the filtration rate has been reported in various bivalve species (Manfrin *et al.*, 2012). This suggests that algal blooms may not be favourable if the abundant species is nutritionally deficient or poorly digested by mussels or harmful to the mussels.

2.3.2 Seston Quantity and Quality

Seston consists of the living microorganisms (e.g. plankton and nekton) and non-living matters (e.g. plant debris or suspended soil particles) moving in the water. As a filter-feeder, the absorption rate of bivalve is also influenced by the changes in both seston quantity and quality. Filter-feeding bivalves are able to compensate for reductions in food quality or quantity by preferential ingestion of organic particles from the filtered matter and selective rejection of the inorganic matter (Newell and Jordan, 1983; Navarro and Iglesias, 1993; Hawkins *et al.*, 1996; Bayne, 1998; Wong and Cheung; 1999). Feeding behaviour of mussels varied by the concentration and mass of organic matter presence in suspended particulate matter (Bayne *et al.*, 1987). Absorption efficiency of *Perna viridis* increases with the ingested organic matter due to lower ingestion rates at higher organic contents (Wong and Cheung, 1999). The absorption rate in *Perna viridis* was reported to reach the maximum at particulate organic matter of 5.30 mg/l, while in *Mytilus edulis* the maximum absorption rate was at 1.9 mg/l (Bayne *et al.,* 1989).