MICROZOOPLANKTON DYNAMICS IN RELATION TO THE MICROBIAL LOOP IN MATANG ESTUARINE WATERS

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FACULTY OF SCIENCE
UNIVERSITI MALAYA
KUALA LUMPUR

2021

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITI MALAYA
KUALA LUMPUR

2021

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **YONG YU LIN**

Matric No: **SHC 120066**

Name of Degree: **DOCTOR OF PHILOSOPHY**

Title of Thesis:

MICROZOOPLANKTON DYNAMICS IN RELATION TO THE MICROBIAL LOOP IN MATANG ESTUARINE WATERS

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MICROZOOPLANKTON DYNAMICS IN RELATION TO THE MICROBIAL LOOP IN MATANG ESTUARINE WATERS

ABSTRACT

Microzooplankton (20-200 μm), along with phytoplankton, bacteria and heterotrophic nanoplankton in a tropical estuary (Matang Mangrove Forest Reserve, MMFR) were investigated to evaluate their abundance in relation to the various environmental and biotic parameters, along with their interactions in the microbial food web to elucidate the role of microzooplankton in the estuarine water. This study looked into the microzooplankton composition, taxonomically and abundance (Chapter 4) and their feeding interaction with the other microbial component in the estuary (Chapter 5). With this information gathered we can thus fill the knowledge gap of the carbon flow in the estuary. A total of 39 microzooplankton taxa comprising of four major groups, i.e. loricate ciliates (37.72 %), aloricate ciliates (29.46 %), dinoflagellates (24.33 %) and meroplanktonic nauplius (8.49 %) were identified. The loricate ciliates were the most diverse group with 31 taxa recorded. Four major species of loricate ciliates were identified, i.e. *Tintinnopsis beroidea*, *Tintinnopsis rotundata*, *Stenosemella avellana* and *Tintinnidium primitivum*, while Strombidiidae and Strobilidiidae dominated the aloricate ciliates. Although small loricate ciliates were ubiquitous, redundancy analysis shows marked shifts in microzooplankton community structure, from one that was dominated by loricate ciliates during the drier South West (SW) monsoon, to aloricate ciliates at the onset of the wet North east (NE) monsoon, and then to dinoflagellates towards the end of the drier NE monsoon period. These shifts were associated with rainfall, dissolved inorganic nutrients, salinity, temperature and microbial food abundance. There was no clear lunar effect on abundance of microzooplankton except for *Favella ehrenbergii* and copepod nauplii that were more abundant during neap than spring tide.

Rain-driven monsoonal effects showed significant impacts on microzooplankton herbivory and primary production; both significantly higher during the drierSouth West monsoon. The MMFR was characterised by high primary production (148 to 4021 µgC $l^{-1}d^{-1}$; 1190 ± 249.6 µgC $l^{-1}d^{-1}$) and low bacterial production (1 to 6.1 µgC $l^{-1}d^{-1}$; 3.7 ± 1.5 µgC $l^{-1}d^{-1}$). There is no coupling observed among bacterial production and primary production. Microzooplankton grazing on primary production was profound (887.02 µgC $l^{-1}d^{-1}$), followed by heterotrophic nanoplankton (1.33µgC $l^{-1}d^{-1}$) and bacterial production (1.28µgC $l^{-1}d^{-1}$). More than 70% of the primary production was transferred to the microzooplankton via grazing, compared to only about 30% of both bacterial production and HNP production. The present study highlighted the role of microzooplankton as an important consumer of phytoplankton production in the highly turbid but productive mangrove estuary.

Keywords: Microzooplankton - tropical estuary – temporal distribution - primary production - microbial dynamic – herbivory

DINAMIK MIKROZOOPLANKTON DI HUTAN PAYA LAUT MATANG SEHUBUNGAN DENGAN LINGKARAN MIKROB

ABSTRAK

Mikrozooplankton (20-200 μm), fitoplankton, bakteria dan nanoplankton heterotrofik di sebuah muara tropika (Hutan Paya Laut Matang, MMFR) telah disiasat untuk menilai kelimpahannya berkaitan dengan pelbagai parameter persekitaran dan biotik, bersama dengan interaksi mereka dalam web makanan mikrob. Sebanyak 39 mikrozooplankton taksa yang terdiri daripada empat kumpulan utama, iaitu *loricate ciliates* (37.72%), *aloricate ciliates* (29.46%), *dinoflagellates* (24.33%) dan meroplanktonic *nauplius* (8.49%) telah dikenal pasti. *Loricate ciliate* adalah kumpulan yang paling pelbagai dengan 31 taksa dicatatkan. Empat spesies utama *loricate ciliate* dikenal pasti, iaitu *Tintinnopsis beroidea*, *Tintinnopsis rotundata*, *Stenosemella avellana* dan *Tintinnidium primitivum*, sementara Strombidiidae dan Strobilidiidae mendominasi *aloricate ciliates*. Struktur komuniti microzooplankton didapati berubah dari semasa ke semasa, di mana analisi redundansi menunjukkan perubahannya yang ketara. Pertaburan *loricate ciliate* yang kecil amat seragam sepanjang penyiasatan. Semasa musim monsoon Barat Daya yang lebih kering, komuniti dikuasai oleh *loricate ciliate*; semasa permulaan musim monsoon Timur Laut yang lebih lembab pula kelihatan lebih *aloricate ciliate*; *dinoflagellate* pula timbul sebagai dominasi semasa perakhiran musim monsoon Timur Laut di mana hujan telah berkurangan. Perubahan komuniti ini dikaitkan dengan hujan, nutrien tak organik terlarut, kemasinan, suhu dan kelimpahan makanan mikroba. Faktor air pasang surut tidak menunjukkan kesan yang jelas terhadap kelimpahan mikrozooplankton kecuali *Favella ehrenbergii* dan copepod nauplii yang lebih banyak pada waktu air surut daripada waktu air pasang.

Musim monsoon yang paling berbeza dari jumlah hujan menunjukkan kesan yang signifikan terhadap kegiatan herbivori mikrozooplankon dan pengeluaran primer; kedua-

duanya jauh lebih tinggi semasa musim monsoon Barat Daya yang kering. Di Hutan Paya Laut Matang, pengeluaran primer yang tinggi (150 hingga 4020 µgC $l^{-1}d^{-1}$) dan pengeluaran bakteria rendah (3.7 ± 1.5 µgC $l^{-1}d^{-1}$) menjadikan ianya keunikan antara ekosistem lain. Mikrozooplankton telah bergantung sangat terhadap pengeluaran primer (887.02 µgC $l^{-1}d^{-1}$), diikuti oleh nanoplankton heterotrofik (1.33µgC $l^{-1}d^{-1}$) dan pengeluaran bakteria (1.28µgC $l^{-1}d^{-1}$). Lebih daripada 70% pengeluaran primer disalurkan ke mikrozooplankton melalui penggembalaan, manakala hanya 30% dari pengeluaran bakteria dan pengeluaran heterotrofik nanoplankton digunakan oleh microzooplankton. Kajian ini menekankan peranan mikrozooplankton sebagai pengguna paling utama bagi pengeluaran fitoplankton di muara bakau yang sangat keruh tetapi produktif.

Kata Kunci: Mikrozooplankton – muara tropika – pertaburan musim – penghasilan primer – dinamik mikrob – herbivori

ACKNOWLEDGEMENTS

First and foremost, I would like to express my utmost gratitude to my supervisor, Associate Professor Dr Lee Choon Weng and for his continuous guidance and encouragement throughout this PhD research. Thank you for the unconditional belief in me. Thanks for the dedicated support, both physical and mentally. Thank you for holding me on track every time I fall.

I would like to express my gratitude to Honorary Professor Dr. Chong Ving Ching (recently retired), who was my first supervisor before retirement. Thanks for the invaluable comments and advice for this study, and most importantly, brought me into this study. I would like to express my gratitude to Dr Chew Li Lee, who guided all my baby steps in the early stage of the research, who gave me precious guidance both in the field and lab, who was the trusted one that I can always look for. Thanks for the kind words and inspiration to sustain my enthusiasm throughout this study,

From the bottom of my heart, I would like to say a big thank you to all my lab mates, from LME and B201. Thanks, Dr Amy, for all the motivations and encouragements (and cookies). Thank you, Cindy, Kean Chong, Soon Loong, Jun Jie, Lee Kien, Joon Hai, Jin Yong, Vivian for helping with my sampling trips. All the trips to Matang were made possible with their kindness. Thank you Yi You, Yvaine, Kian Giap for all the help and guidance in the lab. Cheers to all the good times we had.

I am also grateful to the University of Malaya for providing the lab facilities and financial support in the form of University of Malaya Scholarship Scheme (SBUM) and a Postgraduate Research Grant (PG095-2013A). This study was further supported by the Ministry of Education under a FRGS grant (FP017-2012A) and phase II (IOES-2014D).

My gratitude also goes to several key people; without them, this work would not have come to fruition. They are my fishermen, Mr. Lee Chee Heng, late Mr Alias bin Hamzah and their workers for providing field assistance during sampling. Thank you all the abang driver from the Institute of Biological Sciences for keeping us safe on the road every sampling.

Finally, I would like to dedicate this doctoral thesis to my family. Thanks mom and dad for the support (and even joined my sampling trips), my husband and son, my brothers and sister. And to Ping, please wake up.

TABLE OF CONTENTS

ABSTRACT ..... **iii**

ABSTRAK ..... **v**

ACKNOWLEDGEMENTS ..... **vii**

TABLE OF CONTENTS ..... **ix**

LIST OF FIGURES ..... **xiii**

LIST OF TABLES ..... **xvi**

LIST OF SYMBOLS AND ABBREVIATIONS ..... **xx**

CHAPTER 1: INTRODUCTION……..……1

CHAPTER 2: LITERATURE REVIEW **8**

2.1 Microzooplankton: Classification and Major Groups 8

2.1.1 Ciliated Protozoan 9

2.1.2 Dinoflagellates 12

2.1.3 Metazoans 13

2.2 Ecological Importance of Microzooplankton 14

2.3 Microbial Loop – Microzooplankton Community in the Estuary 16

2.3.1 Bacterial Production and Primary Production 17

2.4 Mangrove Ecosystem 18

CHAPTER 3: METERIALS AND METHOD .....

3.1 Sampling site description 21

3.2 Field sampling 23

3.2.1 Routine sampling of microbial community 23

3.3 Laboratory analysis 24

3.3.1 Chlorophyll *a*, dissolved organic nutrients and dissolved organic carbon ... 24

3.3.2 Bacteria and Heterotrophic Nanoplankton Abundance 24

3.3.3 Phytoplankton and Microzooplankton Abundance 25

3.4 Microbial process rate measurements 26

3.5 Data and statistical analysis 28

CHAPTER 4: RESULTS AND DISCUSSION - MICROZOOPLANKTON ABUNDANCE AND COMMUNITY STRUCTURE IN THE TERUSAN MANGROVE CREEK 30

4.1 Environmental parameters 30

4.2 Bacteria, HNP and phytoplankton abundacnce 35

4.3 Microzooplankton Diversity, Abundance, and Composition 35

4.4 Species-environment relationship 40

4.5 Discussion 47

CHAPTER 5: RESULTS AND DISCUSSION - MICROZOOPLANKTON GRAZING IN RELATION TO MICROBIAL LOOP DYNAMICS IN A HIGHLY TURBID MANGROVE ESTUARY 53

5.1 Biomass of Microbial Components 53

5.2 Microbial Productions 59

5.3 Grazing activities 64

5.4 Discussions 68

5.4.1 Seasonal Changes in Microbial Biomass 68

5.4.2 Primary Production and Microzooplankton Grazing 24

5.4.3 Temperature Dependency of Primary Production versus Microzooplankton Grazing 72

5.4.4 Phytoplankton – Bacteria – HNP coupling 74

5.4.5 Microbial Foodweb in MMFR 74

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION 80

6.1 Microzooplankton of Matang Mangrove Forest Reserve 80

6.2 Microbial loop of Matang Mangrove Forest Reserve 80

6.3 Further studies 81

REFERENCES 82

LIST OF PUBLICATIONS AND PAPERS PRESENTED 102

APPENDIX 103

LIST OF FIGURES

Figure 1.1	Microbial loop as described linking dissolved organic matter to higher trophic levels……	2
Figure 1.2	Detrital food chain and planktonic food web of estuary (Day et al. 2012) ……..	3
Figure 1.3	Size fractions of microbial community in estuary, size classification of plankton base on Sieburth et al. 1978. (Day et al. 2012) ……..	4
Figure 2.1	Literature record of tintinnid distribution (Pierce and Turner, 1993). Note that tropical Southeast Asian water remains unknown in the review…….	10
Figure 3.1	Map of Matang Mangrove Forest Reserve. Filled circle indicates the location of Terusan channel where samples were taken…….	22
Figure 4.1	Monthly total rainfall and number of rainy days recorded from April 2013 to February 2014 at Taiping located 10 km east of the study site. Shaded region indicates SW monsoon period (April-September); unshaded region indicates NE monsoon period (October-Feb)……	31
Figure 4.2	Monthly mean of (a) physical parameters, (b) dissolved inorganic nutrients and (c) microzooplankton potential food components. N denotes neap tide; S denotes spring tide. Error bars (S.E.) are indicated……...	34
Figure 4.3	Monthly abundance and composition of major microzooplankton groups, including species diversity and species evenness index in Terusan channel, from April 2013 to February 2014. Error bars indicate standard error for total abundance. Abbreviations are as given in Fig. 4.2……..	40
Figure 4.4	Scanning Electron Microscope imagae of *Tintinnopsis beroidea*. Upper 2 images we zoomed in to show that shells of tintinnids were made up by diatoms……	43

Figure 4.5 Ordination triplot from RDA of environmental parameters (bold arrows with large arrow heads) and microzooplankton taxa (fine colored arrows with small arrow heads) in Terusan channel, MMFR. Environmental parameters: DO - dissolved oxygen, Phyto-phytoplankton, Chl *a* -chlorophyll *a*, Temp- temperature, HNP-heterotrophic nanoplankton, Rainfall- monthly mean rainfall, Salinity- monthly mean salinity, Secchi – secchi disc depth. Microzooplankton: Blue arrows indicate loricate ciliatesviz. Trot - *Tintinnopsis rotundata,* Tber *T. beroidea,* Tnana *T. nana,* Save *Stenosemella avellana,* , Tacu *T. acuminata*, Fehr *Favella ehrenbergii*, Tnpri *Tintinnidium primitivum*, Ttub *Tintinnopsis tubulosa*, Tninc *Tintinnidium incerta*, Tdir *Tintinnopsis directa*, Tmin *T. minata,* Tmeu *T. meunieri*, Tching *T. chinglanensis*, Ttoc *T. tocatinensis*, Lelo *Leprotintinnus elongatus,* Lbot *Leprotintinnus bottnicus*, Lnor *Leprotintinnus nordqvisti*; red arrows indicate aloricate ciliates viz. Pleuro pleuronematine, Strob stobilidiid, Strom stombidiid Cyclo cycloltrich, Vor vorticella; green arrows indicate dinoflagellates viz. Cer *Ceratium* spp., Dphys *Dinophysis* spp., Pror *Prorocentrum* spp., Perid *Peridinium* spp., Noct *Noctiluca* spp.; black arrows indicate copepod larvae, Naup nauplius. Symbols: ○ SW-spring, □SW-neap, ●NE-spring, ■ NE-neap; numeric letter represents sampling month, e.g. 4 = April. Ellipsoids group the microzooplankton taxa into four categories, large loricate ciliates, small loricate ciliates, aloricate ciliates and dinoflagellates……... 46

Figure 5.1 Monthly biomass of different microbial components in MMFR. 54

Figure 5.2 Monthly production of different microbial components in MMFR……. 55

Figure 5.3 Relationships between biomass of various microzooplankton taxonomic groups (µgC l^{-1}, x-axis) and phytoplankton biomass (µgC l^{-1}, y-axis). Only cases with significant ($p < 0.05$) positive correlation are presented with the regression line…….…… 55

Figure 5.4 Monthly bacterial production from April 2013 to February 2014. X-axis shows time of incubation while y-axis shows bacterial abundance after ln-transformation. First incubation in April showed a negative growth is excluded from subsequent analysis…….……. 61

Figure 5.5 Monthly production of different microbial components in MMFR……..……. 64

Figure 5.6 Dilution experiment from April 2013 to February 2014. X-axis shows dilution factor and y-axis shows phytoplankton growth as measure by chlorophyll *a*…….. 66

Figure 5.7 Temporal variations of primary production and herbivory activity of microzooplankton as estimated by the dilution technique. ……...… 68

Figure 5.8 Temporal variations of bacterial production and bacterivory activity of HNP and microzooplankton (MZP). Note that on some occasions (no reading), there was no significant grazing ($p>0.05$). ……..…….. 68

Figure 5.9 Regression analysis between primary production and grazing by microzooplankton (µg C/l/day). Linear regression slope and coeff of determination (r^2) is shown…….. 72

Figure 5.10 Arrhenius plot of primary production and grazing activity of microzooplankton in the estuary. (μ - phytoplankton growth and g – microzooplankton grazing loss)…… 74

Figure 5.11 Temporal variations of bacterial production and bacterivory activity of HNP and microzooplankton (MZP). Note that on some occasions (no reading), there was no significant grazing ($p>0.05$)……. 75

Figure 5.12 Relationships between primary production, bacterial production, heterotrophic nanoplankton production, microzooplankton production and mesozooplankton production during the southwest monsoon (upper panel) and northeast monsoon (lower panel) seasons……. 79

LIST OF TABLES

Table 2.1 Comparison between maximum abundance (ind l^{-1}) of tintinnids found in different parts of the world's ocean (From Zhang and Wang, 2000)…… 12

Table 2.2 Regional comparisons of oceanic, coastal, and estuarine habitats (upper table), tropical, temperate and polar habitats (lower table). Mean values (± standard errors) are given for initial Chl *a*, phytoplankton growth rate (μ), grazing mortality (m), % Chl *a* grazed day $^{-1}$, and % primary production (PP) grazed day^{-1}. (From Calbet and Landry 2004)…….. 15

Table 2.3 Chlorophyll *a* concentration and primary production in some tropical coastal systems of Asia……. 18

Table 4.1 Summary results of two-way ANOVA on environmental parameters and microbial food components at Terusan channel with respect to monsoon and lunar effects. Interaction between monsoon effect and lunar effect is not significant…..

Table 4.2 Ranked monthly abundance of microzooplankton in Terusan channel, MMFR during the study period April 2013-February 2014. Abbreviations used: percentage occurrence (%Occ), neap (n), spring (s). Individuals per litre: 2 = 11–50; 3 = 51–100; 4 = 101–500; 5 = 501–1000; 6 = 1001–2500; 7 = 2501–5000; 8 = 5001–10,000; 9 = 10,001–20,000 10 ≥ 20,001……. 38

Table 4.3 Summary results of two-way ANOVA on environmental parameters and microbial food components at Terusan channel with respect to monsoon and lunar effects……... 39

Table 4.4 Spearman Rank Order correlation matrix among environmental parameters, microzooplankton potential food components and key taxa of microzooplankton in Terusan channel. Abbreviations used: bacteria (Bact), heterotrophic nanoplankton (HNP), phytoplankton (Phyto), Chlorophyll *a* (Chl *a*), salinity (Sal), rainfall (Rain), temperature (Temp), Secchi depth (Secc), dissolved oxygen (DO). Species arranged with percentage occurrence, from highest to lowest in each category. Only significant correlations are shown; pairwise N = 40…….. 44

Table 5.1 A synoptic account for the microzooplankton recorded in MMFR 2013-2014. TL: total length, OD: oral diameter……... 56

Table 5.2 Seasonal variation of biomass, production, and grazing of microbial components in MMFR. ……. 63

LIST OF SYMBOLS AND ABBREVIATIONS

×	times
%	percentage
℃	Degree Celsius
μ	growth rate
g	Microzooplankton grazing rate
DOM	dissolved organic matter
DOC	dissolved organic carbon
μm	micrometer
mya	million years ago
PP	primary production
MMFR	Matang Mangrove Forest Reserve
NE	Northeast
SW	southwest
HNP	heterotrophic nanoplankton
MZP	microzooplankton
chl *a*	chlorophyll *a*
GF/C	glass fibre filter paper
DIN	dissolved inorganic nutrients
LV	lorica volume
C_t	carbon body weight of tintinnid
BL	body length
μ_{PP}	phytoplankton growth rate
P	production rate
B	carbon biomass

G	empirical-determined instantaneous growth rate
T	average temperature
CVol	cell volume
H’	Shannon-Wiener diversity index
S	species richness
J’	Pielou’s evenness
CV	coefficient of variation
ANOVA	analysis of variance
RDA	redundancy analysis
n	number of observations
MHWN	mean high water neaps
MHWS	mean high water springs
MLWN	mean low water neaps
MLWS	mean low water springs
µgC $l^{-1}d^{-1}$	Microgram Carbon per litre per day
km^2	Kilometre square
ind l^{-1}	Individual per litre
cell ml^{-1}	Cell per litre
µgC l^{-1}	Microgram carbon per litre
fg C $cell^{-1}$	Femtogram carbon per cell
pg C μm^{-3}	picogram carbon per micrometer cube
ng	nanogram
ppt	parts per thousand

CHAPTER 1: INTRODUCTION

Microorganisms are very small yet very abundant in the ocean ecosystem, with a total number of cells of more than 10^{29} that inevitably outnumber macroorganisms; more than 90% of marine biomass is microbial. (Bar-On et al., 2018). Microorganisms have a very important role fulfilling key ecosystem functions. They exhibit a wide diversity of sizes and forms; and by being small, they interact with the environment more rapidly than any due to their high surface area to volume ratio. Hence, the distribution of these very small organisms forms the many critical processes in various ecosystems, including mangrove estuary. Almost exclusively, microbes carry out large-scale ecosystem processes of production, decomposition, and nutrient cycling; and each of these is closely linked together. In turn, they contribute greatly to ecosystem biogeochemical resilience, trophic dynamics, and resistance to invasion (Azam & Malfatti, 2007).

The microbial community forms the microbial loop, which recycles the dissolved organic matter (via bacteria respiration) released by phytoplankton in the water body (Figure 1.1). The microbial loop theory suggests that heterotrophic bacteria are key to control the trophic linkages between dissolved organic matter (DOM), particulate organic matter (POM), and inorganic nutrients in aquatic ecosystems. This ideal model suggested that, i. phytoplankton utilized 10-50% of carbon fixed by photosynthesis and their concentration correlates with bacterial biomass; ii. top down control of bacteria biomass by microzooplankton and other heterotrophic nanoflagellates (Azam et al., 1983). A typical planktonic food web displays two pathways of carbon transfer: particles that are directly consumed by phagotrophic organisms and dissolved organic carbon (DOC) that is consumed by bacteria. DOC in the water is supplied by phytoplankton (through photosynthesis and decomposition) and released during phagotrophy when other microbes feed. Microbial food webs often act as a sink for organic matter – heterotrophic

bacteria remineralize organic nutrients into inorganic forms to be taken up primary producers, and at the same time, converting a portion of the organic matter into new bacterial biomass to be grazed by bacterivores. These microbes together remineralize and oxidise most of the organic matter they consume, giving themselves a critical role in the ecosystem. These trophic linkages are tightly coupled when the main organic matter supply from phytoplankton and nutrients are scarce.

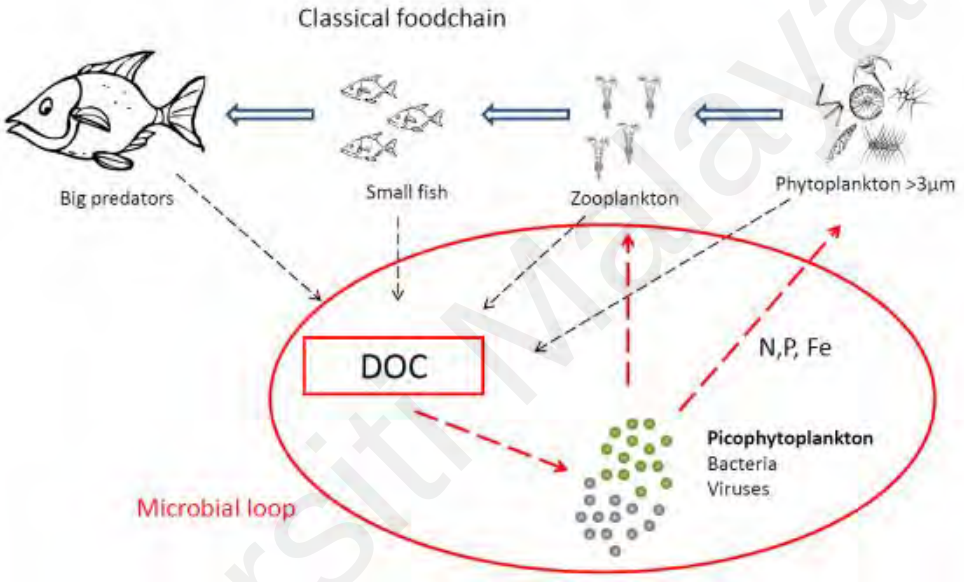


Figure 1.1 Microbial loop as described linking dissolved organic matter to higher trophic levels.

However, in mangrove estuaries where organic matter and nutrients are abundant, the microbial food web linkages may be different. An estuary is defined as a semi enclosed coastal body of water which has a free connection to the open sea and within which sea water is measurably diluted with freshwater derived from land drainage (Pritchard, 1967). With elevated primary productivity, multiple sources of organic matter, limited residence time, changes in the abundance and types of grazers, interactions with sediments and other surfaces, and strong gradient in temperature and salinity, microbial food web

linkages in mangrove estuary should differ greatly from the open ocean paradigm (Figure 1.2).

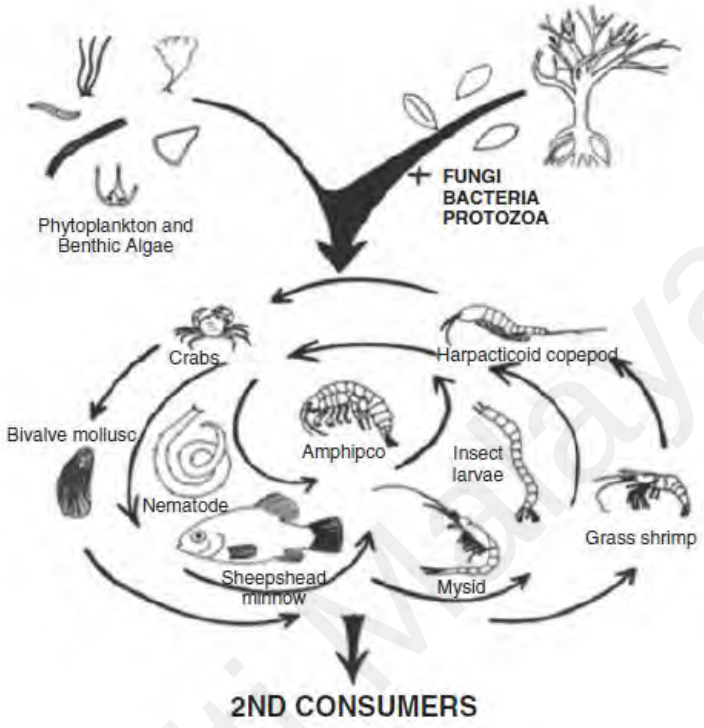


Figure 1.2 Detrital food chain and planktonic food web of estuary (Day et al., 2012)

In the microbial food web, nonlinear changes such as increased average cell size of phytoplankton, elevated growth efficiency of bacteria, and primary production can also affect the trophic linkages in the food web (Day et al., 2012). The microbial community of estuaries made up with all five general groups of microbes: bacteria, algae, protozoa, fungi, and viruses, ranging from 0.1µm to 2000µm (Figure 1.3). The metabolic dominance of bacteria (0.2-0.6 µm) is undeniable as the total mass of heterotrophic bacteria in the ocean exceeds the combined mass of zooplankton and fishes. Flagellates (2-20 µm) are less abundant compared to bacteria and are often found to be heterotrophs or mixotrophs whereas ciliates (20 – 200 µm) are even lesser in abundance than flagellates.

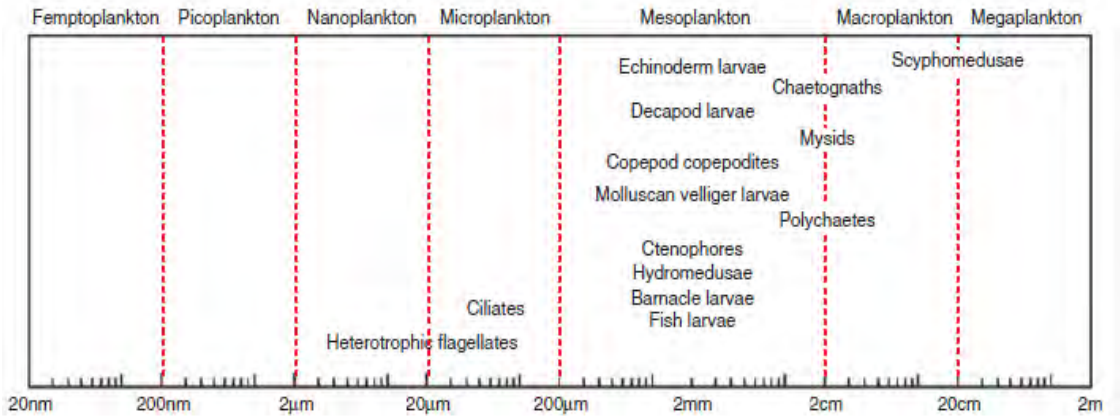


Figure 1.3 Size fractions of microbial community in estuary, size classification of plankton base on Sieburth et al., 1978. (From Day et al., 2012)

Microzooplankton (20-200µm in size) are classified as a group of heterotrophic and mixotrophic organisms and are the top down control factor of microbial food web. Among the microzooplankton, ciliates (subclass Spirotrichea, Oligotrichia and Choreotrichia) and dinoflagellates are predominant ranging between 60-100% (Jyothibabu et al., 2003; Sherr & Sherr, 2007; Sanders, 1987). It also includes foraminiferans, small metazoans, such as copepod nauplii, copepodites, and some meroplanktonic larvae (Calbet, 2008). Ciliates comprise of loricate and aloricate components, for example tintinnids, naked oligotrich ciliates, benthic ciliates, and pelagic ciliates. Dinoflagellates comprise of auto-heterotrophic dinoflagellates, such as the naked dinoflagellates (*Gyrodinium* sp.) and the toxic dinoflagellates (causing red tides).

Together with bacteria and phytoplankton, microzooplankton are recognized as one of the most important groups in marine geochemical cycles of bioactive elements (Sheer & Sheer 2002; Calbet & Landry, 2004). Microzooplankton have higher weight-specific physiological rates such as feeding, respiration, excretion and growth rates as compared to larger metazoans. This enables them to cope better with strong fluctuations in the environment, especially with the constant flushing of estuary and upwelling systems (Calbet, 2008). The smaller microzooplankton are also able to utilize pico- and

nanozooplankton, which are unable to be utilized by larger metazoans. In turn, these organisms act as trophic intermediaries (Uye et al., 1996; Jyothibabu et al., 2003; Calbet, 2008), transferring materials and energy from the microbial loop to higher trophic level metazoans such as mesozooplankton. Grazing of microzooplankton was shown to regulate bacteria and nanophytoplankton population (Verity, 1986; McManus & Fuhrman, 1988). As a major consumer of phytoplankton primary production, Calbet and Landry (2004) showed that microzooplankton grazed an average of 60% primary production, but larger mesozooplankton only consume about 10% of the primary production; in turn, microzooplankton become part of mesozooplankton's diet (Kleppel, 1993; Calbet & Saiz, 2005). Microzooplankton are implicated in the control of harmful dinoflagellates bloom (Sherr & Sherr, 2007).

Although important, there are very few studies on estuarine microzooplankton relative to their truly marine counterparts (Godhantaraman & Uye, 2003). Although the estimated biomass of microzooplankton is always found to be higher than mesozooplankton biomass in the estuary (Buskey, 1993), most of the zooplankton studies have focused on mesozooplankton rather than microzooplankton. This is mainly due to the small size of microzooplankton, limited methods for sample collection and preservation, and difficulties in taxonomic identification (Godhataraman, 2002).

In Malaysia, the Matang Mangrove Forest Reserve (MMFR) has long been the study ground for tropical mangrove in this region and is one of the most sustainable ecosystems in the world despite supporting the community with obvious economic value (Alongi et al., 2004, FAO 2007). Various studies have been conducted in MMFR; from the flora (Gong & Ong, 1990; Eong, 1993) to various fauna such as zooplankton (Chew et al., 2012, 2015; Ramarn et al., 2012), fishes (Ooi & Chong, 2011), mammals (Hoffman et al., 2017), wave impacts (Ismail et al., 2017), and also on its sustainability (Goessens et

al., 2014; Otero et al., 2017). MMFR waters and its sediments are also well-characterised (Alongi et al., 1998a, 2003, 2004, Bong & Lee, 2008). However, the microbial food web and its components remained missing from the above studies.

There are six chapters in the thesis, Chapter 1 is an introduction of the microzooplankton in the microbial loop, Chapter 2 is literature review of this study, Chapter 3 is a methodology description of the community study and grazing relationship, Chapters 4 and 5 are the experimental results, and Chapter 6 is a general conclusion for this study. The major goal of this study is to investigate microzooplankton, the potential key player of the estuary dynamic, thus enable better understanding of the estuary trophodynamics. Therefore, the objectives of this study are

(1) to determine the community structure and abundance of the microzooplankton in Matang mangrove estuaries;

(2) to determine the environmental drivers causing the variations in microzooplankton abundance and community structure, and

(3) to map the flow of carbon sources in the estuary among the microbial components by their production and grazing rates with the focus on microzooplankton.

In this study, the following hypotheses will be tested:

(1) Microzooplankton community structure is dependent on the environmental factors changing with monsoon.

(2) Primary production in the estuary mainly consumed by microzooplankton, thus supported the microbial food web rather than bacterial production.

To achieve the above objectives, the following studies were conducted:

(1) Elucidation of the temporal abundance of microzooplankton in relation to different environmental factors (Chapter 4)

(2) Determining production and biomass of each component of microbial loop (Chapter 5)

CHAPTER 2: LITERATURE REVIEW

2.1 Microzooplankton: Classification and Major Groups

Planktonic communities of the aquatic ecosystem are made up of a diverse collection of organisms. As they are unable to swim against the current, they are adapted physiologically and morphologically to live in the water column. Plankton in the water used to be only in two categories – zooplankton and phytoplankton. Both drifters in the water, phytoplankton produce whereas zooplankton consume. In the light of the advancement in plankton studies, Sieburth et al. (1978) proposed the classification by body size for both zooplankton and phytoplankton (Fig. 1.3). For microzooplankton, the prefix micro- is now added to narrow them to the body size of between 20 and 200µm (Sieburth et al., 1978).

Microzooplankton belongs to the holoplankton – they are planktonic throughout their life cycles. This group of organisms can be mixotrophs or heterotrophs, ranging from unicellular organisms to small metazoan. They are among the most morphologically diverse groups in the plankton community. There are many classifications of microzooplankton and here, we adapt the classification proposed by Calbet (2008). We grouped them into protist and metazoan, protist includes the group of ciliates (Class: Spirotrichea; subclass: Oligotrichia, Choreotrichia), flagellates, dinoflagellates, acantharids, radiolarians, foraminiferans; whereas metazoan includes organisms such as rotifers, and larvae of zooplankton in that size range. This highly diverse group made up a significant component of the plankton community in many marine environments (Olson & Strom, 2002; Leonard & Paerl, 2005; Paterson et al., 2007).

2.1.1 Ciliated Protozoan

Ciliated protozoan is recognised as the main group of the microzooplankton, mainly due to the conspicousness of loricate ciliates – they retain in the plankton nets (Porter et al., 1985; Pomeroy & Wiebe, 1988; Lynn & Montagnes, 1991). Ciliates itself is taxonomically diverse. Morphologically, ciliates can be classified into two major groups, the loricate ciliates, the shell-bearing ciliates (subclass: choreotrich) and aloricate ciliates, the naked ciliates (subclass: oligotrich). Loricate ciliates carry a species-specific external shell or lorica, made of protein with the shape of bowl or vase or tube, hence it can be easily preserved and identified. Fossils record of tintinnids loricate was first recorded by Rüst (1885); of those tintinnids that are from Proterozoic era (~2500mya). Detailed morphology description for identification was published as early as 1929 (Kofoid & Campbell, 1929) whereas aloricate ciliate only came into systematic classification after Corliss (1961). Quantitatively, aloricate ciliates often outnumber loricate ciliates (Leakey et al., 1993; Huang et al., 2012). To date, classification of ciliates still under revision after Corliss (2016) published the latest book on ciliated protozoa.

Among the loricate ciliates, tintinnids (Order: Tintinnida), are a large subgroup of over 1200 species in 75 genera. Lorica of the ciliates could appear to accumulate with foreign particles (Family: Tintinnidiidae, Codonellidae, Codonellopsidae, Coxliellidae), or appear as clean and transparent (Family: Favallidae, Undellidae) (Marshall, 1969). With the easy identification of their lorica morphology, tintinnid emerged as an ideal component to study species distributions, diversity, and changes in the community structure of microzooplankton (Sarkar, 2015). Study of tintinnids are well established; covering major parts of the ocean including North Atlantic (Campbell, 1942; Lindley, 1975), Pacific (Kofoid & Campbell, 1939), Mediterranean Sea (Jörgensen, 1924), Western Arabian Sea (Zeitzschel, 1969, 1982), coast of India (Godhantaraman, 2002;

Rakshit et al., 2014; Sarkar, 2015), the Adriatic Sea (Krsinic, 1988), Japan (Yamamoto et al., 1981; Uye et al., 1996) and China (Wang et al., 2014a; Li et al., 2016; Li et al., 2019). Pierce & Turner (1993) showed that most species of tintinnids are cosmopolitan but in their study, information from tropical Southeast Asia is close to unknown (Fig 2.1, Table 2.1).

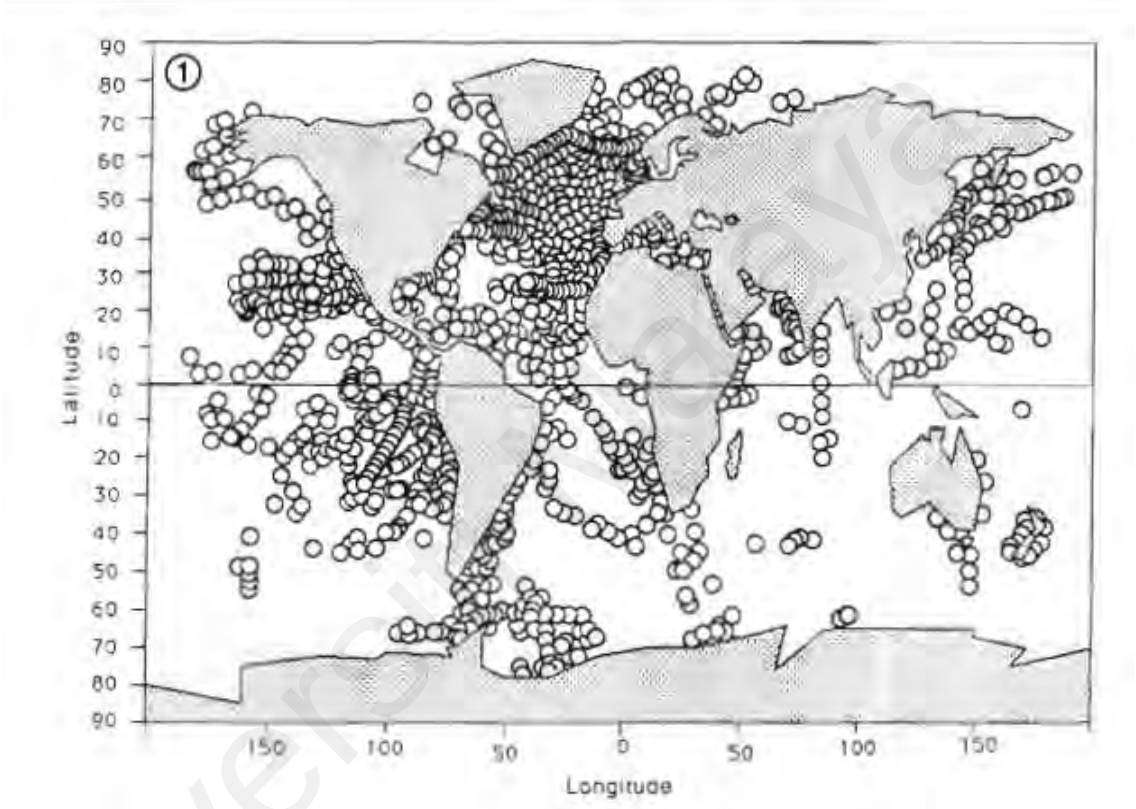


Figure 2.1 Literature record of tintinnid distribution (Pierce and Turner, 1993). Note that tropical Southeast Asian water remains unknown in the review.

Within the phylum Ciliophora Doflein, 1901, there is a vast assemblage of morphologically and ecologically diverse organisms exploiting a variety of food resources, and interacting within the ecosystem (Fauré-Fremiet, 1924). Dolan (1991) showed that ciliates can be classified into three different trophic groups, (a) macrophagous that consumes nanoplankton-size or larger prey), (b) microphagous (consumers of picoplankton—size prey), or (c) predatory (consumer of other ciliates). Each guild plays significant roles in top down and bottom up control. The macrophagous

ciliates (herbivory ciliates), consumes more than 75% of primary production (Putland & Iverson, 2007; Calbet, 2008) and serves as a competitor of herbivory mesozooplankton (Calbet, 2008). The microphagous ciliates, or the bacterivorous ciliates, are important in regulating bacterioplankton populations, both quantitatively (Gast, 1985; Sheer et al., 1986a,b; Albright et al., 1987; Bernard & Rassoulzadegan, 1990; Kalinowska, 2020; Shi et al., 2020; Simo-Matchim, 2020), and qualitatively through selective feeding (Gonzalez et al., 1990; Matz et al., 2002). Finally, the predatory ciliates, or the carnivorous ciliates, with higher clearance rate than mesozooplankton, help to regulate the ciliate community (Robertson, 1983; Stoecker & Capuzzo, 1990). In Dolan (1991), ciliates biomass of Chesapeake Bay consists of 73% macrophagous ciliates, 15% microphagous, and the rest were predatory ciliates. The macrophages ciliates were seen dominating elsewhere, such as Inland Sea of Japan (Uye et al., 1996), Parangipettai, southeast coast of India (Godhantaraman, 2002), São Sebastião Channel, Brazil (Eskinazi-Sant Anna et al., 2006), and many others.

Table 2.1 Comparison between maximum abundance (ind l^{-1}) of tintinnids found in different parts of the world's ocean (From Zhang & Wang, 2000)

Maximum abundance	Location	References
1	Adriatic Sea	Krsinic, 1982
91	Gullmar Fjord, Norway	Hedin, 1975
100	Larzarev Sea	Froneman et al., 1996b
219	Funka Bay, Japan	Dohi, 1982
288	Bay of Mali Ston	Krsinic, 1987a
300	Northern Arabian Sea	Garrison et al., 1998
402	Georges Bank, Northwest Atlantic	Stoecker et al., 1989
422	Adriatic Sea	Krsinic, 1987b
504	Southern Ocean	Froneman & Perissinotto, 1996a,b
600	Bellingshausen Sea, Antarctic	Burkill et al., 1995
780	Western Pacific	Suzuki & Taniguchi, 1998
1000	Solent estuary, England	Burkill, 1982
>1000	Chesapeake Bay, U.S.A.	Coats & Heisler, 1989
1200	Gullmar Fjord, Swedish west coast	Hernroth, 1983
1400	Greenland	Nielsen & Hansen, 1995
1440	Halifax Harbour, Nova Scotia	Gifford, 1988
1500	Irish Sea	Graziano, 1989
2000	Dokai Inlet, Japan	Uye et al., 1998
2000	Washington Coast	Landry & Hassett, 1982
3636	West Coast of Norway	Cordeiro et al., 1997
5000	Southern California, U.S.A.	Beers & Stewart, 1970
5700	The Seto Inland Sea, Japan	Kamiyama & Tsujino, 1996
6700	Laizhou Bay, Bohai Sea, China	Zhang and Wang, 2000
7000	Damariscotta River estuary, U.S.A.	Sanders, 1987
9600a	Long Island Sound	Capriulo & Carpenter, 1980
9765	West coast of Denmark	Cordeiro et al., 1997
11 300	Bahia Blanca Estuary, Argentina	Barria de Cao, 1992
12 600	Long Island Sound, U.S.A.	Capriulo & Carpenter, 1983
15 000	North Coast of Denmark	Cordeiro et al., 1997
23 000	Seto Inland Seam, Japan	Kamiyama, 1994
270 000	Narragansett Bay, U.S.A.	Verity, 1987
729 000	Flodevigen Bay, Norway	Dale & Dahl, 1987

2.1.2 Dinoflagellates

Dinoflagellates are often heterotrophic (Lessard & Swift, 1985; Lessard, 1991; Verity et al. 1993). They were observed feeding on bacteria (Lessard & Swift, 1985), large

diatoms (Hansen, 1992), copepod larvae and nauplii (Sekiguchi & Kato, 1976). Heterotrophic dinoflagellates were often associated with diatom blooms and could make up to 50% of microzooplankton biomass during such events (Sheer & Sheer, 2007). These dinoflagellates are fed by phagocytosis and consume a significant portion of bloom forming diatoms relative to copepods and other mesozooplankton (Sheer & Sheer, 2007). On the other hand, dinoflagellates serve as an important food source for mesozooplankton (Calbet, 2008).

2.1.3 Metazoans

Copepod nauplii make up the greatest number of multicellular organisms of microzooplankton (Calbet, 2008). These are the larval form of copepods and other pelagic and benthic crustaceans, for instance, adults and copepodites of calanoid genera such as *Paracalanus*, *Clausocalanus*, and *Acartia*; cyclopoid genera such as *Oithona*, *Oncaea*, and *Corycaeus*; planktonic harpacticoids of the genus *Microsetella*; and nauplii of almost all copepod species (Turner, 2004). Relative to their adults, the small copepod nauplii are often found to record higher biomass (Hopcroft et al., 1998; Turner 2004). They are predatory and depending on genera, copepod nauplii appear to be opportunistic feeders or show food selectivity (Turner, 2004).

Rotifers were also commonly found within this size range in coastal communities. This group of metazoans often decreases in biomass as salinity increases (Park & Marshall, 2000); they are more commonly seen in freshwater environments. Park & Marshall (2000) demonstrated that rotifers seasonally replace copepod nauplii (achieving over 50% of annual biomass) in Chesapeake Bay. Relative to protists, rotifers have better swimming ability because of the beating cilia around their mouth. These organisms are predators, sharing prey with copepod nauplii and ciliates. Lionard et al. (2005) showed that rotifers are non-selective grazers on phytoplankton.

2.2 Ecological Importance of Microzooplankton

The role of microzooplankton as the primary grazers of marine food webs has emerged in recent years (Calbet & Landry, 2004; Huang et al., 2011; Schmoker et al., 2013; Zhou et al., 2015a,b) especially since Landry and Hasset (1982) developed the dilution technique with minimal equipment requirement. This technique allows the estimation of grazing impact of microzooplankton on natural communities of marine phytoplankton in concurrent with an estimation of primary production. Similar grazing experiments were also conducted on calanoid copepod *Eurytemora affinis*, the cyclopoid copepods *Acanthocyclops robustus* and *Cyclopsvicinus* and the cladocera *Chydorus sphaericus*, *Moina affins* and *Daphnia magna/pulex* that showed no significant grazing (Lionard et al., 2005). This further assures the role of microzooplankton as the main grazer of primary production in the water.

Microzooplankton is widely known for their importance as trophic intermediaries in the marine food web – recycling nutrients, grazing phytoplankton, and linking the microbial loop (Calbet & Landry, 2004). With their small body size and high abundance, microzooplankton stands an advantage of having higher physiological rate. Relative to mesozooplankton, microzooplankton has only about one tenth of the size of mesozooplankton. According to Rubner's Law (Fenchel, 1987), ingestion rate of microzooplankton on primary production is about 178 times lower than mesozooplankton, but because microzooplankton has about 1000-fold higher in abundance than mesozooplankton, microzooplankton can clear up over 5 times more phytoplankton than mesozooplankton (Dolan et al., 2013). With their small body size also, they are able to ingest smaller food particles that is unavailable to mesozooplankton (Robertson, 1983; Stoecker & Capuzzo, 1990).

Microzooplankton removes about half of the primary production (Tsuda et al., 2010; Landry & Calbet, 2004), and they also graze on bacteria and other smaller protozoans (Gonsalves et al., 2017; Rejas et al., 2005; Sherr & Sherr, 1987). With their intense grazing on phytoplankton, microzooplankton is seen as a key control on blooms in many coastal waters (Christaki et al., 2021; Yang et al., 2020; Irigioen et al., 2005). Since Landry and Hasset (1982)'s dilution technique, the microzooplankton grazing is well studied globally but studies in tropical mangrove waters are focused only in India (Gonsalves et al., 2017; Gauns et al., 2015; Jyothibabu et al., 2008a,b) and tropical South America (Conroy et al., 2016; MacManus et al., 2007; First et al., 2007). There is yet a study from tropical Southeast Asia even though Southeast Asia comprises a vast span of tropical estuaries that have high chlorophyll *a* concentration. Tropical estuaries in Southeast Asia have the highest phytoplankton growth rate among other habitats (oceanic and coastal), and with most of the chlorophyll *a* produced grazed by microzooplankton (Table 2.2) (Calbet & Landry, 2004). Of all of the parameters, tropical water showed the highest value whereas polar regions exhibited the lowest (Table 2.2). This marked the intense knowledge gap in the field with the missing information from tropical waters.

Table 2.2 Summary of dilution experiments on regional comparisons of oceanic, coastal, and estuarine habitats (upper table), tropical, temperate and polar habitats (lower table). Mean values (± standard errors) are given for initial Chl *a*, phytoplankton growth rate (μ), grazing mortality (m), % Chl *a* grazed day $^{-1}$, and % primary production (PP) grazed day^{-1} (From Calbet & Landry, 2004)

	Chl *a* (μg L^{-1})	μ (day^{-1})	m (day^{-1})	% Chl *a* grazed	% PP grazed
Oceanic	0.58±0.03	0.59±0.02	0.39±0.01	41.5±1.4	69.6± 1.5
Coastal	3.06±0.53	0.67±0.05	0.40+0.04	47.3±4.4	59.9±3.3
Estuarine	13.0±1.8	0.97±0.07	0.53 +0.04	78.7±7.3	59.7±2.7
Tropical	1.01 ±0.21	0.72±0.02	0.50±0.02	55.1 ±2.3	74.5 +2.0
Temperate	5.18±0.66	0.69±0.03	0.41±0.02	51.4±2.9	60.8±1.8
Polar	0.62±0.06	0.44±0.05	0.16±0.01	19.5±2.1	59.2±3.3

Apart from primary production, microzooplankton were shown to feed on bacteria. Bacterivory in microzooplankton were mainly by oligotrich ciliates (aloricate ciliates), including *Stromnidium* (Fenchel & Jonsson, 1988) and *Strombidium* (Paranjape & Gold, 1982). These oligotrichs consumed approximately 1 to 38% of bacterial production (Pierce & Turner, 1992). However, high levels of bacteria biomass (ranging from 10^6 to 10^8 cell ml^{-1} concentration) are needed to support exclusively bacterivorous ciliates (Gast, 1985); therefore, only estuaries and/or areas polluted by sewage could support such assemblage. In general, bacterivory in microzooplankton are often seen as opportunistic.

Aside from grazing on primary production, microzooplankton is an important food source of mesozooplankton (Calbet & Saiz, 2005). Copepods such as *Acartia*, *Calanus*, *Eucalanus* feed heavily on tintinnids, whereas *Tortanus* feed selectively on tintinnids (Robertson, 1983; Ayukai, 1987; Pierce & Turner, 1992). Aloricate ciliates such as *Strombilidium* are also part of the copepod's diet.

2.3 Microbial Loop - Microzooplankton Community in the Estuary

Before Azam et al. (1983) highlighted the energetic role of microbes and brought up the term 'microbial loop', these highly diverse, actively growing assemblages of Archaea, bacteria and protists were often neglected. Since then, a whole new microbial world was discovered and many questions on marine microbial carbon flux were answered (Azam, 1998; Calbet & Landry, 2004; Lee & Bong, 2006) yet the role of bacteria as a sink or link in the microbial loop is still being debated. Bacteria could package the dissolved organic matter (DOM) as bacterial carbon and transfer it into the higher trophic level, as a link; but metazoans could consume labile particulate organic matter without going through bacteria (Pozzato et al., 2013; Sherr et al., 1987; Ducklow et al., 1986). With that, the role of other microorganism as a link in the place of bacteria in different ecosystem attracted major interest.

2.3.1 Bacterial Production and Primary Production

Traditionally, primary production was thought to be only consumed by the herbivores in the water. Through grazing the organic carbon fixed by photosynthesis transferred to higher trophic levels. But as study advanced, Azam et al. (1983) showed that major flux of organic matter moves via dissolved organic matter into bacteria and the microbial loop; up to 5 to 50% of carbon fixed by primary production is utilised by bacteria. Within the ideal microbial loop model, bacteria are the key component responsible for recycling organic matter, hence bacterial production becomes a key process in determining the carbon flux of the system (Azam et al., 1983).

Bacterial production and phytoplankton production dynamics played a significant role in the trophic interaction. Strong correlation between phytoplankton and bacterial production were shown in most of the water body implying phytoplankton as the most important autochthonous source of bacterioplankton growth substance or both phytoplankton and bacteria grew in response to a common factor (Cole et al., 1988; Almeida et al., 2005; Lee & Bong, 2008). On the other hand, weak coupling suggests the allochthonous source of organic carbon as playing a more important role (Findlay et al., 1991; Tranvik, 1992). However the elevated primary production of estuarine water often complicates this relationship (Table 2.2, Table 2.3).

Table 2.3: Chlorophyll *a* concentration and primary production in some tropical coastal systems of Asia.

Country & Location	Habitat	Chl *a* concentration (μg l^{-1})	Primary Production (μgC l^{-1} day^{-1})	Reference
Malaysia, MMFR	Estuarine mangrove	7 - 45	2 - 751	Alongi et al., 2003
Malaysia, Port Klang	estuarine	0.20 - 4.47	30 - 1380	Lim et al., 2015
Malaysia, Port Dickson	Coastal water	0.14 - 2.76	30 - 2000	Lim et al., 2015
Malaysia, MMFR	Estuarine mangrove	5.1 – 54.7	148 – 4021	Current study
India, Zuari estuary	Estuarine mangrove	4.4 – 39.8	60 - 662	Gomes et al., 1991
India, Cochin Estuary	Estuarine mangrove	1.7 - 47	n/a	Madhu et al., 2007
India,Bay of Bengal	Coastal water	13 - 18	242–265	Jyothibabu et al., 2008b
India, Zuari estuary	Estuarine mangrove	0.18 – 12.78	27.6 - 81	Gauns et al., 2015
Thailand, Sawi Bay	Estuarine mangrove	2 - 12	200 - 600	Ayukai & Alongi, 2000

2.4 Mangrove Ecosystem

Being one of the most dynamic ecosystems on earth; mangrove forests cover approximately 181 000 km^2 of tropical, subtropical, and warm-temperate coastlines (Alongi, 2002). Mangroves are highly variable ecosystems where variations in waves, tides, river flow, and rainfall shape the unique characteristics of each mangrove (Wolanski, 1992). As a result, different salinity regimes generated by advective and longitudinal mixing, and trapping of coastal water encourage different communities of micro and macroorganisms to flourish (Alongi, 2009). Freshwater input during the wet season is a key factor of allochthonous nutrient enrichment (Trott & Alongi, 1999; Wong, 2003) especially phosphorus as atmospheric phosphorus is almost negligible (Tyrell, 1999).

Mangrove estuaries have been known to serve as nursery and feeding grounds for a variety of fish and prawns including those of commercially important species (Chong, 2007). Several hypotheses have been proposed to explain why mangrove habitats are

attractive to juvenile fish and prawns. One hypothesis that has received the most attention states the juvenile fish and prawns are attracted to the food supply from the detritus food web. Based on the examination of fish stomach contents, zooplankton especially copepods and hyperbenthic shrimps (i.e. acetes and mysids) constituted a large proportion of juvenile fish diet in the Matang mangrove estuaries, Malaysia (Chew et al., 2007). Given that zooplankton (Robertson et al., 1988; Chew & Chong, 2011) and benthic animals (Muhammad Ali et al., 1999) are more abundant in mangrove estuaries and nearshore waters compared to offshore waters, it is apparent that mangrove estuaries are food-rich ecosystems supporting various trophic level consumers in the food webs (Robertson & Blaber, 1992; Laegdsgaard & Johnson, 2001). As mangrove detritus contributes a large proportion of the organic matter in mangrove estuaries, Odum & Heald (1975) advanced their idea that mangrove and coastal food webs must be fueled by mangrove carbon directly or indirectly via microorganisms that decompose mangrove detritus. It is on this premise that many subsequent works in the 80s had rested arguments on. With technological advancement, the role of mangrove (versus phytoplankton) as the primary producer or nutritional source for marine consumers has become a bone of contention.

Matang Mangrove Forest Reserve (MMFR) has long been the study ground for tropical mangrove in the region. Various study been conducted in MMFR; from the flora (Gong & Ong, 1990; Eong, 1993), to various fauna such as the zooplankton (Chew et al., 2012, 2014; Ramarn et al., 2012), fishes (Ooi & Chong, 2011), mammals (Hoffman et al., 2017), wave actions (Ismail et al., 2017), the microbe *Escherichia coli* (Ghaderpour et al., 2015), its waters and sediments (Alongi et al., 1998b, 2003, 2004, Bong & Lee, 2008), and also its sustainability (Otero et al., 2017; Goessens et al., 2014). Since Tarutani et al. (2007) showed the lack of relationship between the mesozooplankton production and phytoplankton production in the estuary, there has yet to be studies on the key to the

missing link – microzooplankton production. This study of microzooplankton community and its trophic role alludes to the importance of microzooplankton in the highly productive estuarine mangrove water.

CHAPTER 3: MATERIALS AND METHODS

3.1 Sampling Site Description

The chosen study site was at the Terusan channel, a 6 m-deep mangrove creek located in the Matang Mangrove Forest Reserve (MMFR) in the state of Perak, west coast of Peninsular Malaysia. Terusan channel (4°526'N, 100°341'E) interconnects the Selinsing and Sangga Besar channels downstream of the Matang estuary (Figure 3.1). The MMFR is a silvicultural mangrove production forest planted with mainly the favoured mangrove species, *Rhizophora apiculata* Blume. The large mangrove reserve of approximately 41,000 ha consists of mangrove forests situated on the coast and several deltaic islands drained by the tributaries and interconnecting channels of three main rivers, the Sepetang, Larut and Terong. The tidal regime in the MMFR is semidiurnal, with MHWS, MHWN, MLWN and MLWS of 2.1, 1.5, 0.9 and 0.3 m above Chart Datum, respectively (National Hydrographic Centre, Malaysia). The deltaic estuary is relatively shallow with an average depth of 4.2 m, but depths ranged from 0.5 m to 14.4 m. A strong estuarine stratification in the upper estuary is evident during the northeast (NE) monsoon season (November – March) during heavy rainfall when large quantities of freshwater is flushed into the estuaries. On the other hand, no significant stratification is observed during the southwest (SW) monsoon season (May-September) when the rainfall is relatively lower (Chew et al., 2015). Nevertheless, freshwater inflows and weak vertical mixing during the SW monsoon particularly during neap tide may form a temporary salt wedge that could extend as far as 10 km upstream from the river mouth (Tanaka & Choo, 2000). The water column is generally vertically well mixed during spring tides (Chew et al., 2015).

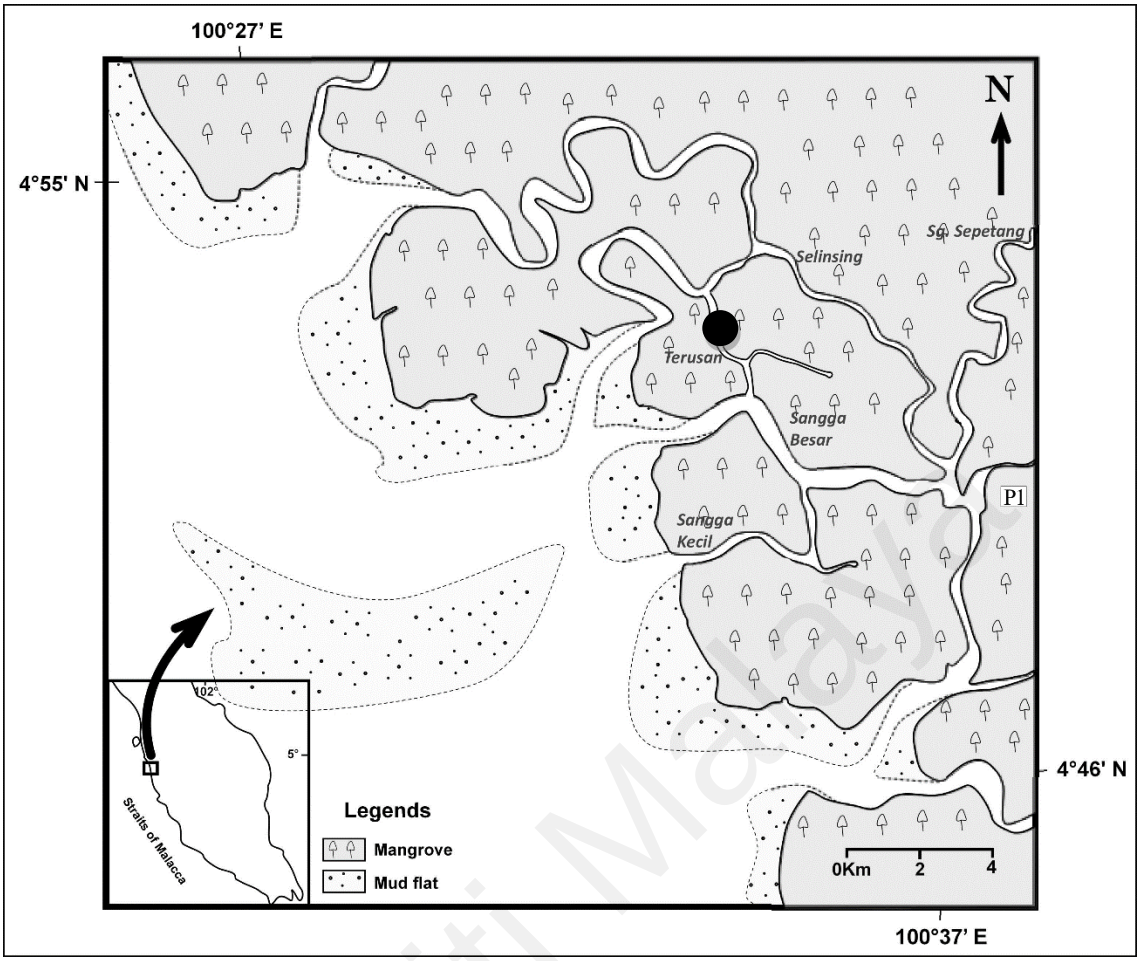


Figure 3.1 Map of Matang Mangrove Forest Reserve. Filled circle indicates the location of Terusan channel where samples were taken.

The *Terusan* channel connects between two large rivers, the Sangga Besar and Selinsing (Figure 3.1). The study site was chosen because of its location away from the main fishing village of Kuala Sepetang and aquaculture (cage fish) activities at the river mouths. There were no other human activities in or around the Terusan including clear-felling of mangrove trees at the time of study.

In the present study, different empirical investigations were carried out in the Matang mangrove estuaries and adjacent coastal waters to elucidate the dynamics and ecological importance of microzooplankton in this mangrove system.

3.2 Field Sampling

Semimonthly samplings (during spring and neap tide) were conducted at sampling station in the deltaic estuary (Fig. 3.1), from April to August 2013 (SW monsoon) and from October 2013 to February 2014 (NE monsoon), to collect both live samples and preserved sample of microzooplankton and their potential food components viz. phytoplankton, bacteria and heterotrophic nanoplankton (HNP). A sampling strategy to collect largely non-active swimmers from a continuous flow of water in opposite directions (flood and ebb flow) within the channel was adopted. No particular tide (flood versus ebb) was favoured for samplings since preliminary studies had indicated no systematic bias between tides.

3.2.1 Routine Sampling of Microbial Community

Water samples at 1 m from the surface were collected using a 4.2-L vertical Van Dorn sampler during daytime (1000 hr to 1400 hr). Surface water sampling was favoured since bottom samples contained less microzooplankton and were subsets of surface samples (Dolan et al., 2002). Five-hundred ml of the collected water sample were poured into a bottle for estimation of chlorophyll *a* and dissolved inorganic nutrients concentrations, 30 ml for bacteria and protists counts and two litre for large phytoplankton and microzooplankton enumeration, respectively. Duplicate samples were made at each sampling station. Samples for bacteria and protists were preserved immediately with filtered glutaraldehyde (0.2 µm pore size) to a final concentration of 4%, while samples for large phytoplankton and microzooplankton were fixed with 0.4% Lugol's iodine. All samples were kept in ice before laboratory analyses.

On top of the above, two 10-l acid washed jerry cans were filled carefully to avoid bubbles with the water collected by Van Dorn water sampler for live samples of the microbials components. Hydrographic measurements, i.e. salinity (ppt), dissolved

oxygen (mg l^{-1}), pH and temperature (°C) were taken using a YSI 556 handheld multiparameter probe. Light transparency of the water column was measured using a Secchi disc. In all, 40 water samples from the Terusan station were examined during the study. Rainfall data from the nearest meteorological station (Taiping Hospital, 10km to the east of MMFR) were obtained from the Malaysian Meteorological Department at Kuala Lumpur.

3.3 Laboratory Analysis

3.3.1 Chlorophyll *a* and Dissolved Inorganic Nutrients

For estimation of chlorophyll *a* concentration, the water sample was filtered through a Whatman GF/C 47-mm filter paper and chlorophyll pigments retained on the filter paper were extracted using 90% acetone. The concentration of chlorophyll *a* was determined by a fluorometric method (Parson et al., 1984) using a Perkin Elmer LS55 spectrofluorometer. Samples were analysed as soon as possible (< 7 days) so as to avoid further pigment degradation. Filtered seawater was analysed for dissolved inorganic nutrients (DIN, i.e. nitrite, nitrate, ammonium, silicate, and phosphate) using standard methods (Parson et al., 1984) and a Hitachi U1900 spectrophotometer.

3.3.2 Bacteria and Heterotrophic Nanoplankton Abundance

Both bacteria and heterotrophic nanoplankton (HNP) were counted using a direct-count method with a U-MWU filter cassette (excitor 330-385 nm, dichroic mirror 400 nm, barrier 420 nm) epifluorescent microscope under 1000x magnification (Olympus BX50, Tokyo, Japan). For bacteria, a 0.5 ml sample was filtered onto a black 0.2 µm pore size isopore filter. The filter was then stained with 4',6-Diamidino-2-Phenylindole (DAPI, 1 µg ml^{-1} final concentration) for seven minutes. A 5 ml sample of HNP was filtered onto a black 0.8 µm pore size isopore filter and then stained with 250 mg l^{-1} primuline for 10

minutes (Caron, 1983). Slides were kept frozen and their microscopy fields were photographed within three days. A minimum of 10 microscope fields or 300 cells were counted for bacteria, and at least 50 microscope fields or 30 cells were observed for HNP. To estimate the photoautotrophs, each microscope field was also viewed under the U-MWG filter cassette (excitor 510-550 nm, dichroic mirror 570 nm, barrier 590 nm) for the autofluorescence of chlorophyll *a*. The abundance of photoautotrophs was subtracted from HNP counts. Later, both biomass of bacteria and heterotrophic nanoplankton were converted into C biomass from the biovolume according to the following conversion factor.

Bacteria: 13.9 ± 6.7 fg C $cell^{-1}$, n=311 (Lee and Bong, 2008)

HNP: biovolume * 0.22 pg C μm^{-3} (Børsheim and Bratbak, 1987)

3.3.3 Phytoplankton and Microzooplankton Abundance

Samples fixed with 0.4% Lugol's iodine were allowed to stand for at least 48 hours at the laboratory before 1.5 l of the clear top liquid was siphoned off. The remaining 500 ml sample was gently tilted back and forth in its bottle to homogenize the mixture before pouring it into a sedimentation chamber (Utermöhl, 1958). The plankton samples were allowed to settle down for at least another 48 hours before 25 ml concentrate was collected from the cavity base. Lugol's iodine (2 ml) was added to the plankton concentrates to obtain a final concentration of 10% iodine for long term preservation (Stoecker, 1994). Enumeration was done by transferring 1 ml of concentrated sample onto a Sedgwick-Rafter chamber and viewed under an inverted microscope (Leica DM IL LED) at 200x. The entire chamber was examined; tintinnids were identified to species based on lorica size and shape whereas the other microzooplankton were identified to the lowest taxa possible under magnification of 200x (Kofoid & Campbell, 1929; Marshall,

1934; Marshall, 1969; Zhang et al., 2012). Phytoplankton, which was enumerated at the same time, were identified to genus level (Tomas, 1997).

Measurements for the microzooplankton (length and width) were taken accordingly. For loricate ciliates, the lorica volume was estimated by using different combinations of geometrical shapes according to Bryansteva & Kurilov, 2003 (see appendix)From lorica volume (LV, μm^3), the carbon body weight of a tintinnid (C_t, pg) was calculated by using the regression equation: $C_t = 444.5 + 0.053LV$ (Verity & Lagdon, 1984). Lorica occupancy was assumed to be 100%. For aloricate ciliates, carbon weight was converted from cell volume by using a factor of 0.14 pg C μm^{-3} (Putt & Stoecker, 1989) whereas for copepod nauplius, carbon content (ng) was calculated from body length (BL, μm) using the regression equation:

$C_c = 1.51 \times 10^{-5} BL^{2.94}$ (Uye unpublished, in Uye et al., 1996).

3.4 Microbial Process Rate Measurements

Concurrent estimations of primary production and microzooplankton grazing rates were carried out according to the dilution method by Landry & Hassett (1982). Seawater was prefiltered with a 200 μm sieve to remove mesozooplankton. Seawater was also filtered through a 0.2-μm membrane filter to generate particle-free seawater which was then used to dilute the prefiltered seawater sample to give the following fractions: 0.2, 0.4, 0.6, 0.8 and 1.0 (undiluted). Each treatment was duplicated and incubated at *in situ* water temperatures and corrected to a 12 h light: 12 h dark cycle (Lim et al., 2015). The growth rate of phytoplankton in each fraction was plotted against dilution factor, and the potential phytoplankton growth rate (μ_{PP}) was determined from the y-intercept. The microzooplankton grazing rate (g) was determined when the linear regression slope was

negative. Apparent production and grazing rate were calculated by multiplying μ_{PP} and g with the *in situ* chlorophyll *a* concentration, which was then converted to C biomass (Carbon:Chlorophyll-*a* conversion factor = 78) according to Lim et al., (2017).

The production rate of the microzooplankton (P, mg C $m^{-3}d^{-1}$, unit equivalent to µg C $l^{-1}d^{-1}$) was estimated as P = B × G where B is the carbon biomass (mg C m^{-3}, unit equivalent to µg C l^{-1}) and G is the empirically-determined instantaneous growth rate (d^{-1}). G is determined according to the following equation from Mülller & Geller (1993): G = 1.52 ln T – 0.27 ln CVol – 1.44, where T is the average temperature of the present study site (29.3°C) and CVol is the cell volume (μm^3).

Bacterial production, HNP production and grazing losses were estimated using a modified method from Wright & Coffin (1984). Plankton in seawater collected were fractionated into its <2-µm, <20-µm and <200-µm components of pico-, nano- and microplankton by using sieves of the appropriate mesh sizes to remove the larger components. It was assumed that the <2-µm sieve-fraction contained only bacteria; <20-µm sieve-fraction contained HNP and bacteria, and the < 200-µm sieve-fraction contained bacteria, HNP and microzooplankton. A 100 ml sample of each filtrate was taken in duplicate to be incubated in the dark at in-situ temperatures for 12 h (Lee & Bong, 2008). An incubation time of 12 h was found to prevent significant microbial and chemical changes that often occurred after 18 h of incubation (Peduzzi & Herndl, 1992; Agis et al., 2007).

In the <2-µm sieve-fraction, bacterial growth rate (µ) was expressed as µ = (In N2- In N1)/T, where N1 and N2 are the bacterial counts at the beginning and end of an incubation period T. HNP increase in the <20-µm sieve-fraction was expressed as HNP growth rate (h^{-1}). HNP production was then estimated from the multiplication of both growth rate and biomass. In the <20-µm sieve-fraction, bacteria grew with HNP grazing pressure ($\mu_{20\text{-}\mu m}$),

and the difference between $\mu_{2\text{-}\mu m}$ and $\mu_{20\text{-}\mu m}$ was calculated as HNP grazing rate. The amount of HNP grazed by the microzooplankton was also estimated via the changes in HNP in the <200-µm ($HNP_{200\text{-}\mu m}$) and <20-µm ($HNP_{20\text{-}\mu m}$) fractions. As microzooplankton grazing should be more pronounced in the <200-µm fraction, the difference between $HNP_{20\text{-}\mu m}$ and $HNP_{200\text{-}\mu m}$ should reflect the HNP grazed by microzooplankton.

3.5 Data And Statistical Analysis

Prior to ANOVA, the abundance of bacteria, HNP, phytoplankton, chlorophyll *a* data were $\log_{10}$ (x+1)-transformed to meet parametric assumptions. Most microzooplankton taxa abundance did not meet parametric assumptions even after data transformation. Hence, non-parametric methods were used to test for significant difference. The Mann-Whitney test was performed to examine separately the monsoonal and lunar effect on the abundance of microzooplankton. One-way Kruskal-Wallis test was then carried out to test the combined effects of monsoon and tide, namely, SW-spring, SW-neap, NE-spring and NE-neap. Spearman rank order correlation was used to determine the significant relationship (if any) among environmental and microzooplankton variables. All statistical tests were performed using the Statistica Version 8 program (StatSoft Inc., 2007).

Redundancy analysis (RDA) is a constrained ordination technique used to construct ordination axes that are also linear combinations of the environmental variables (Ter Braak & Smilauer, 2002). Here, the method was used to relate the microzooplankton abundance to the measured environmental parameters. The top 30 most abundant microzooplankton species and 14 environmental and biological parameters (i.e. rainfall, salinity, dissolved oxygen, temperature, Secchi disc depth, dissolve inorganic ammonia, nitrate, nitrite, phosphate, and silicate, bacteria abundance, HNP abundance, and

phytoplankton abundance) were selected for RDA. All biological data were $\log_{10}$ (x+1)-transformed. RDA was performed by CANOCO 4.5 program (Ter Braak & Smilauer, 2002).

For microbial process rate measurements, the least-square linear regression test was carried out for each dilution test. Correlation analysis was used to show relationships between the different environmental and biological parameters measured. All statistical tests were carried out using STATISTICA 8 (StatSoft, Tulsa, USA).

CHAPTER 4: RESULT AND DISCUSSION - MICROZOOPLANKTON ABUNDANCE AND COMMUNITY STRUCTURE IN THE TERUSAN MANGROVE CREEK

Work done in this chapter is published in

Yong, Y.L., Chew, L.L., Lee, C.W. & Chong, V.C. (2016). Monsoonal and lunar variability in microzooplankton abundance and community structure in the Terusan mangrove creek (Malaysia). Marine Biology Research. 12(3):278-93.

4.1 Environmental Parameters

Environmental parameters in the Matang estuary were strongly influenced by monsoon season. Rainfall, temperature, salinity, pH and chlorophyll *a* concentration showed significant differences between monsoons. The SW monsoon started off with decreasing rainfall from April (308.2 mm) to July (93.8 mm; Fig, 4.1). During this period the Terusan channel was characterized by higher mean salinity (22.2 ± 0.7 ppt), warm water (30.1 ± 0.3 °C), and higher chlorophyll *a* concentration (24.79 ± 3.77 $\mu g\ l^{-1}$) relative to other months. Towards the late SW monsoon (August) and with the onset of NE monsoon (October), the rainfall increased 2 to 3 folds (Figure 4.1), ranging from 204.4 mm (August 2013) to 481.3 mm (November). During the peak NE monsoon (October 2013 to January 2014), the channel become enriched with higher dissolved inorganic nutrients (nitrate and phosphate; $p<0.05$; Table 4.1). Temperature, salinity, and chlorophyll *a* concentration became lower at 28.4 ± 0.2 °C, 18.4 ± 0.1 ppt and 10.57 ± 1.47 $\mu g\ l^{-1}$, respectively. The NE monsoon then ended with a dry spell in February. Rainfall decreased (74.9 mm) and water in the channel turned warmer (30.4 ± 0.3 °C; with higher salinity 27.4 ± 0.3 ppt). Chlorophyll *a* concentration increased relative to the

peak NE monsoon (16.71 ± 2.36 µg l^{-1}). Water transparency as measured by Secchi disc depth and dissolved oxygen concentration showed no difference between monsoons (Table 4.1).

Mean Secchi disc depth was significantly higher during neap than spring tide ($p < 0.01$, Table 4.1). No lunar phase effects ($p>0.05$) were observed for other water parameters. Although there was no significant difference in concentration between lunar phases for all DIN, the concentration of all DIN was comparatively higher during spring tide than neap tide (Table 4.1, Fig. 4.2).

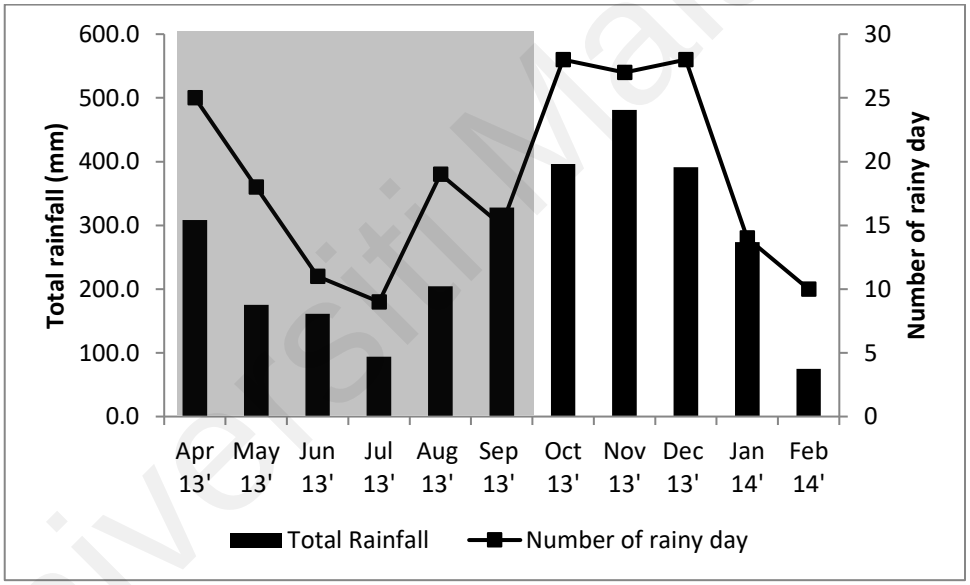


Figure 4.1: Monthly total rainfall and number of rainy days recorded from April 2013 to February 2014 at Taiping located 10 km east of the study site. Shaded region indicates SW monsoon period (April-September); unshaded region indicates NE monsoon period (October-Feb).

Table 4.1: Summary results of two-way ANOVA on environmental parameters and microbial food components at Terusan channel with respect to monsoon and lunar effects. Interaction between monsoon effect and lunar effect is not significant.

Variables	Source of Variations			
	Monsoon effect		Lunar effect	
	SW	NE	Neap	Spring
n	20	20	20	20
Physical Parameters				
Rainfall (mm)	188.52 ± 16.05 ***	323.48 ± 32.3 ***	256 ± 29.38	256 ± 29.83
Temperature (°C)	29.9 ± 0.3 **	28.8 ± 0.2 **	29.5 ± 0.3	29.2 ± 0.2
Secchi depth (m)	0.48 ± 0.05	0.45 ± 0.02	0.54 ± 0.04 ***	0.38 ± 0.02 ***
Salinity (ppt)	23.0 ± 0.7 *	20.0 ± 0.9 *	21.0 ± 0.9	22.0 ± 0.8
DO (mg l^{-1})	2.81 ± 0.27	3.17 ± 0.33	3.22 ± 0.36	2.76 ± 0.22
pH	7.6 ± 0.2 **	7.1 ± 0.1 **	7.3 ± 0.1	7.3 ± 0.1
Chlorophyll *a* (μg l^{-1})	21.82 ± 3.31 *	11.56 ± 1.14 *	16.67 ± 2.97	16.71 ± 2.49
Dissolved inorganic nutrients (μM)				
NH_4^+	2.40 ± 0.5	3.23 ± 0.98	1.98 ± 0.33	3.7 ± 1.04
NO_2^-	2.56 ± 0.34	2.55 ± 0.43	2.50 ± 0.4	2.62 ± 0.36
NO_3^-	1.16 ± 0.32 *	2.84 ± 0.39 *	2.1 ± 0.37	2.51 ± 0.42
PO_4^{3-}	1.08 ± 0.07 **	1.62 ± 0.17 **	1.30 ± 0.11	1.37 ± 0.17
SiO_2	68.19 ± 8.43	88.01 ± 9.72	72.06 ± 9.52	83.72 ± 8.83
Diversity measures				
H'	2.47 ± 0.05 ***	1.94 ± 0.12 ***	2.13 ± 0.13	2.28 ± 0.9
J’	0.82 ± 0.01 ***	0.66 ± 0.04 ***	0.72 ± 0.04	0.77 ± 0.03

Table 4.1 continued

Variables	Source of Variations			
	Monsoon effect		Lunar effect	
	SW	NE	Neap	Spring
n	20	20	20	20
Microbial food components (cell l^{-1})				
Bacteria	$4.80 \pm 0.29 \times 10^9$	$4.65 \pm 0.53 \times 10^9$	$4.94 \pm 0.52 \times 10^9$	$4.51 \pm 0.32 \times 10^9$
HNP	$2.72 \pm 1.26 \times 10^7$ *	$1.24 \pm 0.58 \times 10^7$*	$1.24 \pm 0.49 \times 10^7$	$2.73 \pm 1.29 \times 10^7$
Phytoplankton	$1.24 \pm 0.16 \times 10^5$*	$8.8 \pm 0.6 \times 10^4$*	$9.74 \pm 1.17 \times 10^4$	$1.15 \pm 0.14 \times 10^5$
Microzooplankton	$4.21 \pm 0.53 \times 10^3$***	$1.33 \pm 0.27 \times 10^4$***	$1.09 \pm 0.28 \times 10^4$	$6.6 \pm 1.16 \times 10^3$

Significance: *** $p<0.001$, ** $p<0.01$, * $p<0.1$

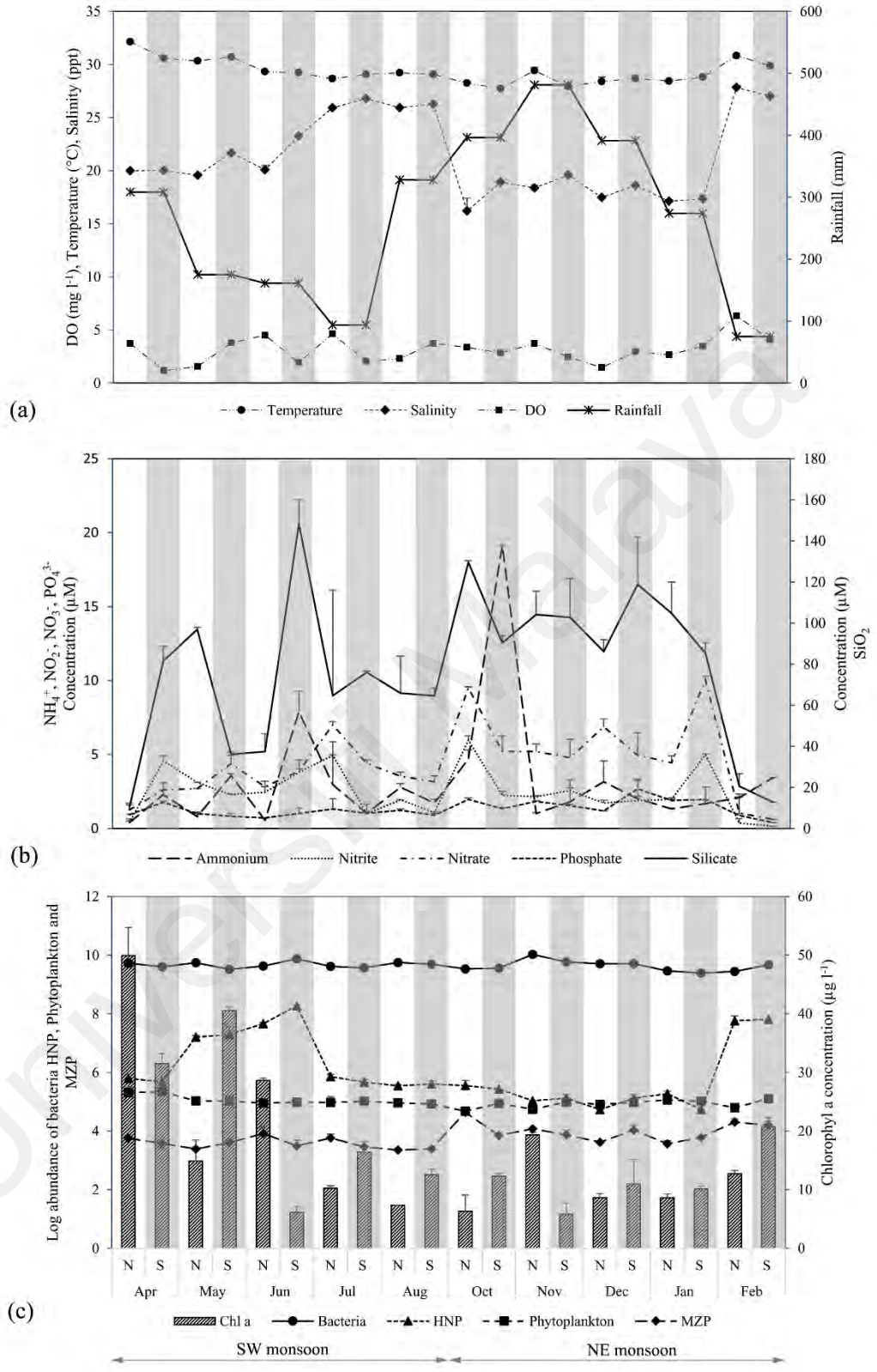


Figure 4.2 Monthly mean of (a) physical parameters, (b) dissolved inorganic nutrients and (c) microzooplankton potential food components. N denotes neap tide; S denotes spring tide. Error bars (S.E.) are indicated.

4.2 Bacteria, HNP, and Phytoplankton Abundance

Bacteria abundance was constant ($p > 0.05$) throughout the sampling period (2.42×10^9 to 1.14×10^{10} cell l^{-1}) despite a slight peak that occurred in November 2013 (Figure 4.2). Compared to bacteria, abundance of HNP was more variable among sampling months with peaks recorded in June 2013 (1.96×10^8 cell l^{-1}) and February 2014 (7.48×10^7 cell l^{-1}) respectively (Figure 4.2). Mean abundance of HNP ($1.98 \times 10^7 \pm 4.38 \times 10^7$ cell l^{-1}) was approximately three orders of magnitude lower than bacteria abundance ($4.72 \times 10^9 \pm 1.88 \times 10^9$ cell l^{-1}) and was significantly more abundant during the SW monsoon than the NE monsoon ($p < 0.05$, Table 4.1). Phytoplankton abundance and chlorophyll *a* concentration ranged from 3.46×10^4 cell l^{-1} to 3.69×10^5 cell l^{-1} and 5.14 µg l^{-1} to 54.71 µg l^{-1}, respectively. Both chlorophyll *a* concentration and phytoplankton abundance were significantly higher during the drier SW monsoon. Phytoplankton community was dominated by *Skeletonema costatum*, composing 64% of phytoplankton abundance. There was no significant difference in abundance of bacteria, HNP and phytoplankton and chlorophyll *a* concentration between lunar phases ($p > 0.05$; Table 4.1)

4.3 Microzooplankton Diversity, Abundance, and Composition

A total of 39 microzooplankton taxa were identified in the sampling period. The highest species richness, S (26 taxa) was recorded during spring tide in both June 2013 and February 2014 and the lowest S (14 taxa) was recorded during neap tide in November 2013. Both Shannon-Wiener diversity index (H') and Pielou's evenness (J') were significantly lower during the NE monsoon as compared to the SW monsoon ($p < 0.01$; Table 4.1) due to the dominance of the aloricate cyclotrichs in October 2013 (> 80% of total abundance; Table 4.2, Fig. 4.3). Both H' and J' were not significantly different between the lunar phases ($p > 0.05$, Table 4.2).

The overall mean microzooplankton abundance was 8.74 ± 9.65 × 10^3 ind l^{-1}. Mean total abundance of microzooplankton was significantly higher during the NE monsoon as compared to the SW monsoon ($p < 0.05$; Table 4.1, Figure 4.2); peak microzooplankton abundance occurred in October 2013 and lowest abundance in August 2013 (Figure 4.3). The microzooplankton were predominated by loricate ciliates during the SW monsoon (50.92 ± 5.82 %), while the aloricate ciliates were predominant during the NE monsoon (38.99 ± 8.83 %; Figure 4.3). Copepod nauplii were always the least abundant component between both monsoons and lunar effects, ranging from 60 ind l^{-1} to 1275 ind l^{-1} across months.

Out of the 39 identified microzooplankton taxa, 29 taxa belonged to the loricate ciliates. Four major species of loricate ciliates (> 95% occurrence; see Table 4.2) were identified, namely, *Tintinnopsis beroidea*, *Tintinnopsis rotundata*, *Stenosemella avellana* and *Tintinnidium primitivum*. *Tintinnopsis rotundata* occurred more during the SW monsoon, while *Tintinnidium primitivum* dominated during the NE monsoon (Table 4.3). Except *T. primitivum* and *T. acuminata*, most of the loricate ciliates were commonly more abundant during the SW monsoon. All three *Leprotintinnus* species were present almost the year round (Table 4.2) and only showed significantly higher abundance during SW monsoon (Table 4.3). Most of the loricate ciliates did not exhibit significant difference in abundance between lunar phases except for *Favella ehrenbergii* and *Tintinnopsis nana*. The large *F. ehrenbergii* was only observed during neap tide while *T. nana* was significantly higher in abundance during spring tide (Table 4.2 and 4.3). There were eight rare species with relatively low abundance of less than 1%, namely, *Tintinnopsis mortenensis, T. vasculum, T. acuminata, T. butschlii, Tintinnidium incerta, Rhizodomus tagatzi*, *Eutintinnus* sp., *Amphorellopsis* spp. (Table 4.2). Interestingly, these rare species were all collected only once during SW monsoon, except for *Eutintinnus* spp. which were collected twice during the spring tide, one each during SW monsoon and NE monsoon.

The aloricate ciliates were represented by five major families from Mesodiniidae, Strombidiidae, Strobilidiidae, Pleuronematidae and Vorticellidae. Aloricate ciliates were more abundant during NE monsoon, with Strombidiidae, Strobilidiidae and Mesodiniidae showing significant difference between monsoon periods. In particular, the monotypic Mesodiniidae in the Cyclotrichiida, was only present during the rainy months of NE monsoon (October to January, Table 4.2). An exceptional peak abundance of cyclotrichs was recorded in October 2013, reaching 36,250 ind l^{-1}. Lunar effect on the aloricate ciliate community was not significant.

Five genera of dinoflagellates (i.e. *Peridinium, Ceratium, Dinophysis, Noctiluca* and *Prorocentrum*) were identified. *Peridinium, Prorocentrum* and *Ceratium* showed the same distribution; they were significantly higher during the NE monsoon, reaching maximum abundance towards the end of NE monsoon as the dry spell began. Of all the dinoflagellates genera, *Ceratium* spp. recorded an exceptional peak (11,313 ind l^{-1}), about 5 folds its average abundance during the end of NE monsoon when the local climate was at its driest (74.9 mm, Table 4.2). No significant lunar phase effect was observed in the dinoflagellate community.

Copepod nauplii were present in all samplings. As opposed to most microzooplankton which did not exhibit lunar phase difference, copepod nauplii recorded significantly higher ($p<0.001$) mean abundance during neap tide.

Table 4.2 Ranked monthly abundance of microzooplankton in Terusan channel, MMFR during the study period April 2013-February 2014. Abbreviations used: percentage occurrence (%Occ), neap (n), spring (s). Individuals per litre: 2 = 11–50; 3 = 51–100; 4 = 101–500; 5 = 501–1000; 6 = 1001–2500; 7 = 2501–5000; 8 = 5001–10,000; 9 = 10,001–20,000 10 ≥ 20,001.

	%Occ	Apr 13'	May 13'	Jun 13'	Jul 13'	Aug 13'	Oct 13'	Nov 13'	Dec 13'	Jan 14'	Feb 14'

Species		n	s	n	s	n	s	n	s	n	s	n	s	n	s	n	s	n	s	n	s
Loricate Ciliate																					
Tintinnopsis. rotundata	100	3	4	4	3	4	4	3	4	2	3	4	3	2	3	4	3	2	2	3	3
Tintinnidium primitivum	100	3	2	4	2	4	4	4	2	4	2	5	4	6	3	4	5	5	5	4	5
T. beroidea	95	4	5	2	4	4	4	3	2		4	5	4	4	4	4	5	4	4	4	2
Stenosemella avellana	95	4	4	3	4		4	4	4	2	4	4	4	8	3	4	4	2	4	6	2
T. minata	90	2	4	2	4	4	4	2	4		3	2	2	4	2		2	2	3	7	3
T. tubulosa	90			4	4	4	3	3	2	4	2	4	3	4	3	3	4	2	3	3	3
T. nana	85	2	3	3	4	2	4		2	2	3	2	2	3	2	2	3	4	5		
L. elongatus	80	2	4	2	2	4	2	2	3	3	3			2		2	2	2	3		4
T. meuneri	75	5	4	4	4	4	2		2	3	2		4				4	4	4	3	2
Leprotintinnus nordqvisti	75	4	3	3	4	4	2	2	4	2	4					2	2		2	3	5
T. tocatinensis	65	4	4	3	4	4	2	2	2	2	3								2	4	4
L. bottnicus	65	3	2	2	3	2	2		4	2	2				2			2		2	3
Codonellopsis spp.	50	4	3	2	3	4		3		2	2									4	2
Tintinnidium incerta	50						3	2	2	2	2		2	2				2		2	2
T. chinglannensis	45	2	3		4	2		2			2					2				2	2
T. mortenensi	40	3	2			3	2					2	2						2		2
T. directa	30	3				2			2		2									2	4
T. acuminata	30											4		2	4	10	2	2			
Favella ehrenbergii	30			2		3						2		3		3				2	
T. tubolosoides	25		4			4	2				2						4				
T. lobianci	10	4	3																		
Rhizodomus tagatzi	10									2	2										
Eutintinnus spp	10						2												2		
T. vasculum	5		2																		
T. annulata	5	4																			
T. butschlii	5	2																			
Amphorellopsis spp.	5																			2	
Aloricate ciliate																					
Strombidiidae	85			4	4	6	4	6	4	4	3	4	6		6	6	6	5	6	7	4
Strobilidiidae	80			2		2	3	4	3	4	2	6	6		4	4	2	2	4	3	2
Pleuronematine	65			2			2	2	4	4		3	3	3	4	3	3			2	2
Mesodiniiidae	30											10			7	4	5	4	4		
Vorticellidae	30		6				4		5									4		5	5
Dinoflagellates																					
Peridinium spp.	100	5	4	4	4	5	4	4	4	4	4	6	5	4	5	4	6	3	3	6	5
Ceratium spp.	95	2		4	4	6	3	6	3	2	4	4	4	2	4	4	7	4	6	7	9
Dinophysis spp.	85			3	4	5	2	3	3		4	2	3	2	3	4	4	2	4	6	4
Prorocentrum spp.	75	3		2			2	4		2	2	4	4	5	5	4	4	2		6	2
Noctiluca spp.	30	4			2	4		2			2										2
Others	65					2	2	2	2		2	3	2		2		2	2	2	2	3
Nauplius																					
Nauplius <200µm	100	6	3	5	4	6	4	6	4	4	4	5	4	6	3	4	4	4	4	6	5

Table 4.3 Summary results of two-way ANOVA on environmental parameters and microbial food components at Terusan channel with respect to monsoon and lunar effects.

	Mann-Whitney Test		Kruskal-Wallis ANOVA	
	Effects	*p*-value	*p*-value	Effects
Loricate ciliates				

Tintinnopsis rotundata	SW > NE	0.029		
Tintinnidium primitivum	NE > SW	<0.001	0.001	NE-s, NE-n > SW-s, SW-n
Stenosemella avellana			0.007	NE-s, NE-n, SW-s > SW-n
T. minata			0.008	SW-s > SW-n, NE-s, NE-n
T. nana	s > n	0.046		
Leprotintinnus elongatus	SW > NE	0.004		
T. meunieri	SW > NE	0.027		
L. nordqvisti	SW > NE	<0.001	0.002	SW-n, SW-s > NE-n, NE-s
T. tocatinensis	SW > NE	0.003		
L. bottnicus	SW > NE	0.007		
Codonellopsis spp.	SW > NE	0.041		
T. acuminata	NE > SW	0.022		
Favella ehrenbergii	n > s	0.001		
T. tubolosoides	SW > NE	0.05		
Stenosemella spp.		0.009		
Aloricate ciliates				
Strombidiidae	NE > SW	0.007	0.008	NE-s > SW-n, SW-s, NE-n
Strobilidiidae	NE > SW	0.033		
Mesodiniidae	NE > SW	<0.001		
Dinoflagellates				
Peridinium spp.	NE > SW	0.037		
Ceratium spp.	NE > SW	0.008	0.015	NE-s > SW-n, SW-s, NE-n
Prorocentrum spp.	NE > SW	<0.001	<0.001	NE-s, NE-n > SW-s, SW-n
Noctiluca spp.	NE > SW			
Nauplius				
Nauplius	NE > SW	<0.001	<0.001	SW-n, NE-n > SW-n, SW-s

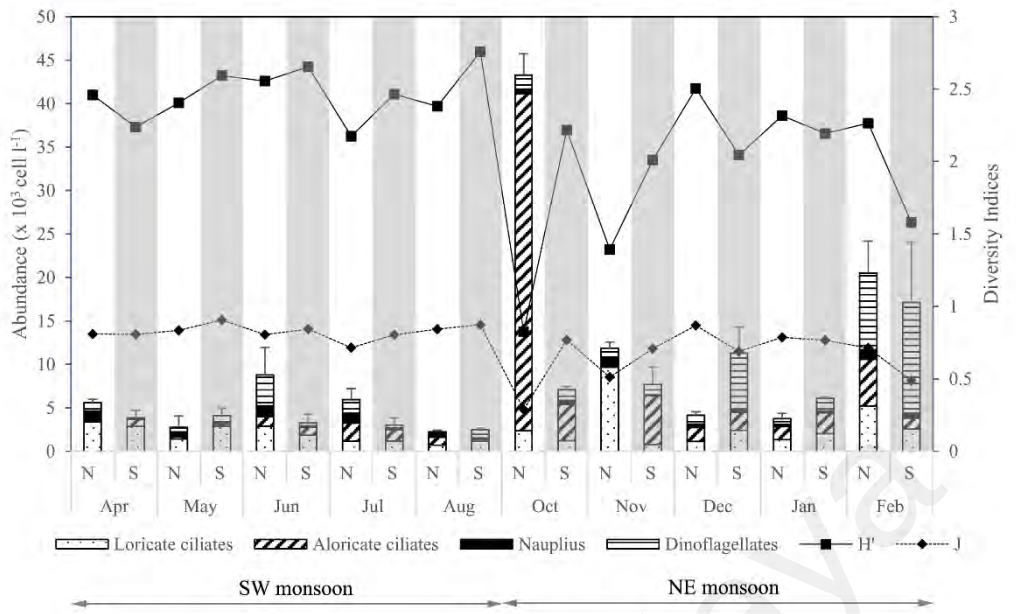


Figure 4.3 Monthly abundance and composition of major microzooplankton groups, including species diversity and species evenness index in Terusan channel, from April 2013 to February 2014. Error bars indicate standard error for total abundance. Abbreviations are as given in Figure 4.2.]

4.4 Species-environment Relationship

The relationship between environmental parameters and 30 microzooplankton species (those with >30% of occurrence) is depicted as an ordination triplot in Figure 4.5, derived from redundancy analysis (RDA). The first two canonical axes explained 44.8% of the total variance in the species data and 55.3% of the species-environment relation. Monthly total rainfall which was relatively higher during the NE monsoon (compared to SW monsoon) was correlated to most DIN concentrations, positively with NO_2^-, PO_4^{3-}, SiO_2, NO_3^- (upper-right quadrant), dissolved oxygen and negatively with salinity (diagonally opposite quadrant). Temperature and chlorophyll *a* concentration were correlated and relatively higher during the SW monsoon. Pearson's correlation test further verified the significant correlations between dissolved inorganic nitrate, phosphate and silicate with rainfall ($0.38 \leq r \leq 0.52$) and salinity ($-0.52 \leq r \leq -0.41$). Among all the dissolved inorganic

nitrogen, nitrate appeared to affect the community to greater extent as compared to ammonium and nitrite (Table 4.4).

For potential food sources of microzooplankton, HNP which had higher abundance during the SW monsoon was positively and highly correlated with salinity (and negatively correlated with rainfall), and to a lesser extent with temperature, chlorophyll *a* concentration and dissolved oxygen (see also Table 4.4). Bacteria abundance as indicated by the short arrow was not obviously related to the physical and chemical parameters, showed no significant changes throughout the sampling. Both bacteria and phytoplankton were not closely related to any particular monsoon period. Except for bacteria, both HNP and chlorophyll *a* exhibited negative correlation with dissolved inorganic nutrients (Table 4.4), i.e. their arrow heads pointed in opposite directions (Figure 4.5). Among all potential food sources, phytoplankton as represented by chlorophyll *a* concentration showed the most significant relationship with microzooplankton (Table 4.4). Scanning electron micrograph (Figure 4.4) further confirms that the microzooplankton do not only rely on phytoplankton for food but also to built their lorica hence the close correlation.

Based on the (monthly) sample distribution and the rainfall arrow on the ordination triplot (Figure 4.5), axis 1 on the right (positive) was interpreted to indicate the trend of increasing rainfall and the dominant effect of the NE monsoon, whereas axis 1 on the left (negative) indicates higher temperature and the dominant effect of the SW monsoon. On the other hand, axis 2 on the top (positive) indicates higher DO and at the bottom (negative) indicates higher salinity. Thus, there is a clear separation in community structure of microzooplankton between the SW and NE monsoon.

Microzooplankton community was represented by large-bodied loricate ciliates with an affinity for higher salinity and temperature during the SW monsoon (negative axis of

RDA1). The large-bodied loricate tintinnid species (ca. 150 µm in total length) included *Leprotintinnus nordqvisti* (Lnor), *L. elongatus* (Lelo*), L. bottnicus* (Lbot)*, Tintinnopsis meuneri* (Tmeu)*, T. tocatinensis* (Ttoc), *T. chinglanensis* (Tching) and *Codonellopsis* spp. (Cod). These species also show strong positive correlations with chlorophyll *a* concentration ($0.38 \leq R \leq 0.64$) and HNP ($0.38 \leq R \leq 0.68$; Table 4.4).

The relatively small loricate tintinnid species (ca. 80 µm) *Stenosemella avellana* (Save), *Tintinnopsis nana* (Tnan), *T. rotundata* (Trot) and *T. beroidea* (Tber) were sampled throughout the sampling months. Except *Stenosemella avellana* which showed significant correlation with phytoplankton, all species did not show any association with changes in their food abundance (Table 4.4). Although loricate ciliates were more abundant during SW monsoon, *Tintinnopsis tubulosa* (Ttub). *T. acuminata* (Tacu) and *Tintinnidium primitivum* (Tnpri) were among the tintinnids that were more abundant during the NE monsoon. The aloricate ciliates, strobilidiids (Strob), strombilidiids (Strom) and Pleuronematine (Pleu) were sampled throughout the sampling period, but they peaked in abundance during the NE monsoon (positive axis 1). These aloricate families were also probably influenced by DIN concentrations. Only *Vorticella* among the aloricate ciliates showed negative correlation with rainfall ($R= -0.41$, $p<0.05$), Dinoflagellates *Ceratium* spp. (Cer), *Dinophysis* spp. (Dphys) and *Prorocentrum* (Pror) were also found to be more abundant during the NE monsoon as compared to the SW monsoon. The former two genera bloomed during the latter part of the NE monsoon when total rainfall decreased. *Noctiluca* was the only dinoflagellate that showed greater abundance during the SW monsoon.

Copepod nauplii, the only metazoan group in the microzooplankton was positioned on the negative side of axis 2; it was neither associated with any potential food nor rainfall.

Abundance of copepod nauplii however showed significant correlation with dissolved oxygen and Secchi disc depth (Table 4.4).

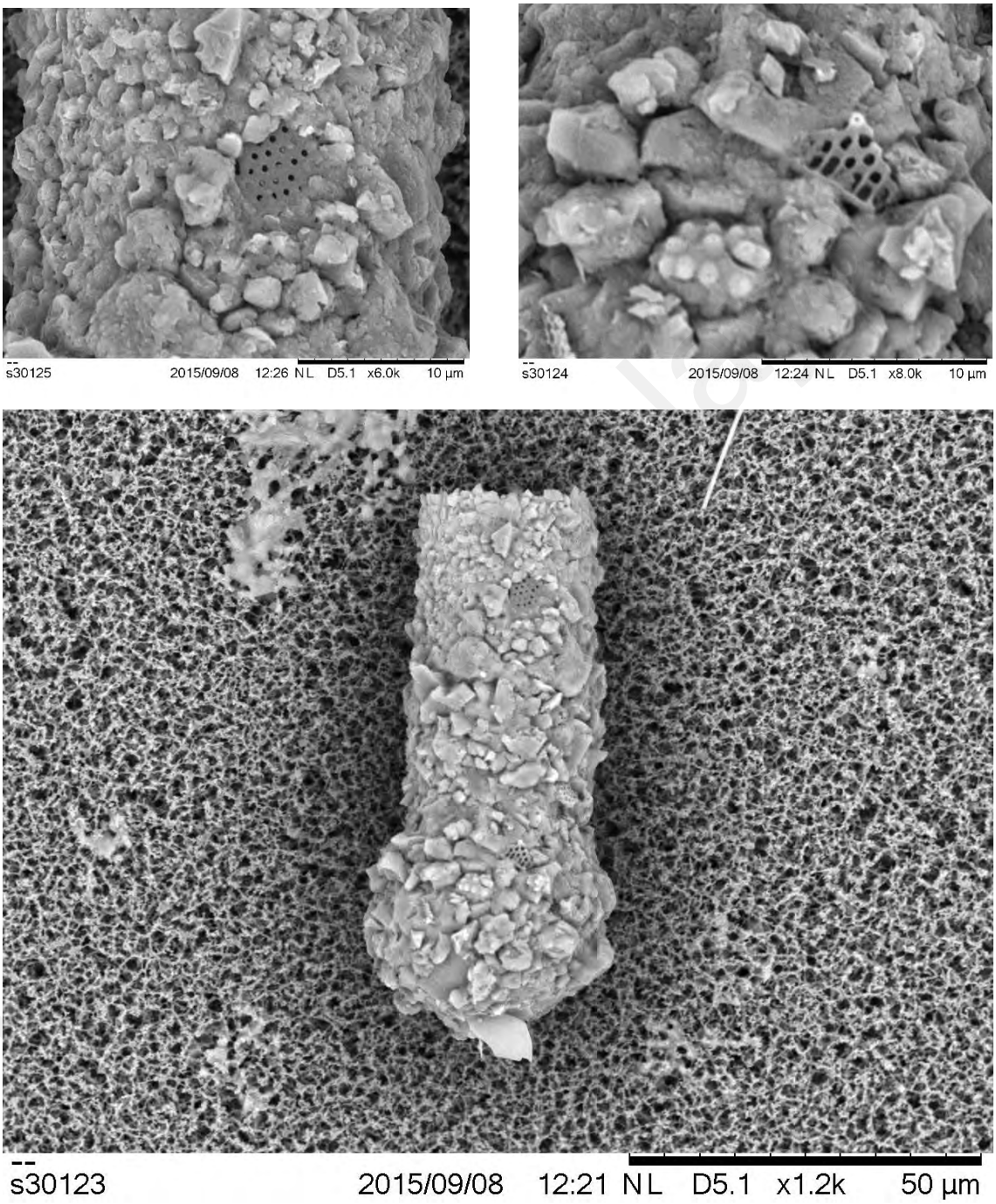


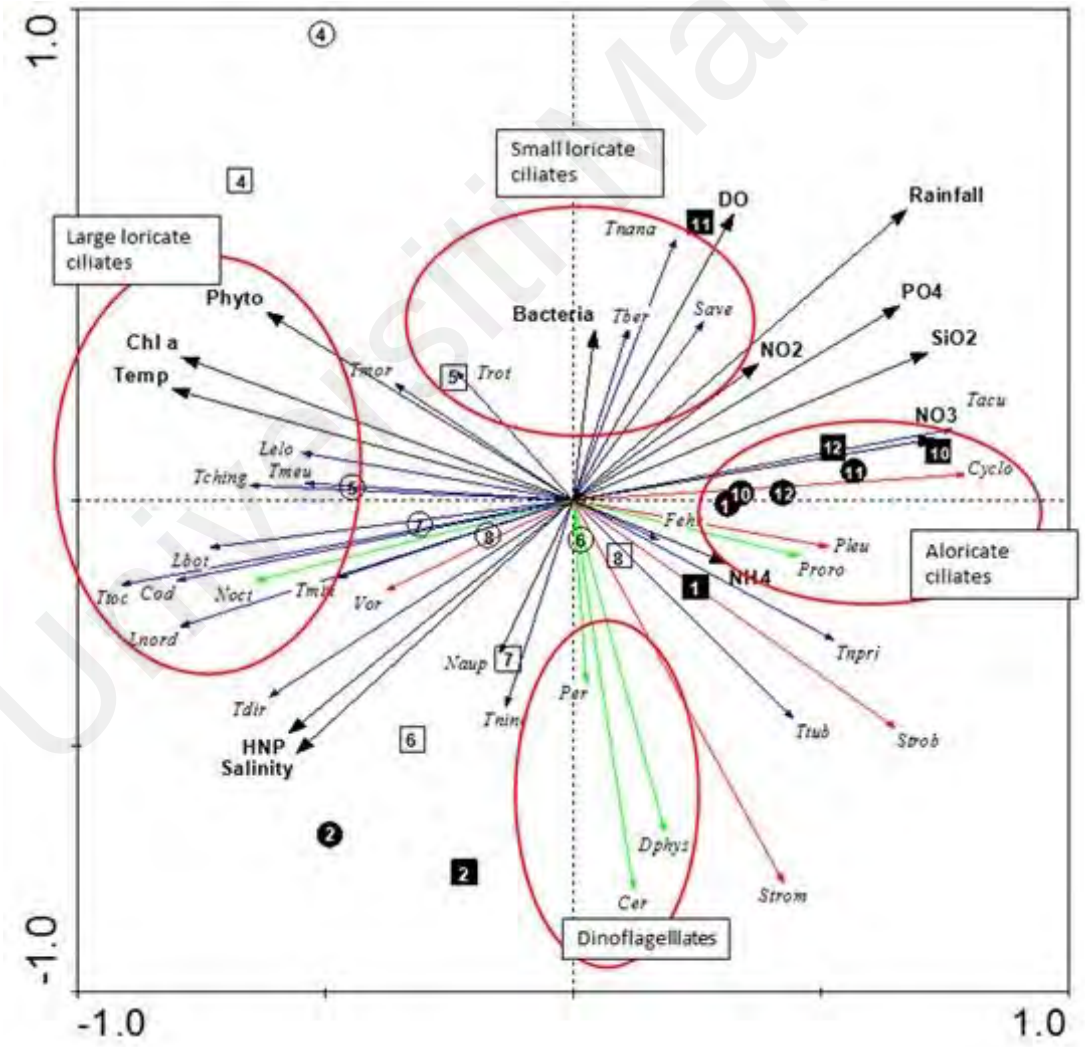
Figure 4.4 Scanning Electron Microscope imagae of *Tintinnopsis beroidea*. Upper 2 images we zoomed in to show that shells of tintinnids were made up by diatoms.

Table 4.4: Spearman Rank Order correlation matrix among environmental parameters, microzooplankton potential food components and key taxa of microzooplankton in Terusan channel. Abbreviations used: bacteria (Bact), heterotrophic nanoplankton (HNP), phytoplankton (Phyto), Chlorophyll *a* (Chl *a*), salinity (Sal), rainfall (Rain), temperature (Temp), Secchi depth (Secc), dissolved oxygen (DO). Species arranged with percentage occurrence, from highest to lowest in each category. Only significant correlations are shown; pairwise N = 40.

	Bact	HNP	Phyto	Chl *a*	H'	J	Rain	Temp	Sal	DO	Secc	NH_4^+	NO_2^-	NO_3^-	PO_4^{3-}	SiO_2
Bact																
HNP				0.34			-0.77	0.64	0.69					-0.75	-0.66	-0.38
Phyto				0.35												
Chl *a*		0.34	0.35					0.68				-0.48	-0.35		-0.42	-0.61
H'						0.91									-0.43	
J					0.91										-0.32	
T. rotundata					0.39	0.37				0.37						
T. primitivum					-0.5	-0.56		-0.34	-0.43		0.37				0.41	0.44
Tintinnopsis beroidea							0.49		-0.38						0.33	
Stenosemella avellana			-0.5													
T. minata		0.45		0.42			-0.39	0.49	0.41							
T. tubulosa			-0.33													
T. nana									-0.37							0.37
L. elongatus				0.38			-0.37	0.38	0.35							
T. meuneri			0.33	0.47	0.37	0.36		0.41				-0.39				
Leprotintinnus nordqvisti		0.67	0.33	0.64	0.31		-0.68	0.66	0.68	-0.35			-0.4	-0.53	-0.68	-0.63
T. tocatinensis		0.68		0.61	0.36		-0.65	0.71	0.63	-0.36				-0.64	-0.6	-0.65
L. bottnicus		0.49	0.34	0.37			-0.48	0.62	0.51				-0.44	-0.43	-0.43	-0.46
Codonellopsis spp.		0.61		0.49			-0.51	0.56	0.51	-0.49				-0.67	-0.48	-0.69
Tintinnidium incerta							-0.37		0.43							

T. chinglannensis				0.46				0.4	0.32					-0.4		-0.4
T. mortenensi				0.48												
T. directa		0.49		0.5			-0.46	0.48	0.55	-0.53		-0.38	-0.64	-0.53	-0.62	-0.69
T. acuminata		-0.46		-0.41			0.55	-0.47	-0.44					0.37	0.42	0.38
Favella ehrenbergii			-0.39								0.53					
Strombidiis				-0.48				-0.49								
Strobilidiids		-0.32		-0.71				-0.71				0.39		0.4		
Pleuronematine	0.32			-0.49				-0.45								
Cyclotrich		-0.53		-0.54	-0.35	-0.33	0.51	-0.55	-0.63					0.46	0.57	0.54
Vorticella		0.38	0.31				-0.41	0.38	0.39							
Peridinium spp.						-0.32										
Ceratium spp.										-0.45						
Dinophysis spp.										-0.36						
Prorocentrum spp.			-0.46	-0.34	-0.37	-0.49	0.37									
Noctiluca spp.		0.42		0.56			-0.32	0.37		-0.5		-0.32		-0.44	-0.53	-0.6
Nauplius <200µm										-0.4	0.6			-0.47		-0.41

Figure 4.5: Ordination triplot from RDA of environmental parameters (bold arrows with large arrow heads) and microzooplankton taxa (fine colored arrows with small arrow heads) in Terusan channel, MMFR. Environmental parameters: DO - dissolved oxygen, Phyto- phytoplankton, Chl *a* -chlorophyll *a*, Temp- temperature, HNP- heterotrophic nanoplankton, Rainfall- monthly mean rainfall, Salinity- monthly mean salinity, Secchi – secchi disc depth. Microzooplankton: Blue arrows indicate loricate ciliatesviz. Trot - *Tintinnopsis rotundata,* Tber *T. beroidea,* Tnana *T. nana,* Save *Stenosemella avellana,* , Tacu *T. acuminata*, Fehr *Favella ehrenbergii*, Tnpri *Tintinnidium primitivum*, Ttub *Tintinnopsis tubulosa*, Tninc *Tintinnidium incerta*, Tdir *Tintinnopsis directa*, Tmin *T. minata,* Tmeu *T. meunieri*, Tching *T. chinglanensis*, Ttoc *T. tocatinensis*, Lelo *Leprotintinnus elongatus,* Lbot *Leprotintinnus bottnicus*, Lnor *Leprotintinnus nordqvisti*; red arrows indicate aloricate ciliates viz. Pleuro pleuronematine, Strob stobilidiid, Strom stombidiid Cyclo cycloltrich, Vor vorticella; green arrows indicate dinoflagellates viz. Cer *Ceratium* spp., Dphys *Dinophysis* spp., Pror *Prorocentrum* spp., Perid *Peridinium* spp., Noct *Noctiluca* spp.; black arrows indicate copepod larvae, Naup nauplius. Symbols: ○ SW-spring, □SW-neap, ●NE-spring, ■ NE-neap; numeric letter represents sampling month, e.g. 4 = April. Ellipsoids group the microzooplankton taxa into four categories, large loricate ciliates, small loricate ciliates, aloricate ciliates and dinoflagellates.



4.5 Discussion

This is the first study in Malaysia and one of a few studies in tropical waters to investigate the temporal dynamics of microzooplankton in mangrove estuary. As in agreement with other studies in tropical (Godhantaraman, 2002; Sarkar, 2015), temperate (Paterson et al., 2007; Asha Devi et al., 2010; Stoecker et al., 2014), polar (Dolan et al., 2013; Dolan & Pierce, 2014) and freshwater (Hambright et al., 2007; Kalinowska, 2004) environments, the microzooplankton in the Matang estuary are dominated by both aloricate and loricate ciliates. Most of the tintinnid ciliate species (e.g. *Tintinnopsis*, *Leprotintinnus, Favella* and *Codonellopsis*) found in the present study are however known to be cosmopolitan species (Pierce & Turner, 1993). Tintinnid and aloricate ciliate concentrations are comparable with those of other tropical estuarine and coastal waters (Sarkar, 2015; Rakshit et al., 2014; Agatha, 2011; Jyothibabu et al., 2008a,b). Thus, the microzooplankton in tropical estuarine waters as in Matang are rich in diversity, although community structure and species abundance are highly variable temporally. The temporal variations of microzooplankton abundance in the Matang estuary appear to be closely linked to rainfall, salinity, temperature and their likely microbial prey. In temperate waters, the abundance of ciliates normally peaks in spring and summer primarily due to high phytoplankton food abundance and temperature (Kamiyama, 1994; Barria de Cao et al., 2005; Bojanic et al., 2004). Unlike temperate waters, tropical mangrove waters like in Matang is conditioned by the monsoonal climate rather than temperature (Godhantaraman, 2002; Jyothibabu et al., 2008; Asha Devi et al., 2010). In Matang, the causal link of the temporal variations of microzooplankton is species-dependent. High salinity, temperature and phytoplankton abundance during the SW monsoon are favourable to the growth of the large-bodied tintinnids (i.e. *Leprotintinnus bottnicus, L. nordqvisti, L. elongatus, Tintinnopsis tocatinensis*). Ciliate biomass is correlated with chlorophyll *a* concentration as phytoplankton serves as one of their major food sources

(Jiang et al., 2013; Yu et al., 2013; Wang et al., 2014b; Sarkar, 2015). Positive correlation of loricate ciliates with phytoplankton or chlorophyll *a* shows a nutritional dependence (Kimor & Golandsky, 1977). Degrading phytoplankton cells and materials are also advantageous to large-bodied tintinnids which are more conspicuous and vulnerable to predators since they need these materials to build their protective lorica (Capriulo, 1982).

In contrast, the small-bodied tintinnids such as *Stenosemalla avellana, Tintinnopsis beroidea, T. nana* and *T. rotundata* are ubiquitous being present throughout the sampling period. This suggests that salinity, temperature and phytoplankton which are variable factors are not the prime factors controlling their temporal abundance (Sarkar, 2015, Dolan & Gallegos, 2001). Apart from being eurythermal and euryhaline (Rakshit et al., 2014), small-bodied tintinnids, with a seemingly lack of temporal variability in their abundance, may depend on the unlimited bacterial food present throughout the year. There are however two exceptions; two low salinity tintinnid species *Tintinnopsis tubulosa* and *Tintinnidium primitivum* were more abundant in low salinity environment during the NE monsoon. Rakshit et al. (2014) demonstrated weak negative correlation between *T. primitivum* abundance and salinity, while *T. tubolosa* predominated in summer with higher rainfall and runoffs (Kamiyama & Tsujino, 1997).

The freshwater input into the estuary significantly increases the amount of ammonium, nitrate and silicate, resulting in higher concentrations of these nutrients during the rainy NE monsoon. The higher rainfall during the NE monsoon apparently triggers the proliferation of aloricate ciliates but not the large-bodied tintinnids in Matang estuary. In particular, cyclotrich ciliates bloom during the early part of the NE monsoon when increased riverine discharges dilute estuarine waters bringing down the salinity but increasing the DIN. Microzooplankton samplings conducted from upstream to downstream of the Sepetang, Selinsing and Sangga Besar channels revealed that

cyclotrich ciliates were low in numbers at the upper and mid sections of Selinsing and absent in the lower section of the Sepetang including all other areas during the SW monsoon (Yong et al. unpublished data). The salinity tolerance of the cyclotrich ciliates ranges from 5 ppt (Crowford & Lindholm, 1997) to 35ppt (Proença, 2004). Thus, the blooms of cyclotrich ciliates in the estuary are unlikely to be ciliates that originated from upstream or those that were flushed downstream during the NE monsoon. Cloern et al. (1994) showed that with heavy precipitation and runoffs, cyclotrichs are very likely to bloom. It has been reported that the endosymbiotic chloroplasts found in the cyclotrich ciliates can uptake the ambient dissolved inorganic nitrogen to bloom (Wilkerson & Grunseich, 1990; Liu et al., 2012). Therefore, the cyclotrich blooms during the NE monsoon is probably triggered by high DIN in the estuary. Given that the cyclotrich ciliates are the main prey for dinoflagellates (Nagai et al., 2008), the gradual disappearance of the cyclotrich ciliates towards the end of the NE monsoon is likely due to such predation and their declining reproductivity as dissolved inorganic nitrogen became limiting for cell replication.

Although most of the microzooplankton abundance appeared not to be influenced by lunar phase, the predatory tintinnid *F. ehrenbergii* was sampled in considerable numbers during neap tide (>17 ind l^{-1}) but was not present during spring tide. Similarly, the abundance of copepod nauplius was significantly higher during neap than spring tide in both SW and NE monsoon. In term of body size, these two groups were the largest among the microzooplankton. Both of them are known to feed on other ciliates (Robertson, 1983; Buskey & Stoecker, 1989), placing them in the upper trophic level of the microzooplankton. These organisms were also the few microzooplankton (2 taxa) that were significantly correlated with water transparency (Secchi reading); all others were not, indicating the generally high water turbidity in Terusan channel (Table 4). Nevertheless, it is not clear why less turbid or clearer water favoured *F. ehrenbergii* and

copepod nauplii; it may due to the higher light intensity that also attracts their prey. Nonetheless, it may explain why *Favella ehrenbergii* and copepod nauplius were more abundant during neap tide (clearer water) than spring tide. However, the lunar difference in abundance may also be explained by their reproductive timing and swimming ability so as to prevent population loss by strong tidal advection during spring tide. Compared to mesozooplankton, planktonic ciliates are less capable of independent swimming movement against currents and tides (Fenchel, 1987; Dolan et al., 2013). They rely more on water diffusion and either swimming at the water boundaries or drift with the flow (Zhu et al., 2013). However, larger ciliates such as *F. ehrenbergii* are capable of directional swimming towards food and against gravity (Jonsson, 1989, Harvey & Manden-Deuer, 2011). Interestingly and in contrast, the adult copepods of *Acartia spinicauda* were more abundant during spring than neap tide at the same sampling site (Kong et al., 2015). Apparently, the need for upstream penetration and population retention necessitates such a behaviour mediated through tidally-induced vertical migration (Schmitt et al., 2011; Chew et al., 2015). However, since larval copepods are weak swimmers, the timing of copepod spawnings during neap tide is clearly an adaptive strategy to avoid more serious advective losses of their young stages during spring tide.

Many studies have been conducted to investigate the correlation between microzooplankton and environmental parameters. Some suggest physicalcontrol, for examples by temperature, salinity and current (Verity, 1986; Sanders, 1987; Cordeiro et al., 1997; Eskinazi-Sant'anna & Björnberg, 2006), whereas others suggest a top-down control, such as by predators (Dolan & Gallegos, 2001; Urrutxurtu et al., 2003). However, both bottom-up and top-down controls such as temperature, light, tidal effect, oxygen concentration, nutrients, prey and predator abundance, can affect the formation of ciliate cysts (Dolan et al., 2012; Lynn, 2010; Kamiyama, 1994; Jonsson, 1994). With the great

environmental fluctuations in the estuary, both encystment and excystment of ciliates could well explain the 'disappearance' and 'bloom' of certain species.

The present study used both cell density and chlorophyll *a* concentration of phytoplankton as measures of the potential autotrophic food source for microzooplankton. Although both variables were highly correlated, only DIN was significantly correlated (albeit negatively) with chlorophyll *a* concentration but not phytoplankton density. This is likely due to the poorer preservation of the smaller-size or soft-bodied phytoplankton other than diatoms by Lugol's iodine. Fresh field samples compared to iodine-preserved samples conserve more of the actual phytoplankton community (Rodríguez-Ramos et al., 2014). Thus, 'phytoplankton' in our study contained more diatoms and much less of the smaller size fraction which included the phytoflagellates and coccoliths; therefore, cell density of the preserved phytoplankton was likely underestimated when viewed under the light microscope. On the other hand, chlorophyll *a* concentration from filtered fresh phytoplankton cells comprising both diatoms and the smaller-size phytoplankton thus appeared accountable for its negative correlation with DIN probably due to rapid uptake and depletion of the nutrients.

The r/K selection theory was applied to tintinnid and aloricate ciliates by Margalef (1982) to explain their adaptation strategies, but he did not provide experimental or field data to show that tintinnids grow slower than aloricate ciliates. However, the growth rates of ciliates tend to decrease with bigger body size but increase with higher temperature and favourable environment (Müller & Geller, 1993). If we assumed that the drier SW monsoon offers a less favourable condition for ciliate growth such as lower DIN, the larger loricate tintinnids would represent *K*-adapted species since they accumulate their biomass (or abundance) slowly but persistently at low nutrient concentrations, while expending substantial energy on lorica building (Dolan et al., 2013). On the other hand,

the aloricate ciliates represent the *r*-adapted species since they are smaller, reproduce and grow rapidly when the environment is favourable such as in the NE monsoon (this study, Figure 4.3). Nonetheless, our study could not provide direct evidence of the higher growth rates of aloricate ciliates except by proxy, i.e. their high abundance. Thus, more studies on how size and environment affect ciliate reproduction and growth are required to verify the hypothesized r/K adaptive strategies in small and large ciliates.

CHAPTER 5: RESULTS AND DISCUSSION - MICROZOOPLANKTON GRAZING IN RELATION TO MICROBIAL LOOP DYNAMICS IN A HIGHLY TURBID MANGROVE ESTUARY.

5.1 Biomass of Microbial Components

The abundance and biomassof phytoplankton (Chl *a*) and other microorganisms in Matang have already been reported (Chapter 4) and their seasonal averages ranged by a few orders. Here, we reported them in carbon biomass so as to more accurately reflect the contribution of each component to the planktonic and microbial food web (Figure 5.1) hence to elucidate the carbon pathway.

The average bacterial biomass observed was similar during the SWM (67.3 ± 17.2 µgC $.l^{-1}$) and NEM (64.6 ± 33.2 µgC l^{-1}) season, whereas the average HNP during the NEM (132 ± 84 µgC l^{-1}) was nearly double that of the SWM (74 ± 26 µgC l^{-1}). Biomass of bacteria was not significantlt higher than HNP. In certain months (May, January, and February) biomass of HNP was higher than bacteria biomass. Although the biomass of phototrophs, as estimated from Chl *a* concentration was nearly double in SWM (1703 ± 1172 µgC l^{-1}) than in NEM (902 ± 390 µgC l^{-1}) , the difference was not statistically significant probably due to the large variance observed. In terms of variation throughout the sampling period, bacterial biomass varied within a smaller range (coefficient of variation or CV = 39%) relative to the other microbes (CVs for HNP: 66%, MZP: 75%, Chl *a*: 94%).

Biomass of microzooplankton was estimated by biovolume. A total of 566 individuals were measured; 37 species were identified, and others were grouped according to shapes and sizes. Dinoflagellates were identified to genus and biovolume was estimated accordingly (Table 5.1). Only the MZP biomass was significantly higher in NEM (152 ± 98 µgC l^{-1}) than SWM (80 ± 59 µgC l^{-1}) ($t=2.49$, $df=14$, $p<0.05$). Biomass of loricate

ciliates was the highest among the microzooplankton, with a maximum record of 59.5 µgC l^{-1}. The aloricate ciliate marked a peak in October (38.6 µgC l^{-1}), dinoflagellates marked its peak in February (169.9 µgC l^{-1}). Difference among categories domination in biomass throughout the study were shown in Figure 5.2. Among categories of microzooplankton, only the loricate ciliates, tintinnids showed a positive correlation with phytoplankton biomass (r^2 = 0.52, $p<0.05$, Figure 5.3) while the rest do not exhibit correlation with phytoplankton biomass.

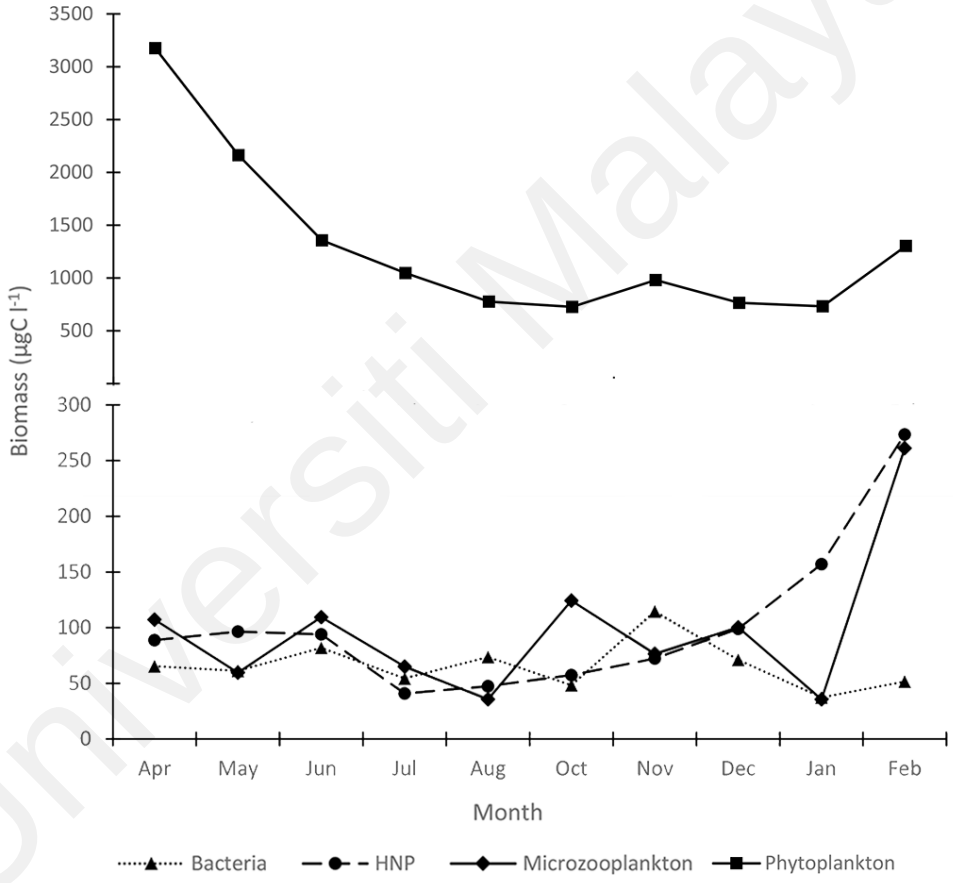


Figure 5.1: Monthly biomass of different microbial components in MMFR. Phytoplankton biomass was one order higher than the rest of the microbial components.

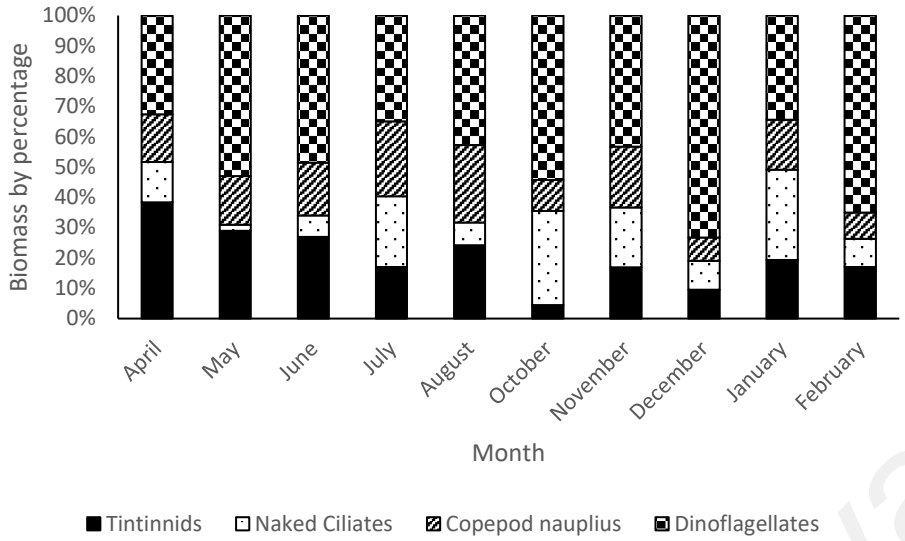


Figure 5.2 Monthly biomass of microzooplankton categories by percentage.

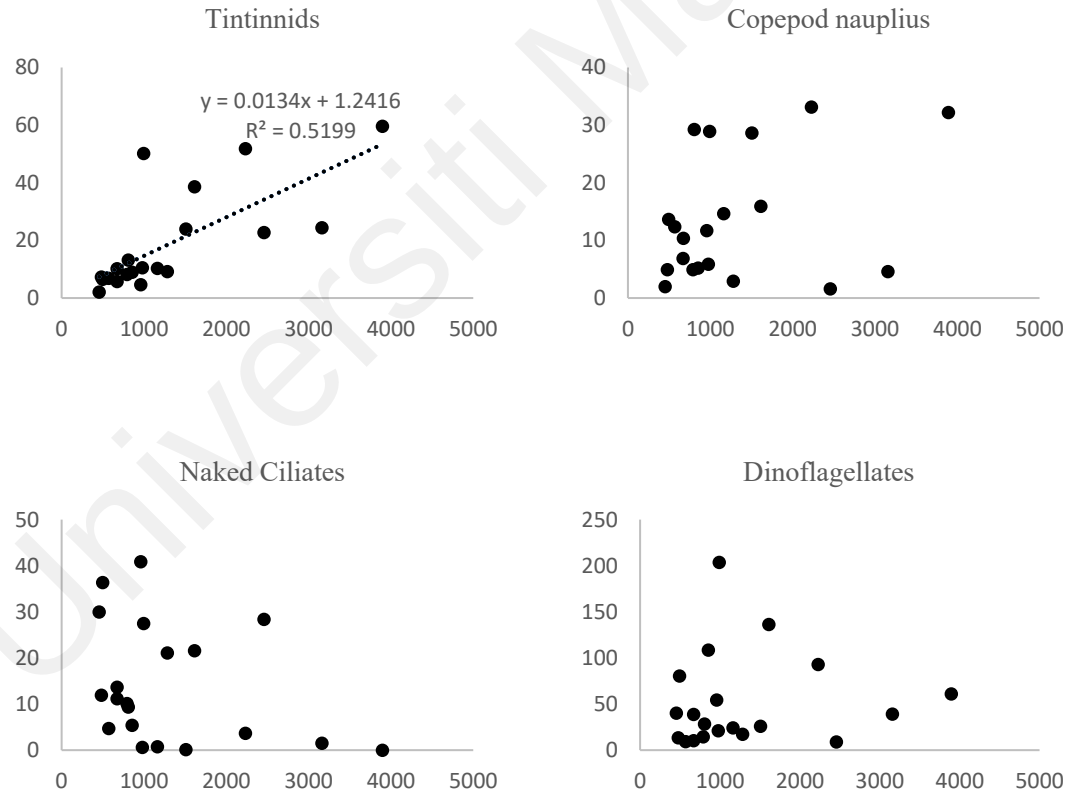


Figure 5.3 Relationships between biomass of various microzooplankton taxonomic groups (µgC l^{-1}, x-axis) and phytoplankton biomass (µgC l^{-1}, y-axis). Only cases with significant ($p < 0.05$) positive correlation are presented with the regression line.

Table 5.1 A synoptic account for the microzooplankton recorded in MMFR 2013-2014. TL: total length, OD: oral diameter.

	n	TL (µm)	OD (µm)	TL/OD	Biovolume (µm^3)	body carbon weight (µg)
Tintinnopsis beroidea	38	58 ± 11	26.2 ± 3.1	2.21 ± 0.32	$3.46 \pm 1.73 \times 10^4$	$2.28 \pm 0.92 \times 10^{-3}$
Tintinnopsis rotundata	19	87.3 ± 8.1	33.4 ± 2.7	2.63 ± 0.27	$8.33 \pm 2.36 \times 10^4$	$4.86 \pm 1.25 \times 10^{-3}$
Tintinnopsis nana	4	42.4 ± 2.5	17 ± 1.3	2.51 ± 0.31	$8.71 \pm 2.12 \times 10^3$	$9.06 \pm 1.12 \times 10^{-4}$
Tintinnopsis minata	22	43.9 ± 2.8	26.7 ± 1.5	1.65 ± 0.15	$8.67 \pm 1.23 \times 10^3$	$9.04 \pm 0.66 \times 10^{-4}$
Tintinnopsis meuneri	18	73.7 ± 11	47.8 ± 4.1	1.54 ± 0.16	$2.97 \pm 0.44 \times 10^5$	$1.62 \pm 0.23 \times 10^{-2}$
Tintinnopsis tocatinensis	11	116.5 ± 12	23.1 ± 2	5.06 ± 0.51	$4.43 \pm 0.78 \times 10^4$	$3.22 \pm 0.41 \times 10^{-3}$
Tintinnopsis mortenensi	4	62.6 ± 4.8	98.6 ± 15	0.65 ± 0.11	$40.66 \pm 2.47 \times 10^5$	$2.52 \pm 1.31 \times 10^{-2}$
Tintinnopsis directa	2	111.5 ± 55.6	102.9 ± 37.8	1.06 ± 0.15	$5.73 \pm 6.89 \times 10^5$	$3.08 \pm 3.65 \times 10^{-2}$
Tintinnopsis tubulosa	38	58 ± 11	26.2 ± 3.1	2.21 ± 0.32	$3.46 \pm 1.73 \times 10^4$	$2.28 \pm 0.92 \times 10^{-3}$
Tintinnopsis tubolosoides	66	98.4 ± 16.4	29.5 ± 6.1	3.38 ± 0.36	$8.01 \pm 3.63 \times 10^4$	$4.69 \pm 1.92 \times 10^{-3}$
Tintinnopsis chinglannensis	12	86.3 ± 5.3	25.5 ± 2.6	3.42 ± 0.43	$8.63 \pm 2.05 \times 10^4$	$5.02 \pm 1.09 \times 10^{-3}$
Tintinnopsis spp1	9	75.4 ± 11.2	25.4 ± 2.4	2.97 ± 0.41	$4.44 \pm 1.94 \times 10^4$	$2.80 \pm 1.03 \times 10^{-3}$
Tintinnopsis vasculum	3	61.9 ± 0.3	39.5 ± 7.2	1.61 ± 0.32	$6.91 \pm 0.4 \times 10^4$	$4.11 \pm 0.21 \times 10^{-3}$
Tintinnopsis lobianci	19	204.7 ± 54.2	61.5 ± 19.6	3.4 ± 0.75	$6.52 \pm 5.54 \times 10^5$	$7.88 \pm 0.16 \times 10^{-3}$
Tintinnopsus acuminata	12	76.5 ± 2.5	25.1 ± 1.4	3.06 ± 0.17	$4.77 \pm 0.43 \times 10^4$	$2.97 \pm 0.23 \times 10^{-3}$
Tintinnopsis annulata	19	204.7 ± 54.2	61.5 ± 19.6	3.4 ± 0.75	$6.52 \pm 5.54 \times 10^5$	$7.88 \pm 0.16 \times 10^{-3}$
Tintinnopsis butschlii	38	58 ± 11	26.2 ± 3.1	2.21 ± 0.32	$3.46 \pm 1.73 \times 10^4$	$2.28 \pm 0.92 \times 10^{-3}$
Tintinnopsis spp2	6	270.3 ± 11.1	62 ± 1.1	4.37 ± 0.25	$6.10 \pm 0.41 \times 10^5$	$3.28 \pm 0.22 \times 10^{-2}$
Rhizodomus tagatzi	6	177.1 ± 24.2	34.1 ± 2.3	5.21 ± 0.77	$1.63 \pm 0.32 \times 10^5$	$8.17 \pm 1.70 \times 10^{-3}$

Table 5.1, continued

Leprotintinnus nordqvisti	8	145.2 ± 55	80.6 ± 9.7	1.82 ± 0.68	$4.92 \pm 1.30 \times 10^{5}$	$2.65 \pm 0.69 \times 10^{-2}$
Leprotintinnus elongatus	11	179.8 ± 32	36.7 ± 6.6	5.01 ± 0.95	$2.01 \pm 0.68 \times 10^{5}$	$1.11 \pm 0.36 \times 10^{-2}$
Leprotintinnus bottnicus	5	254.5 ± 14.2	47.5 ± 5.2	5.39 ± 0.44	$1.71 \pm 0.44 \times 10^{4}$	$9.48 \pm 2.33 \times 10^{-3}$
Stenosemella avellana	5	28.9 ± 2.5	26.7 ± 1.8	1.09 ± 0.1	$1.08 \pm 0.16 \times 10^{4}$	$9.97 \pm 1.01 \times 10^{-4}$
Stenosemella spp	2	34.4 ± 8.4	24.9 ± 6.2	1.47 ± 0.7	$21.4 \pm 5.88 \times 10^{4}$	$9.05 \pm 0.22 \times 10^{-4}$
Eutintinnus spp	3	166.5 ± 14.1	49.1 ± 8.1	3.44 ± 0.49	$3.24 \pm 1.12 \times 10^{5}$	$1.76 \pm 0.59 \times 10^{-2}$
Favella ehrenbergii	19	304.9 ± 54.6	111.7 ± 6.8	2.71 ± 0.36	$2.07 \pm 0.6 \times 10^{6}$	$1.10 \pm 0.32 \times 10^{-1}$
Coxiella spp.	5	230.1 ± 24.7	111.9 ± 5.9	2.06 ± 0.18	$1.92 \pm 0.3 \times 10^{6}$	$1.02 \pm 0.18 \times 10^{-1}$
Codonellopsis spp.	3	109.2 ± 6.2	92 ± 7.5	1.19 ± 0.08	$7.94 \pm 1.90 \times 10^{5}$	$4.25 \pm 1.01 \times 10^{-2}$
Amphorellopsis spp.	2	133.2 ± 18.4	35.5 ± 2.4	3.74 ± 0.27	$1.34 \pm 0.36 \times 10^{5}$	$7.52 \pm 1.91 \times 10^{-3}$
Tintinnidium incerta	3	131.8 ± 40.8	30.6 ± 4	4.28 ± 0.97	$1.01 \pm 0.52 \times 10^{5}$	$5.81 \pm 2.74 \times 10^{-3}$
Tintinnidium primitivum	20	86.7 ± 17.2	21.4 ± 4.4	4.12 ± 0.74	$3.36 \pm 1.87 \times 10^{4}$	$2.22 \pm 0.99 \times 10^{-3}$
Strombidiis	28	77 ± 19.4	40.6 ± 10		$7.16 \pm 4.84 \times 10^{4}$	$1.00 \pm 0.68 \times 10^{-2}$
Strobilidiids	31	68.5 ± 19.4	26.4 ± 6		$2.26 \pm 1.96 \times 10^{4}$	$3.17 \pm 2.75 \times 10^{-3}$
Pleuronematine	6	35.7 ± 6.6	20.9 ± 3		$8.60 \pm 3.49 \times 10^{3}$	$1.20 \pm 0.49 \times 10^{-3}$
Euploites	8	71.5 ± 18.9	28.1 ± 5.2		$2.04 \pm 1.40 \times 10^{4}$	$2.86 \pm 1.95 \times 10^{-3}$
Cyclotrich	14	17.5 ± 3.9	12 ± 3.9		$9.49 \pm 5.53 \times 10^{2}$	$1.33 \pm 0.77 \times 10^{-4}$
Vorticella	6	35.7 ± 6.6	20.9 ± 3		$8.60 \pm 3.49 \times 10^{3}$	$1.20 \pm 0.49 \times 10^{-3}$
Ciliates >50µm	8	75.3 ± 33.6			$3.61 \pm 5.32 \times 10^{5}$	$5.05 \pm 7.45 \times 10^{-2}$
Nauplius <200µm	19	125.5 ± 30.1				2.60 ± 1.90 X 10^{-2}

Table 5.1, continued

Peridinium spp.	3	81 ± 18.1	52.5 ± 20.1	$7.93 \pm 8.37 \times 10^{5}$	$7.32 \pm 7.36 \times 10^{-2}$
Dinophysis spp.	3	80.7 ± 4.3	49.9 ± 2	$2.71 \pm 0.3 \times 10^{4}$	$3.15 \pm 0.33 \times 10^{-3}$
Ceratium spp.	2	314.2 ± 11.7	258.3 ± 6.3	$5.59 \pm 2.27 \times 10^{4}$	$6.18 \pm 2.36 \times 10^{-3}$
Noctiluca spp.	3	81 ± 18.1	52.5 ± 20.1	$7.93 \pm 8.37 \times 10^{5}$	$7.32 \pm 7.36 \times 10^{-2}$
Prorocentrum spp.	3	80.7 ± 4.3	49.9 ± 2	$2.71 \pm 0.3 \times 10^{4}$	$3.15 \pm 0.33 \times 10^{-3}$
	566				

5.2 Microbial Productions

Primary production as estimated by the dilution technique ranged 148 to 4021 µgC $l^{-1}d^{-1}$ and was clearly higher than the production rates of other microbes (Figure 5.5). Although primary production did not exhibit significant differences among moon phases ($p>0.05$), it was significantly higher during the drier SWM than in the NEM ($t=2.42$, $df=18$, $p<0.05$). The highest production estimated was 4021 µgC $l^{-1}d^{-1}$ and occurred at the beginning of the SWM (April 2013) although larger fluctuations occurred during the drier SWM. Production was lower during the wetter NEM, with the lowest recorded in October (148 µgC $l^{-1}d^{-1}$; Figure 5.5). Primary production in this study was found to show correlation with temperature ($r^2=0.42$, $p<0.005$).

A total of 20 sets of incubation were carried out to estimate bacterial production. Of these 20 incubations, only 1 did not produce a significant growth curve (Figure 5.4). The mean bacterial production of 3.7 ± 1.5 µgC $l^{-1}d^{-1}$ was about two order lower than primary production. (Table 5.2). Bacterial production varied within a smaller range (CV: 42%; CV = standard deviation / mean × 100), and with no significant difference between the SWM and NEM season (t-test, $p>0.05$). Bacterial production was highest in May (6.14 µgC $l^{-1}d^{-1}$) and lowest in August (1 µgC $l^{-1}d^{-1}$). In this study, bacterial production showed a significant positive correlation with rainfall ($r^2=0.24$, $p<0.05$; $n=20$).

HNP growth rates were measurable for a total of eight occasions. The other sets of HNP samples (i.e. the <20 µm sieve-fraction) for twelve occasions had negative growth; and the regression line was not significant at the end of the incubation period. HNP production varied within a wide range from 1.55 to 24.24 µgC $l^{-1}d^{-1}$ (CV: 73%), and on average was 12.7 ± 9.3 µgC $l^{-1}d^{-1}$. There was no difference in HNP production between monsoons. HNP production too showed significant correlation with temperature ($r^2=0.59$, $p<0.05$).

Microzooplankton production, as estimated from abundance and biovolume varied widely (CV: 65%), with an average of 121.7±90.8 µgC $l^{-1}d^{-1}$. Among microzooplankton groups, primary production showed positive correlation with tintinnids production (r^2=0.3, $p<0.05$) while showing negative correlation with naked ciliate production (r^2 = -0.31, $p<0.05$). Both nauplius (r^2=0.38, $p<0.05$) and dinoflagellates (r^2=0.44, $p<0.05$) exhibit positive correlation with dissolved oxygen ;.

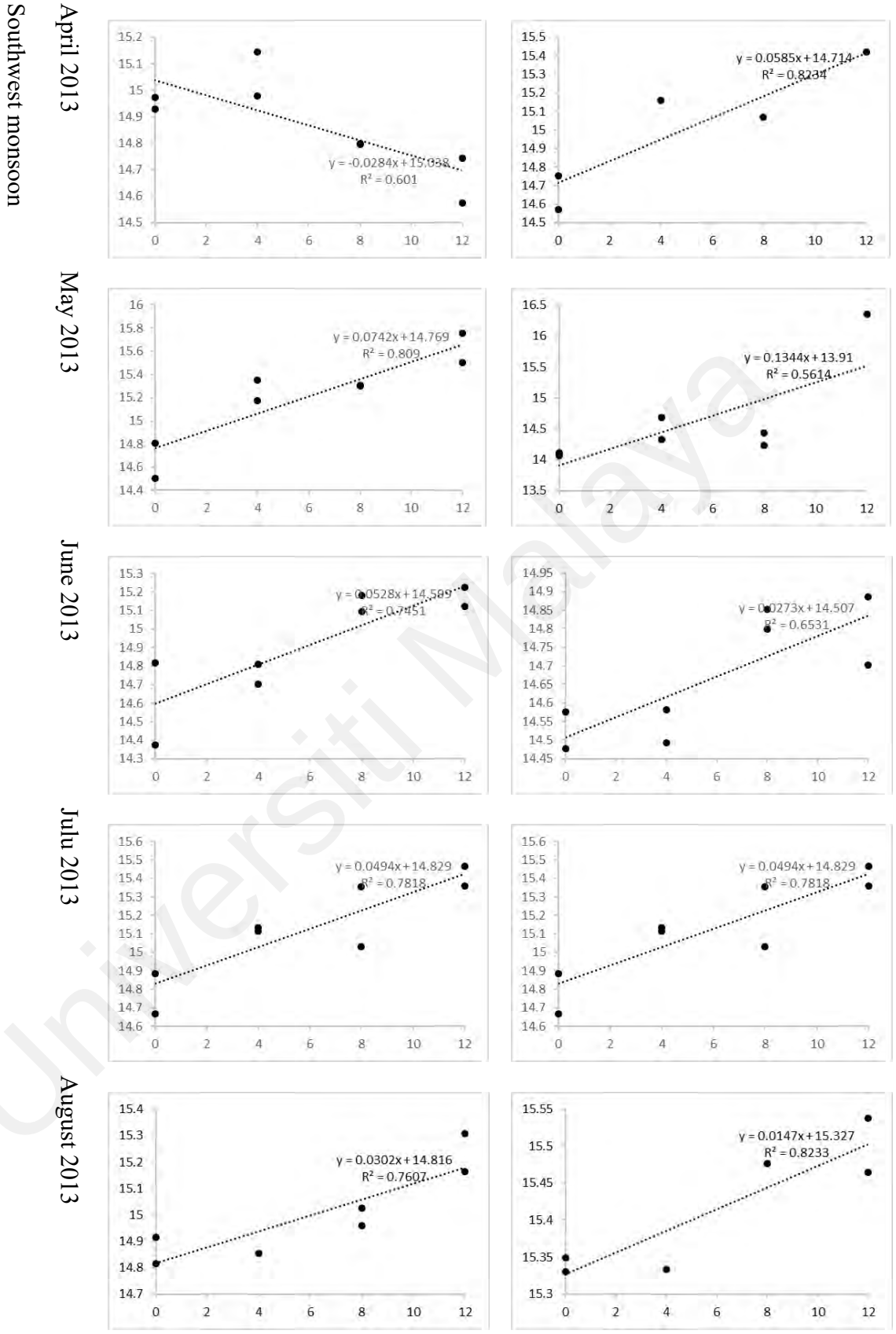


Figure 5.4: Monthly bacterial production from April 2013 to February 2014. X-axis shows time of incubation while y-axis shows bacterial abundance after ln-transformation. First incubation in April showed a negative growth which is excluded from subsequent analysis.

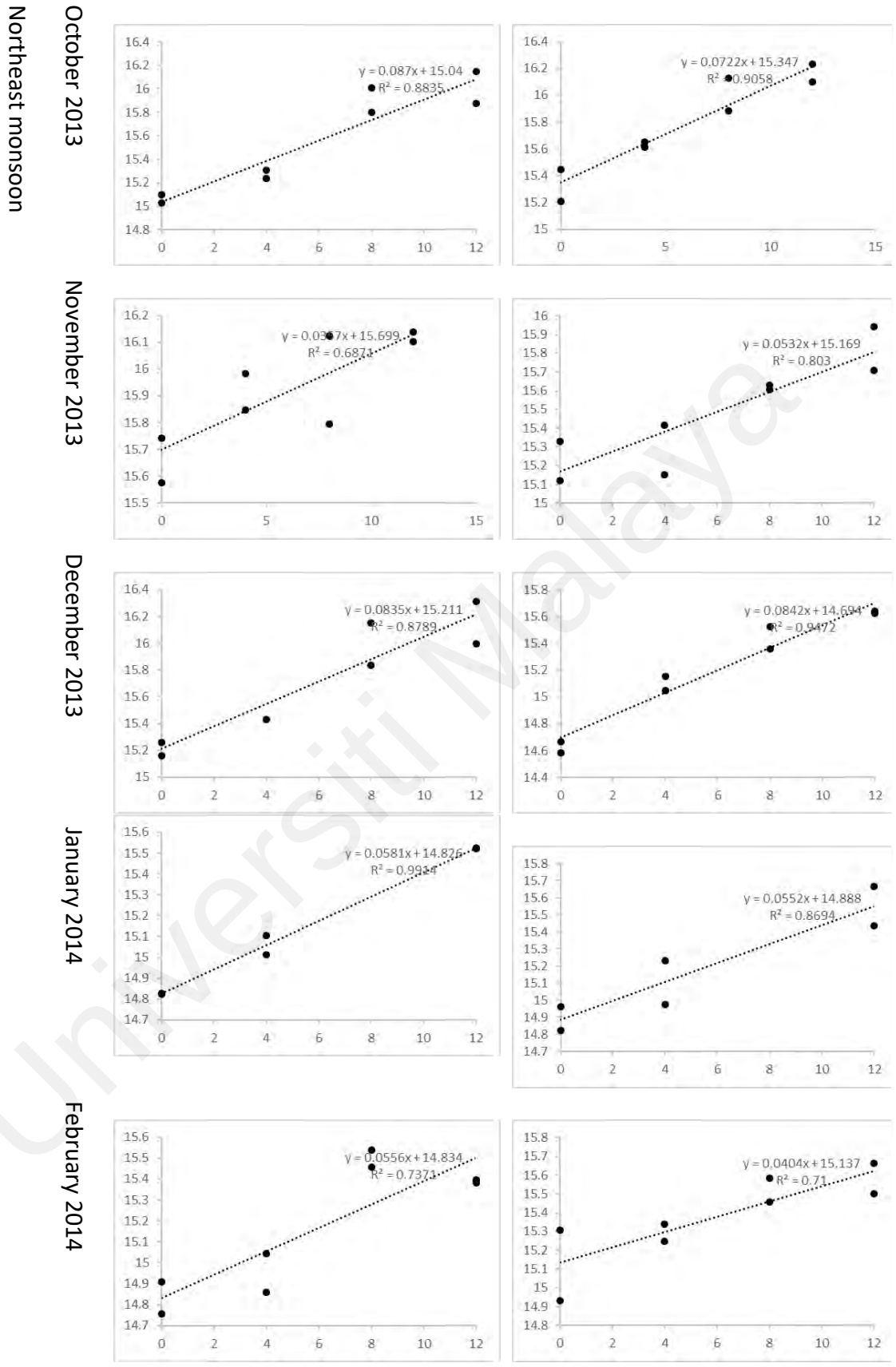


Figure 5.4 continued.

Table 5.2 Seasonal variation of biomass, production, and grazing of microbial components in MMFR.

Biomass, Production and Grazing	Monsoon		
	SW	NE	Average
Bacteria			
Biomass (µgC/l)	67.3	58.0	62.0
Production (µgC/l/d)	3.7	3.7	3.7
Grazing by HNP (µgC/l/d)	1.6	1.2	1.3
Grazing by microzooplankton (µgC/l/d)	1.5	1.2	1.3
Phytoplankton			
Biomass (µgC/l)	1703.0	901.7	1302.3
Production (µgC/l/d)	1682.1**	607.2**	1081.7
Grazing by microzooplankton (µgC/l/d)	1587.4**	387.82**	887.0
HNP			
Biomass (µgC/l)	73.5	102.5	89.9
Production (µgC/l/d)	13.2	6.9	10.7
Grazing by microzooplankton (µgC/l/d)	4.3	1.2	3.5
Microzooplankton			
Biomass (µgC/l)	75.4	119.5	97.4
Production (µgC/l/d)	83.7	159.8	121.7

**p<0.001

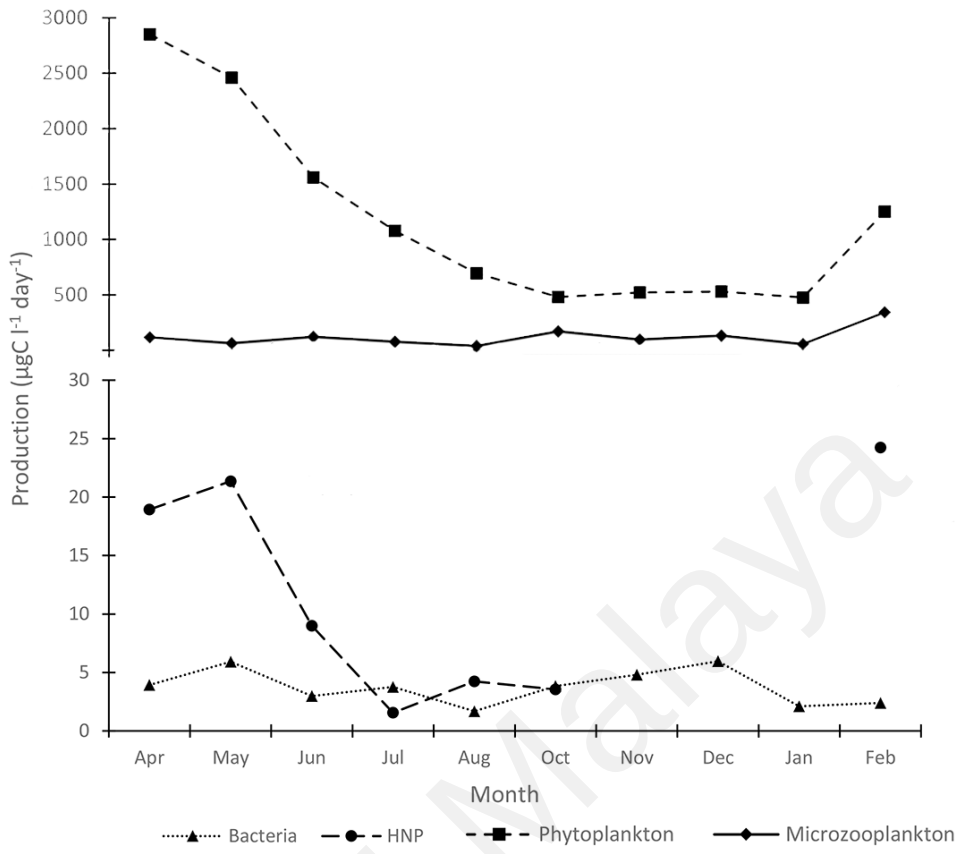


Figure 5.5: Monthly production of different microbial components in MMFR.

5.3 Grazing Activities

The diet of microzooplankton consisted of phytoplankton, HNP and bacteria. In this study, phytoplankton was found to be a more substantial food source for the microzooplankton. A total of 20 dilution experiment were carried out throughout this study (Figure 5.6). As measured from the Landry-Hassett dilution method for herbivory, the microzooplankton grazing rate in the estuary ranged from 106 to 3599 µgC $l^{-1}d^{-1}$, and averaged 1029 ± 1045 µgC $l^{-1}d^{-1}$. Microzooplankton grazing took up a substantial amount of the primary production (83±27%: Fig. 5.7), and the grazing rate was higher in SWM (1636 ± 1188 µgC $l^{-1}d^{-1}$) than in NEM (421 ± 271 µgC $l^{-1}d^{-1}$) ($t=3.15$, $df=10$, $p<0.01$).

In this study, herbivory activity was correlated with chlorophyll *a* concentration (r=0.97), temperature (r=0.8), pH (r=0.78) and SiO_2 (r=-0.55). The HNP's consumption by microzooplankton was estimated at 31% of total HNP production, ranging from 14% to 52% throughout the sampling period (Table 5.2). Of the eight successful incubation sets, only three showed grazing activities of microzooplankton on HNP, ranging from 2.2 to 6.2 $\mu gC\ l^{-1}d^{-1}$.

Bacterivory by microzooplankton and HNP were both examined through a 24-hour incubation. Unfortunately, of the twenty sets of incubation carried out, only six of the microzooplankton grazing experiments were significant, and fourteen of the HNP grazing experiments were significant (Fig. 5.4). There were three additional samplings carried out for bacterivory activities by microzooplankton and HNP during November and December 2015 (wet season), but only one sampling gave significant grazing results. On average, 42±22% of the bacteria production was estimated to be taken up by HNP (1.52 ± 1.17 $\mu gC\ l^{-1}day^{-1}$), with the highest grazing recorded in July (73%, 3.43 $\mu gC\ l^{-1}day^{-1}$). Despite not showing any correlation with the environmental parameters, the highest grazing record coincided with one of the lower rainfall months of the year. An estimated 32% (1.25 ± 0.69 $\mu gC\ l^{-1}day^{-1}$) of the bacteria production was consumed by microzooplankton (ranging from 9% to 52% of the total bacteria production). Bacterivory of microzooplankton showed negative correlation with dissolved oxygen (r=-0.87) but did not correlate with the abundance of microzooplankton groups. Bacterivory by both microzooplankton and HNP did not differ significantly between dry and wet season (T-test, $p>0.05$).

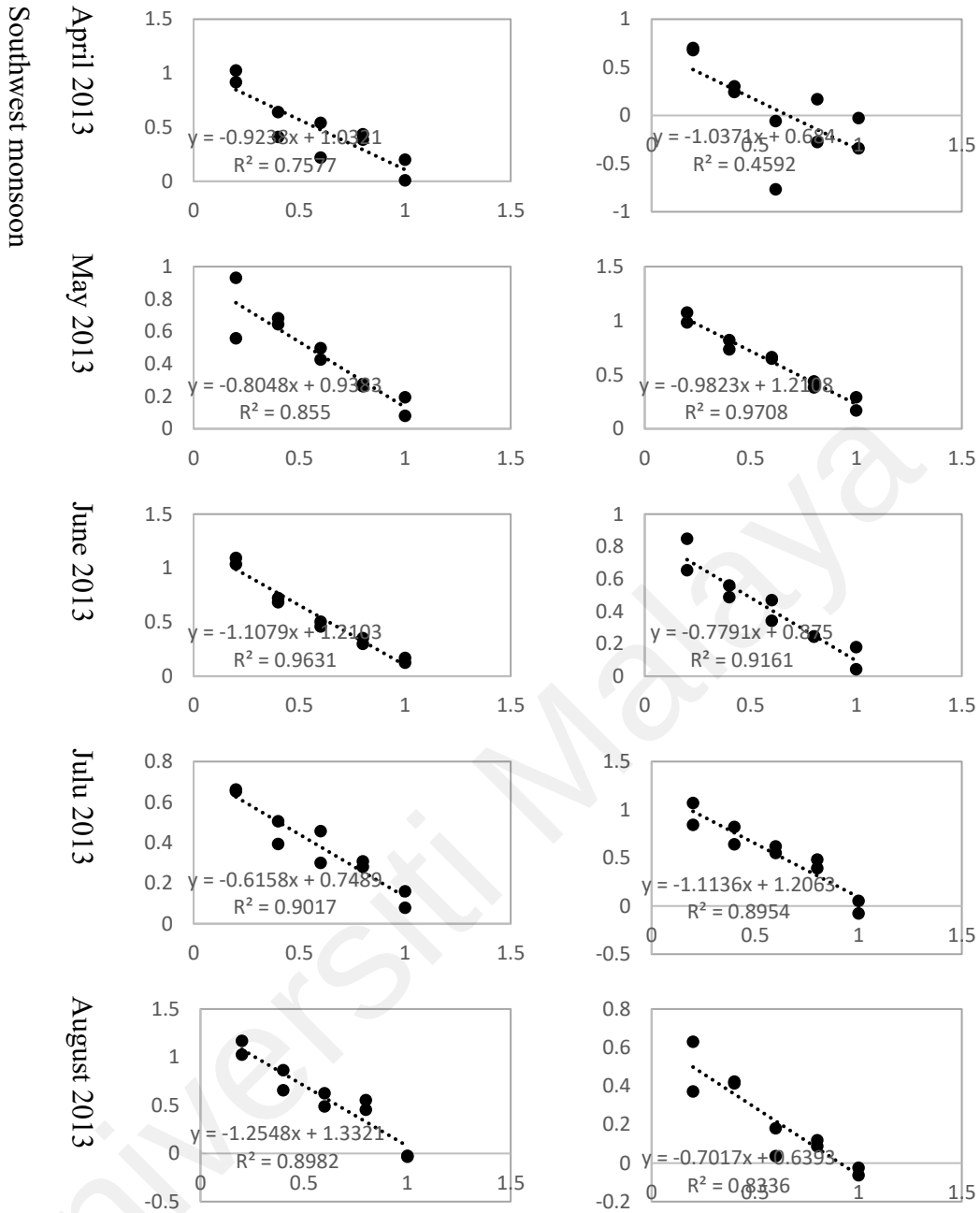
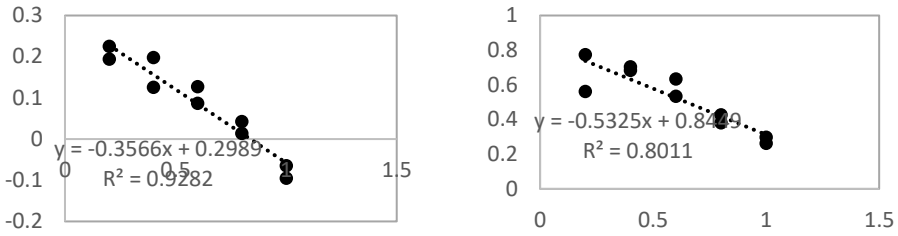


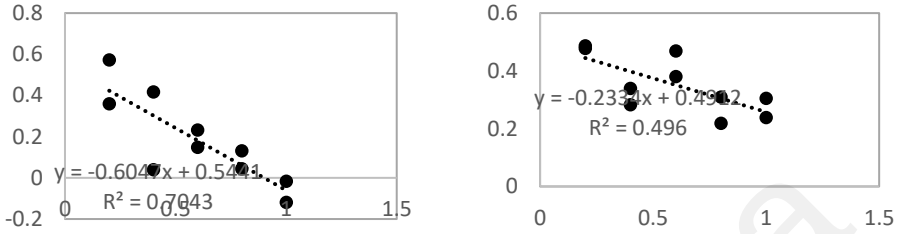
Figure 5.6 Dilution experiment from April 2013 to February 2014. X-axis shows dilution factor and y-axis shows phytoplankton growth as measure by chlorophyll *a*.

Northeast monsoon

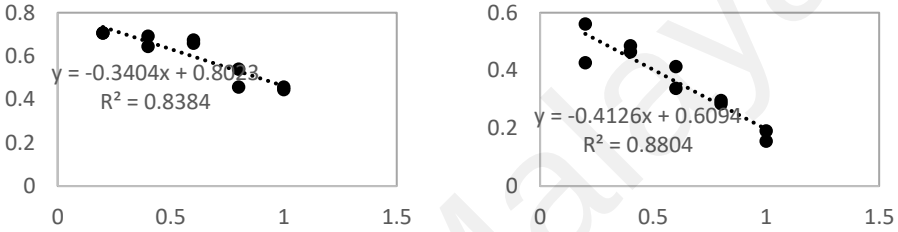
October 2013



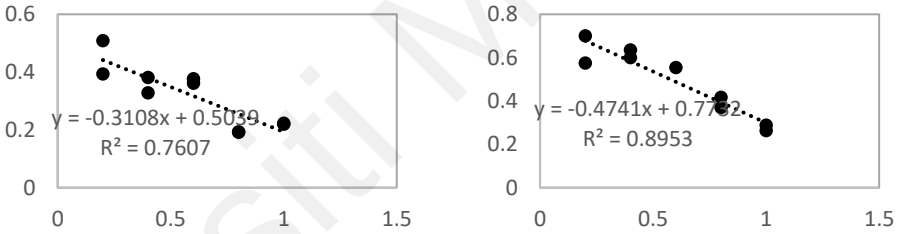
November 2013



December 2013



January 2014



February 2014

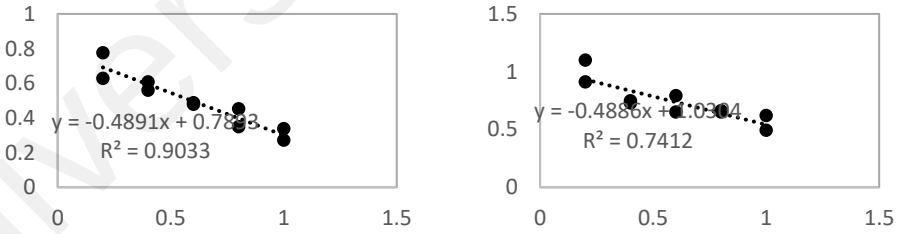


Figure 5.6, continued.

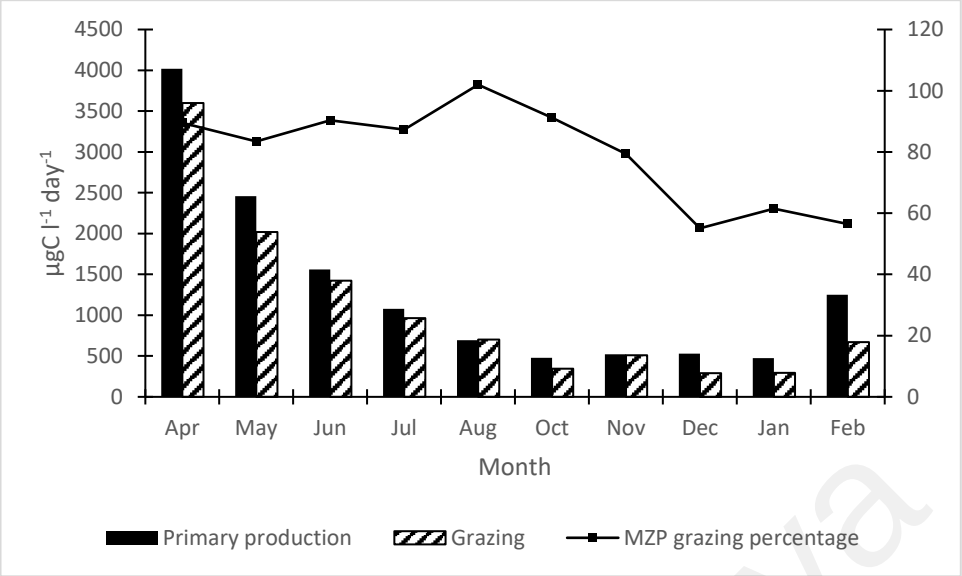


Figure 5.7: Temporal variations of primary production and herbivory activity of microzooplankton as estimated by the dilution technique.

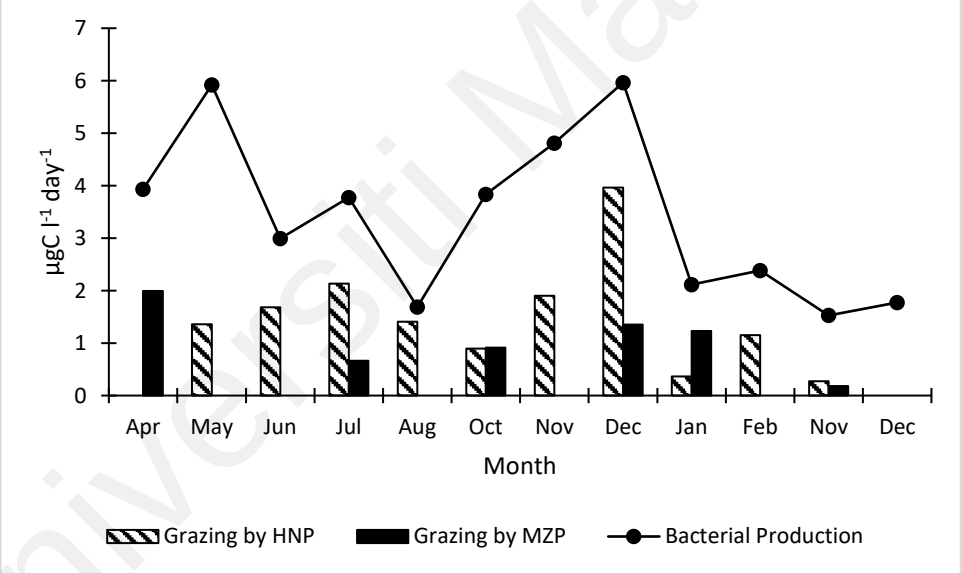


Figure 5.8: Temporal variations of bacterial production and bacterivory activity of HNP and microzooplankton (MZP). Note that on some occasions (no reading), there was no significant grazing ($p>0.05$).

5.4 Discussion

5.4.1 Seasonal Changes in Microbial Biomass

The phototrophic biomass of the highly turbid Terusan estuary in the MMFR was the highest among the microbes measured in this study. As in most studies, phytoplankton production is most important in sustaining the microbial food web, similar to other

tropical estuaries such as in India (Jyothibabu et al., 2008a,b; Gauns et al., 2015), Mexico (Rivera-Monroy et al., 1998) and Costa Rica (Gocke et al., 2002).

The heterotrophic biomass of bacteria, HNP and microzooplankton were only 5%, 8% and 9% of phototrophic biomass, respectively. This huge difference in biomass suggested an overflow of nutrients into the highly productive Matang mangrove as similarly observed in the estuary (Van Meerssche & Pinckney, 2019). In terms of seasonal difference, only the microzooplankton biomass was significantly different being higher in NEM than in SWM. Among the microzooplankton components, ciliated protozoans (tintinnids and aloricate ciliates) were predominant and accounted for >50% of the total microzooplankton biomass (Yong et al., 2016), similar to other tropical estuaries (Beers et al., 1980; Madhu et al., 2007; Gauns et al., 2015).

In this study, it was observed that the phototrophic biomass decreased as NO_3^- and SiO_2 increased (NO_3^-: $R = -0.461$, $p<0.05$; SiO_2: $R = -0.633$, $p<0.01$). A similar trend has been observed in nutrient-rich lakes where phototrophic biomass decreased beyond a threshold level of nutrients (Filstrup & Downing, 2017). Such a decrease in phototrophic biomass under high nutrient condition could occur due to the production of reactive oxygen species that could damage the phytoplankton (Filstrup & Downing, 2017). Other factors responsible for the high nutrient – low chlorophyll waters include shifts in nutrient limitation (Smith & Shapiro, 1981), light availability (Jones et al., 2008), phytoplankton composition and chlorophyll content (Felip & Catalan, 2000), and grazing pressure (Dröscher et al., 2009). However, as phototrophic biomass is the sum of primary production after grazing loss or export, knowing what drives the primary production rates may better explain the observed trend in the phototrophic biomass.

5.4.2 Primary Production and Microzooplankton Grazing

Primary production measured in the present study varied widely from 150 to 4020 µgC $l^{-1}d^{-1}$. However, 65% of the measured readings were less than 1000 µgC l^{-1} d^{-1} (Figure 5.7). Due to the skewed distribution, the median was the best measure of central tendency, i.e. 690 µgC $l^{-1}d^{-1}$. The exceptionally high values that exceeded 2,500 ugC l-1 d-1 (Fig. 5.7) on three occasions are not without precedence since even higher value of 5,000 ugC had been reported for a coastal mangrove lagoon in the Ivory Coast (Iltis, 1984). Although the nearest cage fish farms were located 10km away from the study site, it is not known whether nutrient plumes emanating from farm activities could reach the study site thereby spiking high primary production in April and May. However, a study by Chong (2004) on cage culture effects on water quality of Sangga Besar estuary (see Fig. 3.1) shows that plumes of high nutrient concentration were largely trapped within the farm due to the dense linearly-arranged cage units, while any that escaped outside it quickly dispersed and became diluted by water column mixing. Chlorophyll concentration inside the cages (10-65 ug/L) were not always higher than controls (no cages) (10-45 ug/L) and attributed this to turbidity and zooplankton grazing which could reduce phytoplankton biomass. Another study on cage fish culture effects by Alongi et al. (2003) in the same estuary, at a time when cage culture was more dense than during the present study, shows that nutrient enrichment from aquaculture was not an issue as compared to the higher organic inputs from the large mangrove forest and main village. These authors provided primary production figures on two occasions that ranged from 2-355 mgC $m^{-3}d^{-1}$ (July 1999) and 114 - 751 mgC m^{-3} d^{-1} (April 2000). In the same mangrove estuary, Lee and Bong (2008) gave an estimated mean primary production of 355 µgC $l^{-1}d^{-1}$. Nonetheless, high primary production in tropical estuaries is generally not rare. The upper ranges of the primary production observed in other tropical mangroves e.g. Thailand (200-600 µgC $l^{-1}d^{-1}$), New Guinea (22-693 µgC $l^{-1}d^{-1}$) and Brazil (110-500

µgC $l^{-1}d^{-1}$) (Ayukai & Alongi, 2000, Robertson et al., 1993, Barrera-Alba et al., 2008) are quite similar to the present median estimate for the Matang mangrove estuary. Primary production in the Matang mangrove estuary is higher relative to other coastal sites in Peninsular Malaysia, e.g. Port Dickson (249 ± 33 µgC $l^{-1}d^{-1}$), Port Klang (394 ± 127 µgC $l^{-1}d^{-1}$) and Kuantan (246 ± 88 µgC $l^{-1}d^{-1}$) (Lee & Bong, 2008). Except for Port Klang which is located near mangroves, all others are non-mangrove sites. In MMFR, the high primary production rates explicated the predominant phototrophic biomass, pointing to its highly productive waters.

In this study, primary production was not only correlated with NO_3^-, PO_4^{3-} and SiO_2 (NO_3^-: $R=-0.458$, $p<0.05$; PO_4^{3-}: $R=-0.488$, $p<0.05$; SiO_2: $R=-0.689$, $p<0.001$) but also with temperature ($R=0.777$, $p<0.001$). Using multivariate linear regression, we showed that among the independent variables, temperature was the most important factor that affected primary production rates ($F=8.10$, $p<0.001$). From a comparative study of eutrophic and mesotrophic tropical water systems in Peninsular Malaysia, Lim et al. (2015) suggested a threshold concentration of 4 µM NO_3^- and 20 µM SiO_2 for primary production. Since the average NO_3^- (4.4 ± 2.5 µM) and SiO_2 (76.9 ± 38.3 µM) concentrations at MMFR were above these threshold levels, temperature was probably a more important factor than inorganic nutrient concentration affecting primary production in Matang waters.

Although the primary production rate at MMFR was high, we observed that a substantial amount of primary production was grazed by microzooplankton. Microzooplankton grazing increased as primary production increased ($r^2 = 0.91$, $df=18$, $p<0.001$) (Figure 5.9), and the linear relationship showed that even at the upper ranges of primary production, microzooplankton still grazed >80% of primary production. Among the microzooplankton in this study, tintinnids were the main contributors as tintinnid

production was correlated with primary production (R=0.684, p<0.001). As a herbivorous microzooplankton, the importance of tintinnids has been reported in the Ganges River Estuary (Rakshit 2014, Sarkar 2015), Southeastern Arabian Sea and Cochin backwaters (Jyothibabu et al. 2008b, 2006), and South Coast of India (Godhantaraman 2002). Tintinnids are the main food source for calanoid copepods which predominate in the Matang estuary (Chew & Chong, 2010). Thus, the strong coupling between primary production and microzooplankton grazing suggests the importance of microzooplankton as a trophic link channelling basal energy to higher trophic levels.

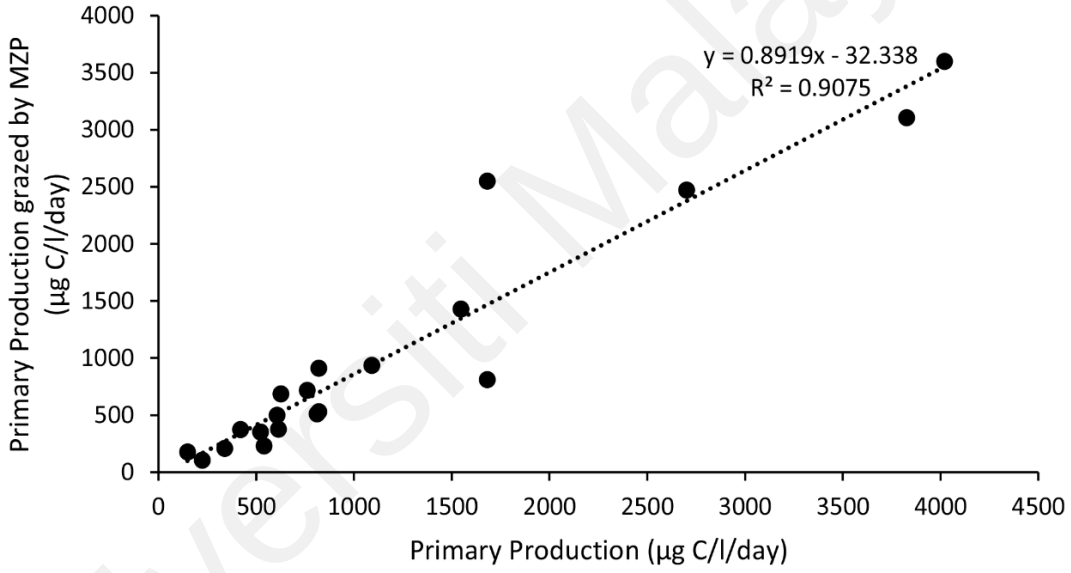


Figure 5.9: Regression analysis between primary production and grazing by microzooplankton (µg C/l/day). Linear regression slope and coeff of determination (r^2) is shown.

5.4.3 Temperature Dependency of Primary Production versus Microzooplankton Grazing

Microzooplankton grazing was also correlated with temperature (R=0.792, p<0.001). Since we found that primary production was correlated to temperature, and microzooplankton grazing is related to primary production (Figure 5.9), we therefore

compared the response of both primary production and microzooplankton grazing to sea temperature rise. Such a comparison would be useful to predict future sea warming scenarios of primary production due to climate change. Temperature dependency is described as activation energy using the Arrhenius equation (Allen et al., 2005). Relationship between natural logarithm of phytoplankton growth (ln μ) and microzooplankton grazing loss (ln g) rates versus seawater temperature (1/kT) were plotted where temperature (T) was in Kelvin (K) and k is the Boltzmann's constant (8.617×10^{-5} eV K^{-1}). In this study, μ increased with temperature ($F = 5.65$, $R = 0.489$, $p<0.05$), and the activation energy (± SE) for μ was 1.29 ± 0.54 eV. g also increased with temperature ($F=10.49$, $R=0.607$, $p<0.01$), and the activation energy (± SE) for g was 2.05 ± 0.63 eV. Activation energy for microzooplankton grazing loss was higher than phytoplankton growth, suggesting that MMFR will trend towards net heterotrophy with sea warming (Chen et al., 2012, Regaudie-de-Gioux & Duarte, 2012). We observed that the heterotrophic process slightly overtook the phototrophic process at the upper temperature range of 38.03°C (Figure 5.10). Therefore, with the projected rise of 2°C by the year 2100 (IPCC 2007), pelagic waters in MMFR is projected to be more heterotrophic in nature.

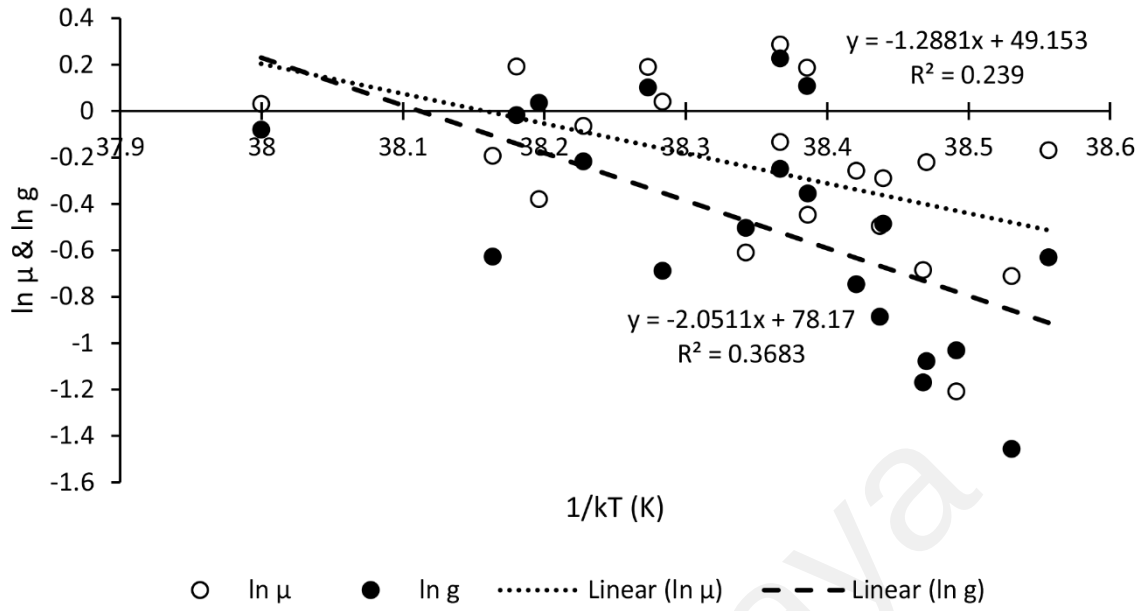


Figure 5.10: Arrhenius plot of primary production and grazing activity of microzooplankton in the estuary. (μ - phytoplankton growth and g - microzooplankton grazing loss)

5.4.4 Phytoplankton − Bacteria – HNP Coupling

Primary production also contributes to the dissolved organic carbon pool which is utilized or repackaged by bacteria into a form utilizable by HNPs. The coupling between phytoplankton – bacteria – HNP forms the microbial loop that drives an alternative food chain alongside the classical phytoplankton – microzooplankton - mesozooplankton food chain (Azam et al., 1983). However, both food chains are interconnected. In most waters, bacteria – phytoplankton coupling either in terms of biomass or process rates are observed (Cole et al., 1988; Lee & Bong, 2008). In selected tropical waters of Peninsular Malaysia, bacterial production correlates with primary production in the range of 170 to 540 µgC $l^{-1}d^{-1}$ (Lee & Bong, 2008). However, in the present study in the MMFR, bacterial production did not correlate with primary production. Primary productivity was on the upper range, from 148 to 4021 µgC $l^{-1}d^{-1}$, and the uncoupling could be due to the highly productive nature of MMFR where the substrate for bacterial production was in excess. Moreover, the DOC pool in MMFR was about 450 µM. Although DOC was measured

once only, it was clearly higher than in other aquatic habitats in Peninsular Malaysia where DOC values ranging from 300 – 390 µM have been obtained (Lee et al., 2009).

In this study, HNP grazing rates were similar to previously reported rates (Bong & Lee, 2011), and HNP grazing rates correlated significantly with bacterial production ($R=0.736$, $p<0.01$) (Figure 5.11). Our observations suggested top-down control regulating bacterial production in the mangrove waters of MMFR. HNP production and microzooplankton grazing on HNP were also measured in this study. However, we were not able to consistently obtain useable rates. As HNP growth rates measured in MMFR ranged from 0.04 to 0.25 d^{-1}, and averaged 0.13 ± 0.08 d^{-1} over a generation time of >7 d, we were not always able to observe significant changes in HNP counts within the 12-h incubation time. HNP growth rates measured in MMFR were in the lower range reported by Wallberg et al. (1999) for tropical coastal systems i.e. from 0.3 to 1.2 d^{-1}.

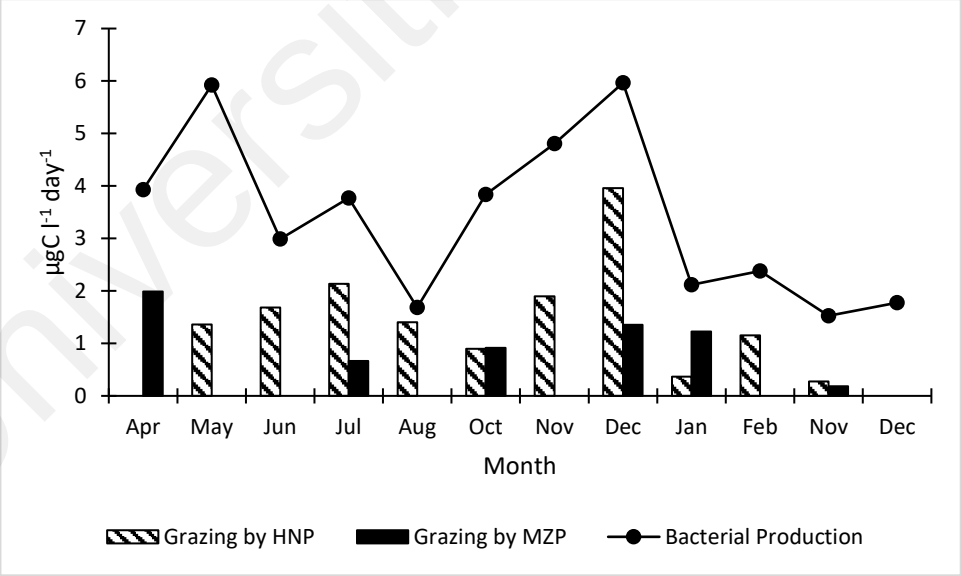


Figure 5.11: Temporal variations of bacterial production and bacterivory activity of HNP and microzooplankton (MZP). Note that on some occasions (no reading), there was no significant grazing ($p>0.05$).

5.4.5 Microbial Foodweb in MMFR

Previously, Tarutani et al. (2007) investigated the relationship between the metazoan zooplankton and primary production in MMFR. However, the primary production was not measured but rather estimated from Chl *a* concentration. Only 5.7% of the primary production was transferred to zooplankton, suggesting that the energy flow from phytoplankton to metazoan zooplanktonis inefficient (Tarutani et al. 2007). The lack of information regarding the role of microzooplankton as an energy-transfer intermediary is obvious from their conclusion, although they suggested that phytoplankton could be consumed by sessile filter feeders on mangrove trees, degraded in the water column, or exported offshore. From the present study, we estimated that >60% of primary production may be transferred to higher trophic levels via microzooplankton production.

In the present study, we showed that the estuarine water of MMFR had very high primary productivity despite its high turbidity. Primary production was especially high in the drier SWM season, and we were able to attribute this to the higher water temperature (29.88 ± 0.3 °C) compared to the NEM season (28.83 ± 0.2°C). Microzooplankton grazing activity recorded in this study is comparable to a study in the Zuari estuary, a tropical monsoon estuary in India (Gauns et al. 2015), and in the western Arabian Sea (Landry et al., 1998). With rapid growth and high ingestion rate (Calbet & Landry, 2004), microzooplankton easily dominated grazing activity over mesozooplankton (Putland & Iverson, 2007). Here, we summarized the data obtained from our study into a schematic flow chart that presents the carbon flow in the estuary (Figure 5.12).

The grazing activity of microzooplankton was different between two monsoons (t-test, $p<0.001$); about 94% of primary production was grazed by microzooplankton during the drier monsoon whereas 64% was grazed during the wetter monsoon (Fig 5.7). Rainfall and nutrient enrichment during the wet monsoon, reduced the grazing pressure of

microzooplankton; hence, primary production was mainly exported. Rainfall also brought in more prey options that might have reduced the grazing pressure of microzooplankton on primary production. During the wetter NEM, the estuary was dominated by bacterivorous aloricate ciliates rather than the herbivorous tintinnids (Yong et al., 2016). There was also reduced primary production concurrent with lower herbivory activity during the wetter NEM. In addition, the herbivorous copepod species in the estuary, *Pseudodiaptomus annandalei* peaked in abundance throughout the NEM during this study (Kong et al., 2015). Since *P. annandalei* was the only estuarine copepod species found in abundance during the wetter season, it presumably served as a perfect competitor to the herbivorous tintinnids in the estuary. Despite, the view held by others that microzooplankton diversity is more closely related with resources rather than competitive interaction or predation (Dolan et al., 2002; Löder, 2011), we contend that with abundant food resource, dominant competitors in abundance may supress tintinnid abundance. Thus, our study shows that microzooplankton are functionally both prey and competitor to mesozooplankton.

The higher primary production and higher temperature during the drier SWM thus provides ample food supply that appears to promote the growth of loricate ciliates or tintinnids during this season (Yong et al., 2016). Herbivory activity is thus significantly higher in the SWM season since the larger, herbivorous loricate ciliates were reported to outnumber the small, bacterivorous aloricate ciliates (Yong et al., 2016). Hence, the higher population of herbivorous species amongst the microzooplankton leads to higher grazing rate during SWM. As suggested by Schmoker et al. (2013), higher grazing by microzooplankton in the estuary was due to the lower biomass of mesozooplankton in warmer waters (Chew & Chong, 2010).

Our incubation to measure grazing rate of microzooplankton on HNP generated a rather low success rate. This might be due to the community structure of the microzooplankton. Predatory ciliates, such as *Favella* sp. and *Tintinnopsis lobioncoi* that were found in this study (Yong et al., 2016) are known to ingest flagellates (Stoecker & Capuzzo, 1990) of the HNP. As flagellates -consuming species were not dominant in the estuary, only 3.5% of HNF production were grazed by microzooplankton with no significant difference between the monsoon (Fig 5.12). This shows that HNP is not a major supporter of microzooplankton production in the Matang mangrove estuary.

Although the main pelagic food chain of 'phytoplankton – microzooplankton – mesozoplankton' is found to be substantially different between monsoons, we did not observe any significant monsoonal difference in the microbial loop. Bacterial production was more than two order lower than primary production; and was consistent throughout the year. Around 84% (SWM) and 65% (NEM) of the bacterial production were consumed within the loop; this showed that there is some other minor channel that took up the bacterial production in the estuary. The amount of carbon recycled through the microbial loop was minimal relative to the classical food chain. The minor role played by bacteria might be a reflection of the highly productive nature of MMFR as phytoplankton – bacteria decoupling was observed.

In future studies, more investigations on zooplankton growth and grazing processes including microzooplankton to mesozooplankton predation in highly variable meteo-hydrological settings, will greatly benefit the understanding of pelagic carbon flow processes and dynamics in tropical estuaries.

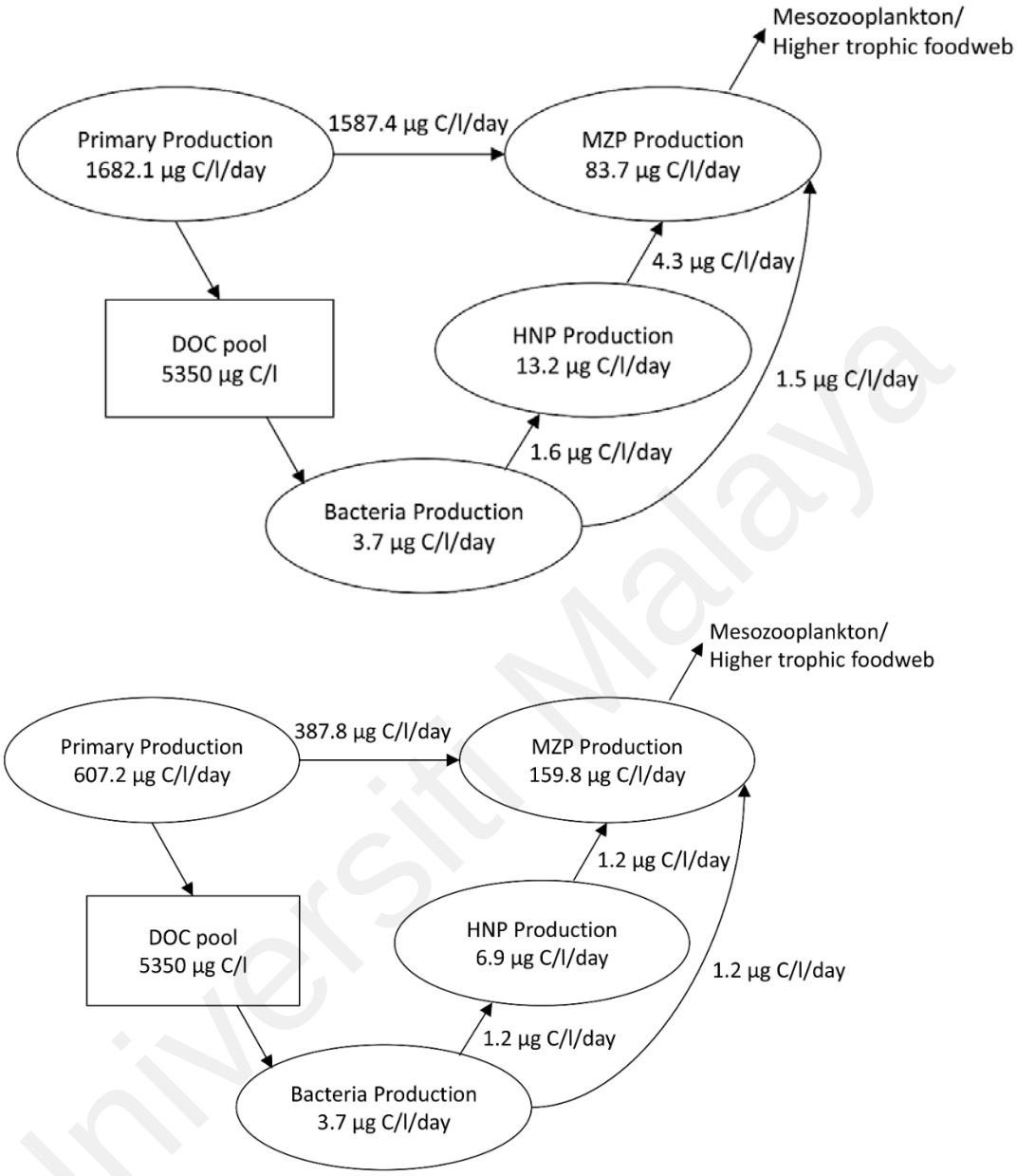


Figure 5.12: Relationships between primary production, bacterial production, heterotrophic nanoplankton production, microzooplankton production and mesozooplankton production during the southwest monsoon (upper panel) and northeast monsoon (lower panel) seasons.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION

6.1 Microzooplankton of Matang Mangrove Forest Reserve

Microzooplankton were diverse and numerically very abundant in the Matang estuary as compared with similar studies in tropical estuary (Sarkar, 2015; Jyothibabu et al., 2008a,b; Godhantaraman, 2002). Shifts in microzooplankton community structure between monsoons, are apparently associated with rainfall, salinity, temperature, DIN and microbial food concentrations. Lower rainfall and higher chlorophyll *a* concentration during the SW monsoon, favoured the loricate ciliates. With the increase in rainfall and dissolved inorganic nutrients during the NE monsoon, aloricate ciliates then dominate. Except for a few taxa, lunar phase which affects the strength of tidal current (neap and spring tide) has no effect microzooplankton abundance. Given their high abundance, it is likely that Matang's microzooplankton channel substantial trophic energy to higher trophic levels.

6.2 Microbial loop of Matang Mangrove Forest Reserve

In this study, we ascertained that MMFR is characterised by high primary production but low bacteria production; primary production contributed to the main productivity of the mangrove water. This high or excess primary production driven by monsoon showed no correlation with bacterial production suggesting the importance of phototrophy relative to heterotrophy. With the uncoupling between primary production and bacterial production, the ecological significance of microzooplankton grazing on phytoplankton to channel carbon (energy) to higher trophic levels became more obvious. From the production and biomass of each component of the microbial loop measured in both monsoons, this study shows the importance of microzooplankton grazing in the microbial food web in MMFR, and how they differed between NEM and SEM.

6.3 Future Studies

a) As microzooplankton shown to have prominent role as trophic intermediaires but related study is close to nothing in Malaysia, further studies in different ecosystem in Malaysia will be interesting to shed more light on our understanding in microzooplankton. Dependency / choice on bacterial production or primary production will be a key to further understand the carbon flow of the ecosystem.

b) This study, however, did not identified microzooplankton with molecular technique. Cultures and sequencing of microzooplankton in elsewhere (Saccà & Giuffrè, 2013; Wallin, 2019) showed variation and hence redefining species of microzooplankton. Certain species in this study showed slight variation of morphological characteristic might spark interesting new finding.

c) Microzooplankton showed food selectivity in the current study. Further study with culture to examine food selectivity on microzooplankton will better exhibit these trophic intermediaries behave in different environment, ie, when the preferred food is scarce and will the herbivory microzooplankton shift to bacterivory.

d) Impact of climate change on microzooplankton community structure, or even morphological change through cultures of microzooplankton should be included in future studies. Impact of climate changes was shown to depend heavily on responses of microorganisms – production, consumption of green house gasses (Cavicchioli et al., 2019).

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