VIRAL LYSIS AS TOP-DOWN CONTROL OF BACTERIAL PRODUCTION IN TROPICAL WATERS OF PENINSULAR MALAYSIA

STANLEY CHAI CHOON YIP

INSTITUTE FOR ADVANCED STUDIES
UNIVERSITI MALAYA
KUALA LUMPUR

2022

VIRAL LYSIS AS TOP-DOWN CONTROL OF BACTERIAL PRODUCTION IN TROPICAL WATERS OF PENINSULAR MALAYSIA

STANLEY CHAI CHOON YIP

DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PHILOSOPHY

INSTITUTE FOR ADVANCED STUDIES
UNIVERSITI MALAYA
KUALA LUMPUR

2022

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **STANLEY CHAI CHOON YIP**

Matric No: **HMA170018 / 17036642**

Name of Degree: **MASTER OF PHILOSOPHY**

Title of Thesis ("this Work"):

VIRAL LYSIS AS TOP-DOWN CONTROL OF BACTERIAL PRODUCTION IN TROPICAL WATERS OF PENINSULAR MALAYSIA

Field of Study:

MARINE SCIENCES

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation:

VIRAL LYSIS AS TOP-DOWN CONTROL OF BACTERIAL PRODUCTION IN TROPICAL WATERS OF PENINSULAR MALAYSIA

ABSTRACT

Viruses are the most abundant biological entities in the biosphere. Viruses infect host cells for replication and survival. Hence, viruses play important roles in the nutrient cycles and genetic transfer. Viral lytic and lysogenic life cycles have different effects to the ecosystem as lytic cycle leads to production of more virus particles and death of host cells whereas lysogenic cycle produces prophage that reproduce along with the host cell. However, the environmental changes such as temperature increment and eutrophication may cause the shift of the lysogenic to lytic life cycle which may affect the host cell community. The changes of viral life cycle and community structure of bacteria and phytoplankton due to environmental change will bring significant consequences onto biogeochemical cycles. The water samples were collected from nearshore (Port Klang and Port Dickson) and offshore waters (Yan and Kuala Rompin) surrounding Peninsular Malaysia. Bacterial production, virus production and protist grazing at each sampling sites were measured. Microcosm experiment were carried out to determine the relationship of seawater warming and eutrophication with viral dynamics. Mitomycin C was used to induce viral lytic life cycle. Observations revealed site-dependent differences in dissolved inorganic nutrient concentration, TSS (43.3 – 106.7 mg L^{-1}), dissolved oxygen (148 – 265 µM), Chl *a* (0.21 – 3.54 µg L^{-1}) and bacterial abundance (4.09×10^5 – 9.41×10^5 cells mL^{-1}). However viral abundance (measured as virus-like particles or VLP) (1.04×10^6 – 2.39×10^6 VLP mL^{-1}) and virus production rates (0.59×10^5 – 4.55×10^5 VLP mL^{-1} h^{-1}) were not coupled to neither bacterial abundance nor production (0.85×10^4 – 6.42×10^4 cells mL^{-1} h^{-1}). The uncoupling was probably due to the bacterial replete state as suggested by the low virus to bacteria ratio (1.8 – 4.3). *Ex situ* experiments

were carried out to investigate the effects of seawater warming (25 – 37°C) and eutrophication (0.1 – 1.0× marine broth concentration) on viral dynamics. Both seawater warming and eutrophication were able to induce lysogen to switch from lysogenic to lytic cycle as the result showed increased virus production ($R^2 > 0.537$) and decreased lysogeny ($R^2 > 0.896$). From this study, eutrophication was a more important driver for virus production. Therefore, with the increasing eutrophication at the coastal waters, the role of viruses in the marine microbial food web will gain importance as virus production increases. Enhanced virus production will drive the habitat towards heterotrophy as increased viral lysis will promote organic matter supplies for the bacterial growth, and will further affect biogeochemical cycling.

Keywords: Viral lysis, lysogeny, bacterial production, protist grazing, top-down control, Kill-the-Winner

VIRUS LITIK SEBAGAI KAWALAN ATAS-BAWAH UNTUK PRODUKSI BAKTERIA DI PERAIRAN PANTAI TROPIKA SEMENANJUNG MALAYSIA

ABSTRAK

Virus adalah entiti biologi yang terbanyak di biosfera. Virus menjangkiti sel perumah untuk replikasi dan kelangsungan hidup. Oleh itu, virus memainkan peranan yang penting dalam kitar nutrien dan pemindahan materi genetik. Kitaran litik dan lisogeni membawa kesan yang berbeza terhadap ekosistem. Kitaran litik menghasilkan lebih banyak virus dan meyebabkan kematian sel perumah sedangkan kitaran lisogeni menghasilkan profaj yang akan membiak bersama dengan sel perumah. Namun begitu, perubahan alam sekitar seperti penambahan suhu dan eutrofikasi akan meyebabkan pertukaran kitaran lisogeni kepeda kitaran litik dan akan mempengaruhi komuniti sel perumah. Perubahan dalam kitaran hidup virus dan struktur komuniti bakteria dan fitoplankton disebab oleh perubahan alam sekitar akan membawa akibat yang signifikan kepada kitaran biogeokimia. Sampel air telah diambil dari lokasi dekat pesisir (Port Klang dan Port Dickson) dan lokasi luar pesisir (Yan dan Kuala Rompin). Produksi bakteria, produksi virus dan peragutan protist telah diukur di setiap tapak persampelan. Eksperimen mikrokosma telah dilaksanakan untuk menentukan perhubungan antara peningkatan suhu laut dan perhubungan antara eutrofikasi dengan dinamik virus. Mitomisin C digunakan untuk mendorong pertukaran kitaran lisogeni kepada kitaran litik. Pemerhatian teleh mendedahkan perbezaan antara nutrien tak organik terlarut, TSS (43.3 – 106.7 mg L^{-1}), oksigen terlarut (148 – 265 μM), Chl *a* (0.21 – 3.54 μg L^{-1}) dan kelimpahan bakteria ($4.09 \times 10^5 – 9.41 \times 10^5$ cells mL^{-1}) adalah bergantung kepada lokasi. Tetapi kelimpahan virus (diukur sebagai zarah seperti virus atau VLP) ($1.04 \times 10^6 – 2.39 \times 10^6$ VLP mL^{-1}) dan kadar produksi virus ($0.59 \times 10^5 – 4.55 \times 10^5$ VLP mL^{-1} h^{-1}) adalah tidak berpasangan dengan kelimpahan bakteria dan produksi bakteria ($0.85 \times 10^4 – 6.42 \times 10^4$ cells mL^{-1} h^{-}

[1]). Ketidak-berpasangan ini mungkin disebabkan daripada keadaan bakteria yang banyak seperti yang dicadangkan oleh nisbah virus ke bakteria yang rendah (1.8 – 4.3). Eksperimen *ex situ* telah dilaksanakan untuk menyiasat kesan pemanasan air laut (25 – 37 °C) dan eutrofikasi (0.1 – 1.0 × kepekatan kaldu marin) terhadap dinamik virus. Kedua-dua pemanasan air laut dan eutrofikasi mampu mendorong lisogen untuk menukar dari kitarab lisogeni ke litik kerana keputusan telah menunjukkan peningkatan produksi virus ($R^2 > 0.537$) dan penurunan lisogeni ($R^2 > 0.896$). Daripada kajian ini, eutrofikasi merupakan pemacu yang lebih utama untuk produksi virus. Oleh demikian, dengan bertambahan eutrofikasi di kawasan pinggir laut, peranan virus di jaringan makanan mikrob lautan akan berganda mustahak sebab produki virus bertambah. Produksi virus yang tertingkat akan memacu habitat terhadap heterotrofi oleh kerana lisis virus akan memperbanyakkan bekalan jirim organik untuk pertumbuhan bakteria, dan akan mempengaruhi kitaran biogeokimia dengan selanjutnya.

Kata kunci: lisis virus, lisogeni, produksi bakteria, ragutan protist, kawalan atas-bawah, Kill-the-Winner

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to every person who made my journey of Master degree of Philosophy in University of Malaya possible.

First of all, I would like to thank my parents for their infinite encouragement and support during my study and laboratory experiment periods.

I would like to express my gratitude to my supervisor, A/P Dr Lee Choon Weng, for his guidance, support and encouragement in my laboratory experiments, manuscript writing and thesis writing.

I would also like to thank my co-supervisor, A/P Dr Bong Chui Wei, for her help especially in preparation for presentations.

I am also like to give my appreciation to University of Malaya for providing the grant (FG027-17AFR) and research facilities to aid my study.

I am really grateful for the guidance and help provided by my seniors, Lim Joon Hai, Wong Yi You, Thiang Ee Lean and Low Kyle Young, in Laboratory of Marine Ecology (LME) throughout my study.

Last but not least, I would like to thank my friends who gave me support and encouraging words throughout my study.

TABLE OF CONTENTS

ORIGINAL LITERARY WORK DECLARACTION…… ii

ABSTRACT…….. iii

ABSTRAK…… v

ACKNOWLEDGEMENTS…… vii

TABLE OF CONTENTS…… viii

LIST OF FIGURES…… xi

LIST OF TABLES……. xiii

LIST OF SYMBOLS AND ABBREVIATIONS……. xiv

LIST OF APPENDICES……... xviii

CHAPTER 1: INTRODUCTION…… 1

1.1 Background…… 1

1.2 Problem statement…….. 3

1.3 Research objectives…… 5

1.3.1 General objective…….. 5

1.3.2 Specific objectives…… 5

CHAPTER 2: LITERATURE REVIEW…… 6

2.1 Marine food web…… 6

2.2 Protist grazing…… 10

2.3 Virus……... 11

2.3.1 Virus lytic and lysogenic cycle……. 14

2.4 Environmental change…… 18

2.4.1 Climate change…….…… 18

2.4.2 Eutrophication…….……. 23

CHAPTER 3: METHODOLOGY…… 27

3.1 Sampling…… 27

3.2 Environmental parameters……. 28

3.2.1 Dissolved oxygen concentration.…….. 29

3.2.2 Dissolved inorganic nutrient concentration…….. 30

3.2.3 Chlorophyll *a* concentration……. 32

3.2.4 Total suspended solids…….. 32

3.2.5 Trophic state index…… 33

3.3 Bacterial and viral abundance…… 33

3.4 Virus production rate……. 34

3.5 Bacterial production and protist grazing rate……. 35

3.5 Microcosm experiments on viruses…… 36

3.7 Statistical analysis…….. 38

CHAPTER 4: RESULTS…….. 39

4.1 Environmental parameters……. 39

4.2 Biological parameters…… 41

4.3 Microbial processes…… 46

4.4 Virus dynamics with increasing temperature and nutrients…… 49

CHAPTER 5: DISCUSSION…… 54

5.1 Environmental conditions……. 54

5.2 Virus and microbial distribution…… 55

5.3 Comparison of viral lysis and protist grazing as fates for bacterial production. 56

5.4 Effects of seawater warming and eutrophication on viral dynamics……. 60

CHAPTER 6: CONCLUSION……..... 70

REFERENCES……..... 71

LIST OF PUBLICATIONS AND PAPERS PRESENTED……... 83

APPENDICES……..... 84

LIST OF FIGURES

Figure 2.1: The line shows the energy in marine environment flowing through a classic food chain and microorganisms whereas the broken line shows the energy flow through virus via viral infection. Direction of the energy flow is indicated by the arrowhead…9

Figure 2.2: Viral life cycles extracted from Howard-Varona et al., 2017. The solid lines show the flow of lytic cycle whereas the broken lines show the flow of lysogenic cycle. The thickened lines show the switch from lysogenic to lytic cycle…… 15

Figure 2.3: Graph shows the observed globally averaged combined land and ocean surface temperature anomaly 1850–2012 extracted from IPCC (2014). Different coloured lines indicating different data sources……..... 19

Figure 2.4: Graph of sea ice extent (a) and global mean sea level change 1900–2010 (b) extracted from IPCC (2014). Different coloured lines indicating different data sources……. 21

Figure 2.5: Eutrophication in the sea caused by nutrient runoff resulted in bacterial growth and algal bloom that lead to hypoxia. Harmful algal bloom will lead to the release of algal toxin into the water body. Both bacterial growth and algal bloom promote viral growth…… 26

Figure 3.1: Map of the location of sampling sites. '—'show the sampling transects at offshore sites. Lower left inset shows the sampling transect at Yan ranged from 5 km to 25 km from shore whereas the right inset shows the sampling transect at Kuala Rompin which was from 5 km to 30 km from the shore……... 28

Figure 4.1: Histogram showing the distribution of bacterial biovolume measured in this study…… 43

Figure 4.2: Contour plots showing the temporal and spatial distribution of bacterial abundance (cell mL^{-1}) at both Yan and Kuala Rompin sampling transects. Each panel shows the bacterial distribution for the different months sampled whereas x-axis shows depth (m) and y-axis shows distance from the shore (km)…….. 44

Figure 4.3: Contour plots showing the temporal and spatial distribution of viral abundance (VLPs mL^{-1}) at both Yan and Kuala Rompin sampling transects. Each panel shows the bacterial distribution for the different months sampled whereas x-axis shows depth (m) and y-axis shows distance from the shore (km)…… 44

Figure 4.4: Regression slopes of virus growth rates (h^{-1}) with temperature increase for both untreated (line with round symbols) and mitomycin-C treated (broken line with triangle symbols) samples at Port Klang (left panel) and Port Dickson (right panel).. 50

Figure 4.5: Regression slopes of virus growth rates (h^{-1}) with nutrient concentration (× marine broth strength) increase for both untreated (line with round symbols) and mitomycin-C treated (broken line with triangle symbols) samples at Port Klang (left panel) and Port Dickson (right panel)……. 51

Figure 4.6: Regression slopes of lysogeny (% of lysogen) with temperature (upper panel) and nutrient concentration (× marine broth strength) (lower panel) at Port Klang (line with round symbols) and Port Dickson (broken line with triangle symbols)…….. 53

Figure 5.1: Correlation analysis between bacterial abundance and chlorophyll *a* concentration (Chl *a*). The linear regression slope is also shown…… 55

Figure 5.2: Correlation analysis between bacterial production and chlorophyll *a* concentration (Chl *a*). The linear regression slope is also shown…… 57

Figure 5.3: Correlation analysis between protist grazing and bacterial production. For nearshore sites (Port Dickson and Port Klang), the linear regression slope is shown. Data when protist grazing was nought (not detectable) was not included…… 58

Figure 5.4: Regression slopes of lysogeny (% of lysogen) with temperature (upper panel) and nutrient concentration (× marine broth strength) (lower panel) at Port Klang (line with round symbols) and Port Dickson (broken line with triangle symbols)…….. 65

Figure 5.5: Regression slopes of lysogeny (% of lysogen) against bacterial growth rate (h^{-1}) in (A) the temperature experiments (upper panel) and (B) nutrient experiments. Data from Port Klang are shown as round symbols whereas data from Port Dickson are shown as triangle symbols. Regression slopes shown are for Port Klang (line) and Port Dickson (broken line)……. 66

Figure 5.6: Scatter plot of virus production rates (log VLP ml^{-1} h^{-1}) against bacterial abundance (log cells ml^{-1}). The filled symbols are the data points from the *ex situ* experiments (A) for temperature and (B) for nutrients. Symbols of darker shades indicate increasing temperature or nutrient concentration……. 68

LIST OF TABLES

Table 4.1: Environmental parameters at Port Dickson, and Port Klang (mean ± standard deviation). Comparison of measurements was carried out using ANOVA, and when significant, pair-wise comparison was carried via Tukey's-HSD. * indicated significant ANOVA at $p<0.05$ whereas ** indicated significant ANOVA at $p<0.001$. Samples with the same letters are different significantly…… 40

Table 4.2: F values of two-way ANOVA for surface water variables against the following factors: station selection (S) and distance from shore (D). Significant effects are indicated with * at $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$…… 41

Table 4.3: Range and mean ± SD of bacterial and virus abundance (as VLP), and the virus to bacteria ratio (VBR) among the four sites. Comparison of means were carried out via ANOVA, and *** is significance at $p < 0.001$. The same superscript letters indicated significant pair-wise comparison after Tukey's-HSD test…….... 42

Table 4.4: F values of three-way ANOVA for column water bacterial (BA) and virus (VA) abundance against the following factors: sampling month, distance from shore (D) and sample depth (Dh). Significant effects are indicated with * at $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$……..……. 46

Table 4.5: Range and mean ± SD of bacterial growth, bacterial production, protist grazing rate and virus production rate among the four sites. ND is not detectable. Comparison of means were carried out via ANOVA, and * is significance at $p < 0.05$. The same superscript letters indicated significant pair-wise comparison after Tukey's-HSD test. 48

Table 4.6: Range and mean ± SD of viral lysis (estimated using a virus burst size of 44.8), and the proportion of viral lysis and protist grazing over bacterial production (%). The sum proportion of both grazing and lysis over bacterial production is also shown. Comparison of means were carried out via ANOVA, and * is significance at $p < 0.05$. The same superscript letters indicated significant pair-wise comparison after Tukey's-HSD test…… 48

Table 4.8: Range and mean ± SD of viral lysis (estimated using a virus burst size of 44.8), and the proportion of viral lysis and protist grazing over bacterial production (%). The sum proportion of both grazing and lysis over bacterial production is also shown. Comparison of means were carried out via ANOVA, and * is significance at $p < 0.05$. The same superscript letters indicated significant pair-wise comparison after Tukey's-HSD test…… 47

Table 5.1: Univariate correlation matrix for temperature experiment. Significance for correlation analysis is as shown: ** is $p < 0.01$, *** is $p < 0.001$…… 61

Table 5.2: Univariate correlation matrix for nutrient experiment. Significance for correlation analysis is as shown: * is $p < 0.05$, ** is $p < 0.01$, *** is $p < 0.001$…….. 62

LIST OF SYMBOLS AND ABBREVIATIONS

<	:	less than
>	:	greater than
=	:	equal
×	:	multiply
±	:	plus–minus
%	:	percentage
°C	:	degree Celsius
&	:	and
–	:	to
ANCOVA	:	Analysis of Covariance
ANOVA	:	Analysis of Variance
CH_4	:	methane
Chl *a*	:	chlorophyll *a*
CO_2	:	carbon dioxide
CO_3^{2-}	:	carbonate ion
DAPI	:	4', 6-diamidino-2-phenylindole
DNA	:	deoxyribonucleic acid
DO	:	dissolved oxygen
DOC	:	dissolved organic carbon
DOM	:	dissolved organic matter

et al.	:	and others
FCCC	:	Framework Convention on Climate Change
h	:	hour
H^+	:	hydrogen ion
HAB	:	harmful algal bloom
HCO_3^-	:	bicarbonate ion
H_2O	:	water molecule
IPCC	:	Intergovernmental Panel on Climate Change
kDa	:	kilo Dalton
km	:	kilometre(s)
L	:	litre(s)
log	:	base 10 logarithm
MAGIC	:	magnesium-induced coprecipitation
m	:	meter(s)
mL	:	millilitre(s)
µ	:	bacterial growth rate
µg	:	microgram(s)
µL	:	microliter(s)
µm	:	micrometre(s)
µM	:	micromolar(s)
nm	:	nanometre(s)

NH_4	:	ammonium
N_2O	:	nitrous oxide
NOAA	:	National Oceanic and Atmospheric Administration
NO_2	:	nitrite
NO_3	:	nitrate
O_2	:	oxygen
O^{2-}	:	oxide ion
p	:	significant level
PAST	:	Paleontological Statistics
PO_4	:	phosphate
ppt	:	parts per thousand
π	:	Pi
RNA	:	ribonucleic acid
SD	:	standard deviation
SOS	:	save our ship
Spp.	:	species
SiO_4	:	silicate
TSI	:	trophic state index
TSS	:	total suspended solids
UK	:	United Kingdom
USA	:	United States of America

UV	:	ultraviolet
VLP	:	virus-like particle

LIST OF APPENDICES

Appendix A: Picture of running AMICON Stirred Ultrafiltration Cell Millipore Model 8400 to obtain virus-free filtrate……... 84

Appendix B: Picture of sample stained with SYBR Green 1 captured via epifluorescence microscopy. Viruses (red-circled) were smaller and dimmer…….. 85

Appendix C: Date of sampling and GPS coordinates of the sampling location…….. 86

Appendix D: Compilation of physical and chemical data measured from offshore sites……... 87

Appendix E: Compilation of physical and chemical data measured from nearshore sites…….. 88

CHAPTER 1: INTRODUCTION

1.1 Background

Ocean is a large water mass that makes up most of the Earth's surface. Ocean regulates the global climate and stores carbon dioxide (CO_2). Different sections of the ocean such as coastal waters, open waters, seafloor and zones including photic zone, aphotic zone, and benthic zones harbour a variety of species. The study of food web is important to understand the flow of energy from primary production to consumer at high trophic level in the ocean. Bacteria, phytoplankton and viruses are ubiquitous and act as the pivotal driving force of energy cycles in the oceanic environment (Karl, 2007; Jacquet et al., 2010; Mojica & Brussaard, 2014). Hence, study of the relationship and function of microorganisms and viruses involved provides insight regarding the ecological and biogeochemical mechanisms in the ocean.

Bacteria play an important role in the food web by consuming the energy generated by primary producers and transferring the energy to consumers at higher trophic level in the food web (Azam et al., 1983; Stocker & Seymour, 2012). The energy in bacteria is transferred when they are grazed by protists. Viral infection also causes bacterial mortality. When bacterial host is lysed by virus, the nutrient is recycled as the organic content in the host cell is released into the environment (Okazaki et al., 2019) and becomes available for other organisms. Both protists and viruses are the main top-down controls of bacteria (Sherr et al., 2007; Suttle, 2007; Li et al., 2019). Viral infection has consequences on bacterial abundance, bacterial diversity, and gene transfer in the bacterial community (Wommack et al., 1999; Clasen et al., 2008; Evans et al., 2009). However, different viral life cycles, whether lytic or lysogenic cycles, have different impact on bacteria (Chen et al. 2019).

The importance of virus is generally site-dependent. Bacterial mortality due to viral lysis can be a minor component (Binder, 1999; Lee & Bong, 2012) or even similar proportion to grazing (Fuhrman & Noble, 1995; Hwang & Cho, 2002). In some habitats, viral lysis can be more important than grazing (Almeida et al. 2001; Bettarel et al. 2002; Choi et al., 2003). Understanding the contribution of protist grazing and viral lysis on bacterial production provides knowledge of the energy flow in a particular habitat, whether energy move up the food web or recycles within the microbial community (Tsai et al., 2013a).

As viruses are obligate parasites that depend on bacterial hosts for survival (Wommack & Colwell, 2000; Jacquet et al., 2010; Mojica & Brussaard, 2014), viral processes in offshore waters should differ from nearshore waters due to differences in microbial community composition, metabolism and environmental parameters (Mojica & Brussaard, 2014; Wang et al., 2019). Similarly, protist grazing on bacteria should be different between nearshore and offshore waters due to the availability of bacteria (Li et al., 2019). According to Almeida et al. (2001), bacterial mortality due to viral lysis in an estuarine system range from 49 % to 74 %, and is higher nearshore. However, Tsai et al. (2013c) reported no difference in viral lysis at both nearshore and offshore waters. The differences reported could be due to the influence of both bacteria and environmental gradients on virus and viral processes (Almeida et al., 2001; Tsai et al., 2013c). These differences also suggest that the contribution of viral lysis between nearshore and offshore waters should be investigated further.

Climate change especially seawater warming and eutrophication can bring consequences to the biogeochemical cycles that occurred in the oceanic environment. Seawater warming and eutrophication alter the physical and chemical characteristics of the marine environment, which will lead to disrupted primary production and phototrophic community composition, bacterial abundance, metabolism and community

compositions (Larsen et al., 2008; Danovaro et al., 2011; Demory et al., 2017). These changes which affect the host cells and can have a dramatic impact on viral abundance, diversity and processes (Danovaro et al., 2011; Cabral et al., 2017). Another major impact of seawater warming and eutrophication on viruses is the lysogenic induction where viruses switch from lysogenic to lytic life cycle. The factors that causes lysogenic induction include UV irradiation, nutrient addition, temperature increase, presence of hydrogen peroxide, vigorous cell growth and pollutants such as polynuclear aromatic hydrocarbons, polychlorinated biphenyls, and pesticides (Weinbauer & Suttle, 1999). Lysogeny induction has implications towards the microbial food web dynamics as it affects the rate of mortality of the hosts.

1.2 Problem statement

At present, most studies on viral dynamics are carried out in temperate waters, less is known about viral processes and the lysogenic to lytic dynamics in the tropics. Very few studies on the ecology of viruses are available from the south Southeast Asian waters of the Sunda Shelf although it is one of the most productive coastal water in the world (Bettarel et al., 2011; Lee & Bong, 2012; Pradeep Ram et al., 2018). Coastal waters have important geological function in biogeochemical cycles as exchanging of products between atmosphere, land, and open ocean occurs in coastal waters (Mackenzie et al., 2000). It has been reported that the two most crucial environmental change in the Malaysian waters are warming and eutrophication (Bush & Fairbanks, 2003; Cheevaporn & Menasveta, 2003; Lim et al., 2018; Lim et al., 2019; Lee et al., 2020) which may lead to disastrous ecological and environmental risks (Li et al., 2017). Malaysia is a developing country, with swift industrial and economic growth (Kaw & Kannan, 2016; Kozaki et al., 2017), and experiences eutrophication in the form of anthropogenic pollutants from rapid

development, urbanisation, tourism, industrialisation, and agricultural activities are discharged into the seawater surrounding the country (Masood et al., 2016; Mwaura et al., 2017). Howarth et al. (2011) also reported that tropical coastal waters were more susceptible to eutrophication than temperate coastal waters.

In order to better understand how the marine microbial food web in these waters function in the face of environmental change, it is crucial to assess the effects of warming and eutrophication on viral processes. The relatively warm and stable tropical waters in Malaysia should give better insight to the possible factors affecting the top-down controls of bacterial production in both nearshore and offshore waters as opposed to the wide ranging temperature that act as the main driver for many biological processes in temperate waters (Lee et al., 2001). In this study, it is hypothesized that both warming and eutrophication will increase viral lysis. Furthermore, as temperatures in the tropics are already high and relatively stable (Lee et al., 2009), eutrophication should be a more important driver for virus production.

1.3 Research objectives

1.3.1 General objective

To understand the effects of environmental change on the viral dynamics in tropical coastal waters of Peninsular Malaysia.

1.3.2 Specific objectives

a) To investigate the impact of viral lysis relative to protist grazing on bacterial production

b) To determine how seawater warming and eutrophication affect the lysogenic-lytic dynamics in tropical coastal waters of Peninsular Malaysia

CHAPTER 2: LITERATURE REVIEW

2.1 Marine food web

Ocean is a large mass of salt water that covers about 71 % of the Earth's surface (Karl, 2007). The ocean has its role to play as the major component that makes up the Earth's surface. The ocean is the largest habitat available on Earth (Karl, 2007), housing millions of species across the domain of life. Microscopic organisms such as bacteria to multicellular, enormous organisms including sea grasses, fishes, and marine mammals resides in the ocean. Ocean life can be found from the epipelagic zone to the hadopelagic zone of the oceans, and from cold waters to hot thermal vents. The interactions between the marine organisms and environment in a food web maintain a balanced ecosystem where energy and material cycling sustain the ocean.

A food web is made up of numerous interconnecting food chains where a food chain consists of primary producer and multiple levels of consumers (Reece et al., 2011). Each component of the food chain is also known as trophic level. The organisms in the food chain have a prey-predator relationship where organisms at higher trophic level prey on organisms at lower trophic level (Reece et al., 2011). Energy transfer up from one trophic level to another level can be observed via food chain. When different food chains are combined into a food web, a complex network of prey-predator interactions in certain ecosystem can be organised where the flow of energy within the ecosystem can be studied (Dunne et al., 2002). Every single species in the food web is essential and decline of populations or extinction of a single species will have detrimental effect on the energy cycle. With a fundamental knowledge about the food web, one can understand the ecosystem better.

In a classic marine food web, marine algae particularly diatoms are the primary producers. The energy flows from diatoms to zooplankton such as copepods and krill as primary consumers. The energy then moves up the trophic level to planktivorous fishes and then predatory fishes. However, the energy flowing through the classic food web might be a small part of the total energy cycle (Pomeroy, 1974). Intensive studies of microorganisms have brought new insight into the studies of marine food web, suggesting that other pathway may be present involving the major part of energy. Large, multicellular photosynthetic organisms such as seaweeds, sea grasses, and kelps are estimated to account for only 10 % of total primary production in the marine environment. Scientists believe that the major primary producers are small-sized phytoplankton, notably in the size range between 1 – 10 μm (Sherr & Sherr, 1994; Karl, 2007). This is due to the high abundance and high activity of these phytoplankton available in the ocean (Tsai et al., 2018). Worden et al. (2004) reported that picophytoplankton accounted for 76 % of net carbon produced per day. *Synechococcus* spp., a ubiquitous member of picophytoplankton, is capable to account for up to 60 % of total primary production in subtropical water (Tsai et al., 2018). The high contribution of picophytoplankton to total primary production (42 – 55 %) in the estuary (Gaulke et al., 2010) also showed that picophytoplankton plays a pivotal role in primary production. In temperate water, picophytoplankton accounted for 5.1 – 73.9 % of total primary production (Steitz & Velimirov, 1999). On the other hand, bacterial communities are metabolically active and has a high growth rate in the ocean. These bacteria consumed substantial energy from primary producers (Rivkin & Legendre, 2001; Kirchman et al., 2009) for metabolic processes and growth which leads to a suggestion that bacteria play an important role in the energy cycling in marine environment (Azam et al., 1983; Fuhrman & Noble, 1995). These discoveries of phytoplankton and bacteria led to the development of a pathway that involves microorganisms.

The proposed pathway (Azam et al., 1983) begins with nanophytoplankton as the primary producers that carry out photosynthesis for metabolism. The absorption of CO_2 via primary producers is one of the pathway for ocean to uptake and store CO_2 (Watson & Liss, 1998; Takahashi et al., 2002; Juranek et al., 2019). Dissolved organic carbon (DOC) is introduced into the ocean via exudation of fixed carbon or dead phytoplankton. The DOC is a significant part of primary production (Pomeroy, 1974). If not utilised, a major portion of ocean's primary production will be lost. The DOC is consumed directly by marine bacteria as energy for metabolism and growth. The uptake of DOC by bacteria is an important role because DOC is not an accessible carbon source to most marine organisms at higher trophic levels. Bacteria are grazed by heterotrophic protists whereas the protists are prey of filter feeders or planktivorous fishes. While the DOC goes through bacteria and protists, DOC is repackaged into the classic food web, resulting in additional energy becoming available to the higher trophic levels in the food chain such as plankton feeders and predatory fishes (Sarmento et al., 2010). As the energy flows through the microbes, nanophytoplankton, bacteria and protist formed a "loop" before entering the classic food web (Figure 2.1). This pathway is also known as the "microbial loop", a term first used by Azam et al. (1983). It is believed that up to 50 % of total primary production enters the microbial loop (Wommack & Colwell, 2000). Hence, microbial loop practically allows a large portion of primary production to become available to the whole marine food web.

Other than grazing loss, it is no longer possible to estimate the contribution from bacteria without considering the other top-down control on bacteria i.e. viral lysis (Sherr et al., 2007; Suttle, 2007; Li et al., 2019). Phytoplankton and bacteria can be infected and lysed by virus (Bratbak et al., 1994; Cabral et al., 2017). When bacteria and phytoplankton were lysed by viruses, the cellular components rich in carbon, nitrogen and phosphorus are released back into the environment in the form of DOC and inorganic nutrients. This

process is known as the "viral shunt" (Weitz & Wilhelm, 2012). The shunted DOC serves as other source of food for bacteria whereas the inorganic nutrients can be utilised by primary producers. Viral shunt reduces the food availability to organisms at higher trophic level (Tsai et al., 2015; Zhang et al., 2021). The understanding of bacteria and their function in marine food webs have increased significantly since Pomeroy's seminal paper in 1974 (Karl, 2007).

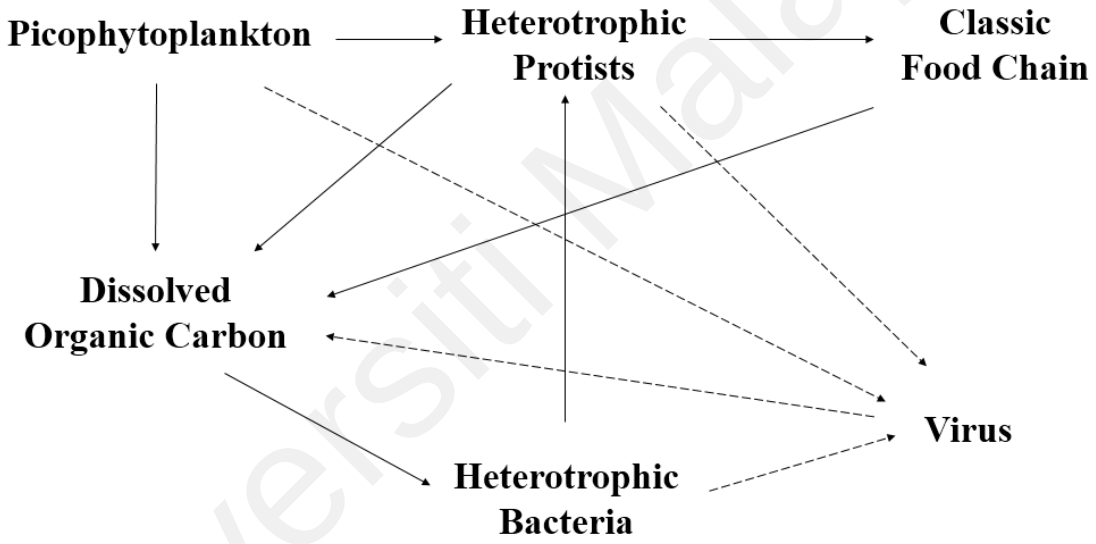

Figure 2.1: The line shows the energy in marine environment flowing through a classic food chain and microorganisms whereas the broken line shows the energy flow through virus via viral infection. Direction of the energy flow is indicated by the arrowhead.

2.2 Protist grazing

Protists are eukaryotes grouped under the Kingdom Protista. Protists are very diverse, and ranged from unicellular to multicellular. There are also protists that form colonies. Sizes of protists vary broadly, from microscopic to colossal. There are photosynthetic protists that make their own food and heterotrophic protists that feed on organic compounds (Paffenhöffer et al., 2007; Sherr et al., 2007). Mixotrophic protists that are capable to obtain energy via both photosynthesis and feeding can also be found in the environment (Sherr et al., 2007; Fenchel, 2008). Protists can be found in various habitats including ocean. Photosynthetic protists in the ocean are also known as marine algae. Brown, green, red algae, dinoflagellates, and diatoms are well-known marine algae. Most marine algae are microscopic, however some, including seaweeds and kelps, are macroscopic. Photosynthetic protists are the major primary producers in the oceanic environment (Sherr et al., 2007).

Heterotrophic protists consume organic compounds such as bacteria in the environment for energy. The process of bacterial grazing by protists is very crucial for energy cycling in the ocean. As bacteria require large amount of energy to maintain high metabolic rate and growth, bacteria are expected to consume a majority of primary production in the ocean. Bacteria with high metabolic rate and that are larger in size are usually targeted by protists (Sherr & Sherr., 1994). When bacteria are consumed by protists, the energy will be transferred up the trophic level in the ocean food web. Although there are other bacteria feeders such as microscopic crustacean and filter feeders, protists are the main predator of bacteria (Sherr et al., 2007). Protists act as the bridge that allows energy to flow from producers to food chain by preying on bacteria (Zhang et al., 2021). When protists are consumed by organisms at higher trophic levels such as filter feeders and plankton feeders, the major part of energy from primary production can be channelled into food web instead of being lost to the environment.

Besides transferring energy into food web, protist grazing on bacteria regulates bacterial abundance in a habitat. Protists are not species-selective feeders of bacteria which means that protists feed on the bacteria that comes in contact with it, regardless of the bacterial species. However, there are several factors affecting the protist grazing on bacteria. One of the factors is bacterial abundance where high bacterial abundance supports high protist grazing rate as plenty of prey is available as food. Size of the prey can affect protist grazing too. Bacteria with extreme size either smaller or larger than the prey size range will be ignored by protists (Paffenhöffer et al., 2007) as protists determine the suitability for capture and the food value of the prey by size. On the other hand, certain bacterial metabolites such as toxin and dimethylsulphide can act as protective agents to prevent protist grazing. A grazing threshold is available to maintain the balance of microbial community where the grazing activity of protists is reduced or ceased once the bacterial abundance fall below a certain level (Paffenhöffer et al., 2007).

2.3 Virus

Viruses are the most abundant (Evans et al., 2009; Cabral et al., 2017) and most diverse (Cissoko et al., 2008) biological entities in the biosphere, ranging from 10^5 to 10^7 virus-like particles (VLPs) mL^{-1} in the oceanic environment (Paul & Kellogg, 2000; Hewson et al., 2003). Viruses are made up of genetic information as the core, either in DNA or RNA, enveloped by protein coat. Viruses are obligate parasites that require other organisms for replication and survival (Wommack & Colwell, 2000; Mojica & Brussaard, 2014). Viruses infect a host cell, hijack and reprogram the host cell's metabolism to produce virus progeny. Viruses can infect all living organisms including humans, animals, plants and bacteria (Evans et al., 2009; Anderson et al., 2013; Okazaki et al., 2019). Viruses actively infect host to maintain its persistence in the environment (Aylward et al., 2017).

Viruses are so diverse that every single species can be infected by at least one type of virus. As viruses are abundant and ubiquitous, viruses have essential role on Earth (Cissoko et al., 2008; Clasen et al., 2008; Aylward et al., 2017). First of all, viruses are important in horizontal gene transfer in oceanic life. Viruses infect other living organisms such as bacteria by transporting viral genetic material into the host cells. Besides viral genetic information, viruses can carry virulent factor genes such as antibiotic resistance and toxin genes to the host cells as viruses incorporate into host genome for replication. Hence, viruses also serve as the genetic reservoir in the ocean. Through acquiring new genes and metabolic functions, viruses also promote co-evolution of the virus and its host (Anderson et al., 2013).

Viruses play an important role in regulating host community composition (Hewson et al., 2003; Zeigler Allen et al., 2017). Viruses frequently infect and produce new virus progeny to maintain a steady-state concentration of viruses and hosts in an environment. Virus exerts significant control on bacterial mortality, and can account for 5% to 80% of bacterial mortality in various aquatic environments (Almeida et al., 2001; Bettarel et al., 2002; Choi et al., 2003; Tsai et al., 2013c). Viruses infect and kill the host to reduce the host population. At high abundance of hosts, the chance of contact between virus and host increases which leads to higher viral infection rate (Weinbauer, 2004). Viral infection is often observed in host population with high metabolism activity and larger cell size. These active host cells consumed large amount of energy to carry out essential metabolism where the energy can be utilised by viruses for replication. As a parasite, viruses have unexpectedly narrow range of host (Hewson et al., 2003; Junger et al., 2018) because viruses recognised and attached to host via unique receptors on surface of the host cell. Hence, the viral infection and mortality of host are species specific. The species specific infection and mortality lead to the observation of the oscillating abundance of single type of virus and host species whereas the total viral and host abundance remain

constant in an environment (Weinbauer, 2004). This is due to the repeated event where reduction of one host abundance reduce its virus but promote the abundance of other virus and host species. As viral lysis kills the competitors for resources, the abundance and production of non-infected populations increase (Weinbauer, 2004). Therefore, unlike protist grazing that regulates prey abundance, viruses are capable of regulating host diversity.

Viruses also play pivotal role in influencing ecosystems and biogeochemistry of ocean via nutrient cycles (Weitz & Wilhelm, 2012; Anderson et al., 2013). Viruses take part in the nutrient cycles by infecting the microbes involved in these cycles and affect the microbial community size, diversity and metabolism. Viruses are known to contain host-derived auxiliary metabolic genes which can stimulate host metabolic pathways (Brum et al., 2016; Okazaki et al., 2019) so that the host can respond swiftly to environmental change. Viral lysis of bacterial cells, also known as the viral shunt, releases cellular content of the host into the environment as dissolved organic matter (DOM) (Okazaki et al., 2019). These released cellular contents, often rich in carbon, nitrogen and phosphorus, are important source of nutrients to bacteria and primary producers especially in oligotrophic environment (Weinbauer, 2004). As viral lysis released most of original cellular contents into the DOM pool, viral lysis has extreme efficiency in converting biomass to DOM (Wommack & Colwell, 2000). This showed that viruses play an important role in nutrient cycles such as nitrogen and phosphorus cycle (Weinbauer, 2004; Zimmerman et al., 2020). Viruses also lyse primary producers which may affect the carbon fixation rates, suggesting that viruses have significant role in carbon cycle (Weinbauer, 2004). The release of organic content from bacterial lysis provides additional energy and stimulates the growth of bacteria and primary producers (Jacquet et al., 2010; Matteson et al., 2012) but is “shunted” away from protist grazers and higher trophic levels (Zimmerman et al., 2020). Viruses can also utilise these organic content uptake by hosts

to produce new viral progenies (Weinbauer, 2004). On the other hand, viral shunt allows the aggregation of organic matter to sink into the deep sea. The sinking of organic matter, also known as marine snow, transports the energy from producers in the surface to the consumers in the deep sea where food is scarce (Weitz & Wilhelm, 2012; Zhang et al., 2021). Viruses are beneficial to viral hosts such as prokaryotes and phytoplankton as viruses help in maintaining the biomass and productivity of the hosts (Weinbauer, 2004). Overall, viruses show strong influences on the performance of microbial food webs, host diversity and biogeochemical cycles. The total impact of viruses on the marine ecosystems depends on the viral life cycle, either lytic or lysogenic cycle, as the fate of bacterial host is different depending on the type of life cycle (Weinbauer et al., 2003; Jacquet et al., 2010; Chen et al., 2019).

2.3.1 Virus lytic and lysogenic cycle

In lytic life cycle, the genetic information of the virus enters the host cell and utilise the host metabolism to produce replicas. Viral protein is also being produced using the host's system. Viral protein and genetic information are then assembled into new virus progeny. Once the concentration of viruses exceeded the host cell capacity, the host cell is lysed and the virus progenies are released, allowing the viruses to infect other cells (Jacquet et al., 2010; Anderson et al., 2013). Viral lytic life cycle is heavily dependent on host cell abundance, host metabolic rates and the trophic status of the environment (Wommack & Colwell, 2000; Almeida et al., 2001; Chen et al., 2011; Anderson et al., 2013; Mojica & Brussaard, 2014). High number of bacterial host leads to high lytic virus production (Wommack & Colwell, 2000) whereas active host cells in eutrophic environments are also prone to viral lysis (Jacquet et al., 2010). Different from lytic life cycle where the host is killed, lysogenic life cycle is a long-term relationship between the

virus and the host. The genetic information of the virus integrated into the host genome upon entering the host cell or remain extrachromosomal as circular or linear plasmid (Howard-Varona et al., 2017). The host cell containing the virus genetic information is known as lysogen where the virus is known as prophage. The prophage replicates simultaneously with the host, and is dormant and will not cause damage to the host. However, the prophage can be induced and enter the lytic cycle and in turn kill the host (Jacquet et al., 2010; Anderson et al., 2013; Howard-Varona et al., 2017; Chen et al., 2019). There are hosts developed antiphage mechanism, such as adsorption inhibition, DNA injection blocking, restriction-modification and abortive infective, to prevent viral infection (Weinbauer, 2004).

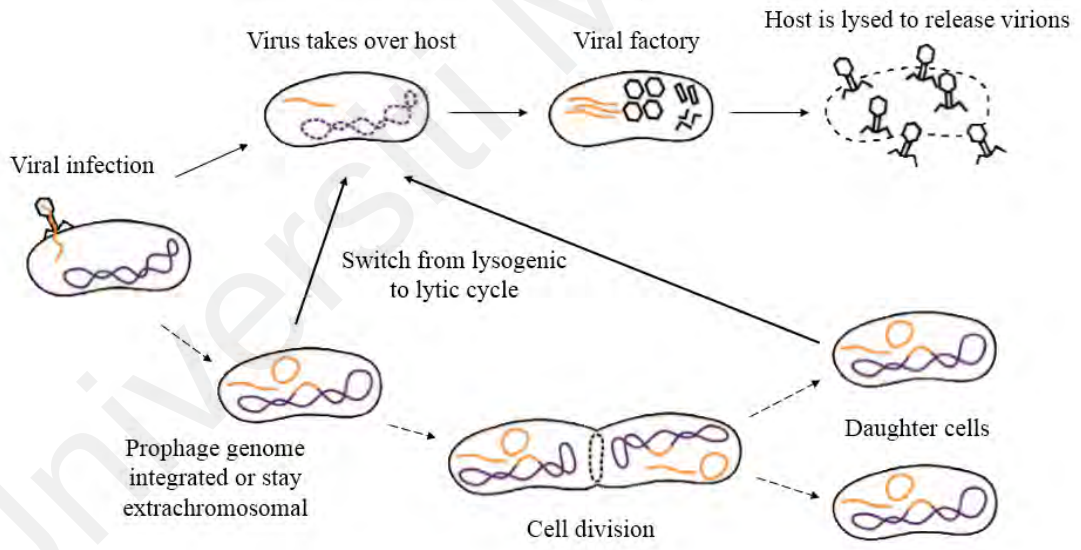

Figure 2.2: Viral life cycles extracted from Howard-Varona et al., 2017. The solid lines show the flow of lytic cycle whereas the broken lines show the flow of lysogenic cycle. The thickened lines show the switch from lysogenic to lytic cycle.

There are various factors that will affect the decision of temperate virus to enter lysogeny, such as host physiology and environmental conditions (Paul & Kellogg, 2000; Howard-Varona et al., 2017). Increase of lysogeny occurrence can be observed when the host density and activity are low, and in oligotrophic environment (Paul & Kellogg, 2000; Weinbauer et al., 2003; Brum et al., 2016; Howard-Varona et al., 2017). Jiang & Paul (1994) reported that lysogeny ranged from 0.1 % to 43.4 % in the marine environment where eutrophic environments have a lower percentage of lysogens compared to oligotrophic environments. Hence, lysogeny is often described as a strategy of viruses to survive low host abundance and maintain its persistence in the environment during unfavourable conditions (Jacquet et al., 2010; Bettarel et al., 2011; Tsiola et al., 2016). However, lysogeny does not only favour survival and existence of virus but hosts also gain benefit from the prophage. Lysogenic hosts will gain new phenotypic characteristics (Wommack & Colwell, 2000). The auxiliary genes in virus are able to activate host metabolic pathway during lysogeny. The activation of metabolic pathway (Weinbauer & Suttle, 1999) allows the host to utilise different types of nutrient as energy, allowing the host to thrive at harsh environment. Host cells also gained additional characteristics such as production of virulence factor and antibiotic resistance genes that increase the survivability of the host (Wommack & Colwell, 2000). Lysogenic infection also provides lytic immunity or superinfection immunity to the host to prevent a secondary infection by other same or similar viruses (Weinbauer & Suttle, 1999; Wommack & Colwell, 2000). Hence, lysogenic infection improves host's fitness and leads to a mutualistic relationship (Jacquet et al., 2010; Chen et al., 2019).

Under certain circumstances, lysogenic induction will occur where lysogenic life cycle is switched to lytic life cycle. A lysogen consists of a repressor that prevent lysogenic induction. Once the repressor is inactivated, lysogenic induction can occur (Howard-Varona et al., 2017). Lysogenic induction can happen spontaneously in nature where

some of the prophage switch to lytic life cycle under ideal conditions. Spontaneous lysogenic induction occurs in an extremely low frequency (Howard-Varona et al., 2017; Morris et al., 2020). Lysogens are susceptible to anthropogenic pollutants (Mojica & Brussaard, 2014). Environmental stress that triggers the host cell's DNA damage response or the SOS response and inactivate the repressor will initiate the lysogeny induction (Howard-Varona et al., 2017; Morris et al., 2020). Drastic changes in nutrient concentration, pH and temperature can cause lysogeny induction (Howard-Varona et al., 2017). Other stresses such as antibiotics, hydrogen peroxide, and ultraviolet irradiation that cause damage to host cell's DNA will lead to lysogeny induction too (Howard-Varona et al., 2017). "Kill-the-Winner" hypothesis is proposed to describe that high bacterial abundance cause lysogeny induction where temperate phages switch to lytic cycle and lyse bacterial host (Weinbauer et al., 2003; Weitz & Wilhelm, 2012). By reducing the abundance of the dominant species in a habitat (Jacquet et al., 2010; Okazaki et al., 2019), other species with lower competitive capability are able to thrive and survive, in turn promoting species evenness (Matteson et al., 2012; Thurber et al., 2017). In contrast, "Piggyback-the-Winner" hypothesis describe that lysogeny increases at high bacterial abundance, such as beyond 1×10^6 cells mL^{-1}, which reflects the increased contribution of temperate viruses in ecosystems with high host abundance (Knowles et al. 2016). Temperate viruses enter lysogeny to protect the dominant species from lytic viruses and protists predation (Knowles et al., 2016; Thurber et al., 2017; Vaqué et al., 2019).

2.4 Environmental change

2.4.1 Climate change and its effect on microbial processes

Climate change is a global phenomenon where the general weather pattern of the Earth undergoes a gradual transformation over a long period of time. According to Pielke (2004), the Framework Convention on Climate Change (FCCC) defined climate change as "a change of climate that is attributed directly or indirectly to human activity, that alters the composition of the global atmosphere, and that is in addition to natural climate variability over comparable time periods." Generally, it is believed that climate change began around mid-18th century when industrial revolution began. Climate change is often linked with global warming which is the increase in average temperature on the Earth's surface particularly due to human activities. The Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) (2014) reported that Earth's surface temperature had increased for 0.85°C from 1880 to 2012. Global warming is mainly due to accumulation of greenhouse gases such as CO_2, methane (CH_4) and nitrous oxide (N_2O) in the atmosphere due to human activities (Beman et al., 2010; Tsiola et al., 2016). These greenhouse gases absorb and prevent the release of heat from the Earth's surface, increasing the global average temperature. The emission of anthropogenic greenhouse gases has increased since industrial revolution era and reached 49 gigatonne of CO_2-equivalent per year in 2010 (IPCC, 2014). The increase in anthropogenic greenhouse gas emission accelerated global warming. It is expected that the global average surface temperature will increase between 0.3 °C to 4.8 °C by the end of 21st century (IPCC, 2014). As global climate change will affect all components in an ecosystem, it is expected that climate change will impact microorganisms and viruses (Zhang et al., 2021).

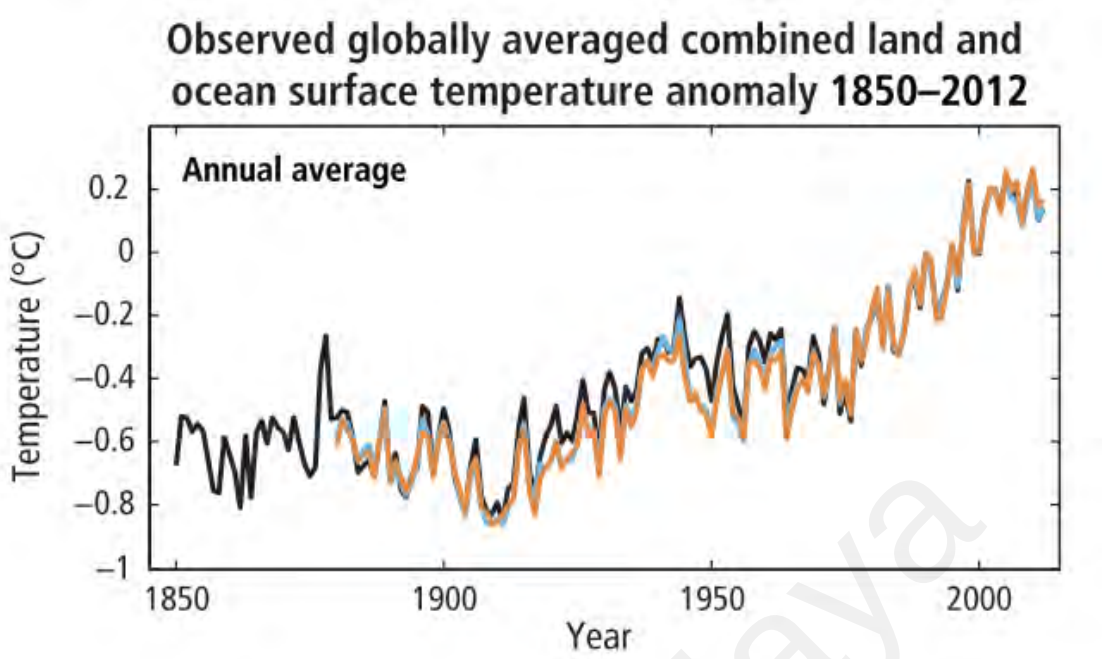

Figure 2.3: Graph shows the observed globally averaged combined land and ocean surface temperature anomaly 1850–2012 extracted from IPCC (2014). Different coloured lines indicating different data sources.

Oceans play an important role in the regulation of the climate. To regulate the climate, the ocean stores, distributes and dissipates the heat energy absorb from solar radiation. The absorbed heat energy is distributed towards Northern and Southern poles and will further drive global climate patterns (Chang et al., 2006). As the oceans have the capacity to store more than 1000 times of heat energy compare to the atmosphere, the oceans are the main reservoir of heat energy (Reid, 2016). However, climate change caused the oceans to absorb too much of heat energy and lead to seawater warming, which brings great consequences to the environment. Seawater warming alters the physicochemical characteristics including temperature, salinity, turbulence and nutrient concentration of the ocean (Mojica & Brussaard, 2014). The altered physicochemical characteristics affect the viral host abundance, distribution and metabolism and thus influence the oceanic viral dynamics (Danovaro et al., 2011; Mojica & Brussaard, 2014; Tsiola et al., 2016). As oceans are dominated by microbial ecosystems that play pivotal role in the

biogeochemical cycles on Earth, any significant change in microbial metabolism caused by seawater warming has the possibility to alter these biogeochemical cycles (York, 2018; Zhang et al., 2021). For instance, biological processes take part for about 80 % in the carbon cycle where small disruption on the biological components will strongly affect the carbon cycle (U.S. DOE., 2008).

One of the most obvious consequences from seawater warming is the loss of polar ice as climate change-related impacts are most prominent in polar regions (Zhang et al., 2021). The polar ice sheets in Greenland and Antarctic are losing mass where Artic sea ice extent is decreasing (IPCC, 2014) and leads to the increase of sea level (Thompson, 2010; IPCC, 2014). The increase of sea level will cause flooding or even loss of land masses, especially coastal area and islands. Both seawater warming and loss of sea ice improve the growth condition and lengthen the growing period of phytoplankton which lead to the extensive carbon fixation at polar regions (Moore et al., 2018; Kim & Kim, 2021). The phytoplankton bloom will further cause redistribution of nutrients in the intermediate and deep ocean layers which deprived the nutrients concentration for bacteria at ocean surfaces (York, 2018). The reduction of sea ice extent affects the ice algal abundance and disrupts the food web in polar regions (Kim & Kim, 2021).

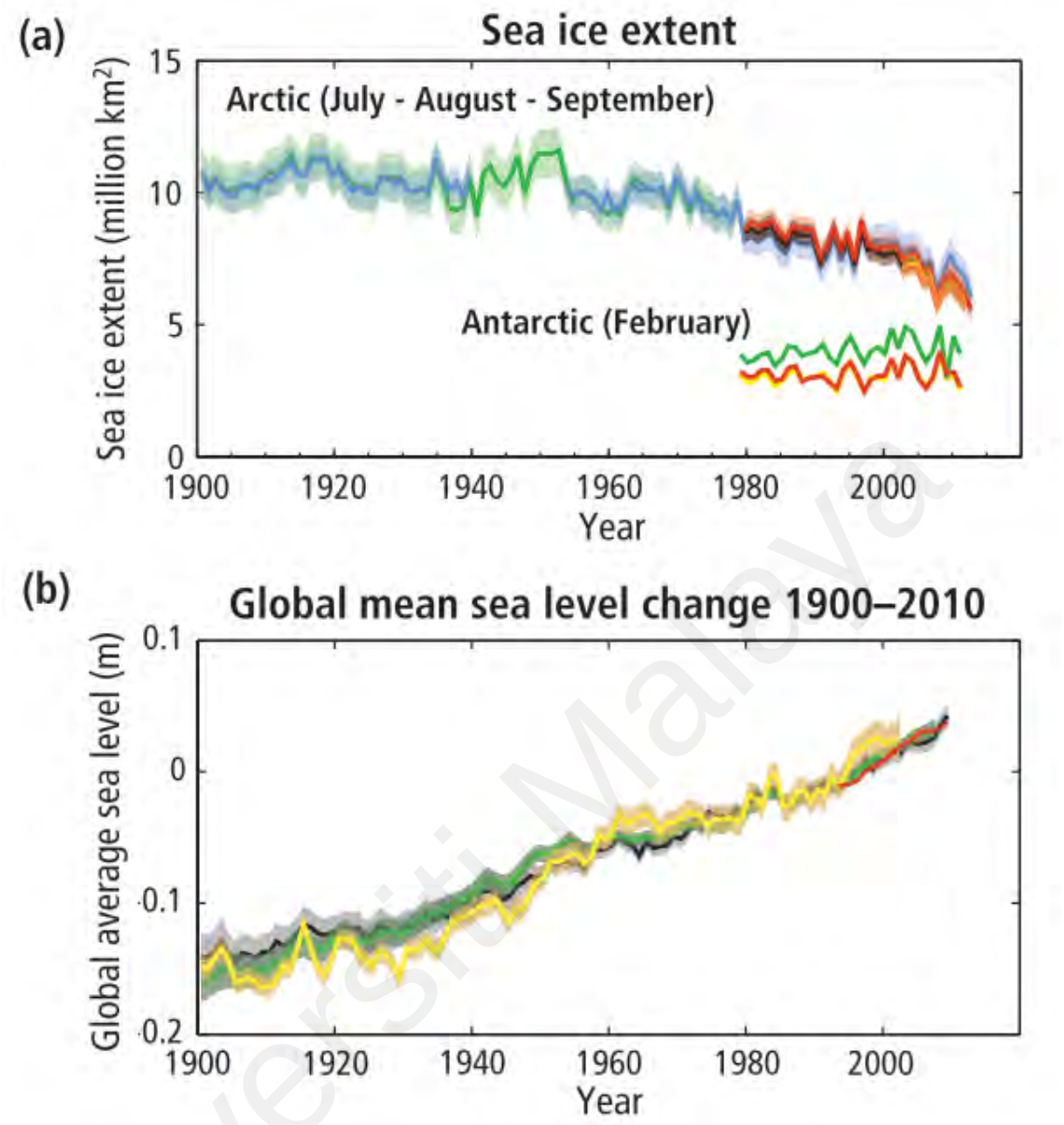

Figure 2.4: Graph of sea ice extent (a) and global mean sea level change 1900–2010 (b) extracted from IPCC (2014). Different coloured lines indicating different data sources.

Ocean also plays a role as the primary storage medium of CO_2 (Henson et al., 2017). Ocean is expected to absorb up to 25 % anthropogenic CO_2 that has decreased the pH of the ocean water, resulting in ocean acidification (Beman et al., 2010; Duarte et al., 2013; Tsiola et al., 2016). Hydrogen ions (H^+), the indicator of acidic condition, can bond with CO_2 to form bicarbonate ions (HCO_3^-) and reduce the concentration of carbonate ions (CO_3^{2-}) (Beman et al., 2010) which were utilised by calcifying organisms for calcification

(Duarte et al., 2013). Ocean acidification further suppress nitrification in the ocean (Beman et al., 2010) which may disrupt nitrogen cycle and lead to a lack of nitrogen source in open sea. Ocean acidification also leads to the formation of oxygen minimal zones as H^+ attracts oxide ion (O^{2-}) to form water molecule (H_2O) (IPCC, 2014). In oxygen minimal zones where microbes carried out anaerobic respiration, nitrate and sulphate are being utilised by microbes to gain energy instead of oxygen (Bianchi et al., 2018). Anaerobic respiration promotes denitrification in the ocean which affect the pattern of global nitrogen cycle (Bianchi et al., 2018). Denitrification further limits the availability of nitrates for phytoplankton, thus limiting the primary productivity (York, 2018).

Seawater warming also cause an increase in vertical stratification of ocean, preventing water mixing and disrupting the circulation of nutrient and oxygen from deep sea to surface (Mackenzie et al., 2000). Vertical stratification may reduce the nutrient availability to the phytoplankton in ocean surfaces and lead to decrease in abundance and production rate of phytoplankton (Moore et al., 2018). Danovaro et al. (2011) reported that the decrease of net primary production might be due to vertical stratification that occur in the low-latitude ocean. The decrease of nutrient concentrations in Indian Ocean is caused by vertical stratification due to seawater warming (Kim & Kim, 2021). The lack of nutrient concentration will decrease the growth of primary producers and further affect the bacterial growth (Kim et al., 2020). Vertical stratification also reduced the carbon export and lead to the decline of biological production in the deep sea (Mackenzie et al., 2000; York, 2018).

Increase in seawater temperature might restructure the bacterial community as new bacterial species that adapted to the warmer temperature replace the old ones (Lara et al., 2013). Smale et al. (2017) reported that increased in seawater temperature led to the increase of both bacterial abundance and community richness values. The shifts in

community structure might alter ecological functions (Smale et al., 2017). Modifications on viral and microbial communities caused by climate change may also have a cascading effect on marine food web as minor changes at the base of a food web might be amplified over trophic levels (Sarmento et al., 2010).

Seawater warming also promotes bacterial respiration and leads to intensive heterotrophy (Kim et al., 2020; Simone et al., 2021). Due to seawater warming, the carbon flow in marine ecosystem shifts from grazing towards viral lysis (Evans et al., 2021). An enhanced heterotrophy will reduce the efficiency of energy transferring up the trophic levels of a food web (Kim et al., 2020). As seawater warming reduced the production of DOC, enhanced heterotrophic respiration will lead to an environment with limited nutrient (Simone et al., 2021).

In general, bacterial host growth rate and abundance will increase along with the increase of temperature (Sarmento et al., 2010) which will shorten the lytic cycle and increase virus production (Lara et al., 2013; Zhang et al., 2021). Increase in bacterial growth caused by increasing temperature is certain to induce the viruses to switch from lysogenic to lytic cycle (Ashy & Agustí, 2020). Both Lara et al. (2013) and Evans et al. (2021) reported that viral lysogenic cycle switch to lytic cycle at high temperature due to warming. As warming affects host growth and abundance, viral life strategies can be shifted and may impact the biogeochemical cycles (Zhang et al., 2021).

2.4.2 Eutrophication and its effect on microbial processes

The Oceanic Service of National Oceanic and Atmospheric Administration (NOAA), USA described eutrophication as a nutrient-enriched environment that promotes the abundance of aquatic plant and algae in estuaries and coastal waters which lead to harmful algal blooms (HAB), formation of dead zones and death of fishes. The death of

overpopulated plants and algae lead to decomposition where decomposers consume O_2 and produce large quantity of CO_2. Eventually, the water becomes hypoxic, or even anoxic and kills large aquatic organisms such as fishes (Meyer-Reil & Köster, 2000; Hewson et al., 2003). The area of anoxic waters and dead zones is increasing globally (Howarth et al., 2011). Anoxic waters lead to loss of habitat, changes in ecological structure and loss of biodiversity (Howarth et al., 2011). Anoxic usually start at bottom waters, which will change the benthic fauna species and facilitate sulphate reduction to sulphide (Howarth et al., 2011). High sulphide concentrations will inhibit nitrifying bacteria and affect nitrogen cycle (Howarth et al., 2011). HAB is often accompanied with algal toxin which can be deadly to humans and other living organisms (Smith, 2003; Kroeze et al., 2013). HAB also increase water turbidity that block sunlight, which affect the life of submerged aquatic plants (Meyer-Reil & Köster, 2000). Besides, HAB reduces the transfer of energy to higher trophic levels as most of HAB species are not fully grazed and in turn disrupt the marine food web (Gilbert et al., 2010).

Human population growth and food production result in excessive nutrient input via sewage discharges and runoffs that are the main factor of eutrophication (Meyer-Reil & Köster, 2000; Kroeze et al., 2013; Piroddi et al., 2021). Moreover, the intensifying usage of chemical fertiliser has increased the accumulation of nutrients especially nitrogen and phosphorus in the aquatic environment (Moore et al., 2013). Nitrogen often acts as a limiting factor of primary production in marine environment (Howarth et al., 2011; Cha et al., 2016). However, chemical fertiliser introduced an over-level nitrogen concentration into the environment and facilitated the bloom of plants and algae (Meyer-Reil & Köster, 2000). It is estimated that two-fold greater of nitrogen and three-fold greater of phosphorus were discharged into the ocean, causing eutrophication (Howarth et al., 2011). Hence, eutrophication is one of the most influential anthropogenic effect on aquatic environment (Piroddi et al., 2021)

Eutrophication affects viral communities indirectly as eutrophication has impact on viral host communities where the increase in nutrient concentration affects the growth, abundance, and metabolism of microbes (Danovaro et al., 2003; Mojica & Brussaard, 2014). Cabral et al. (2017) reported that eutrophication may structure the bacterial abundance and consequently viral abundance in marine waters. The change in microbial community will affect the viral dynamics as viruses strongly rely on the host to carry out necessary functions. During phytoplankton bloom in conjunction with eutrophication, the increase in viral abundance was observed (Wommack & Colwell, 2000; Cabral et al., 2017; Coello-Camba et al., 2020). The increase in bacterial abundance was also closely followed by an increase in frequency of infected bacteria and the abundance of free viruses (Wommack & Colwell, 2000). These observations are strong evidence for close coupling between virus production and production of bacterial communities. Nutrient addition also facilitates the growth of bacterial host cells that can cause the viruses to switch from lysogenic to lytic cycle and increases virus production (Danovaro et al., 2003; Mojica & Brussaard, 2014; Malits et al., 2021). Mojica and Brussaard (2020) reported that prophage induction decreased along with increase in nutrient concentrations. Similar to bacterial host, phytoplankton growth is stimulated by eutrophication which will lead to lysogenic induction where cyanophage production is increased (Coello-Camba et al., 2020).

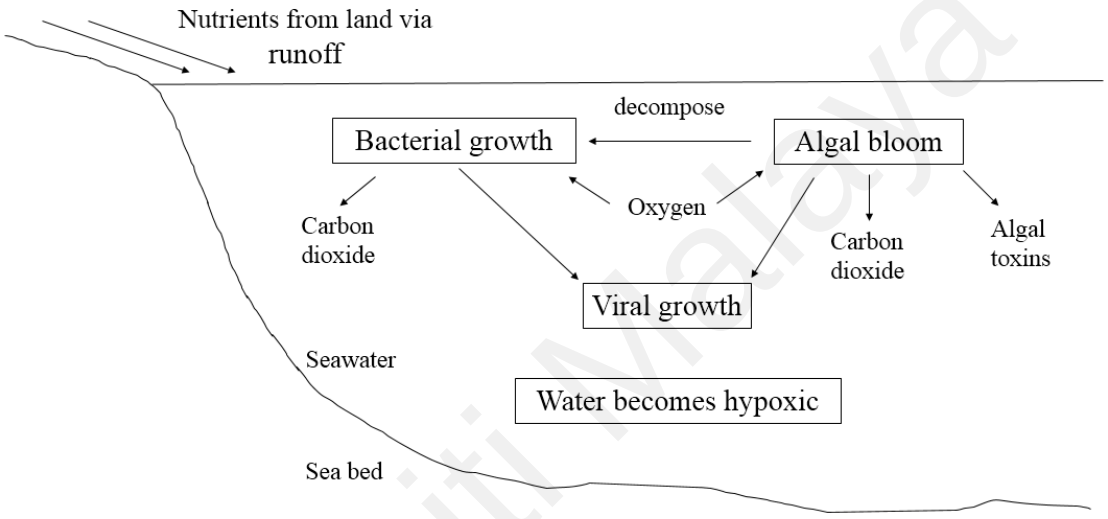

Figure 2.5: Eutrophication in the sea caused by nutrient runoff resulted in bacterial growth and algal bloom that lead to hypoxia. Harmful algal bloom will lead to the release of algal toxin into the water body. Both bacterial growth and algal bloom promote viral growth.

CHAPTER 3: METHODOLOGY

3.1 Sampling

Seawater sampling was carried out at two nearshore sites, which were Port Dickson, Negeri Sembilan (2°29'N, 101°47'E) and Port Klang, Selangor (3°00'N, 101°24'E); and for offshore sites, along two transects at Yan, Kedah from 5°49'N 100°22'E to 5°49'N 100°8'E and Kuala Rompin, Pahang from 2°49'N 103°28'E to 2°49'N 103°45'E (Figure 3.1). Surface water was collected three times at each nearshore sites, from October to November 2017. At the offshore sites, sampling was carried out every 5 km in a linear transect up to 25 km from shore at Yan from March to May 2017, and up to 30 km from shore at Kuala Rompin from June to September 2017. At both offshore transects, surface water samples and water samples at every 5 m depth until 20 m depth were collected. Due to logistic constraints, virus production, bacterial production and protist grazing rates were only measured for surface water samples collected at nearshore sites and at 5 km, 15 km and 25 km offshore sites. *Ex situ* microcosm experiments to assess temperature and nutrient effects on virus processes were also carried out but with surface water samples collected at nearshore sites only from May to October, 2018; and from April to July, 2019.

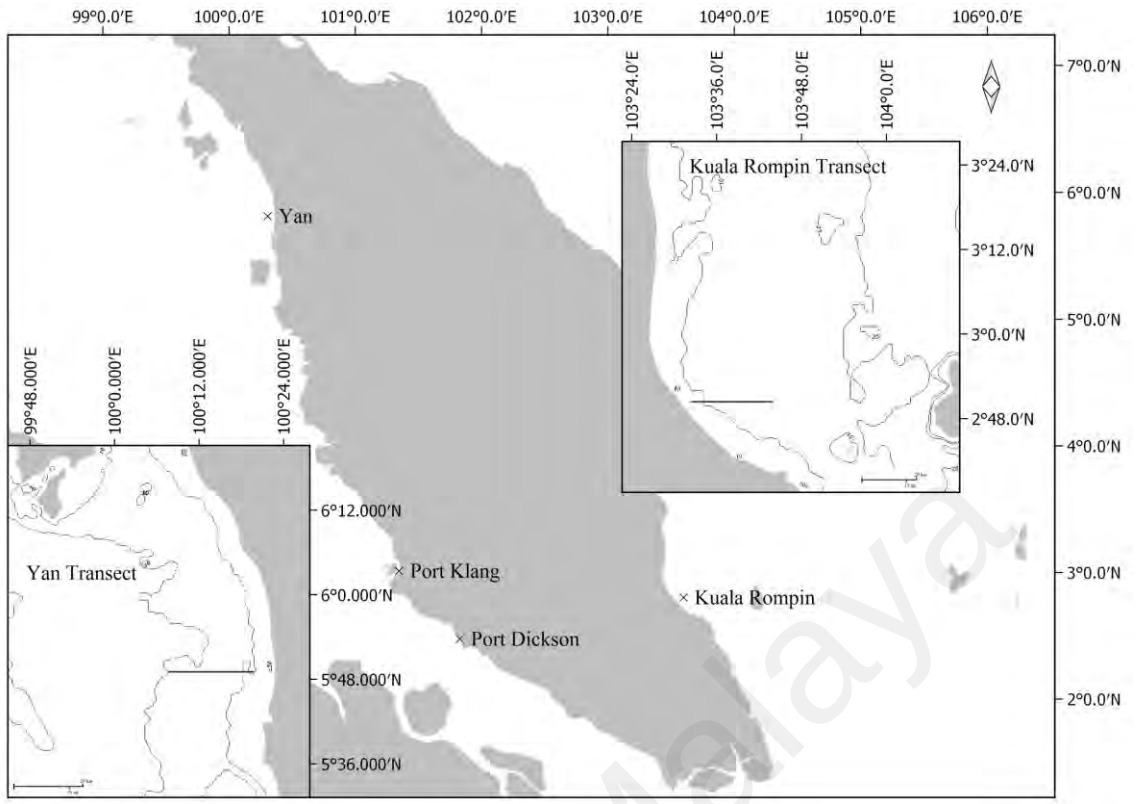

Figure 3.1: Map of the location of sampling sites. '—'show the sampling transects at offshore sites. Lower left inset shows the sampling transect at Yan ranged from 5 km to 25 km from shore whereas the right inset shows the sampling transect at Kuala Rompin which was from 5 km to 30 km from the shore.

3.2 Environmental parameters

In situ water temperature and salinity were measured using conductivity meter (YSI Pro30, USA) whereas Secchi disc depth was measured to estimate water transparency. Water samples for microbial abundance measurement were preserved with 1 % glutaraldehyde (final concentration) (Merck, Germany). Water samples for other analysis were stored in an icebox and transported back to the laboratory for further processing.

3.2.1 Dissolved oxygen concentration

Dissolved oxygen (DO) concentration was measured via Winkler titration method (Grasshoff et al., 1999). In a DO bottle, seawater sample was mixed with reagent A, which was 60% manganese chloride (Systerm, Malaysia) and reagent B, which was alkaline iodide solution prepared from 60 % potassium iodide (Univar, USA) and 30% potassium hydroxide (BDH, USA). The DO bottle was inverted repeatedly to ensure the seawater sample and reagents were mixed thoroughly. The DO bottle was left to stand to form precipitate. Then, sulphuric acid (Univar, USA) was added to the DO bottle to dissolve the precipitate formed. The seawater sample with dissolved precipitate was transferred to a titration flask. The seawater sample was titrated with 0.01 M sodium thiosulphate (Systerm, Malaysia) where starch solution (Systerm, Malaysia) was used as indicator.

$$DO\ (\mu M) = [(0.01 \times 250 \times 10^3 \times T) / (V - 0.6)] \times f$$

where T is the total titrant used; V is the volume of DO bottle; f is the standardise value of thiosulphate

To determine the f value, distilled water was first mixed sulphuric acid, and then with reagent A and reagent B in a DO bottle. Potassium iodate standard solution was added into the mixture. The mixture was titrated with sodium thiosulphate until colourless, by using starch solution as indicator.

$$f = 5.001 / t$$

where t is the total titrant used

3.2.2 Dissolved inorganic nutrient concentration

Dissolved inorganic nutrient [ammonium (NH_4), nitrite + nitrate (NO_2 + NO_3), phosphate (PO_4) and silicate (SiO_4)] were measured according to Parsons et al. (1984). For ammonium concentration, seawater sample was mixed with 10 % phenol (Merck, Germany). Then, the seawater sample was mixed with 0.5 % sodium nitroprusside (Unilab, Philippines). Oxidising solution prepared by using sodium hypochlorite (Clorox, USA) and alkaline reagent [sodium citrate (Systerm, Malaysia), sodium hydroxide (Systerm, Malaysia)] was added into the seawater sample. The water sample was left to stand in dark for one hour for the formation of blue indophenol colour. The absorbance was measured using U-1800 spectrophotometer (Hitachi, Japan) at 640 nm.

For nitrate concentration, a cadmium reduction column was prepared. Cadmium pellets was activated by washing with hydrochloric acid (HCl) (Univar, USA). The pellets was washed with distilled water to remove excess HCl. These pellets was then coated with copper sulphate solution (BDH, USA) Seawater sample was ran through the column to reduce nitrate to nitrite for measurement. For nitrite concentration, seawater sample was mixed with sulphanilamide (Scharlau, Spain) and left to stand for five minutes. Then, 0.1 % N-(1-naphthyl)-ethylenediamine (Sigma, USA) was added into the seawater sample. The seawater sample was left to stand for 30 minutes for the formation of coloured azo dye. The absorbance was measured using spectrophotometer at 543 nm. As nitrate is reduced to nitrite for measurement, the concentration of nitrate and nitrite was recorded as a sum of nitrate + nitrite (NO_2 + NO_3).

For phosphate concentration, seawater sample was mixed with ascorbic acid (Sigma, USA). A mixed reagent was prepared from 10 % ammonium molybdate tetrahydrate (BDH, USA), 25 % sulphuric acid, ultra-pure water and 2.5 % potassium antimonyl-tartrate solution (Sigma, USA). The mixed reagent was added into the seawater sample and left to stand for 20 minutes for the formation of molybdenum blue. The absorbance was measured using spectrophotometer at 880 nm. For offshore samples, PO_4 was concentrated via MAGIC method (Karl & Tien, 1992) before measurement.

For silicate concentration, seawater sample was mixed with 25 % sulphuric acid and left to stand for one minute. An acid molybdate reagent was prepared from ammonium molybdate tetrahydrate, 25 % sulphuric acid, and ultra-pure water and added into the seawater sample. The seawater sample was left to stand for 10 minutes. The seawater sample was then mixed with 10 % oxalic acid solution (BDH, USA) and ascorbic acid. The seawater sample was left to stand for 20 minutes for the formation of molybdenum blue. The absorbance was measured using spectrophotometer at 810 nm.

3.2.3 Chlorophyll *a* concentration

Chlorophyll *a* (Chl *a*) concentrations were measured according to Parsons et al. (1984). For Chl *a*, the seawater sample was filtered through pre-combusted glass fibre filter (GF/F) paper (Whatman, England). Then, the filter paper was soaked in 90 % acetone (Merck, Germany) in an amber vial and kept at -20 °C overnight to extract Chl *a*. The fluorescence of acetone was measured using LS55 spectrofluorometer (Perkin Elmer, USA) at excitation/emission wavelength of 440/680 nm. Chl *a* concentrations were then calculated using the following formula:

$$\text{Chl } a \text{ (µg/L)} = (0.0023 \times F) \times (v / V)$$

where F is the fluorescence of acetone; v is the volume of acetone used in mL; and V is the volume of water sample filtered in L

3.2.4 Total suspended solids

Total suspended solids (TSS) was measured by gravimetric method. The seawater sample was filtered through pre-weighed glass fibre filter paper (W1). The filter paper was dried at 60 °C for a week. The weight of filter paper was measured for a few days until the weight was constant (W2).

$$\text{Total dissolved solids (mg / L)} = (W2 - W1) / V$$

where V is the volume of water sample filtered.

3.2.5 Trophic state index

The trophic state index (TSI) at every sampling site was calculated based on Chl *a* concentration and Secchi disc depth measurement according to Carlson (1977),

$$TSI(\text{Chl } a) = 9.81 \times \ln(\text{Chl } a) + 30.6$$

$$TSI(\text{Secchi}) = 60 - 14.41 \times \ln(\text{Secchi disc depth})$$

where TSI(Chl *a*) is the value of TSI based on Chl *a* concentration in μg L^{-1} and TSI(Secchi) is the value of TSI based on Secchi disc depth in m.

The average of the two TSI values was used to characterize the trophic state of the sampling site where the value <40 = oligotrophic, 40-50 = mesotrophic, 50-70 = eutrophic and >70 = hypereutrophic.

3.3 Bacterial and viral abundance

Bacterial and viral were measured by direct count methods. To measure bacterial abundance, 2 mL of water sample was stained with 100 μL working concentration of DAPI (10 μg mL^{-1} 4', 6-diamidino-2-phenylindole) (Sigma, USA) for seven minutes (Kepner & Pratt, 1994). The stained water sample was filtered onto a black polycarbonate filter paper (0.2 μm pore size). The filter paper was then placed on a glass slide and viewed under epifluorescence microscope (Olympus BX60, Japan) with a U-MWU filter cassette (Olympus, Japan) (excitor 330-385 nm, dichroic mirror 400 nm, barrier 420 nm). A minimum of 30 randomly selected microscopic fields were captured using digital imaging system (analySIS® version 5.0, Soft Imaging System, Germany). The viral abundance is expressed as virus-like particles (VLPs). To enumerate VLPs, 2 mL of sample was stained with 100 μL, 5× working concentration of SYBR Green I Nucleic Acid stain (Lonza, Switzerland) for 15 minutes and filtered through 0.02 μm pore size

Anodisc (Whatman, United Kingdom). The Anodisc was placed on top of one drop of anti-fading mountant (50:50:1 solution of glycerol, phosphate-buffered saline and p-phenylenediamine) before viewing via epifluorescence microscope (Noble & Fuhrman, 1998) with a U-MWU filter cassette (Olympus, Japan) (excitor 330-385 nm, dichroic mirror 400 nm, barrier 420 nm). At least 20 randomly selected microscopic fields were captured for enumeration.

3.4 Virus production rate

Virus production rate was determined by the virus reduction with continuous cell resuspension approach (Weinbauer et al., 2010). Virus-free filtrate was obtained by filtering the seawater sample via AMICON Stirred Ultrafiltration Cell Millipore Model 8400 (Merck, Germany) with a 30 kDa filter. A total of 300 mL surface seawater sample was filtered through a 0.2 μm polycarbonate filter (Merck, Germany). Then, 900 mL volume of virus-free filtrate were gradually added into the filtering sample until about 200 mL of mixture was left in the filtration funnel. The mixture (50 mL each) was then distributed into triplicate bottles and incubated at *in situ* temperature. The enumeration of VLPs were carried out at 0, 4, 8, and 12 hour of incubation. The virus production rate was determined from the slope of viral abundance against incubation time. The virus production rate was corrected for the loss of bacterial cells during the experimental set up by multiplying the virus production rate with the ratio of *in situ* bacterial abundance to the experimental bacterial abundance at 0 hour of incubation.

The burst size is estimated from the bacterial cell volume throughout the study as the burst size and bacterial host cell volume are correlated significantly (Wommack & Colwell, 2000). The bacterial cell volume was calculated using the formula,

$$\text{Volume of spherical cell} = (\pi A^3) / 6$$

where A is the diameter of the cells, and

$$\text{Volume of ellipsoidal cell} = (\pi AB^2) / 6$$

where A is the cell length and B is the width of the cell (Lee & Bong, 2008). The burst size was then calculated by applying the regression equation according to Weinbauer & Peduzzi (1994),

$$\text{Burst size} = 41.24 + 70.70 \text{ (cell volume)}$$

The virus-inferred bacterial lysis was then calculated by dividing the virus production rate with burst size (Weinbauer et al., 2010). Since virus production rate was measured at the surface waters, biovolume measurement was carried out for surface water samples only.

3.5 Bacterial production and protist grazing rate

One hundred mL of seawater sample was each fractionated into <0.7 µm and 20 µm fractions using GF/F filter with 0.7 µm pore size and nylon mesh with 20 µm pore size, respectively (Bong & Lee, 2011). The fractionated sample was incubated in the dark at *in situ* temperature for 12 hours. Subsamples were collected at 0 h and 12 h incubation for enumeration of bacterial abundance. The bacterial growth rates (µ) were then calculated as natural logarithmic increase in bacterial abundance over time. The bacterial growth rate in the <0.7 µm fraction ($\mu_{0.7}$) was assumed without protist grazing loss

whereas the bacterial growth rate in the <20 μm fraction (μ_{20}) was the result of both bacterial growth and grazing. Hence, the protist grazing rate was calculated as follows,

$$\text{Protist grazing rate} = BA \times (\mu_{0.7} - \mu_{20})$$

where BA is the *in situ* bacterial abundance.

3.6 Microcosm experiments on viruses

Two types of microcosm experiment involving temperature and nutrient concentration were carried out to investigate the environmental effect on viral dynamics. Virus production rate was measured whereas lysogeny was determined through lysogenic induction via mitomycin C. Mitomycin C was chosen as the inducing agent of lysogens in this study since mitomycin C is one of the most effective and widely used inducing agents (Weinbauer & Suttle, 1996; Payet & Suttle, 2013). Mitomycin C inhibits DNA synthesis by forming a crosslink between two double helical DNA strands which will interfere DNA replication and transcription (Verweij & Pinedo, 1990). However, mitomycin C does not inhibit the DNA repair mechanism of cells. Viruses are able to activate DNA repair mechanism of bacteria with active metabolism (Jiang & Paul, 1996; Weinbauer & Suttle, 1999). Hence, mitomycin C inhibits DNA replication which will trigger the prophage to activate the DNA repair mechanism and lead to the lysogenic induction where the prophage switch from lysogenic to lytic cycle (Weinbauer & Suttle, 1996). Although UV irradiation is frequently being used, the result of lysogenic induction produced by mitomycin C was similar to UV irradiation (Levine, 1961). Thus, mitomycin C is suitable to be used for the study of lysogenic induction.

For temperature-regulated experiment, 50 mL of seawater samples were distributed into five pairs of 100 mL sterile Schott bottles. Each pair of the Schott bottles were labelled with C for control, and T for treatment. Mitomycin C (Sigma, USA) with a final concentration of 1 µg/mL (Paul & Weinbauer, 2010) was added into the samples labelled with T. The samples were incubated at 25 °C, 28 °C, 31 °C, 34 °C, and 37 °C water baths for 12 hours. For experimental manipulation of nutrient concentration, marine broths (Difco, USA) of 0.1×, 0.25×, 0.5×, 0.75× and 1.0× concentration were prepared with distilled water. The salinity of the diluted marine broths was adjusted to the salinity of the marine broth with 1.0× concentration by sodium chloride (BDH, UK). These marine broth were sterilised by autoclaving at 121 °C for 15 min before use. Then, 5 mL of seawater samples were mixed with 45 mL of the nutrient-adjusted marine broth. A pair of untreated C and mitomycin C treated T bottles were prepared for each nutrient concentrations. The samples were incubated at *in situ* temperature for 12 hours.

For both experiments, subsamples were collected at 0 and 12 hour of incubation for the enumeration of VLPs. Natural logarithmic increase of VLPs after incubation was calculated as virus growth rate whereas the calculation of percentage of lysogens was adapted from Paul & Weinbauer (2010) as follows,

$$\% \text{ of lysogens} = \{[(VLP_{T12} - VLP_{T0}) - (VLP_{C12} - VLP_{C0})] / BS\} / BA$$

where VLP_{T12} is the VLP of T at 12 hour, VLP_{T0} is the VLP of T at 0 hour, VLP_{C12} is the VLP of C at 12 hour, VLP_{C0} is the VLP of C at 0 hour, BS is the burst size and BA is the *in situ* bacterial abundance.

Bacterial growth rate in C bottles were also measured as natural logarithmic increase in bacterial abundance over time.

3.7 Statistical analysis

All data were reported as mean ± standard deviation (SD) unless stated. Count data was log-transformed before carrying out parametric statistics. Two-factor and three-factor analysis of variance (ANOVA) was carried out with R-studio version 1.1.456 (RStudio Team 2020) and R version 4.0.3 (R Core Team 2013) to determine the factors that influenced the variables measured and whether the interaction among the factors existed. Single factor ANOVA was also carried out for comparison of all sites, and if significantly different, a post-hoc Tukey's pair-wise comparison was carried out. Correlation tests were carried out to determine the significance of the relationships among the measured variables. Student's t-test were carried out to determine the difference between the top-down controls at nearshore and offshore sites, and the difference between treated and untreated samples in the experimental setup. Analysis of covariance (ANCOVA) were performed for slope comparison. Unless mentioned otherwise, all statistical analyses were carried out using the software PAST version 4.03 (Hammer et al., 2001). The significance level used was 0.05 unless when Bonferroni correction was applied.

CHAPTER 4: RESULTS

4.1 Environmental parameters

The surface water temperature measured among the four sampling sites ranged from 29.4 to 30.4°C (Table 4.1). Via two-way ANOVA, surface water temperature did not differ among the stations (S) and with distance from the shore (D) (Table 4.2). The salinity measured ranged from 25.2 to 33.8 ppt. Salinity was higher at offshore sites (Yan and Kuala Rompin), and clearly differed between offshore and nearshore sites. Through two-way ANOVA, the distance from the shore was another factor contributing to the variability in salinity. The two-way interaction (S × D) did not show any significant impact on salinity. The Secchi disc depth ranged from 0.4 to 0.7 m in the nearshore sites (Port Dickson and Port Klang) and 3.0 to 15.0 m in the offshore sites. Similar to the distribution patterns of salinity, both station selection and the distance from shore were key factors contributing to Secchi disc variability.

The TSS measured at the nearshore sites (91.3 – 106.7 mg L^{-1}) was higher than offshore sites (43.3 – 95.3 mg L^{-1}). The DO concentration at Port Klang (148 – 163 μM) was the lowest among all sampling stations (148 – 265 μM). For dissolved inorganic nutrients, NH_4 concentration ranged from not detectable to 16.20 μM. NO_2 + NO_3 concentration ranged from 0.12 to 8.90 μM. The PO_4 concentration ranged from 0.01 to 2.80 μM whereas the SiO_4 concentration ranged from 0.14 to 8.40 μM. The concentrations of dissolved inorganic nutrients were highest at Port Klang. Both NH_4 and SiO_4 were also higher at Port Dickson compared to offshore sites. For all the mentioned variables, both station selection and distance from shore were primary factors driving the difference (see Table 4.2). Chl *a* concentration was measured as a proxy for phototrophic biomass and ranged from 0.21 to 3.54 μg L^{-1}. Chl *a* concentration was higher at nearshore sites, and highest at Port Klang. Similarly, both station selection and distance from shore

were factors contributing to the variability in Chl *a* concentration. The TSI measured ranged from 18.1 – 58.1. TSI was remarkably different between offshore stations which were oligotrophic (18.1 – 36.1) and nearshore stations which were eutrophic (52.2 – 58.1) in nature.

Table 4.1: Environmental parameters at Port Dickson, and Port Klang (mean ± standard deviation). Comparison of measurements was carried out using ANOVA, and when significant, pair-wise comparison was carried via Tukey's-HSD. * indicated significant ANOVA at p<0.05 whereas ** indicated significant ANOVA at p<0.001. Samples with the same letters are different significantly.

Physical and Chemical Parameters	Yan (n=6)	Kuala Rompin (n=12)	Port Dickson (n=3)	Port Klang (n=3)
Temperature (°C)	30.4 ± 0.62	29.6 ± 0.88	30.1 ± 1.46	29.4 ± 0.72
Salinity (ppt)* **	31.1 ± 0.83^{ab}	31.6 ± 1.71^{cd}	26.6 ± 1.18^{ac}	29.3 ± 1.61^{bd}
Secchi disc depth (m)*	10.2 ± 4.8^{ab}	8.5 ± 2.2^{cd}	0.5 ± 0.1^{ac}	0.6 ± 0.1^{bd}
Total suspended solids (mg L^{-1})* **	60 ± 7.7^{ab}	70 ± 15.8^{cd}	95 ± 5.0^{ac}	160 ± 3.8^{bd}
Dissolved oxygen (µM)* **	240 ± 8^{a}	220 ± 8^{b}	220 ± 27^{c}	153 ± 12^{abc}
NH_4 (µM)* **	0.54 ± 0.11^{ab}	0.37 ± 0.09^{cd}	5.33 ± 0.61^{ace}	17.29 ± 2.76^{bde}
NO_2 + NO_3 (µM)* **	0.54 ± 0.03^{a}	0.79 ± 0.03^{b}	1.21 ± 0.36^{c}	8.33 ± 0.67^{abc}
SiO_4 (µM)* **	0.77 ± 0.03^{ab}	0.70 ± 0.05^{cd}	2.69 ± 0.36^{ace}	7.28 ± 1.40^{bde}
PO_4 (µM)* **	0.15 ± 0.01^{a}	0.04 ± 0.00^{b}	0.33 ± 0.12^{c}	2.16 ± 0.71^{abc}
Chlorophyll *a* (µg L^{-1})* **	0.75 ± 0.10^{a}	0.62 ± 0.02^{b}	1.53 ± 0.02^{abc}	2.90 ± 0.03^{abc}
Trophic State Index (TSI)* **	26.6 ± 7.2^{ab}	27.5 ± 3.4^{cd}	52.3 ± 1.2^{ac}	53.5 ± 2.0^{bd}

Table 4.2: F values of two-way ANOVA for surface water variables against the following factors: station selection (S) and distance from shore (D). Significant effects are indicated with * at $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Surface water variables	Station (S)	Distance (D)	S × D
Temperature	2.079	0.931	0.644
Salinity	4.931*	35.879***	1.121
Secchi disc depth	5.694**	51.640***	0.846
Dissolved oxygen	6.265**	6.869*	0.127
Total suspended solid	5.080*	16.746***	0.229
Ammonium (NH_4)	170.825***	248.692***	0.172
Nitrate + Nitrite (NO_3+NO_2)	41.467***	63.611***	1.965
Phosphate (PO_4)	45.211***	48.112***	0.278
Silicate (SiO_4)	29.72***	55.95***	0.510
Chlorophyll *a* (Chl *a*)	19.929***	66.340***	3.971
Log bacterial abundance	15.536***	4.336	3.220
Log viral abundance	1.233	1.034	2.031
Log virus production	3.216*	2.786	1.216
Log bacterial production	0.747	16.292***	0.001
Log protist grazing	0.901	1.612	0.013
Biovolume	1.849	0.843	0.102

4.2 Biological parameters

The bacterial abundance measured ranged from 5.48×10^5 to 9.41×10^5 cells mL^{-1} in the nearshore sites and ranged from 3.51×10^5 to 6.53×10^5 cells mL^{-1} in the offshore sites. Bacterial abundance at offshore sites were lower than Port Klang. From a two-way ANOVA, bacterial abundance was driven by station selection but not distance from shore. On the other hand, the viral abundance measured ranged from 1.39×10^6 to 2.11×10^6 VLPs mL^{-1} in the nearshore sites and ranged from 1.01×10^6 to 2.39×10^6 VLPs mL^{-1} in

the offshore sites. In contrast to bacterial abundance, viral abundance showed no significant difference among the four sampling sites and with distance from the shore. Virus to bacteria ratio (VBR) ranged from 1.8 – 4.3 and was not different among all sampling sites.

Bacterial cell biovolume ranged from 0.004 to 1.206 μm^3 $cell^{-1}$ in all sampling sites. There was no difference in biovolume with distance from the shore and among the different stations (see Table 4.2). Interaction between two factors were also not significant. Therefore, the total measured bacterial cell biovolume (n = 2400) was combined for analysis (Figure 4.1). As the biovolume distribution was skewed to the right, the median was chosen as a better measure of central tendency. Median biovolume was 0.050 μm^3 with a lower to upper quartile range of 0.041 – 0.067 μm^3.

Table 4.3: Range and mean ± SD of bacterial and virus abundance (as VLP), and the virus to bacteria ratio (VBR) among the four sites. Comparison of means were carried out via ANOVA, and * is significance at p < 0.001. The same superscript letters indicated significant pair-wise comparison after Tukey's-HSD test.**

	Yan (n=6)	Kuala Rompin (n=12)	Port Dickson (n=3)	Port Klang (n=3)
Bacterial abundance ($\times 10^5$ cells mL^{-1})* **	5.2 – 5.7 (5.4 ± 0.1)^a	3.5 – 6.5 (5.3 ± 0.3)^b	5.8 – 7.3 (6.7 ± 0.5)	8.1 – 9.4 (8.6 ± 0.4)^{ab}
VLP abundance ($\times 10^6$ VLPs mL^{-1})	1.0 – 2.4 (1.5 ± 0.2)	1.1 – 1.8 (1.5 ± 0.6)	1.4 – 2.0 (1.8 ± 0.2)	1.7 – 2.0 (1.8 ± 0.9)
Virus : Bacteria Ratio (VBR)	1.8 – 4.3 (2.8 ± 0.4)	2.3 – 3.3 (2.8 ± 0.1)	1.9 – 3.5 (2.7 ± 0.5)	2.0 – 2.4 (2.2 ± 0.1)

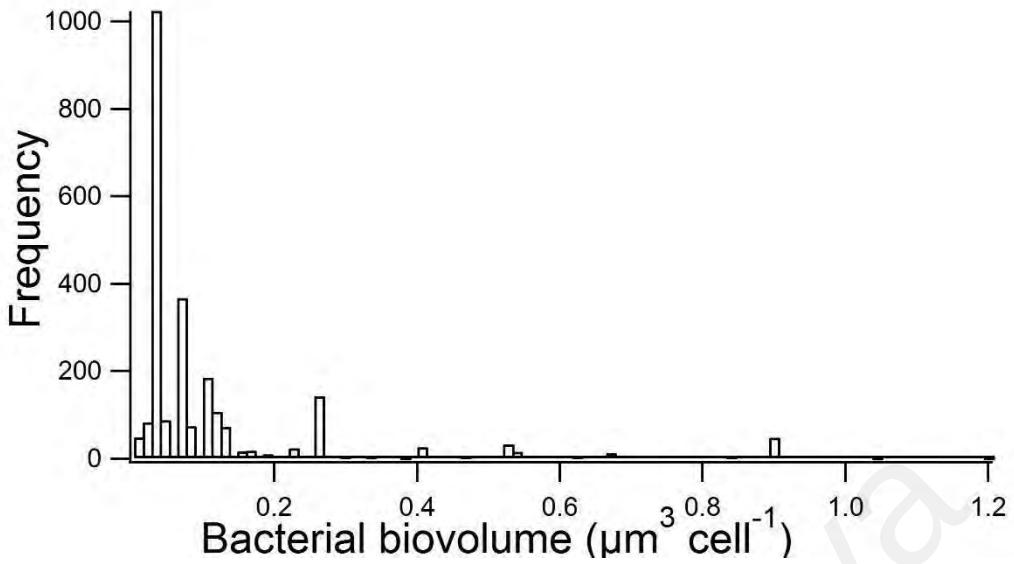

Figure 4.1: Histogram showing the distribution of bacterial biovolume measured in this study.

In this study, the bacterial and viral abundance throughout the water column (at 5 m interval until 20 m depth) at both offshore Yan and Kuala Rompin transects were also measured. Bacterial abundance ranged from 2.57×10^5 to 6.30×10^5 cells mL^{-1} at the offshore Yan transect and ranged from 2.67×10^5 to 7.70×10^5 cells mL^{-1} at the Kuala Rompin transect (Figure 4.2).

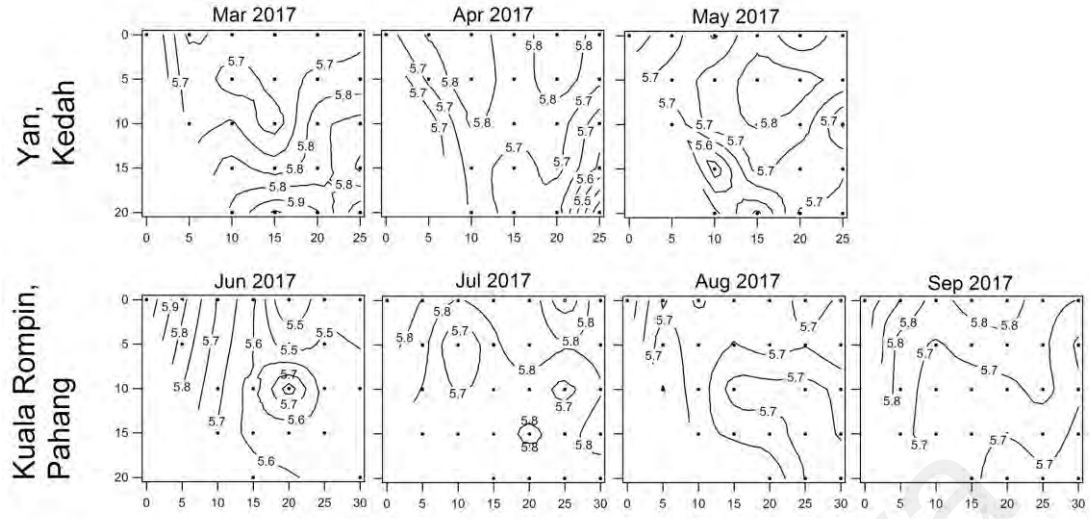

Figure 4.2: Contour plots showing the temporal and spatial distribution of bacterial abundance (cell mL^{-1}) at both Yan and Kuala Rompin sampling transects. Each panel shows the bacterial distribution for the different months sampled whereas x-axis shows depth (m) and y-axis shows distance from the shore (km).

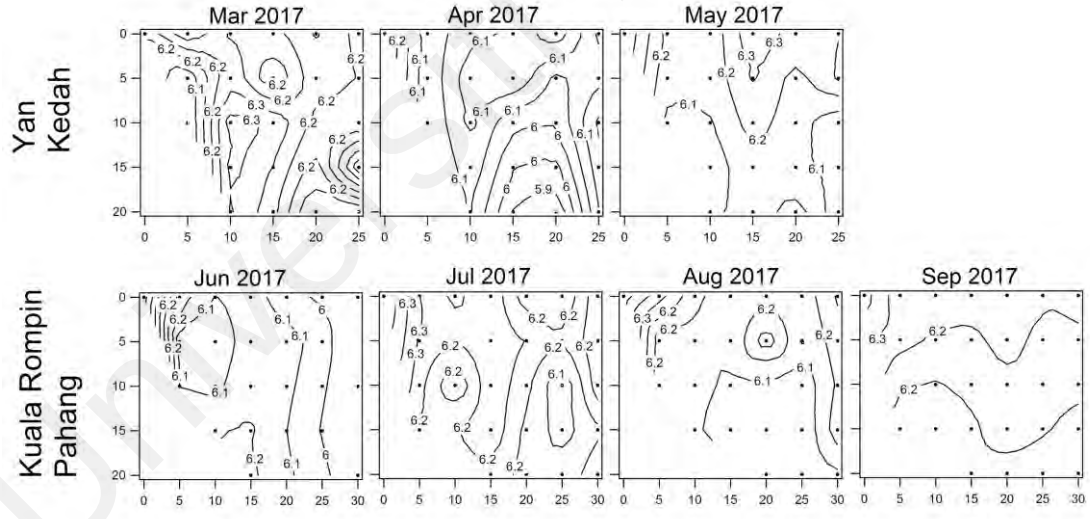

Figure 4.3: Contour plots showing the temporal and spatial distribution of viral abundance (VLPs mL^{-1}) at both Yan and Kuala Rompin sampling transects. Each panel shows the bacterial distribution for the different months sampled whereas x-axis shows depth (m) and y-axis shows distance from the shore (km).

From a three-way ANOVA, bacterial abundance at Yan was not influenced by the factors alone which are sampling month (M), distance from shore (D) or depth (Dh). However, the interactions between the factors were significant (M × D × Dh, M × D and M × Dh) (Table 4.4). The sampling month was the common factor among all three interactions, which suggested the relative importance of sampling month in contributing to the bacterial distribution at Yan. A more in-depth investigation is probably required in order to make conclusion from the interaction patterns. In contrast, sampling month, distance from shore and depth were individually crucial factors influencing the bacterial abundance at Kuala Rompin. Two-way interactions (M × D and D × Dh) also had significant impacts on bacterial distribution, where bacteria generally decreasing when the distance from the shore increased.

At offshore Yan transect, viral abundance measured ranged from 0.50×10^6 to 3.06×10^6 VLPs mL^{-1}. On the other hand, viral abundance measured at offshore Kuala Rompin transect ranged from 0.99×10^6 to 2.97×10^6 VLPs mL^{-1}. Viral abundance measured throughout the water column (Figure 4.3) at the offshore Yan transect revealed that both sampling month and depth were significant factors contributing to virus distribution (see Table 4.4). However there was no interaction among the factors. For the offshore Kuala Rompin transect, all three factors (sampling month, distance from shore and depth) influenced the virus distribution significantly. Two-way interactions (M × D and D × Dh) were also important where viral abundance decreased when the distance from shore increased. The viral abundance was generally higher in the surface water for both transects in this study.

Table 4.4: F values of three-way ANOVA for column water bacterial (BA) and virus (VA) abundance against the following factors: sampling month, distance from shore (D) and sample depth (Dh). Significant effects are indicated with * at p < 0.05, ** p < 0.01 and *** p < 0.001.

	Time (T)	Distance (D)	Depth (Dh)	T × D	T × Dh	D × Dh	T × D × Dh
log BA at Yan	1.625	0.671	0.563	5.905**	13.981***	1.534	4.772*
log BA at Kuala Rompin	24.952***	54.347***	17.316***	14.366***	2.633	9.103**	2.579
log VA at Yan	15.124***	0.314	10.173**	0.046	2.470	0.042	1.097
log VA at Kuala Rompin	12.182***	34.117***	21.424***	3.568*	2.407	9.323**	0.787

4.3 Microbial processes

Bacterial growth rates at both Yan and Kuala Rompin were in the range of 0.014 to 0.095 h^{-1} whereas bacterial growth rates ranged from 0.046 to 0.068 h^{-1} at both Port Dickson and Port Klang (Table 4.5). The bacterial growth rates measured were then expressed in bacterial production. The bacterial production at both Yan and Kuala Rompin ranged from 8.5×10^3 to 37.9×10^3 cells mL^{-1} h^{-1}. Bacterial production at both nearshore sites was ranged from 33.3×10^3 to 64.2×10^3 cells mL^{-1} h^{-1}, which was clearly higher than the bacterial production at offshore sites. Two-way ANOVA revealed that bacterial production was driven by distance from shore (see Table 4.2).

Protist grazing measured ranged from not detectable to 20.0×10^3 cells mL^{-1} h^{-1} at the offshore sites and ranged from 8.7×10^3 to 20.2×10^3 cells mL^{-1} h^{-1} at the nearshore sites. In contrast with other variables, protist grazing was neither driven by station selection nor distance from shore (see Table 4.2). Protist grazing at both Yan and Kuala Rompin sites accounted for 25.8 ± 24.0% of bacterial production which was slightly lower than the

protist grazing at both nearshore stations Port Dickson and Port Klang that accounted for 29.3 ± 5.6% of bacterial production (Table 4.5).

The virus production rates measured at offshore sites ranged from 0.59×10^5 to 4.55×10^5 VLPs mL^{-1} h^{-1}. Tukey's pairwise comparison showed that the virus production rates at Yan and Kuala Rompin were different ($p < 0.05$). At nearshore sites, the virus production rates measured was in the range between 1.82×10^5 and 3.64×10^5 VLPs mL^{-1} h^{-1}. The virus production rates at both nearshore and offshore were similar. The virus production rates were driven by station selection (Table 4.2).

The calculated burst size based on median biovolume measured was 44.8, and this burst size was used to calculate the virus-inferred bacterial lysis. Virus-inferred bacterial lysis will be referred to as viral lysis. The viral lysis at offshore sites ranged from 1.3×10^3 to 10.2×10^3 cells mL^{-1} h^{-1} with the mean of $5.4 \pm 2.5 \times 10^3$ cells mL^{-1} h^{-1} whereas the viral lysis at Port Dickson and Port Klang ranged from 4.6×10^3 to 8.1×10^3 cells mL^{-1} h^{-1} with the mean of $5.9 \pm 1.3 \times 10^3$ cells mL^{-1} h^{-1} (see Table 4.6). Similar to virus production rate, viral lysis at Kuala Rompin was statistically higher than at Yan. Virus lysis accounted for 21.3 ± 9.7% of bacterial production offshore and 23.0 ± 5.0% nearshore.

Table 4.5: Range and mean ± SD of bacterial growth, bacterial production, protist grazing rate and virus production rate among the four sites. ND is not detectable. Comparison of means were carried out via ANOVA, and * is significance at $p < 0.05$. The same superscript letters indicated significant pair-wise comparison after Tukey's-HSD test.

	Yan (n=6)	Kuala Rompin (n=12)	Port Dickson (n=3)	Port Klang (n=3)
Bacterial growth (h^{-1})	0.020 – 0.053 (0.042 ± 0.005)	0.014 – 0.095 (0.048 ± 0.007)	0.046 – 0.066 (0.058 ± 0.006)	0.050 – 0.068 (0.057 ± 0.006)
Bacterial production ($\times 10^3$ cells mL^{-1} h^{-1})*	10.3 – 28.5 (22.8 ± 2.6)	8.5 – 37.9 (23.7 ± 2.9)[a]	33.3 – 43.9 (38.3 ± 3.1)	40.9 – 64.2 (49.2 ± 7.5)[a]
Protist grazing rate ($\times 10^3$ cells mL^{-1} h^{-1})	ND – 11.9 (3.7 ± 1.9)	ND – 20.0 (7.3 ± 2.2)	8.7 – 9.8 (9.4 ± 0.3)	13.6 – 20.2 (16.5 ± 2.0)
Virus production rate ($\times 10^5$ VLP mL^{-1} h^{-1})*	0.6 – 2.9 (1.7 ± 0.3)[b]	1.5 – 4.6 (2.8 ± 0.3)[b]	2.0 – 2.6 (2.3 ± 0.2)	2.4 – 3.6 (3.0 ± 0.4)

Table 4.6: Range and mean ± SD of viral lysis (estimated using a virus burst size of 44.8), and the proportion of viral lysis and protist grazing over bacterial production (%). The sum proportion of both grazing and lysis over bacterial production is also shown. Comparison of means were carried out via ANOVA, and * is significance at $p < 0.05$. The same superscript letters indicated significant pair-wise comparison after Tukey's-HSD test.

	Yan	Kuala Rompin	Port Dickson	Port Klang
Viral lysis ($\times 10^3$ cells mL^{-1} h^{-1})*	1.3 – 6.5 (3.7 ± 0.8)[a]	3.4 – 10.2 (6.3 ± 0.7)[a]	4.5 – 5.9 (5.2 ± 0.4)	5.3 – 8.1 (6.6 ± 0.8)
Virus lysis : Bacterial production (%)	5.2 – 25.5 (14.5 ± 3.0)	13.4 – 39.8 (24.7 ± 2.6)	17.8 – 23.1 (20.2 ± 1.5)	20.7 – 31.8 (25.8 ± 3.2)
Protist grazing : Bacterial production (%)	0 – 51.9 (15.2 ± 8.3)	0 – 85.7 (31.1 ± 9.6)	21.9 – 26.2 (24.7 ± 1.4)	31.5 – 37.1 (33.9 ± 1.6)
Grazing+Lysis : Bacterial production (%)	11.9 – 74.6 (33.3 ± 8.9)	10.2 – 122.2 (64.5 ± 10.9)	33.4 – 43.9 (38.5 ± 3.0)	44.2 – 52.1 (47.5 ± 2.4)

4.4 Virus dynamics with increasing temperature and nutrients

Figure 4.4 shows the virus growth rates in both untreated (or control) and mitomycin-C treated samples as temperature increased from 25 °C to 37 °C. The virus growth rates in the untreated samples increased with temperature at both Port Klang ($F = 30.44$, $p < 0.001$) and Port Dickson ($F = 27.99$, $p < 0.001$). However, the rate of increase with temperature at both Port Klang (0.0006 VLP mL^{-1} h^{-1} $°C^{-1}$) and Port Dickson (0.0003 VLP mL^{-1} h^{-1} $°C^{-1}$) were different ($F = 7.81$, $p < 0.01$). In contrast, virus growth rates in the mitomycin-C treated sample fluctuated and did not vary significantly with temperature ($p > 0.05$).

Similar to temperature-regulated microcosm experiment, virus growth rates in the untreated sample increased with nutrient concentration at both Port Klang ($F = 32.6$, $p < 0.001$) and Port Dickson ($F = 64.38$, $p < 0.001$) (Fig. 4.5). The slope of increase at Port Dickson (0.0144 VLP ml^{-1} h^{-1} marine broth $strength^{-1}$) was higher than at Port Klang (0.0115 VLP ml^{-1} h^{-1} marine broth $strength^{-1}$) ($F = 8.81$, $p < 0.01$). Virus growth rates in mitomycin C treated samples at both Port Klang and Port Dickson did not show any trends ($p > 0.40$).

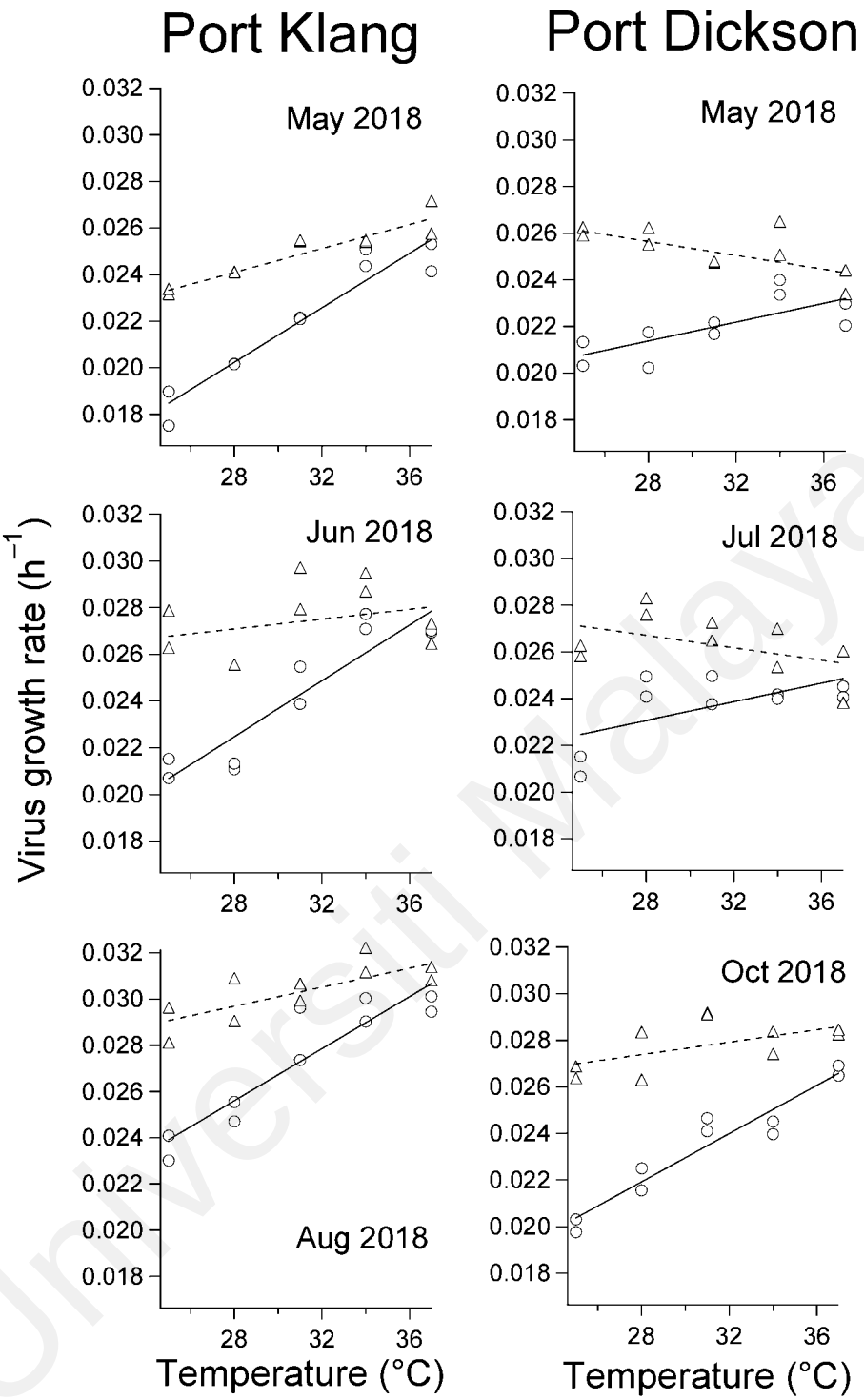

Figure 4.4: Regression slopes of virus growth rates (h^{-1}) with temperature increase for both untreated (line with round symbols) and mitomycin-C treated (broken line with triangle symbols) samples at Port Klang (left panel) and Port Dickson (right panel).

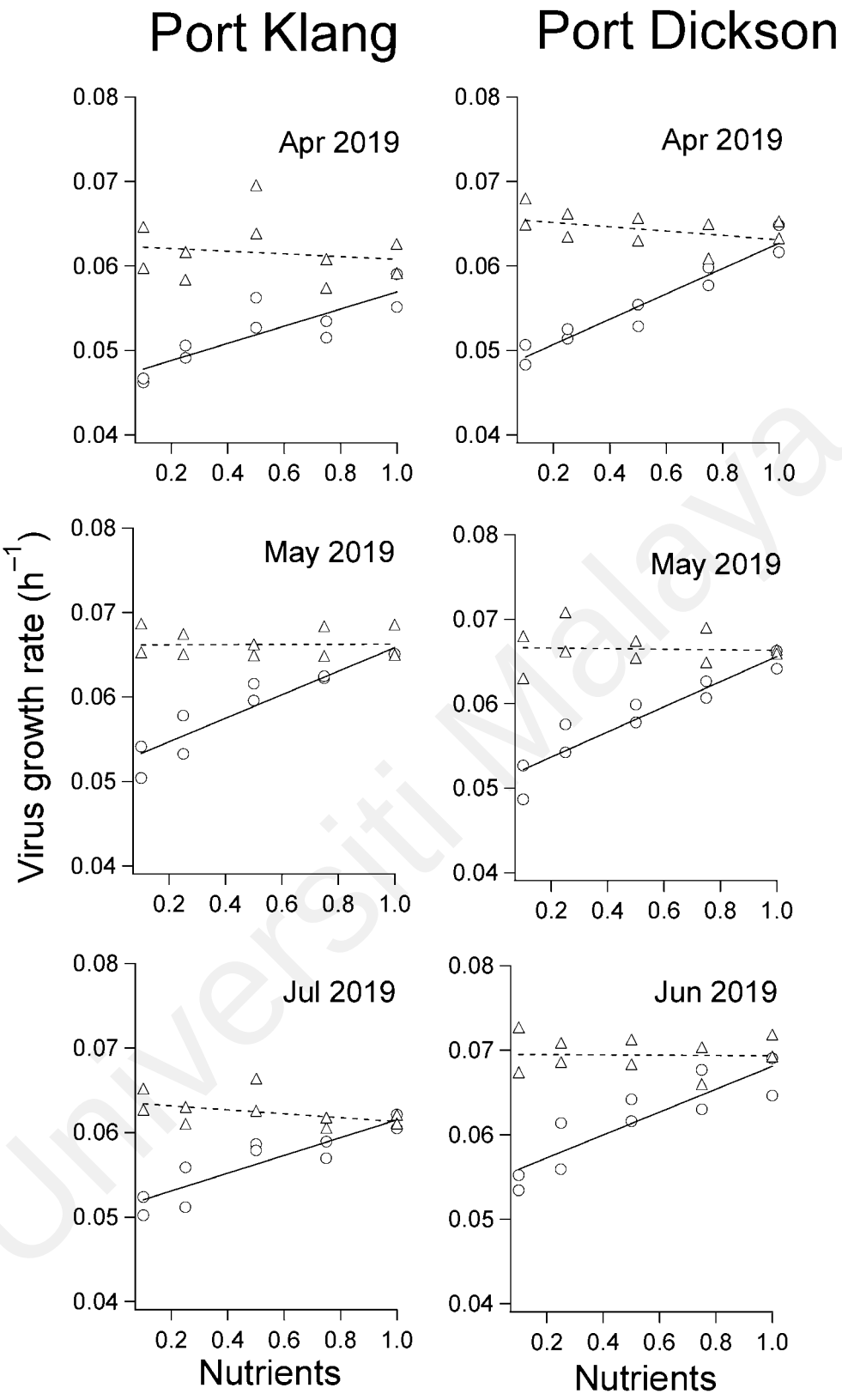

Figure 4.5: Regression slopes of virus growth rates (h^{-1}) with nutrient concentration (× marine broth strength) increase for both untreated (line with round symbols) and mitomycin-C treated (broken line with triangle symbols) samples at Port Klang (left panel) and Port Dickson (right panel).

At high temperature and nutrient concentration, the virus production rate in control and treated microcosms were similar (Figures 4.4 and 4.5). This suggested that most lysogenic phages in control switched to lytic cycles to produce more viral progeny. Therefore, the percentages of lysogens decreased when temperature and nutrient concentration increased (Figure 4.6).

Paired t-test was carried out to compare the slopes of virus production rate between control and treated microcosms at each experimental temperature and nutrient concentration. After Bonferroni correction ($p < 0.01$), the slopes are significantly different for all temperature and nutrient concentration except 37 °C and 1 × nutrient concentration at both Port Dickson and Port Klang sites ($p < 0.01$). It can be concluded that lysogeny is not significant at 37 °C and 1 × nutrient concentration.

As the differences observed between the untreated and mitomycin C treated samples were probably the result of lysogenic induction, the percentage of lysogen was estimated at each point of the microcosm experiments. In the temperature experiments, lysogen ranged from 0.03% to 1.60% at Port Klang and from 0.39% to 2.48% at Port Dickson. For nutrient experiments, lysogen ranged from 0.41% to 5.60% at Port Klang and from 0.56% to 7.40% and Port Dickson. Lysogeny decreased with increasing temperature and increasing nutrient concentration at both Port Klang and Port Dickson (Fig. 4.6).

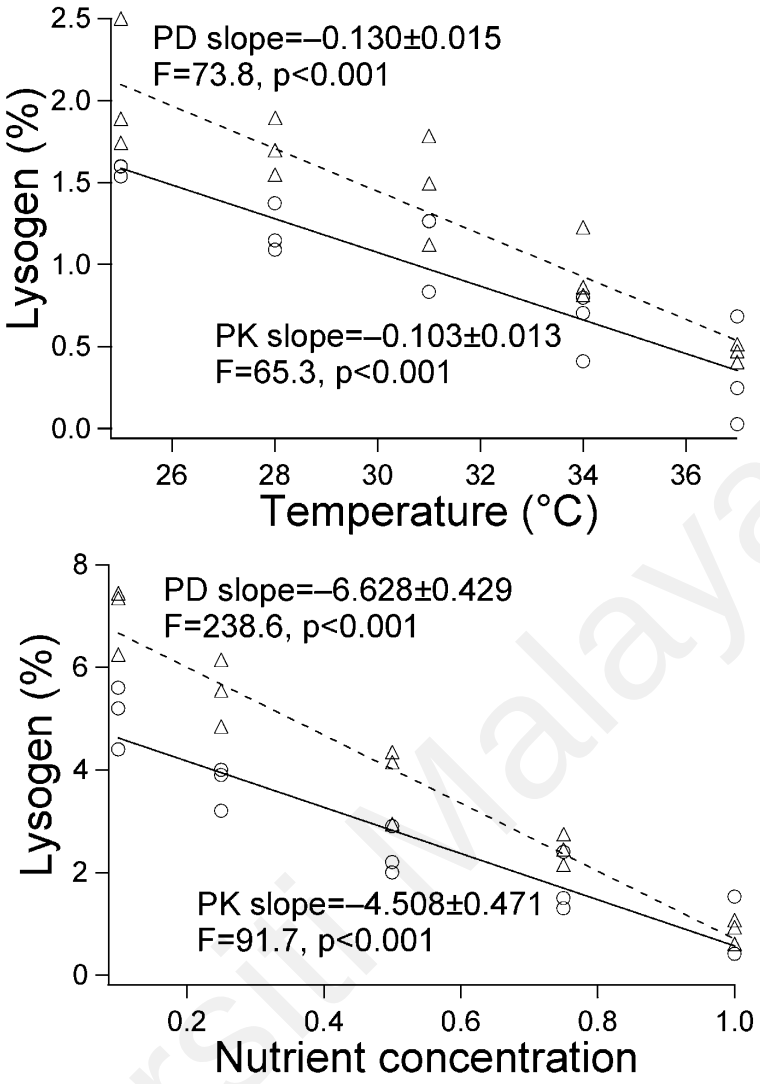

Figure 4.6: Regression slopes of lysogeny (% of lysogen) with temperature (upper panel) and nutrient concentration (× marine broth strength) (lower panel) at Port Klang (line with round symbols) and Port Dickson (broken line with triangle symbols).

The bacterial growth in the temperature-regulated microcosm experiment increased from 0.048 h^{-1} to 0.060 h^{-1} in Port Klang and 0.049 h^{-1} to 0.062 h^{-1} in Port Dickson when the incubation temperature increased from 25 °C to 37 °C in the control sets. The bacterial growth in the eutrophication experiment increased from 0.110 h^{-1} to 0.151 h^{-1} in Port Klang and 0.105 h^{-1} to 0.139 h^{-1} in Port Dickson when the concentration increased from 0.1 × to 1.0 × in the control sets.

CHAPTER 5: DISCUSSION

5.1 Environmental conditions

In this study, the seawater temperatures measured were relatively high and stable, and reflective of the tropical systems in this study (Lee & Bong, 2008). For the other variables measured, both station selection and distance from shore were important factors that drove most of the differences observed. Salinity increased towards the open sea as offshore sites were located further from the influence of freshwater whereas nearshore sites (Port Dickson and Port Klang) exhibited anthropogenic influence with lower DO, higher TSS and higher dissolved inorganic nutrient concentrations. Among nearshore sites, water quality at Port Klang was poorer (Lim et al., 2019; Lim et al., 2021).

When using TSI as a proxy for the state of eutrophication, both Port Dickson and Port Klang were in the eutrophic state whereas Yan and Kuala Rompin transects were oligotrophic. Although a long-term study of Port Klang had previously categorized it as a hypereutrophic station (Lim et al., 2018), the sampling in this current study did not cover the intense rainfall periods when nutrient input into the Port Klang estuary tends to spike (Lee et al., 2020). The eutrophic conditions in the nearshore stations also lead to a generally higher phototrophic biomass (Lim & Lee, 2017).

5.2 Bacterial and viral abundance

Bacterial abundance measured in this study was within the range of tropical waters (Lee & Bong, 2008; Jasna et al., 2018), and its distribution was also driven by station selection. Bacterial abundance at Port Klang was highest among the four stations. As Port Klang contained the highest concentrations of dissolved inorganic nutrient and primary producers (Lim et al., 2020), the high phototrophic biomass probably supported the high bacterial abundance observed. In this study, bacterial abundance correlated positively with Chl *a* (Fig. 5.1). Although correlation does not indicate causality, this phototrophic – heterotrophic coupling is often observed in a wide variety of aquatic habitats (Cole et al., 1988; Lee & Bong, 2008), and probably reflected the bacterial utilization of dissolved organic matter supplied by primary production (Azam et al., 1983).

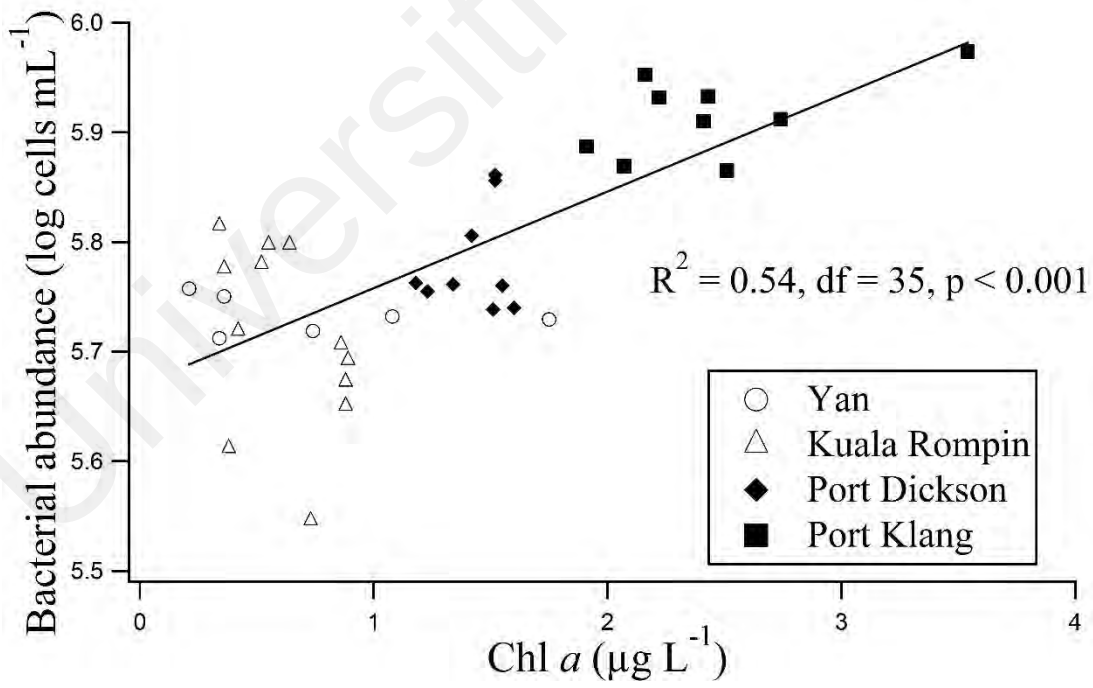

Figure 5.1: Correlation analysis between bacterial abundance and chlorophyll *a* concentration (Chl *a*). The linear regression slope is also shown.

Viral abundance was generally one order higher than bacterial abundance, and is within the range of tropical waters (Cissoko et al., 2008; Dinsdale et al., 2008; Lee & Bong, 2012; Jasna et al., 2018) but in the lower end of the range reported for temperate coastal waters ($10^7 – 10^8$ VLP ml^{-1}, Clasen et al., 2008). There was no difference observed in viral abundances among the sampling sites, and concurred with the observation by Wilhelm and Suttle (1999) that viral abundance is known to be generally quite constant within a location. At nearshore sites where TSS levels were higher, viral abundance could be underestimated as TSS is known to passively adsorb virus particles (Chen et al., 2011, Mojica & Brussaard, 2014). However, the effect of absorption should be minimal as viral abundance did not correlate with TSS ($R^2 = 0.05$, $p > 0.30$) in this study.

Virus to bacteria ratio (VBR) is often used to indicate the relationship between viruses and bacteria. In this study, VBR ranged from 1.8 to 4.3 (Table 4.3), and was in the range for tropical waters (1.4 – 4.8, Patten et al., 2008) but in the lower end of the range for subtropical waters (1.1 – 44, Jasna et al., 2019). VBR in tropical waters is typically lower relative to temperate and polar waters (Lee & Bong, 2012). Moreover, a VBR of 5 was recently suggested as a cut-off value to have an effect on microbial community in lakes (Keshri et al., 2018).

5.3 Microbial process rates

Bacterial growth rates was from 0.014 to 0.095 h^{-1} whereas bacterial production ranged $8.5 \times 10^3 – 37.9 \times 10^3$ cells mL^{-1} h^{-1}. Bacterial production rates measured in this study was within the range of tropical waters (Lee & Bong, 2008; Lee & Bong, 2012; Pradeep Ram et al., 2014; Li et al., 2018). Bacterial production also increased with Chl *a* concentration (Fig. 5.6), and is reflective of the bacterial dependency on primary producers for food (Cole et al., 1988; Lee & Bong, 2008). Bacterial growth rate

measurements seemed to concur with the bacterial abundance distribution where both bacterial biomass and activity were generally higher nearshore. As nearshore sites were eutrophic, the high nutrient concentrations supported the abundance of both primary producers and bacteria. The availability of nutrients were sufficient to support and enhance the bacterial growth rates.

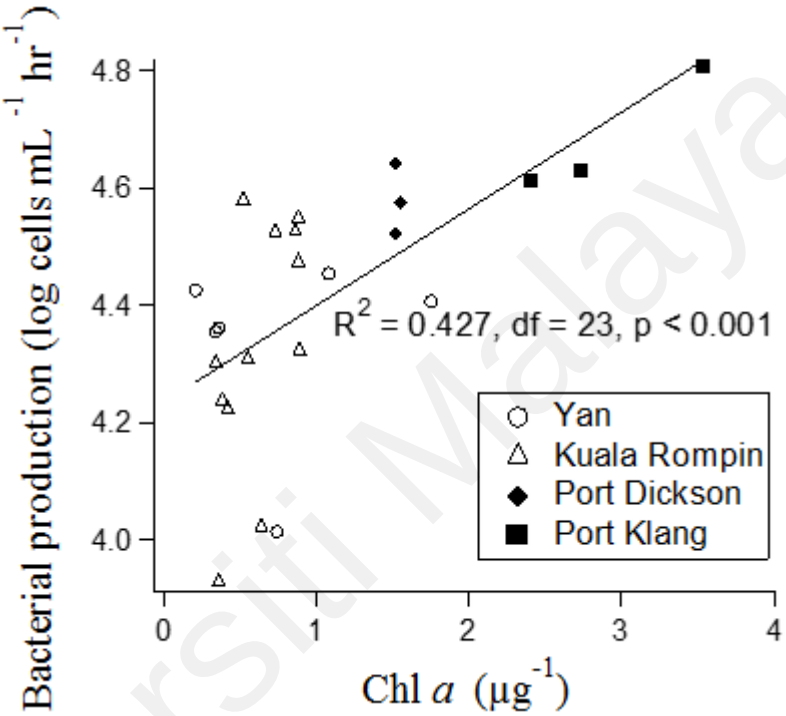

Figure 5.2: Correlation analysis between bacterial production and chlorophyll *a* concentration (Chl *a*). The linear regression slope is also shown.

Protist grazing is one of the two major fates for bacterial production in aquatic habitats (Miki & Jacquet, 2008, Tsai et al., 2013a). Protist grazing rates measured in this study ranged from not detectable to 20.2 × 10^3 cells mL^{-1} h^{-1}, and were within the range reported for tropical waters (Bong & Lee, 2011; Zhang et al., 2018). Although there is usually a predator-prey coupling between protists and bacteria (Sherr et al., 2007; Bong & Lee, 2011), protist grazing rates correlated with bacterial production only for nearshore sites in this study ($R^2 = 0.685$, $p < 0.05$). At offshore sites, the predator-prey relationship between protists and bacteria showed an irregular pattern. Although this study did not

measure the protist abundance, protist abundance at offshore sites might be low and could lead to a lower protist grazing rate as the encounter rate between the protist and bacteria decreases. Besides, the bacterial community at offshore sites might have grazing-resistant characteristics that prevent protist grazing (Jürgens & Güde, 1994; Evans et al., 2021).

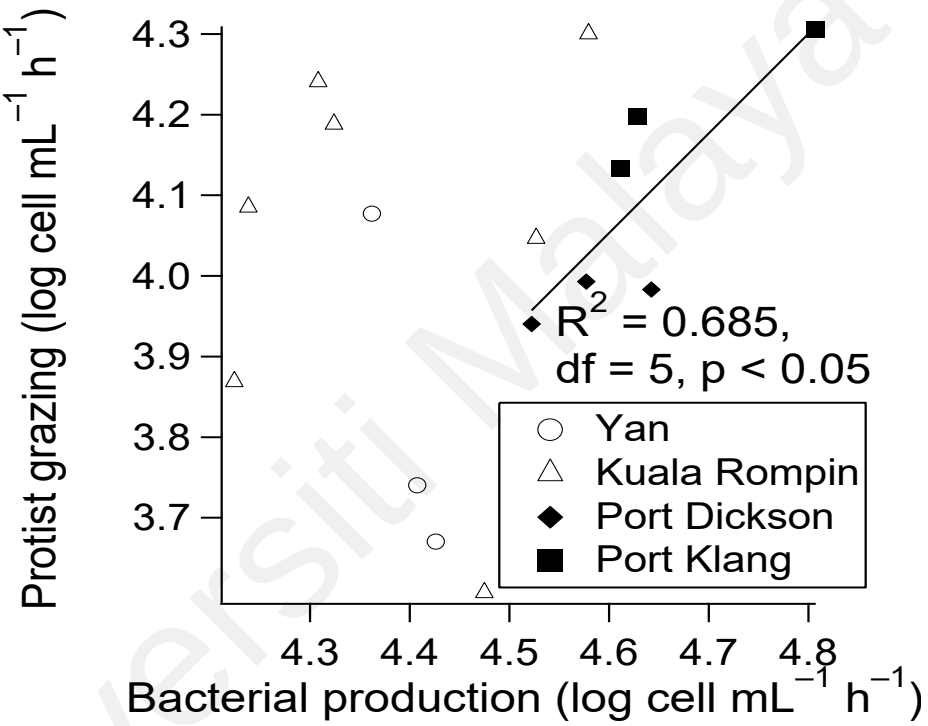

Figure 5.3: Correlation analysis between protist grazing and bacterial production. For nearshore sites (Port Dickson and Port Klang), the linear regression slope is shown. Data when protist grazing was nought (not detectable) was not included.

The virus production rates measured were within the range for tropical waters (Chen et al., 2011, Jasna et al., 2019). In contrast to protist grazing, virus production rates did not correlate with bacterial production at either nearshore or offshore sites. The lack of discernible pattern between virus production and bacterial production could be a reflection of the uncoupling between virus and bacteria when there was a replete of

bacterial hosts (Miki & Jacquet, 2008; Tsai et al., 2013a), which was suggested by the generally low VBR in this study.

In order to calculate viral lysis rate, virus burst size was estimated from the bacterial biovolume. The virus burst size estimated was 44.8, and was within the range of other studies (Bettarel et al., 2006; Parada et al., 2006; Jasna et al., 2019). Viral lysis rates ranged from 1.3×10^3 to 10.2×10^3 cells mL^{-1} h^{-1}. Viral lysis accounted for 21.3 ± 9.7 % and 23.0 ± 5.0 % of bacterial production at offshore and nearshore sites, respectively. These were within the range of viral-induced bacterial mortality in aquatic systems, which is 10 – 30 % and can be up to 72 % (Heldal & Bratbak, 1991). On all occasions, bacterial production were adequate to support viral lysis (see Table 4.8).

On the other hand, protist grazing accounted for 0 to 85.7% of bacterial production (see Table 4.8). At nearshore sites, a higher proportion of bacterial production was grazed (29.3 ± 5.6%) as opposed to viral lysis (23.0 ± 5.0%). The eutrophication at nearshore sites promoted bacterial production (Miki & Jacquet, 2008) that stimulated protist grazing (Baltar et al., 2016) but not viral lysis. Although viruses will also likely infect active bacterial cells, these infected cells, including the viruses, could be grazed and removed by grazers (Miki & Jacquet, 2008; Tsai et al., 2013b; Malits et al., 2021). The virus component in these waters were rather muted in their response to environmental differences and bacterial processes. This was probably due to the more than adequate availability of bacterial hosts that had the potential to mask any viral response. After comparing offshore and nearshore sites, the proportion of bacterial production grazed by protists and lysed by viruses revealed no differences neither offshore nor nearshore sites. Although the impact of these processes showed no spatial pattern, they will have cascading effects on the overall biogeochemical cycling of organic matter in these waters (Proctor & Fuhrman, 1990).

Bacterial loss caused by top-down control (i.e. sum of grazing and viral lysis) ranged from 10.2% to 71.3% of bacterial production, and averaged about 52.4 ± 9.1% nearshore and 47.0 ± 33.0% offshore. Only half of bacterial production could be accounted for by both these top-down controls. As bacterial abundance does not change substantially year-on-year (Lee & Bong, 2008), other loss pathways could play a role e.g. sunlight, attachment, sinking, horizontal and vertical transportation (Alonso-Sáez et al., 2006; Stocker & Seymour, 2012; Tsai et al., 2015; Li et al., 2018).

5.4 Effects of seawater warming and eutrophication on viral dynamics

In general, the increment of the slope of virus production rate can be observed in control when the incubation temperature and nutrient concentration increased (Figures 4.4 & 4.5). The increase in the slope showed that temperature and nutrient concentration promotes virus production rate and triggered the shift from lysogenic to lytic cycle, and lead to the increase in virus production rate. However, the slopes of control in Port Dickson and Port Klang were statistically different ($p < 0.05$), and suggested that the virus production rate at both sites responded differently. This may be due to higher nutrient concentrations at Port Klang as there is eutrophication at Port Klang (table 4.1).

Correlation analyses of experimental data showed that as temperature increased, both virus production and bacterial growth increased whereas lysogeny decreased (Table 5.1). Warming is reported to stimulate bacterial growth and virus production (Steenhauer et al., 2016; Demory et al., 2017; Maat et al., 2017). In this study, warming resulted in stronger viral control as virus production increased at a higher rate than bacterial production at both Port Klang (ANCOVA: $F = 12.26$, $p < 0.01$) and Port Dickson (ANCOVA: $F = 5.647$, $p < 0.05$). Our results concurred with interaction studies employing single isolates (e.g. Demory et al., 2017; Maat et al., 2017). Although Frenken et al. (2020) suggested that in

complex food webs, warming may not lead to stronger viral control; only viral and bacterial abundance were measured and compared. As abundance is a product of both top-down and bottom-up pressures, patterns observed may not have reflected *in situ* interactions (Lee & Bong 2008). The use of natural bacterial and viral community in this present study, and the measurement of production rates clearly showed that warming will result in stronger viral control, and increase the importance of the viral loop (Weinbauer et al., 1993).

Table 5.1: Univariate correlation matrix for temperature experiment. Significance for correlation analysis is as shown: ** is $p < 0.01$, * is $p < 0.001$.**

	Virus production (VLP mL^{-1} h^{-1})	**Lysogeny (%)**	**Bacterial growth rate (h^{-1})**
Temperature (°C)	0.744***	-0.869***	0.891***
Virus production (VLP mL^{-1} h^{-1})		-0.835***	0.500**
Lysogeny (%)			-0.626***

Table 5.2 shows the univariate correlation matrix of variables measured in the nutrient experiments. Similar to the observations from temperature experiments, as nutrients increased, both virus production and bacterial growth increased whereas lysogeny decreased. Nutrients have also been shown to increase bacterial growth and stimulate virus production (Liu et al., 2015; He et al., 2019). When nutrient concentration increased, virus production at Port Klang increased at a higher rate than bacterial production (ANCOVA: $F = 45.36$, $p < 0.001$), and resulted in stronger viral control. However at Port Dickson, bacterial production increased at a higher rate than virus production (ANCOVA:

F = 96.36, $p < 0.001$). As Port Klang waters are already eutrophic in nature, the level of nutrients in the marine broth might not be sufficient to accelerate bacterial growth at Port Klang whereas the bacteria at Port Dickson were stimulated. Although nutrients increased both bacterial growth and virus production, bacterial response differed probably as bacterial community was different between these two stations (Lee et al., 2015).

Table 5.2: Univariate correlation matrix for nutrient experiment. Significance for correlation analysis is as shown: * is $p < 0.05$, ** is $p < 0.01$, * is $p < 0.001$.**

	Virus production (VLP mL^{-1} h^{-1})	**Lysogeny (%)**	**Bacterial growth rate (h^{-1})**
Nutrient concentration (× marine broth strength)	0.537***	-0.904***	0.903***
Virus production (VLP mL^{-1} h^{-1})		-0.231	0.435*
Lysogeny (%)			-0.802***

The microcosm experiments showed that lysogeny exist in the tropical coastal waters of Malaysia although the extent of lysogeny might not be as high as in temperate waters (Payet & Suttle, 2013). In temperate waters, lytic cycle dominates during productive season such as summer whereas lysogenic cycle is more important during unproductive season such as winter (Payet & Suttle, 2013). Unlike temperate waters that have unproductive periods, tropical waters have a stable and warm temperature that are optimum for primary production and bacterial growth throughout the year (Cabral et al., 2017; Ashy & Agustí, 2020). Adequate and active host cells are available to support lytic virus production in tropical waters (Bettarel et al., 2008). On the other hand, some prophage might be induced before experiment as bacterial cells in tropical waters are

exposed to high level of UV radiation, which is a strong environmental inducing agent (Bettarel et al., 2008). Hence, a large portion of viruses found in this study were lytic viruses instead of lysogenic.

For lysogeny estimations, lysogen ranged from 0.03% to 2.48% in the temperature experiment (see Figure 5.4). This was in the lower end of the range previously reported for the tropical Red Sea i.e. from undetectable to 29.1% (mean = 5.0%) (Ashy & Agustí 2020). The lysogen estimated also matched the report by Weinbauer et al. (2003) where lysogens were below 15 % in surface waters. Lysogeny can range from 50% (Jiang & Paul 1994) to 90% (Freifelder 1987), and is higher in cold waters (Jin et al., 2020). However, mitomycin C does not induce all lysogens to switch from lysogenic to lytic life cycle (Weinbauer et al., 2003; Payet & Suttle, 2013). Hence, the total lysogens might be underestimated in this study.

As lysogeny was estimated from the differences between untreated and mitomycin-C treated samples, there were significant differences in the virus production rates between untreated and treated samples at all temperatures ($t > 5.07$, $p < 0.004$) except at 37°C ($t < 3.83$, $p > 0.01$, not significant after Bonferroni correction) (see Figure 4.4). Therefore in tropical waters, warming induced lysogen, and significantly reduced lysogeny at least until 37°C. Lysogeny also decreased as bacterial growth rate increased (Demory et al., 2017; Maat et al., 2017) (Figure 5.5). Lysogeny decreased at a similar rate at both Port Dickson and Port Klang ($F = 0.02$, $p > 0.80$). However the average lysogeny at Port Klang was significantly lower ($F = 24.27$, $p < 0.001$). As eutrophication is known to drive viruses towards lytic cycle (Weinbauer et al., 1993), the eutrophic Port Klang waters probably contained less lysogen and explained the lower lysogeny at Port Klang.

In the nutrient experiment, lysogen ranged from 0.04% to 7.40% (see Figure 5.4). The increase in nutrient concentration induced more lysogen relative to temperature.

Differences between untreated and treated samples were also significant at all nutrient concentrations ($t > 4.87$, $p < 0.005$) except at 0.75× strength at Port Dickson ($t = 3.21$, $p = 0.024$, not significant after Bonferroni correction) and at 1.0× strength at both stations ($t > 1.97$, $p > 0.05$). Therefore eutrophication was a more potent lysogen inducer relative to temperature, and significantly reduced lysogeny at least until 0.75× strength marine broth (see Figure 4.5). In nutrient experiments, lysogeny decreased as bacterial growth rate increased (Demory et al., 2017; Maat et al., 2017) (see Figure 5.5).

Lysogens were inversely correlated to bacterial growth rate in both temperature and eutrophication microcosm experiment. This correlation was similar to the other findings (Weinbauer et al., 2003; Payet & Suttle, 2013). However unlike the pattern observed in the temperature experiment, lysogen induction occurred at a faster rate at Port Dickson ($F = 11.58$, $p < 0.01$), and the average lysogeny at Port Dickson and Port Klang was not different ($p > 0.05$). The different response observed could again be due to different bacterial community at Port Dickson and Port Klang (Lee et al., 2015).

The *ex situ* experimentation revealed that both warming and eutrophication promoted bacterial growth and induced lysogen that resulted in an increase in virus production. Although Knowles et al. (2016) suggested a Piggyback-the-Winner hypothesis where beyond 1×10^6 cells ml^{-1}, lysogeny is favoured, bacterial abundance in this study was constantly $<1 \times 10^6$ cells ml^{-1}. Thus, the virus dynamics in this study followed the Kill-the-Winner framework where warming and eutrophication accelerate the temperate to lytic shift (Thingstad & Bratbak, 2016).

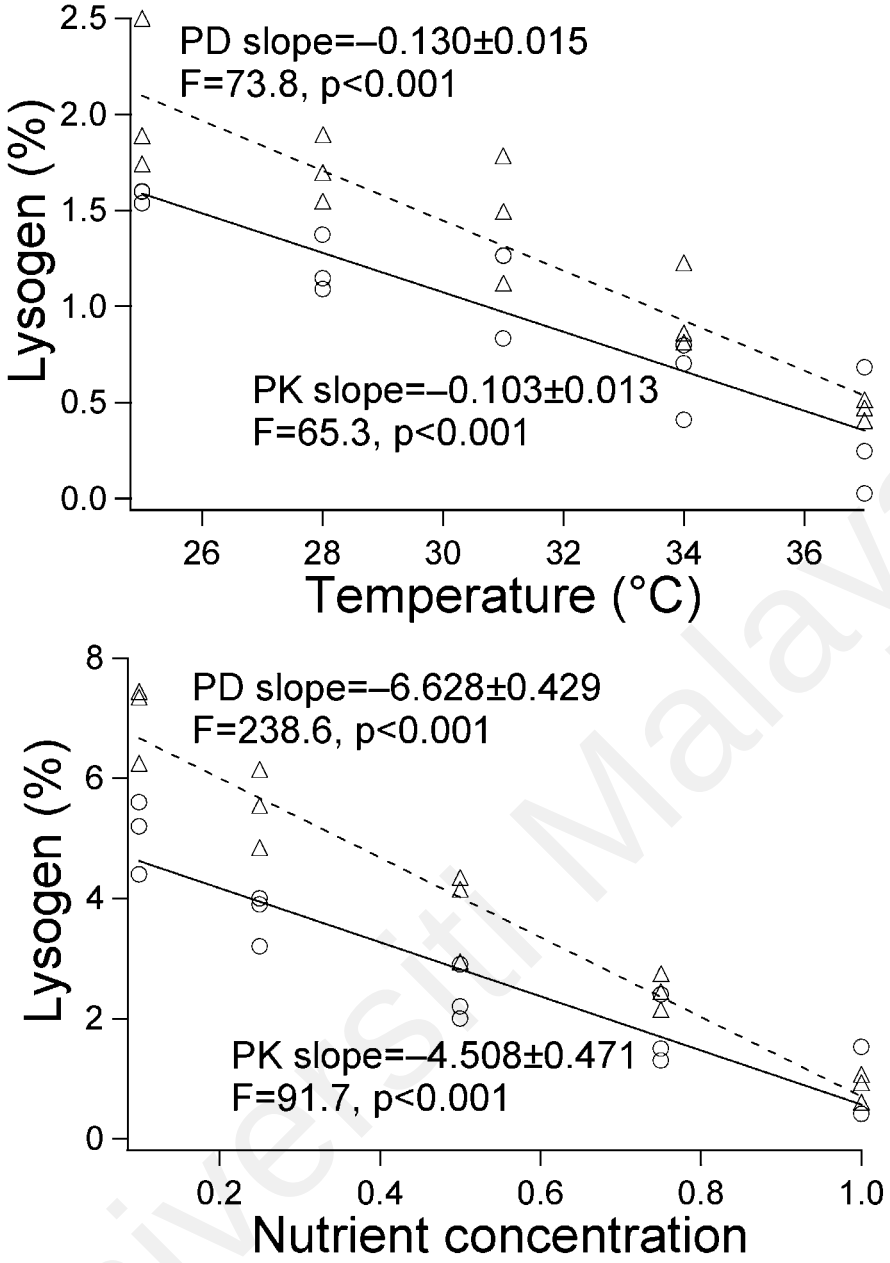

Figure 5.4: Regression slopes of lysogeny (% of lysogen) with temperature (upper panel) and nutrient concentration (× marine broth strength) (lower panel) at Port Klang (line with round symbols) and Port Dickson (broken line with triangle symbols).

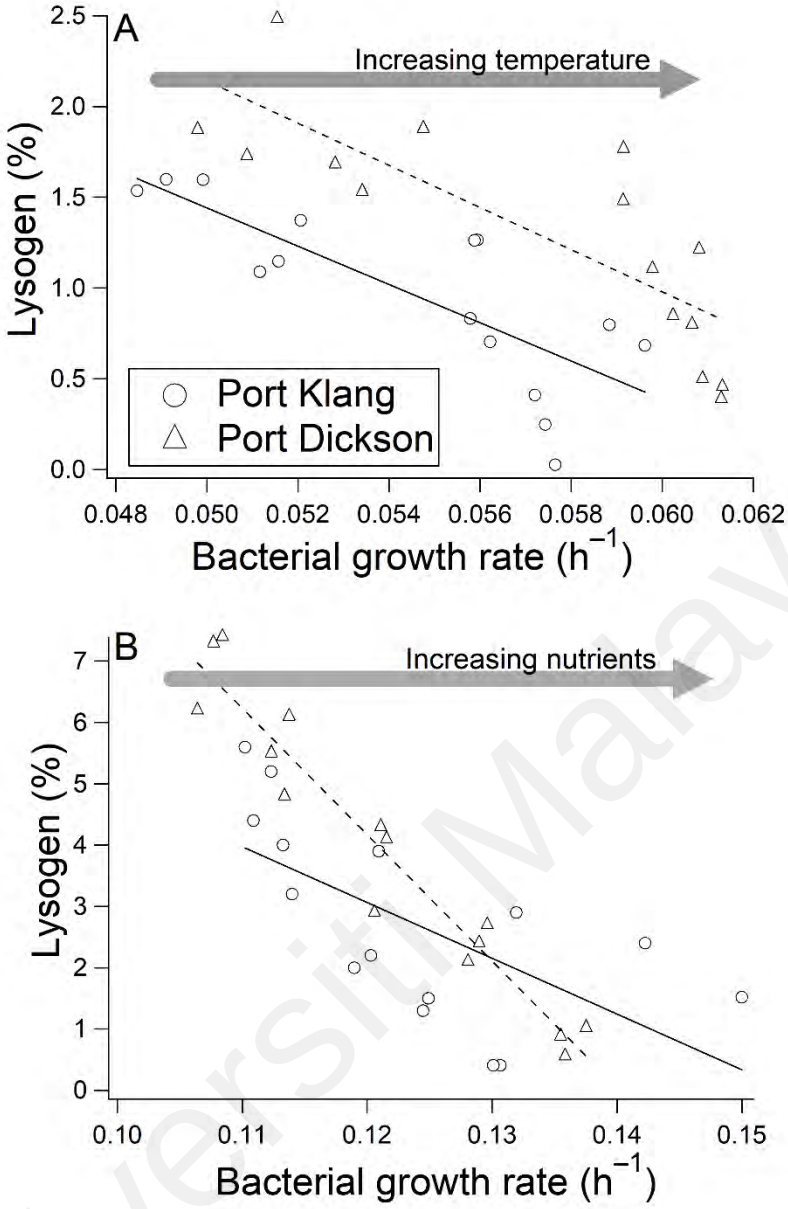

Figure 5.5: Regression slopes of lysogeny (% of lysogen) against bacterial growth rate (h^{-1}) in (A) the temperature experiments (upper panel) and (B) nutrient experiments. Data from Port Klang are shown as round symbols whereas data from Port Dickson are shown as triangle symbols. Regression slopes shown are for Port Klang (line) and Port Dickson (broken line)

In contrast to the laboratory experiment, our field study did not reveal similar trends. Possible reasons for this included the relatively stable *in situ* temperature (Lee et al., 2009) that negated temperature variability as a factor. For the eutrophication factor, nutrient concentrations at each station might not vary enough to reveal significant microbial response although stations of different trophic status were selected.

In an effort to synthesize the observations from the *ex situ* experiments with the field data measured, both experimental and field data were overlaid (Figure 5.6). The virus production rates from the temperature experiment (25°C – 37°C) were in the lower range of the field data. Even at the average *in situ* temperature of about 31°C, experimental virus production rates were in the lower ranges of the field measurements suggesting that warming had minor additive effect on *in situ* virus production rates. Although studies in temperate waters have reported strong warming effect on virus production (Steenhauer et al., 2016; Cheng et al., 2017; Maat et al., 2017), microbial process rates in tropical waters are generally independent of temperature (Lee et al., 2009). Moreover, most organisms in the tropics already function at their temperature optimum (Pomeroy & Wiebe, 2001).

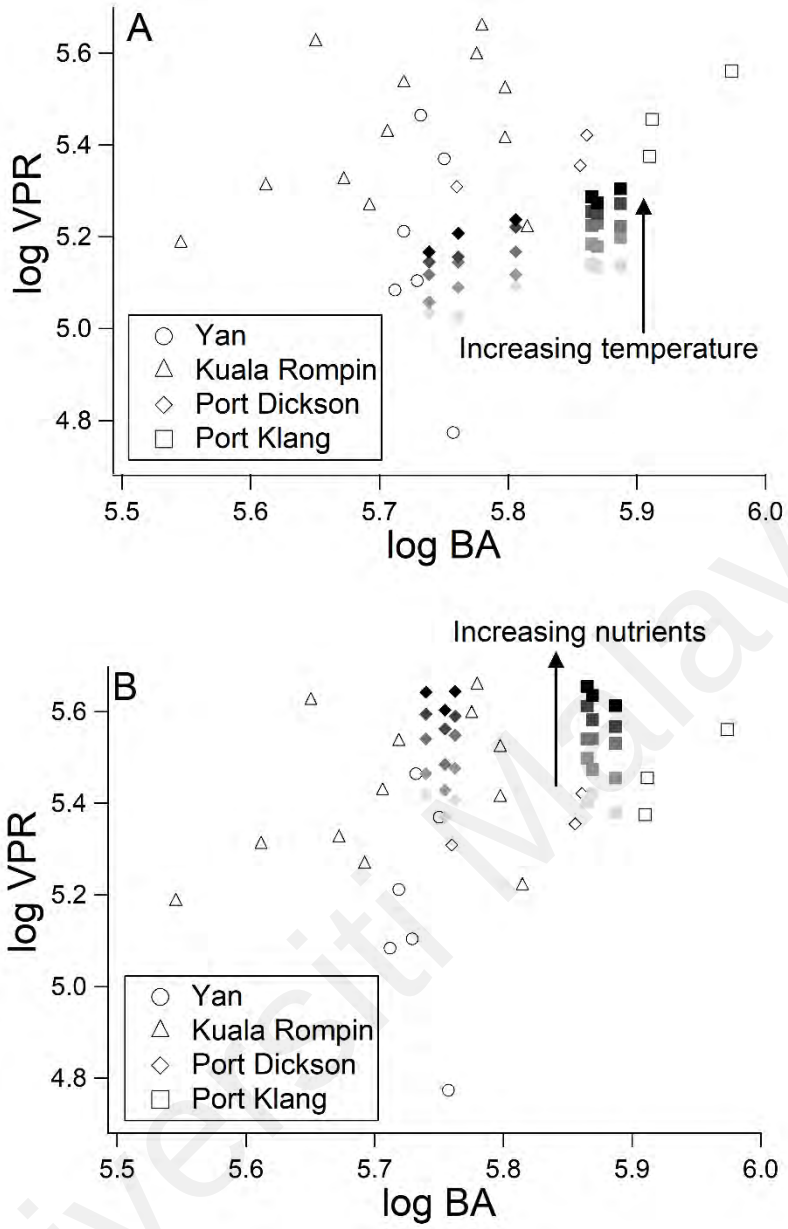

Figure 5.6: Scatter plot of virus production rates (log VLP ml^{-1} h^{-1}) against bacterial abundance (log cells ml^{-1}). The filled symbols are the data points from the *ex situ* experiments (A) for temperature and (B) for nutrients. Symbols of darker shades indicate increasing temperature or nutrient concentration.

In contrast, *ex situ* virus production rates from eutrophication experiment were in the upper ranges of field measurements (see Figure 5.6). At high tropical temperature that facilitates bacterial growth, the rate is limited by other factors such as substrate and nutrient concentration (Tsai et al., 2015). Hence, relative to temperature increase, the increase in nutrient concentration seemed a more important factor that will increase virus production, and subsequently virus control (Liu et al., 2015).

Eutrophication also reduces lysogeny that further increases virus production (Maat et al. 2016; Maat & Brussaard 2016; Cheng et al. 2019). Nutrient recycling via viral lysis is another important source of nutrient. These nutrients are mainly used to sustain the viability of primary producers and bacteria, rather than becoming an energy to transfer up the food web (Fuhrman & Noble, 1995). In complex food web systems, enhanced virus production will drive the habitat towards heterotrophy especially as increased viral lysis will further supply organic matter for the bacterial growth, and will affect biogeochemical cycling (Jin et al., 2020).

CHAPTER 6: CONCLUSION

Viruses and microorganisms are the key players in marine food web and other biogeochemical cycles. Both top-down controls, protist grazing and viral lysis, on bacteria are important pathways of carbon flow within marine ecosystem. Protist grazing on bacteria channels carbon from primary producers to consumers at higher trophic level. Viral lysis of bacteria ensures that carbon is recycled and reused for the growth of primary producer and bacterial communities. Field observations revealed site-dependent differences at the level of primary producers and bacteria but the virus component was not coupled to bacteria. The uncoupling could be due to the generally low VBR which suggested a replete bacterial state. In this study, the loss of bacterial production by top-down controls was 46.1 % ± 18.1 %. Therefore, attachment, sinking, horizontal and vertical transportation could have accounted for the remaining bacterial production.

Lysogeny provides benefits to both bacterial hosts and viruses in terms of adaptability and survivability. From *ex situ* experiments, lytic life cycle was favoured over lysogenic. Both warming and eutrophication increased virus production and decreased lysogeny according to the Kill-the-Winner hypothesis. This study showed that both seawater warming and eutrophication were able to induce lysogen to switch from lysogenic to lytic cycle. Although warming elicited a uniform response, viral response towards eutrophication seemed to be site-dependent and could be due to the different bacterial community structure at the different sites. From the synthesis of both experimental data and field observations, eutrophication should be a more important driver for virus production. In this study, an essential data gap was filled in tropical waters and suggested that with environmental change especially eutrophication, virus production will increase, and the role of viruses in the marine microbial food web will become more important.

REFERENCES

Almeida, M. A., Cunha, M. A., & Alcantara, F. (2001). Loss of estuarine bacteria by viral infection and predation in microcosm conditions. *Microb Ecol*, *42*, 562–571.

Alonso-Sáez, L., Gasol, J. M., Lefort, T., Hofer, J., & Sommaruga, R. (2006). Effect of natural sunlight on bacterial activity and differential sensitivity of natural bacterioplankton groups in Northwestern Mediterranean coastal waters. *Appl Environ Microbiol*, *72*, 5806–5813.

Anderson, R. E., Brazelton, W. J., & Baross, J. A. (2013). The deep viriosphere: assessing the viral impact on microbial community dynamics in the deep subsurface. *Reviews in Mineralogy & Geochemistry*, *75*, 649–675.

Ashy, R. A., & Agustí, S. (2020). Low host abundance and high temperature determine switching from lytic to lysogenic cycles in planktonic microbial communities in a tropical sea (Red Sea). *Viruses*, *12*. https://doi.org/10.3390/v12070761.

Aylward, F. O., Boeuf, D., Mende, D. R., Wood-Charlson, E. M., Vislova, A., Eppley, J. M., … DeLong, E. F. (2017). Diel cycling and long-term persistence of viruses in the ocean's euphotic zone. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(73), 11446–11451.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., & Thingstad, F. (1983). The ecological role of water-column microbes in the sea. *Mar Ecol Prog Ser*, *10*, 257–263.

Baltar, F., Palovaara, J., Unrein, F., Catala, P., Horňák, K., Šimek, K., … Pinhassi, J. (2016) Marine bacterial community structure resilience to changes in protist predation under phytoplankton bloom conditions. *ISME Journal*, *10*, 568–581.

Beman, J. M., Chow, C., King, A. L., Feng, Y., Fuhrman, J. A., Andersson, A., … Hutchins, D. A. (2010). Global declines in oceanic nitrification rates as a consequence of ocean acidification. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 208–213.

Bettarel, Y., Sime-Ngando, T., Amblard, C., Carrias, J-F., Sargos, D., Garabetian, F., & Lavandier, P. (2002). The functional importance of bacteriophages in the microbial loop of an oligomesotrophic lake over a diel cycle. *Intl J Limnol*, *38*, 263–269.

Bettarel, Y., Bouvy, M., Dumont, C., & Sime-Ngando, T. (2006). Virus-bacterium interactions in water and sediment of West African inland aquatic systems. *Appl Environ Microbiol*, *72*, 5274–5282.

Bettarel, Y., Arfi, R., Bouvier, T., Bouvy, M., Briand, E., Colombet, J., … Sime-Ngando, T. (2008). Virioplankton distribution and activity in a tropical eutrophicated bay. *Est Coast Shelf Sci*, *80*(3), 425–429.

Bettarel, Y., Bouvier, T., Agis, M., Bouvier, C., Chu, T. V., Combe, M., … Tran, H. Q. (2011). Viral distribution and life strategies in the Bach Dang Estuary, Vietnam. *Microb Ecol*, *62*, 143–154.

Binder, B. (1999). Reconsidering the relationship between virally induced bacterial mortality and frequency of infected cells. *Aquat Microb Ecol*, *18*, 207–215.

Bong, C. W., & Lee, C. W. (2011). The contribution of heterotrophic nanoflagellate grazing towards bacterial mortality in tropical waters: comparing estuaries and coastal ecosystems. *Mar Freshw Res*, *62*, 414–420.

Bianchi, D., Weber, T. S., Kiko, R., & Deutsch, C. (2018). Global niche of marine anaerobic metabolisms expanded by particle microenvironment. *Nature Geosci*, *11*, 263–268.

Bratbak, G., Thingstad, F., & Heldal, M. (1994). Viruses and the microbial loop. *Microb Ecol*, *28*(2), 209–211.

Brum, J. R., Hurwitz, B. L., Schofield, O., Ducklow, H. W., & Sullivan, M. B. (2016). Seasonal time bombs: dominant temperate viruses affect Southern Ocean microbial dynamics. *ISME Journal*, 10, 437–449.

Bush, A. B., & Fairbanks, R. G. (2003). Exposing the Sunda shelf: Tropical responses to eustatic sea level change. *Journal of Geophysical Research*, *108*(15). https://doi.org/10.1029/2002jd003027.

Cabral, A. S., Lessa, M. M., Junger, P. C., Thompson, F. L., & Paranhos, R. (2017). Virioplankton dynamics are related to eutrophication levels in a tropical urbanized bay. *PLoS ONE*, *12*(3), e0174653.

Carlson, R. E. (1977). A trophic state index for lakes. *Limnol Oceanogr*, *22*(2), 361–369.

Cha, Y., Alameddine, I., Qian, S. S., & Stow, C. A. (2016). A cross-scale view of N and P limitation using a Bayesian hierarchical model. *Limnol Oceanogr*, *61*, 2276–2285.

Chang, P., Yamagata, T., Schopf, P., Behera, S. K., Carton, J., Kessler, W. S., … Xie, S. (2006). Climate fluctuations of tropical coupled systems – the role of ocean dynamics. *Journal of Climate*, *19*, 5122–5174.

Chen, X., Liu, H., Weinbauer, M., Chen, B., & Jiao, N. (2011). Viral dynamics in the surface water of the western South China Sea in summer 2007. *Aquat Microb Ecol*, *63*, 145–160.

Chen, X., Ma, R., Yang, Y., Jiao, N., & Zhang, R. (2019). Viral regulation on bacterial community impacted by lysis-lysogeny switch: a microcosm experiment in eutrophic coastal waters. *Front Microbiol*.https://doi.org/10.3389/fmicb.2019.01763

Cheng, K., Van de Waal, D. B., Niu, X. Y., & Zhao, Y. J. (2017). Combined effects of elevated pCO_2 and warming facilitate cyanophage infections. *Front Microbiol*. 8:1096. https://doi.org/10.3389/fmicb.2017.01096

Cheng, K., Frenken, T., Brussaard, C., & Van de Waal, D. B. (2019). Cyanophage propagation in the freshwater Cyanobacterium *Phormidium* is constrained by phosphorus limitation and enhanced by elevated pCO_2.. *Front Microbiol*. 10:617. https://doi.org/10.3389/fmicb.2019.00617

Cheevaporn, V., & Menasveta, P. (2003). Water pollution and habitat degradation in the Gulf of Thailand. *Mar Poll Bull*, *47*, 43-51.

Choi, D. H., Hwang, C. Y., & Cho, B. C. (2003). Comparison of virus- and bacterivory-induced bacterial mortality in the eutrophic Masan Bay, Korea. *Aquat Microb Ecol*, *30*, 117–125.

Cissoko, M., Desnues, A., Bouvy, M., Sime-Ngando, T., Verling, E., & Bettarel, Y. (2008). Effects of freshwater and seawater mixing on virio- and bacterioplankton in a tropical estuary. *Freshw Biol*, *53*, 1154–1162.

Clasen, J. L., Brigden, S. M., Payet, J. P., & Suttle, C. A. (2008). Evidence that viral abundance across oceans and lakes is driven by different biological factors. *Freshw Biol*, *53*, 1090–1100.

Coello-Camba, A., Diaz-Rua, R., Duarte, C. M., Irigoien, X., Pearman, J. K., Alam, I. S., & Agusti, S. (2020). Picocyanobacteria community and cyanophage infection responses to nutrient enrichment in a mesocosms experiment in oligotrophic waters. *Front Microbiol*, *11*, 1153. https://doi.org/10.3389/fmicb.2020.01153.

Cole, J. J., Findlay, S., & Pace, M. I. (1988). Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar Ecol Prog Ser*, *43*, 1–10.

Danovaro, R., Armeni, M., Corinaldesi, C., & Mei, M. L. (2003). Viruses and marine pollution. *Mar Poll Bull*, *46*, 301–304.

Danovaro, R., Corinaldesi, C., Dell'Anno, A., Fuhrman, J. A., Middelburg, J. J., Noble, R.T., & Suttle, C. A. (2011). Marine viruses and global climate change. *FEMS Microbiology Review*, 993–1034.

Demory, D., Arsenieff, L., Simon, N., Six, C., Rigaut-Jalabert, F., Marie, D., … Baudoux, A. (2017). Temperature is a key factor in Micromonas-virus interactions. *International Society for Microbial Ecology*, 601–612.

Dinsdale, E. A., Pantos, O., Smriga, S., Edwards, R. A., Angly, F., Wegley, L., … Rohwer, F. (2008). Microbial ecology of four coral atolls in the Northern Line islands. *PLoS ONE*, *3*(2). https://doi.org/10.1371/journal.pone.0001584.

Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Stechbauer, A., Ramajo, L., … McCulloch, M. (2013). Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawter pH. *Estuaries and Coasts*, *36*, 221–236.

Dunne, J. A., Williams, R. J., & Martinez, N. D. (2002). Food-web structure and network theory: the role of connectance and size. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(20), 12917–12922.

Evans, C., Pearce, I., & Brussaard, C. (2009). Viral-mediated lysis of microbes and carbon release in the sub-Antarctic and Polar Frontal zones of the Australian Southern Ocean. *Environ Microbiol*, *11*, 2924–2934.

Evans, C., Brandsma, J., Meredith, M. P., Thomas, D. N., Venables, H., J., Pond, D. W., & Brussaard, C. (2021). Shift from carbon flow through the microbial loop to the viral shunt in coastal Antarctic waters during Austral Summer. *Microorganisms*, *9*, 460. https://doi.org/10.3390/microorganisms9020460.

Fenchel, T. (2008). The microbial loop – 25 years later. *Journal of Experimental Marine Biology and Ecology*, *366*, 99–103.

Freifelder, D. (1987). Molecular Biology. Boston: Jones and Bartlett Inc.

Frenken, T., Brussaard, C., Velthuis, M., Aben, R., Kazanjian, G., Hilt, S., ... Van de Waal, D. B. (2020). Warming advances virus population dynamics in a temperate freshwater plankton community. *Limnology and Oceanography Letters*, *5*, 295–304.

Fuhrman, J. A., & Noble, R. T. (1995). Viruses and protists cause similar bacterial mortality in coastal seawater. *Limnol Oceanogr*, *40*, 1236–1242.

Gaulke, A. K., Wetz, M., & Paerl, H. (2010). Picophytoplankton: A major contributor to planktonic biomas and primary production in a eutrophic, river-dominated estuary. *Est Coast Shelf Sci*, *90*(1), 45–54.

Gilbert, P. M., Allen, J. I., Bouwman, A. F., Brown, C. W., Flynn, K. J., Lewitus A. J., & Madden, C. J. (2010). Modeling of HABs and eutrophication: Status, advances, challenges. *Journal of Marine Systems*, *83*, 262–275.

Grasshoff, K., Kremling, K., & Ehrhardt, M. (1999). Methods of seawater analysis (3rd ed). Weinheim, Germany.

Hammer, O., Harper, D. A., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electron*, *4*, 1–9.

He, L., Yin, K., & Yuan, X. (2019). Double maximum ratios of viruses to bacteria in the water column: Implications for different regulating mechanisms. *Front Microbiol*. 10:1593. https://doi.org/10.3389/fmicb.2019.01593

Heldal, M., & Bratbak, G. (1991). Production and decay of viruses in aquatic environments. *Mar Ecol Prog Ser*, *72*, 205–212.

Henson, S. A., Beaulieu, C., Ilyina, T., John, J. G., Long, M., Séférian, R., … Sarmiento, J. L. (2017). Rapid emergence of climate change in environmental drivers of marine ecosystems. *Nat Commun*, *8*, 14682. https://doi.org/10.1038/ncomms14682.

Hewson, I., Vargo, G. A., & Fuhrman, J. A. (2003). Bacterial diversity in shallow oligotrophic marine benthos and overlying waters: effects of virus infection, containment, and nutrient enrichment. *Microb Ecol*, *46*(3), 322–336.

Howard-Varona, C., Hargreaves, K. R., Abedon, S. T., & Sullivan, M. B. (2017). Lysogeny in nature: mechanisms, impact and ecology of temperate phages. *ISME Journal*, 1–10.

Howarth, R., Chan, F., Conley, D. J., Garnier, J., Doney, S. C., Marino, R., & Billen, G. (2011). Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Front Ecol Environ*, *9*(1), 18–26.

Hwang, C. Y., & Cho, B. C. (2002). Virus-infected bacteria in oligotrophic open waters of the East Sea, Korea. *Aquat Microb Ecol*, *30*, 1–9.

IPCC. (2014).*Climate Change 2014: Synthesis Report*. IPCC, Geneva, Switzerland, 151 pp.

Jacquet, S., Miki, T., Nole, R., Peduzzi, P., & Wilhelm, S. (2010). Viruses in aquatic ecosystems: important advancements of the last 20 years and prospects for the future in the field of microbial oceanography and limnology. *Advances in Oceanography and Limnology*, *1*(1), 97–141.

Jasna, V, Ram, P, Parvathi, A, & Sime-Ngando, T (2018) Differential impact of lytic viruses on prokaryotic morphopopulations in a tropical estuarine system (Cochin estuary, India). *PLoS ONE*, *13*(3). https://doi.org/10.1371/journal.pone.0194020.

Jasna, V., Parvathi, A., Aswathy, V. K., Aparna, S., Dayana, M., Aswathy, A. J., & Madhu, N. V. (2019). Factors determining variations in viral abundance and viral production in a tropical estuary influenced by monsoonal cycles. *Reg Stud Mar Sci*, *28*, 100589. https://doi.org/10.1016/j.rsma.2019.100589.

Jiang, S., & Paul, J. (1994). Seasonal and diel abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the marine environment. *Mar Ecol Prog Ser*, *104*, 163–172.

Jiang, S., & Paul, J. (1996). Occurrence of lysogenic bacteria in marine microbial communities as determined by prophage induction. *Mar Ecol Prog Ser*, *142*, 27–38.

Jin, M., Cai, L., Ma, R., Zeng, R., Jiao, N., & Zhang, R. (2020). Prevalence of temperate viruses in deep South China Sea and western Pacific Ocean. *Deep-Sea Res I*, *166*:103403. https://doi.org/10.1016/j.dsr.2020.103403

Junger, P. C., Amada, A. M., Paranhos, R., Cabral, A. S., Jacques, S. M., & Farjalla, V. F. (2018). Salinity drives the virioplankton abundance but not production in tropical coastal lagoons. *Microb Ecol*, *75*, 56–63.

Juranek, L., Takahashi, T., Mathis, J., & Pickart, R. (2019). Significant biologically-mediated CO_2 uptake in the Pacific Arctic during the late open water season. *JGR Oceans*, *124*(2), 821–843.

Jürgens, K., & Güde, H. (1994). The potential importance of grazing-resistant bacteria in planktonic systems. *Mar Ecol Prog Ser*, *112*, 169–188.

Karl, D. M., & Tien, G. (1992). MAGIC: a sensitive and precise method for measuring dissolved phosphorus in aquatic environments. *Limnol Oceanogr*, *37*(1), 105–116.

Karl, D. M. (2007). Microbial oceanography: paradigms, processes and promise. *Nat Rev Microbiol*, *5*, 759–769.

Kaw, H. Y., & Kannan, N. (2016). A review on polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in South Asia with a focus on Malaysia. In: P. de Voogt (Eds) Reviews of Enviromental Contaminations and Toxicology Volume 242 (pp.153–181). Cham, Switzerland: Springer International Publishing.

Kepner, R., & Pratt, J. (1994). Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. *Microbiol Rev*, *58*, 603–615.

Keshri, J., Pradeep Ram, A. S., & Sime-Ngando, T. (2018). Distinctive patterns in the taxonomical resolution of bacterioplankton in the sediment and pore waters of contrasted freshwater lakes. *Microb Ecol*, *75*, 662–673.

Kim, B., An, S., Kim, T., & Hyun, J. (2020). Uncoupling between heterotrophic bacteria and phytoplankton and changes in trophic balance associated with warming of seawater in Gyeonggi Bay, Yellow Sea. *Estuaries and Coasts*, *43*, 535–546.

Kim, S., & Kim, K. (2021). Impact of climate change on the primary production and related biogeochemical cycles in the coastal and sea ice zone of the Southern Ocean. *Science of the Total Environment*, *751*, 141678–141692.

Kirchman, D. L., Hill, V., Cottrell, M. T., Gradinger, R., Malmstrom, R. R., & Parker, A. (2009). Standing stocks, production and respiration of phytoplankton and heterotrophic bacteria in the western Arctic Ocean. *Deep-Sea Res II*, *56*, 1237–1248.Knowles, B., Silveira, C. B., Bailey, B. A., Barott, K., Cantu, V. A., Cobían-

Güemes, A. G., … Rohwer, F. (2016). Lytic to temperate switching of viral communities. *Nature*, *531*, 466-470.

Kozaki, D., Harun, N., Rahim, M., Mori, M., Nakatani, N., & Tanaka, K. (2017). Determination of water quality degradation due to industrial and household wastewater in the Galing River in Kuantan, Malaysia using ion chromatograph and water quality data. *Environments*, *4*(2), 35-49.

Kroeze, C., Hofstra, N., Ivens, W., Löhr, A., Strokal, M., & Wijnen, J. (2013). The links between global carbon, water and nutrient cycles in an urbanizing world – the case of coastal eutrophication. *Current Opinion in Environmental Sustainability*, *5*, 566-572.

Lara, E., Arrieta, J. M., Garcia-Zarandona, I., Boras, J. A., Duarte, C. M., Agustí, S, … Vaqué, D. (2013). Experimental evaluation of the warming effect on viral, bacterial and protistan communities in two contrasting Arctic systems. *Aquat Microb Ecol*, *70*, 17-32.

Larsen, J. B., Larsen, A., Thyrhaug, R., Bratbak, G., & Sandaa, R. A. (2008). Response of marine viral populations to a nutrient induced phytoplankton bloom at different pCO_2 levels. *Biogeosciences*, *5*, 523-533.

Lee, C. W., Kudo, I., Yanada, M., & Maita, Y. (2001). Bacterial abundance and production and heterotrophic nanoflagellate abundance in subarctic coastal waters (Western North Pacific Ocean). *Aquat Microb Ecol*, *23*, 263–271.

Lee, C. W., & Bong, C. W. (2008). Bacterial abundance and production, and their relation to primary production in tropical coastal waters of Peninsular Malaysia. *Mar Freshw Res*, *59*, 10–21.

Lee, C. W., Bong, C. W., & Hii, Y. S. (2009). Temporal variation of bacterial respiration and growth efficiency in tropical coastal waters. *Appl Environ Microbiol*, *75*(2), 7594–7601.

Lee, C. W., & Bong, C. W. (2012). Relative contribution of viral lysis and grazing to bacterial mortality in tropical coastal waters of Peninsular Malaysia. *Bull Mar Sci*, *88*, 1–14.

Lee, C. W., Lim, J. H., Heng, P. L., Marican, N. F., Narayanan, K., … Bong, C. W. (2020). Influence of elevated river flow on hypoxia occurrence, nutrient concentration and microbial dynamics in a tropical estuary. *Environ Monit Assess*, *192*, 660

Lee, S. W., Lee, C. W., Bong, C. W., Narayanan, K., & Sim, E. U. (2015). The dynamics of attached and free-living bacterial population in tropical coastal waters. *Mar Freshw Res*, *66*, 701–710.

Li, H., Lin, L., Ye, S., Li, H., & Fan, J. (2017). Assessment of nutrient and heavy metal contamination in the seawater and sediment of Yalujiang Estuary. *Mar Poll Bull*, *117*, 499–506.

Li, Y., Sun, L. L., Su, H. N., Zhang, X. Y., Xie, B. B., Chen, X. L., … Qin, Q. L. (2018). Vertical and horizontal biogeographic patterns and major factors affecting bacterial communities in the open South China Sea. *Sci Rep*, *8*(1), 8800. https://doi.org/10.1038/s41598-018-27191-w.

Li, X., Xu, J., Shi, Z., Xu, D., Li, R., Li, Q., & Liu, H. (2019). Regulation of protist grazing on bacterioplankton by hydrological conditions in coastal waters. *Est Coast Shelf Sci*, *218*,1–8.

Lim, J. H., & Lee, C. W. (2017). Effects of eutrophication on diatom abundance, biovolume and diversity in tropical coastal waters. *Environ Monit Assess*, *189*, 432–441.

Lim, J. H., Lee, C. W., Bong, C. W., Affendi, Y. A., Hii, Y. S., & Kudo, I. (2018). Distributions of particulate and dissolved phosphorus in aquatic habitats of Peninsular Malaysia. *Mar Poll Bull*, *128*, 415–427.

Lim, J. H., Wong, Y. Y., Lee, C. W., Bong, C. W. & Kudo, I. (2019). Long-term comparison of dissolved nitrogen species in tropical estuarine and coastal water systems. *Est Coast Shelf Sci*, *222*,103–111.

Lim, J. H., Lee, C. W., & Bong, C. W. (2020). Investigating factors driving phytoplankton growth and grazing loss rates in waters around Peninsular Malaysia. *J Ocean Limnol*. https://doi.org/10.1007/s00343-020-9227-1.

Lim, J. H., Lee, C. W., Bong, C. W., & Kudo, I. (2021). The impact of eutrophication towards selected bacterial process rates in tropical coastal waters. *Mar Poll Bull*, *169*, 112524. https://doi.org/10.1016/j.marpolbul.2021.112524.

Liu, H., Yuan, X., Xu, J., Harrison, P. J., He, L., & Yin, K. (2015). Effects of viruses on bacterial functions under contrasting nutritional conditions for four species of bacteria isolated from Hong Kong waters. *Sci Rep*, *5*, 14217. https://doi.org/10.1038/srep14217

Maat, D. S., & Brussaard, C. (2016). Both phosphorus- and nitrogen limitation constrain viral proliferation in marine phytoplankton. *Aquat Microb Ecol*, *77*, 87–97.

Maat, D. S., de Blok, R., & Brussaard, C. (2016). Combined phosphorus limitation and light stress prevent viral proliferation in the phytoplankton species *Phaeocystis globosa*, but not in *Micromonas pusilla*. *Front Mar Sci*. 3:160. https://doi.org/10.3389/fmars.2016.00160

Maat, D. S., Biggs, T., Evans, C., Bleijswijk, J., Wel, N., Dutilh, B. E., & Brussaard, C. (2017). Characterization and temperature dependence of Arctic *Micromonas polaris* viruses. *Viruses*, *9*, 134-153.

Mackenzie, F. T., Ver, L. M., & Lerman, A. (2000). Coastal-zone biogerochemical dynamics under global warming. *International Geology Review*, *42*(3), 193-206.

Malits, A., Boras, J. A., Balagué, V., Calvo, E., Gasol, J. M., Marrasé, C., … Vaqué, D. (2021). Viral-mediated microbe mortality modulated by ocean acidification and eutrophication: consequenses for the carbon fluxes through the microbial food web. *Front Microbiol*, *12*, 635821. https://doi.org/10.3389/fmicb.2021.635821.

Masood, N., Zakaria, M. P., Halimoon, N., Aris, A. Z., Magam, S. M., Kannan, N., … Al-Odaini, N. A. (2016). Anthropogenic waste indicators (AWIs), particularly PAHs and LABs, in Malaysian sediments: Application of aquatic environment for identifying anthropogenic pollution. *Mar Poll Bull*, *102*, 160-175.

Matteson, A. R., Loar, S. N., Pickmere, S., DeBruyn, J. M., Ellwood, M. J., Boyd, P. W., … Wilhelm, S. W. (2012). Production of viruses during a spring phytoplankton

bloom in the South Pacific Ocean near of New Zealand. *FEMS Microbiol Ecol*, *79*, 709–719.

Meyer-Reil, L., & Köster, M. (2000). Eutrophication of marine waters: effects on benthic microbial communities. *Mar Poll Bull*, *41*, 255–263.

Miki, T., & Jacquet, S. (2008). Complex interactions in the microbial world: underexplored key links between viruses, bacteria and protozoan grazers in aquatic environments. *Aquat Microb Ecol*, *51*, 195–208.

Mojica, K. D., & Brussaard, C. (2014). Factors affecting virus dynamics and microbial host-virus interactions in marine environments. *FEMS Microbiol Ecol*, *89*, 495–515.

Mojica, K. D., & Brussaard, C. (2020). Significance of viral activity for regulating heterotrophic prokaryote community dynamics along a Meridional gradient of stratification in the Northeast Atlantic Ocean. *Viruses*, *12*(11), 1293.

Moore, C. M., Mills, M. M., Arrigo, K. R., Berman-Frank, I., Bopp, L., Boyd, P. W., … Ulloa, O. (2013). Processes and patterns of oceanic nutrient limitation. *Nature Geosci*, *6*, 701–710.

Moore, J. K., Fu, W., Primeau, F., Britten, G. L., Lindsay, K., Long, M., … Randerson, J. T. (2018). Sustained climate warming drives declining marine biological productivity. *Science*, *359*, 1139–1143.

Morris, R. M., Cain, K. R., Hvorecny, K. L., & Kollman, J. M. (2020). Lysogenic host-virus interactions in SAR11 marine bacteria. *Nat Microbiol*, *5*, 1011–1015.

Mwaura, J., Umezawa, Y., Nakamura, T., & Kamau, J. (2017). Evidence of chronic anthropogenic nutrient within coastal lagoon reefs adjacent to urban and tourism centers, Kenya: A stable isotope approach. *Mar Poll Bull*, *119*, 74-86.

NOAA. What is eutrophication? Retrieved on March 22, 2021 from https://oceanservice.noaa.gov/facts/eutrophication.html.

Noble, R. T., & Fuhrman, J. A. (1998). Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquat Microb Ecol*, *14*, 113–118.

Okazaki, Y., Nishimura, Y., Yoshida, T., Ogata, H., & Nakano, S. (2019).Metagenomic exploration of viral diversity and virus-host interactions in a deep freshwater lake. *bioRxiv*. https://doi.org/10.1101/655167.

Paffenhöffer, G., Sherr, B. F., & Sherr, E. B. (2007). From small scales to the big picture: persistence mechanisms of planktonic grazers in the oligotrophic ocean. *Marine Ecology*, 211–246.

Parada, V., Herndl, G. J., & Weinbauer, M. G. (2006). Viral burst size of heterotrophic prokaryotes in aquatic systems. *J Mar Biol Assoc UK*, *86*, 613–621.

Parsons, T. R., Maita, Y., & Lalli, C. M. (1984). A manual of chemical & biological methods for seawater analysis. Oxford, United Kingdom.

Patten, N. L., Mitchell, J. G., Middelboe, M., Eyre, B. D., Seuront, L., … Glud, R. N. (2008). Bacterial and viral dynamics during a mass coral spawning period on the Great Barrier Reef. *Aquat Microb Ecol*, *50*, 209–220.

Paul, J., & Kellogg, C. (2000). Ecology of bacteriophages in nature. *Viral Ecology*, *28*, 243–253.

Paul, J., & Weinbauer, M. (2010). Detection of lysogeny in marine environments. In: S. W. Wilhelm, M. G. Weinbauer, & C. A. Suttle (Eds.), Manual of aquatic viral ecology (pp. 30–33). Texas, US: ASLO.

Payet, J. P., & Suttle, C. A. (2013). To kill or not to kill: The balance between lytic and lysogenic viral infection is driven by trophic status. *Limnol Oceanogr*, *58*(2), 465–474.

Pielke, R. A. (2004). What is climate change? *Energy and Environment*, *15*(3), 515–520.

Piroddi, C., Akoglu, E., Andonegi, E., Bentley, J. W., Celic, I., Coll, M., … Tsikliras, A. C. (2021). Effects of nutrient management scenarios on marine food webs: a pan-European assessment in support of the marine strategy framework directive. *Front Mar Sci*. https://doi.org/10.3389/fmars.2021.596797.

Pomeroy, L. R. (1974). The ocean's food web, a changing paradigm. *BioScience*, *24*(9), 499–504.

Pomeroy, L. R., & Wiebe, W. J. (2001). Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquat Microb Ecol*, *23*, 187–204.

Pradeep Ram, A. S., Palesse, S., Colombet, J., Thouvenot, A., & Sime-Ngando, T. (2014). The relative importance of viral lysis and nanoflagellate grazing for prokaryote mortality in temperate lakes. *Freshw Biol*, *59*, 300–311.

Pradeep Ram, A. S., Mari, X., Brune, J., Torréton, J. P., Chu, V. T., Raimbault, P., … Sime-Ngando, T. (2018). Bacterial-viral interactions in the sea surface microlayer of a black carbon-dominated tropical coastal ecosystem (Halong Bay, Vietnam). *Elem Sci Anth*, *6*(1), 13–32.

Proctor, L. M., & Fuhrman, J. A. (1990). Viral mortality of marine bacteria and cyanobacteria. *Nature*, *343*, 60–62.

Reece, J., B., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., & Jackson, R. B. (2011). Campbell Biology Ninth Edition. United States of America: Pearson.

Reid, P. C. (2016). Ocean warming: setting the scene. In: D. Laffoley & J. M. Baxter (Eds.), Explaining ocean warming: causes, scale, effects and consequences (pp. 17–46). Gland, Switzerland: IUCN.

Rivkin, R. B. & Legendre, L. (2001). Biogenic carbon cycling in the upper ocean: effects of microbial respiration. *Science*, *291*, 2398–2400.

Sarmento, H., Montoya, J. M., Vázquez-Domínguez, E., Vaqué, D., & Gasol, J. M. (2010). Warming effects on marine microbial food web processes: how far can we go when it comes to predictions? *Phil. Trans. R. Soc. B*, *365*, 2137–2149.

Sherr, B. F., Sherr, E. B., Caron, D. A., Vaulot, D., & Worden, A. Z. (2007). Oceanic protists. *Oceanography*, *20*, 130–134.

Sherr, E. B., & Sherr, B. F. (1994). Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. *Microb Ecol*, *28*, 223–235.

Simone, M. N., Schulz, K. G., Oakes, J. M., & Eyre, B. D. (2021). Warming and ocean acidification may decrease estuarine dissolved organic carbon export to the ocean. *Biogeosciences*, *18*, 1823–1838.

Smale, D. A., Taylor, J. D., Coombs, S. H., Moore, G., & Cunliffe, M. (2017). Community responses to seawater warming are conserved across diverse biological groupings and taxonomic resolutions. *Proc R Soc B*, *284*, 20170534. https://dx.doi.org/10.1098/rspb.2017.0534.

Smith V. H. (2003). Eutrophication of freshwater and coastal marine ecosystems: a global problem. *Environ Sci & Pollut Res*, *10*(2), 126–139.

Steenhauer, L. M., Wierenga, J., Carreira, C., Limpens, R., Koster, A. J., Pollard, P. C., & Brussaard, C. (2016). Isolation of cyanophage CrV infecting *Cylindrospermopsis raciborskii* and the influence of temperature and irradiance on CrV proliferation. *Aquat Microb Ecol*, *78*, 11–23.

Steitz, A., & Velimirov, B. (1999). Contribution of picocyanobacteria to total primary production and community respiratory losses in a backwater system. *J Plankton Res*, *21*(12), 2341–2360.

Stocker, R. & Seymour, J. R. (2012). Ecology and physics of bacterial chemotaxis in the ocean. *Microbiology and Molecular Biology Reviews*, *76*(4), 792–812.

Suttle, C. A. (2007). Marine viruses - major players in the global ecosystem. *Nat Rev Microbiol*, *5*, 801–812.

Takahashi, T., Sutherland, S. C., Sweeney, C., Poisson, A., Metzl, N., Tilbrook, B., … Nojiri, Y. (2002). Global sea-air CO_2 flux based on climatological surface ocean pCO_2, and seasonal biological and temperature effects. *Deep-Sea Res II*, *49*, 1601–1622.

Thingstad, T., & Bratbak, G. (2016). Viral strategies at sea. *Nature*, *531*, 454–455.

Thompson, L. G. (2010). Climate change: The evidence and our options. *Nat Rev Microbiol*. https://doi.org/10.1038/nrmicro.2016.176.

Thurber, R. V., Payet, J. P., Thurber, A. R., & Correa, A. M. S. (2017). Virus-host interactions and their roles in coral reef health and disease. *Nat Rev Microbiol*. https://doi.org/10.1038/nrmicro.2016.176.

Tsai, A. Y., Gong, G. C., Huang, J. K., & Lin, Y. C. (2013a). Viral and nanoflagellate control of bacterial production in the East China Sea summer 2011. *Est Coast Shelf Sci*, *120*, 33–41.

Tsai, A. Y., Gong, G. C., & Hung, J. (2013b). Seasonal variations of virus- and nanoflagellate-mediated mortality of heterotrophic bacteria in the coastal ecosystem of subtropical western Pacific. *Biogeosciences*, *10*, 3055–3065.

Tsai, A. Y., Gong, G. C., Sanders, R. W., & Huang, J. K. (2013c). Contribution of viral lysis and nanoflagellate grazing to bacterial mortality in the inner and outer regions of the Changjiang River plume during summer 2011. *J Plankton Res*, *35*, 1283–1293.

Tsai, A. Y., Gong, G. C., & Shiau, W. (2015). Viral lysis and nanoflagellate grazing on prokaryotes: effects of short-term warming in a coastal subtropical marine system. *Hydrobiologia*, *751*, 43–54.

Tsai, A. Y., Gong, G. C., Chung, C. C., & Huang, Y. T. (2018). Different impact of nanoflagellate grazing and viral lysis on *Synechococcus* spp. and picoeukaryotic mortality in coastal waters. *Est Coast Shelf Sci*, *209*, 1–6.

Tsiola, A., Pitta P., Giannakourou, A., Bourdin, G., Marro, S., Maugendre, L., … Gazeau, F. (2016). Ocean acidification and viral replication cycles: frequency of lytically infected and lysogenic cells during a mesocosm experiment in the NW Mediterranean Sea. *Est Coast Shelf Sci*, *186*, 139–151.

U.S. DOE. (2008). *Biogeochemical cycling of carbon in oceans and climate change*. (Report No. DOE/SC-108). U.S. Department of Energy Office of Science. https://genomicscience.energy.gov/carboncycle/report/biogeocycle.pdf

Vaqué, D., Lara, E., Arrieta, J. M., Holding, J., Sà, E. L., Hendriks, I. E., … Duarte, C. M. (2019). Warming and CO_2 enhance arctic heterotrophic microbial activity. *Front Microbiol*. https://doi.org/10.3389/fmicb.2019.00494

Verweij, J. & Pinedo, H. M. (1990). Mitomycin C: mechanism of action, usefulness and limitations. *Anti-Cancer*, *1*, 5–13.

Wang, Z., Juarez, D. L., Pan, J. F., Blinebry, S. K., Gronniger, J., Clark, J. S., … Hunt, D. E. (2019). Microbial communities across nearshore to offshore coastal transects are primarily shaped by distance and temperature. *Environ Microbiol*, *21*, 3862–3872.

Watson, A. J., & Liss, P. S. (1998). Marine biological controls on climate via the carbon and sulphur geochemical cycles. *Philos Trans R Soc Lond B Biol Sci*, *353*, 41–51.

Weinbauer, M. G., Fuks, D., & Peduzzi, P. (1993). Distribution of viruses and dissolved DNA along a coastal trophic gradient in the northern Adriatic Sea. *Appl Environ Microbiol*, *59*, 4074–4082.

Weinbauer, M. G., & Peduzzi, P. (1994). Frequency, size and distribution of bacteriophages in different marine bacterial morphotypes. *Mar Ecol Prog Ser*, *108*, 11–20.

Weinbauer, M. G., & Suttle, C. A. (1996). Potential significance of lysogeny to bacteriophage production and bacterial mortality in coastal waters of the Gulf of Mexico. *Appl Environ Microbiol*, *62*(12), 4374–4380.

Weinbauer, M. G., & Suttle, C. A. (1999). Lysogeny and prophage induction in coastal and offshore bacterial communities. *Aquat Microb Ecol*, *18*(3), 217–225.

Weinbauer, M. G., Brettar, I., & Hofle, M. G. (2003). Lysogeny and virus-induced morality of bacterioplankton in surface, deep, and anoxic marine waters. *American Society of Limnology and Oceanography*, 1457–1465.

Weinbauer, M. G. (2004). Ecology of prokaryotic viruses. *FEMS Microbiology Review*, *28*, 127–181.

Weinbauer, M. G., Rowe, J. M., & Wilhelm, S. W. (2010). Determining rates of virus production in aquatic systems by the virus reduction approach. In: S. W. Wilhelm, M. G. Weinbauer, & C. A. Suttle (Eds.), Manual of aquatic viral ecology (pp. 1–8). Texas, US: ASLO.

Weitz, J. S. & Wilhelm, S. W. (2012). Ocean viruses and their effects on microbial communities and biogeochemical cycles. *F1000 Biology Reports*, *4*, 17–24.

Wilhelm, S. W., & Suttle, C. A. (1999). Viruses and nutrient cycles in the sea. *BioScience*, *49*, 781–788.

Wommack, K. E., Ravel, J., Hill, R. T., Chun, J., & Colwell, R. R. (1999). Population dynamics of Chesapeake Bay virioplankton: total community analysis by pulsed-field gel electrophoresis. *Appl Environ Microbiol*, *65*, 231–240.

Wommack, K. E., & Colwell, R. R. (2000). Virioplankton: viruses in aquatic ecosystems. *Microbiol Mol Biol Rev*, *64*, 69–114.

Worden, A. Z., Nolan, J. K., & Palenik, B. (2004). Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component. *Limnol Oceanogr*, *49*(1), 168–179.

York, A. (2018). Marine biogeochemical cycles in a changing world. *Nat Rev Microbiol*, *16*(5), 259.

Zeigler Allen, L., McCrow, J. P., Ininbergs, K., Dupont, C. L., Badger, J. H., Hoffman, J. M., … Venter, J. C. (2017). The Baltic Sea virome: diversity and transcriptional activity of DNA and RNA viruses. *mSystems*. https://doi.org/10.1128/mSystems.00125-16.

Zhang, Y., Wang, X., & Yin, K. (2018). Spatial contrast in phytoplankton, bacteria and microzooplankton grazing between the eutrophic Yellow Sea and the oligotrophic South China Sea. *J Oceanology Limnol*, *36*, 92–104.

Zhang, R., Weinbauer, M. G., & Peduzzi, P. (2021). Aquatic viruses and climate change. *Curr Issues Mol Biol*, *41*, 357–380.

Zimmerman, A. E., Howard-Varona, C., Needham, D. M., John, S. G., Worden, A. Z., Sullivan, M. B., … Coleman, M. L. (2020). Metabolic and biogeochemical consequences of viral infection in aquatic ecosystems. *Nat Rev Microbiol*, *18*(1), 21–34.