THE INFLUENCE OF ENVIRONMENTAL FACTORS ON PHYTOPLANKTON COMPOSITION AND DYNAMICS ALONG THE JOHOR STRAIT USING CONVENTIONAL AND METABARCODING APPROACHES

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ABSTRACT

Johor Strait is a narrow strait located between Malaysia and Singapore; and serves as the main marine aquaculture area for the two countries. The Strait was subjected to anthropogenic activities that led to eutrophication and an increase in harmful algae blooms (HAB). However, the information associated with spatial-temporal bloom dynamic, and the environmental constraints affecting the phenology of the harmful phytoplankton species in the Johor Strait is still lacking. Hence, a field study was conducted from January 2017 to September 2019 as an effort to continuously monitor the water quality and occurrence of HAB in the Strait. Phytoplankton and water samples were collected monthly during high tide along the Johor Strait and *in situ* physical and chemical parameters such as temperature, salinity, pH, dissolved oxygen, nitrate-nitrogen (NO_3-N), ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), silicate-silica (SiO_3-Si), phosphate-phosphorus (PO_4-P) and chlorophyll *a* were determined. The phytoplankton compositions were identified microscopically. A metabarcoding approach targeting the 18S ribosomal RNA (rRNA) gene marker has also been employed to study the spatial-temporal structure of HAB community assemblages between May 2018 and September 2019, covering 19 stations across the Johor Strait. In this study, a total of 48 genera of phytoplankton were identified morphologically. The most diverse group was diatom (32 genera) followed by the dinoflagellate (15 genera). Bloom-forming diatoms included *Chaetoceros*, *Coscinodiscus*, *Eucampia*, *Guirnadia*, *Navicula*, *Pseudo-nitzschia*, *Rhizosolenia*, *Skeletonema* and *Thallasiosira*; and dinoflagellate *Blixaea quinquecornis* and *Scrippsiella* were succeeded by predominantly single species or co-dominated by more than a single species throughout the study period. Nitrate and silicate were found to be the triggering factor for the bloom-forming of diatoms, while phosphate was important for sustaining dinoflagellates succession in the Strait. An attempt has been made to determine the cause of hypoxia-anoxia condition in the inner part of Johor Strait, suggesting that high chlorophyll-*a* concentration (up to 48.8 µg/L) and high biomass blooms of *Skeletonema*, *Chaetoceros*, *Rhizosolenia*, and *Thalassiosira* observed at the inner part of the strait to be the main cause. A hypoxic to anoxic dead zone, with the dissolved oxygen levels ranging from 0.19 to 1.7 mg/L, was identified in the inner Johor Strait, covering an estimated area of 10.3 km^2. The occurrence of high biomass diatom blooms has likely induced the formation of the hypoxic-anoxic zone along the inner part of Johor Strait. The operational taxonomic units (OTUs) of HAB taxa retrieved from the metabarcoding datasets revealed a much higher number of taxa (26 OTUs) than before, with 12 new taxa identified. The most common and abundant HAB taxa in the strait (frequency of occurrences >70%) are *Heterosigma akashiwo*, *Fibrocapsa japonica*, *Pseudo-nitzschia pungens*, *Dinophysis* spp., *Gymnodinium catenatum*, *Alexandrium leei*, and *Alexandrium tamiyavanichii*. This study illustrated that the nutrient dynamic influenced by environmental variables were the key factor in the succession of the phytoplankton community and HAB assemblages in the tropical eutrophic of Johor Strait.

ABSTRAK

Selat Johor adalah selat sempit terletak di antara Malaysia dan Singapura; berfungsi sebagai kawasan utama untuk akuakultur laut bagi dua negara tersebut. Selat itu juga mengalami pelbagai aktiviti antropogenik yang menyebabkan eutrofikasi dan peningkatan kejadian ledakan alga berbahaya (HAB) di kawasan tersebut. Walau bagaimanapun, maklumat tentang dinamik ledakan berhubungkan ruang-masa dan faktor persekitaran yang mempengaruhi fenologi fitoplankton berbahaya di kawasan itu masih berkurangan. Justeru, penyelidikan lapangan telah dilakukan dari Januari 2017 hingga September 2019 sebagai usaha untuk terus memantau kualiti air dan kejadian ledakan alga berbahaya di Selat tersebut. Sampel fitoplankton dan air diambil setiap bulan ketika air pasang di sepanjang Selat Johor dan *in situ* parameter fizikal serta kimia seperti suhu, kemasinan, pH, oksigen terlarut, nitrat-nitrogen (NO_3-N), ammonia-nitrogen (NH_3-N), nitrit-nitrogen (NO_2-N), silikat-silika **(**SiO_3-Si), fosfat-fosforus **(**PO_4-P) dan klorofil *a* ditentukan. Komposisi fitoplankton telah dikenalpasti secara mikroskopik. Pendekatan metabarkod (*metabarcoding*) penanda gen 18S ribosom RNA (rRNA) juga telah digunakan untuk mengkaji struktur komuniti spesies HAB berhubungkait ruang dan masa meliputi 19 stesen di seluruh Selat Johor antara Mei 2018 hingga September 2019. Dalam kajian ini, sebanyak 48 genera fitoplankton telah dikenalpasti secara morfologi. Kumpulan yang paling pelbagai adalah diatom (32 genera) diikuti oleh dinoflagelat (15 genera). Diatom yang dominan membentuk ledakan adalah *Chaetocero*s, *Coscinodiscus*, *Eucampia*, *Guirnadia*, *Navicula*, *Pseudo-nitzschia*, *Rhizosolenia*, *Skeletonema* dan *Thallasiosira*; dan dinoflagelat *Blixaea quinquecornis* dan *Scrippsiella* hadir secara bersilih-ganti atau serentak pada masa yang sama sepanjang kajian dijalankan. Hasil kajian menunjukkan bahawa nitrat dan silikat merupakan faktor pencetus pembentukan ledakan diatom, manakala fosfat penting untuk sesaran dinoflagelat. Satu usaha juga telah dilakukan untuk mengenalpasti penyebab keadaan hipoksik-anoksik di kawasan dalaman Selat Johor. Bacaan klorofil *a* tinggi (hingga 48.8 µg/L) serta ledakan berkepadatan sel yang tinggi oleh *Skeletonema*, *Chaetoceros*, *Rhizosolenia*, dan *Thalassiosira* di kawasan dalaman selat telah menjadi penyebab utama. Keadaan hipoksik ke zon mati anoksik dengan kandungan oksigen terlarut antara 0.19 hingga 1.7 mg/L telah dikenal pasti meliputi kawasan seluas 10.3 km^2 di bahagian dalaman Selat Johor. Kejadian ledakan diatom berkepadatan sel yang tinggi merupakan penyebab pembentukan zon hipoksik-anoksik di sepanjang bahagian dalaman Selat Johor. Unit taksonomi operasi (OTU) untuk taksa HAB telah diperolehi daripada set data metabarkod menunjukkan bilangan taksa HAB (26 OTU) lebih tinggi daripada sebelumnya di mana 12 OTU adalah merupakan rekod baru di selat tersebut. Taksa HAB yang paling biasa ditemui dan paling banyak di Selat (kekerapan kemunculan > 70%) terdiri daripada *Heterosigma akashiwo*, *Fibrocapsa japonica*, *Pseudo-nitzschia pungens*, *Dinophysis* spp., *Gymnodinium catenatum*, *Alexandrium leei*, dan *Alexandrium tamiyavanichii*. Kajian ini telah menunjukkan bahawa dinamik nutrien yang dipengaruhi oleh pembolehubah persekitaran adalah faktor utama untuk sesaran bagi perubahan struktur komuniti fitoplankton dan kumpulan HAB di ekosistem eutrofik perairan Selat Johor.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOSIM	-	one-way analysis of similarity
CCA	-	canonical correspondence analysis
Chl *a*	-	chlorophyll *a*
CFP	-	ciguatera fish poisoning
DA	-	domoic acid
DO	-	dissolved oxygen
DNA	-	deoxyribonucleic acid
eDNA	-	environmental DNA
HAB	-	Harmful Algae Bloom
HTS	-	high-throughput sequencing
IM	-	inter-monsoon
OTU	-	operational taxonomic unit
nMDS	-	non-metric multidimensional scaling
N	-	Nitrogen
NO_3	-	nitrate
NO_2	-	nitrite
NH_3	-	ammonia
NEM	-	Northeast Monsoon
PEAR	-	Paired-End read merger
PERMANOVA	-	permutational analysis of variance
P	-	Phosphorus
PO_4	-	phosphate
PCR	-	Polymerase chain reaction
PSP	-	paralytic shellfish poisoning
PR^2	-	Protist Ribosomal Reference
rRNA	-	ribosomal RNA
Si	-	Silica
SiO_3	-	silicate
SIMPER	-	similarity percentages
STX	-	Saxitoxin

SWM - Southwest Monsoon

18S - small-subunit

LIST OF SYMBOLS

≈ - approximate
bp - base pair
cm - centimetre
℃ - degree Celsius
fmol - femtomole
km - kilometre
L - litre
m - metre
µg - microgram
µL - microlitre
µm - micrometre
µM - micromolar
mM - milimolar
mg - milligram
mL - millilitre
mm - millimetre
min - minute(s)
ng - nanogram
nm - nanometre
% - percentage
psu - practical salinity unit
s - second
×*g* - times gravity
U - unit of enzyme activity

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Marine phytoplankton also known as microalgae are primary producers in the ocean. They contribute approximately 50% to global primary production (Käse and Geuer, 2018), and have adapted to different marine environments with great diversity in morphology, size, metabolism, life cycle and pigments (Matsunaga et al., 2005). Phytoplankton is widely used as an indicator to measure the changes of ecological and biogeochemical processes in the marine ecosystems due to the fact that phytoplankton is highly sensitive to various environmental changes, with a high growth rate and measurable photosynthetic responses (Paerl et al., 2007). Human activities responsible for anthropogenic input in aquatic systems and resulted in increasing nutrient content accompanied by a variation in nutrient ratios which greatly affect the phytoplankton composition and production and altered the ecosystem structure and function (Spatharis et al., 2007; McQuatters-Gollop et al., 2009).

As a result, the proliferation of selected harmful phytoplankton species may result in the formation of harmful algal blooms (HABs) incidence, which is disruptive to aquatic ecosystem health, fisheries production, and tourism in many coastal areas worldwide (Hallegraeff, 2004; Anderson et al., 2012). Phytoplankton that is considered harmful is divided into two main groups: 1) the toxin producers; which can produce various types of algal toxin that contaminate shellfish mollusks or responsible for the mortality of cultured or wild fishes; 2) high-biomass blooms, which cause hypoxia in the surrounding waters and subsequently cause mortalities of marine life (Zingone and Enevoldsen, 2000).

It is still a matter of debate as to the causes of HABs. The possibility of human activities such as nutrient enrichment (from industrial waste, human settlements, agricultural and aquaculture runoff and discharge), climate changes, or transport of algal species via ship ballast water (Maso and Garce, 2006; Anderson et al., 2012; Li et al., 2014). Anthropogenic activities such as aquaculture activities, agricultural fertilizer, urban runoff, and municipal sewage caused the plankton composition shifting and might cause some of the harmful algal species to bloom as well (Buyukates and Roelke, 2005; Spatharis et al., 2007). Generally, it is well accepted human activities in coastal systems increase nutrient loading and consequently affect the phytoplankton composition (Wang et al., 2008). However, phytoplankton species respond differently to various environmental factors. Changes in temperature, precipitation, nutrient stress or availability, and the physical structure of the water column all influence the productivity, composition, and global range of phytoplankton assemblages, but large uncertainty remains about how the integration of these drivers might shape future HABs (Wells et al., 2015; Wells et al., 2020).

The frequency, duration, and geographic extent of harmful blooms and toxic algae are increasing throughout Asia and the world (Glibert et al., 2006; Heisler et al., 2008; Furuya et al., 2010; Glibert et al., 2012). The countries of Asia with particular concern over increasing HABs are China, Philippines, Malaysia, Hong Kong, Japan, and Korea (Furuya et al., 2010, Furuya et al., 2018; Yñiguez et al., 2020). One of the most common environmental effects of HABs is fish kills in the expanding aquaculture and fisheries industry (Anderson et al., 2012; Sanseverino et al., 2016). Damages to the aquaculture industry have been estimated in the tens to hundreds of millions of dollars for blooms that have occurred in Korea, Japan, and the Philippines (Glibert et al., 2014). Single HAB fish-kill events in Korea and Japan have been estimated to have cost more than 100 million dollars in lost aquaculture and wild fish kills (Furuya et al., 2010). Toxic phytoplankton blooms may also lead to shellfish poisoning, which can have severe human health implications including death (Berdalet et al., 2015; Dorantes-Aranda et al., 2015).

Bloom events in Southeast Asia (eg., Philippines, Singapore, Malaysia) are of concern due to its intense dependence on coastal production as a source of livelihood and economy (Hishamunda et al., 2009; Ferrer, 2016, Yñiguez et al., 2020). Tropical bloom dynamics are complex and, unlike some temperate environments, involve a variety of species without a clear bloom pattern (Lim et al., 2012; Leong et al., 2015). Under temperate climates, periods of harsh winters suppress primary productivity, creating empty and low competition niches which become available during spring or summer (e.g., Garneau et al., 2011; Tobin et al., 2013). In contrast, tropical weather variables tend to be consistent throughout the year. Annual seasonality is provided by the monsoons, with nutrients and input elevated by rainfall (Sundarambal et al., 2012) and regulated accumulation of nutrients and pollutants in the marine system (Dippner et al., 2011; Taufikurahman and Hidayat, 2017). Considering the HAB occurrences of *Pyrodinium bahamense* blooms and fish kills events occurrences in Southeast Asia, it seems that transitions from dry to rainy conditions promote increased nutrient enrichment (eutrophication) and a more stable water column during El Niño (e.g., warmer sea surface temperatures) can be the triggering factors (Azanza and Taylor, 2001; Villanoy et al., 2006; Ferrera et al., 2016; Yñiguez et al., 2018).

The rapid advancement of technology in deoxyribonucleic acid (DNA) sequencing and bioinformatics analysis allowed researchers to analyse phytoplankton community structure via massive sequencing of DNA sequences from environmental samples, a process known as “metabarcoding” (Yoon et al., 2016). The DNA metabarcoding technique has also been applied in many global marine environment projects including the Tara Oceans Expedition (Sunagawa et al., 2015) and the Ocean Sampling Day (Kopf et al., 2015). This highly sensitive approach has been widely demonstrated in environmental monitoring in terms of revealing the seasonal phytoplankton community pattern (Gran-Stadniczeñko et al., 2019; Sildever et al., 2019) and capability to detect some previously unknown species (De-Vargas et al., 2015; Egge et al., 2015), novel species/genera (e.g., Nagai et al., 2017) and HABs species (Liu et al., 2020) from the field.

This study sits in Johor Strait, which served as the border between Singapore and Malaysia. The area has developed intensively for marine port activities, urban development (Sakari et al., 2010). Johor Strait is also the major coastal aquaculture area for the two countries, with 85% of the aquaculture cage concentrated along the Johor Strait (Lim, 2016). The construction of the causeway in the year 1923 divided the strait into East and West sides, and water flow was limited between both halves of the strait (Koh, 1991). The causeway across the strait linking Malaysia to Singapore has led to poor flushing regimes (Maren and Gerritsen, 2012; Lee and Zaharuddin, 2014), which has caused the accumulation of pollutants (Wood et al., 1997) and nutrients (Gin et al., 2006). Economy activities differed between the two sides of the Johor strait. The eastern part of the Johor Strait is occupied with shipping and aquaculture farming of green shellfish mussel, *Perna viridis*. Meanwhile, the western Johor Strait served as the main finfish aquaculture site, shipping cargo container port at the outer part of the strait (i.e., Tanjung Pelepas port) and area for a new economic zone of Johor (i.e., Iskandar Malaysia) (Zulkifli et al., 2010). Hence, the strait has been experiencing huge ecosystem damages due to rapid economic development involving various human activities such as seafloor dredging for shipping lane maintenance and land reclamation for residential/commercial properties (Zulkifli et al., 2010). Hydrology has also been altered due to damming of river mouths to create coastal reservoirs (eg., Kranji reservoir, Singapore side) that discharge large volumes of freshwater algae to the inner part of Johor Straits at unpredictable intervals. Some of these reservoirs experience high eutrophication rates due to watershed pollution (Gin et al., 2011; Xing et al., 2014) and therefore have a major influence upon the nutrient status of the coastal waters (e.g., Khoo, 1991). The massive expansion of coastal development and aquaculture activities at both sides of the strait has led to an increase in anthropogenic impacts, increased pollution and eutrophication of coastal waters (Leong et al., 2012, 2015; Zulkifli et al., 2010; Mohd-Din et al., 2020).

HABs events have been documented in Johor Strait water resulted in mass mortalities of cultured fish and shellfish since the 1980s (Khoo, 1985; Usup et al., 2003; Lim et al., 2014a; Teng et al., 2016), with causative species involved in some of these bloom events remained unclear. Bloom of *Chattonella marina* was first reported in the year 1983 and subsequently in the year 1985 along the Johor Strait, and led to shrimp mass mortality during both events (Khoo, 1985). In 2002, the bloom of *Prorocentrum minimum* was confirmed for the first time along the Johor Strait, causing massive water discolouration, but no fish kills were reported during the event (Usup et al., 2003). An unprecedented massive fish kill was reported in 2014 along the Western Johor Strait due to the bloom of *Karlodinium australe* that caused about four tonnes of caged fish killed during the incident (Lim et al., 2014a). The bloom has recurred in the subsequent year, from February to March 2015, with even greater mariculture losses (Teng et al., 2016).

1.2 Problem Statement

HABs events were not new in the Johor Strait, with the incident records that could be traced back to the 1980s and caused mass mortalities to aquaculture and wild of fish and/or shellfish which also led to notable industrial disasters in the mariculture industries of Malaysia and Singapore. The occurrence of harmful algal species in aquaculture areas has posed serious threats not only to the economy but also to the seafood safety of the affected countries. Therefore, understanding the phytoplankton community assemblages and their ecological interaction is crucial to better detect and predict HABs outbreak in the tropical eutrophic of Johor Strait.

To date, data spatial and temporal changes on phytoplankton composition and community assemblages of harmful algal bloom species with regulated environmental drivers in the Johor Strait remained scarce. Taking this into account, further and deeper investigation in this study is crucial to evaluate the bloom dynamic of the phytoplankton including the harmful phytoplankton assemblages and its relationship with environmental factors. This information will contribute better insight into the spatial-temporal pattern, and the phenology of bloomed phytoplankton and HABs species in Johor Strait water.

1.3 Research Questions

The finding obtained in this study was used to address the following questions:

(a) What are the environmental factors that influence the algal bloom dynamic and HAB community assemblages in Johor Strait?

(b) What causes the hypoxia-anoxia condition in the inner part of the Strait?

(c) How diverse are the HAB community assemblages considering the spatial and temporal distributions in the strait?

1.4 Research Hypotheses

(a) Environmental parameters and nutrient dynamics affect the phenology of the phytoplankton community and harmful algal species abundance in Johor Strait.

(b) High biomass of algal blooms induces the formation of hypoxia-anoxia condition in the inner part of the strait.

(c) Different HAB functional groups in the Johor Strait water occupy and occurred in different environmental niches across space and time.

1.5 Research Objective

The objectives of the research are:

(a) To determine the bloom dynamic of diatoms and dinoflagellates community succession.
(b) To determine the environmental parameters and nutrient dynamics affecting the phenology of the phytoplankton community.

(c) To investigate the prolonged high biomass diatom blooms induced formation of hypoxic-anoxic zones in the inner part of Johor Strait

(d) To characterize phytoplankton composition shift using next-generation sequencing with metabarcoding approach emphasizing on HABs species.

1.6 Scope of Study

Sampling stations in this study were selected based on previously known algal bloom hotspots, areas of aquaculture activities (fish cages, mussel farms), and areas with anthropogenic disturbances (human settlements, coastal reclamation) along the Johor Strait. In this work, a field investigation was conducted from January 2017 to September 2019, to evaluate the bloom dynamic of the phytoplankton community and its relationship with environmental drivers in Johor Strait water. Phytoplankton and water samples were collected fortnightly during high tide in the proposed ten stations located along the Johor Strait. Environmental parameters such as dissolved oxygen (DO), temperature, pH and salinity were measured *in situ*; chlorophyll *a* (Chl *a*) and nutrients i.e., [(nitrate-nitrogen (NO_3-N)], [ammonia-nitrogen (NH_3-N)], [nitrite-nitrogen (NO_2-N)], [silicate-silica (SiO_3-Si)] and [phosphate-phosphorus (PO_4-P)] were also determined spectrophotometrically. An attempt has been made to investigate the prolonged high biomass diatom blooms induced formation of hypoxic-anoxic zones in the inner part of Johor Strait as an effort to continuously monitor the water quality and occurrence of algal bloom in the area.

Currently, the spatial and temporal dynamics of HAB community assemblages and the regulating environmental drivers remain elusive. Furthermore, previous studies relied mainly upon a conventional microscopic approach in determining phytoplankton composition and abundance, which was labour-intensive, time-consuming, and required taxonomic expertise in species identification. Hence, we explore the spatial-temporal structure of HAB community assemblages along the Johor Strait by applying a metabarcoding approach targeting the 18S ribosomal RNA (rRNA) gene marker. A total of 19 sampling stations spanning ≈65 km long at the Johor Strait was selected to represent the water property gradients. The responses of HAB community assemblages were presented by the changes in composition and abundances of HAB taxa based on the metabarcoding assay. Hence, this study is vital to a better understanding of the bloom formation and dynamics of HABs in this unique ecologicallyand economically important ecosystem.

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