COMMUNITY STRUCTURE ANALYSIS OF PHYTOPLANKTON AS AN INDICATOR OF MANGROVE ECOSYSTEM HEALTH

By

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Mangrove ecosystems are substantial to water quality management as it helps to improve water quality by absorbing pollutants from runoff. Unfortunately, deforestation of mangroves in Malaysia are in a critical state due to the development of urban population and currently prompted to several issues. Lack of sustainable mangrove management in addition to high anthropogenic pollutants from runoff could lead to marine eutrophication and subsequently distressed the biological communities. The occurrence of phytoplankton is particularly an important ecological indicator in accessing mangrove ecosystem health since phytoplankton react as a key role for mangrove productivity that form essential base of oceanic food webs. Phytoplankton community species can define the major functional groups that ultimately influence ecosystem pollution due to the occurrence of harmful algae blooms. This study was aim to identify the phytoplankton communities uses as bioindicators of mangrove estuarine areas. In this study, phytoplankton communities and its regional physicochemical parameters were explored to identify the potential uses of phytoplankton as a reliable indicator of mangrove ecosystem, as well as to formulate a phytoplankton-based index to access mangrove pollution. Two major locations of the Matang mangroves of Perak and the Pendas mangroves of Johor had been selected, representing various mangrove disturbance and pollution levels. Rivers namely Tiram Laut, Tinggi and Sepetang (located in the Matang mangroves of Perak) while Pendas (located in the Pendas mangroves of Johor) were classified based on ground truth to represent the pristine mangroves of the least disturbance (Tiram Laut), moderately disturbance (Tinggi), high disturbance (Sepetang) and highly degraded area with very high disturbance (Pendas). *In situ* water parameters and water samples for phytoplankton enumeration, chlorophyll *a*, total solids and water nutrients were collected monthly for one-year cycle using 5L Niskin water sampler. Phytoplankton abundance and composition to the lowest taxa were investigated using Sedgewick Rafter counting chamber, while water nutrients analyses were done based on standard methods using spectrophotometric analysis. Among phytoplankton groups, diatoms and dinoflagellates were the main phytoplankton groups that represented as the highest densities contributed >60% of the total phytoplankton community in all stations. A centric diatom, *Skeletonema costatum* was a major diatom species found in the moderately disturbed, highly disturbed and highly degraded area, blooms at the higher rate constituting 64.6% of the mean total phytoplankton which showed increased densities with the increasing level of disturbance. The survivals of *Skeletonema* species in the moderately disturbed area (1.14 x 10^5 cells/L) were associated to temperature and total nitrogen ($p < 0.05$), while turbidity was significantly in highly reclaimed area as the blooms occurred during dry weather of the northeast monsoon season. On the other hand, *Cyclotella choctawhatcheeana* has indicated the pristine area, controlled by transparency and salinity, while small temperature elevation and eutrophication had led to dinoflagellates blooms of *Protoperidinium acutum* in the highly disturbed mangrove area. Dissimilar phytoplankton community structure was noted in the highly degraded mangrove area of the Pendas mangrove Johor, due to the occurrence of numerous harmful dinoflagellates blooms including *Peridinium quinquecorne*, *Karenia* sp., *Prorocentrum lima*, and *Karlodinium australe*. The harmful unarmored *Karlodinium australe* and a centric diatom *Skeletonema costatum* were main phytoplankton group occurred in the highly degraded mangrove area associated to the total dissolved solids and nutrients ($p < 0.05$). The present study suggested that the phytoplankton biodiversity declines and the abundance of harmful species increased with increasing disturbance levels. Based on the phytoplankton community data within different levels of mangrove disturbance, the best four metrics were concluded using PCA tool for the development of the Phytoplankton-Blooms Class Index (*PbCI*). Phytoplankton indicators based on cells formation or types of species blooms recorded were ranked from 1 to 5 in order to assess mangrove ecosystem health. This study illustrated the changes in phytoplankton community which influenced by disturbance that significantly important to indicate the status of mangrove ecosystem health.

Keywords: mangroves, phytoplankton, harmful algae blooms, pollution, health index

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
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ANALISIS STRUKTUR KOMUNITI FITOPLANKTON SEBAGAI INDIKATOR KESIHATAN EKOSISTEM HUTAN PAYA BAKAU

Oleh

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Ekosistem hutan paya bakau adalah sangat penting bagi pengurusan kualiti air kerana ia dapat memperbaiki kualiti air dengan menyerap bahan pencemar yang dibawa melalui kelimpahan air sungai. Malangnya, penebangan hutan paya bakau di Malaysia telah berada pada tahap membimbangkan terutamanya bagi pembangunan bandar yang kini telah mengakibatkan pelbagai isu. Kelemahan pengurusan hutan bakau yang tidak mampan serta pencemaran antropogenik yang tinggi menyebabkan berlakunya eutrofikasi dan seterusnya mengganggu keseluruhan komuniti biologi. Kelimpahan komuniti spesis fitoplankton merupakan petunjuk yang penting sebagai indikasi kesihatan bagi ekosistem hutan paya bakau kerana ia mempengaruhi produktiviti hutan paya bakau yang bertindak sebagai unsur asas dalam siratan makanan hidupan marin. Penentuan spesis fitoplankton dapat menjelaskan kumpulan komuniti utama yang mempengaruhi pencemaran ekosistem terutamanya dengan kelimpahan spesis berbahaya. Kajian ini dijalankan dengan matlamat untuk mengenalpasti komuniti fitoplankton sebagai penunjuk biologi di perairan hutan paya bakau. Dalam kajian ini, kelimpahan komuniti fitoplankton dan unsur fizikokimia dalam lingkungannya telah dikaji bagi mengenalpasti potensi penggunaan fitoplankton sebagai penunjuk biologi yang jitu bagi ekosistem hutan paya bakau, justeru dapat merumuskan indeks berasaskan fitoplankton untuk megakses pencemaran hutan paya bakau. Dua lokasi utama iaitu di Hutan Paya Laut Matang, Perak dan Hutan Paya Bakau Pendas, Johor telah dipilih bagi mewakili pelbagai tahap ancaman dan pencemaran. Sungai yang dikenali sebagai Tiram Laut, Tinggi dan Sepetang (terletak di Hutan Paya Laut Matang, Perak), serta Pendas (terletak di Hutan Paya Bakau Pendas, Johor) telah diklasifikasikan berdasarkan kaedah pemerhatian setempat, menggambarkan kawasan tidak tercemar iaitu paling kurang terganggu (Tiram Laut), kawasan sederhana terganggu (Tinggi), kawasan yang banyak terganggu (Sepetang), dan kawasan yang sangat banyak terganggu (Pendas). Pengukuran parameter secara *in situ* dan sampel air untuk penentuan kandungan fitoplankton, klorofil a, jumlah ampaian dan kadar nutrien air telah diambil pada setiap bulan selama setahun

menggunakan alatan persampelan Niskin 5L. Kelimpahan dan komposisi fitoplankton mengikut taxa yang terendah telah disiasat dengan metod perhitungan langsung menggunakan slaid Sedgewick Rafter, manakala analisis kandungan nutrient air telah disempurnakan dengan metod yang standard menggunakan alat spektrofotometer. Berdasarkan pemerhatian, kumpulan fitoplankton yang dikenalpasti mempunyai kepadatan tertinggi adalah dalam kelompok diatom dan dinoflagelat, yang telah menyumbang >60% daripada jumlah komuniti di semua stesen. *Skeletonema costatum* adalah diatom berbentuk sentrik yang merupakan spesis umum yang utama dijumpai, melimpah dengan kadar yang tinggi sebanyak 64.6% daripada jumlah purata fitoplankton, dan telah meningkat selari dengan peningkatan ancaman pencemaran. Kepadatan species *Skeletonema* di kawasan gangguan sederhana (1.14×10^5 cells/L), telah dipengaruhi oleh suhu air dan jumlah kandungan nitrogen ($p < 0.05$), manakala di kawasan yang ditebus guna, dipengaruhi oleh kekeruhan air, dan ia telah berkembang biak pada musim monsoon timur laut yang kering. Tambahan pula, kepadatan *Cyclotella choctawhatcheeana* telah menjadi penunjuk kepada kawasan tidak tercemar, yang dikawal oleh faktor ketelusan dan kemasinan air, manakala perubahan peningkatan suhu air dan eutrofikasi telah menyebabkan kelimpahan dinoflagelat *Protoperidinium acutum* di kawasan yang sangat terganggu. Kelimpahan komuniti fitoplankton yang berbeza telah direkodkan di kawasan hutan paya yang ditebus guna di Pendas, dengan kehadiran pelbagai spesis dinoflagelat berbahaya yang terdiri daripada *Peridinium quinquecorne*, *Karenia* sp., *Procentrum lima* dan *Karlodinium australe*. Spesis dinoflagelat berbahaya, *Karlodinium australe* dan diatom *Skeletonema costatum* merupakan species ledakan utama yang ditemui di kawasan yang ditebus guna, dikawal oleh faktor jumlah larutan terampai dan nutrien air ($p < 0.05$). Kajian ini telah membuat kesimpulan bahawa biodiversiti fitoplankton akan merundum dan kepadatan spesis berbahaya akan meningkat selari dengan peningkatan kadar ancaman pencemaran. Berdasarkan data komuniti fitoplankton ini, empat pembolehubah telah disimpulkan melalui teknik PCA bagi penghasilan indeks ‘Phytoplankton-Blooms Class Index’ (*PbCI*). Indikator fitoplankton yang direkodkan adalah berdasarkan kategori atau jenis formasi spesis yang melimpah, dan telah dikelaskan pada julat 1 sehingga 5 bagi mengenalpasti tahap kesihatan hutan paya bakau. Kajian ini menggambarkan perubahan komuniti fitoplankton yang telah dipengaruhi oleh ancaman dan gangguan pencemaran yang amat penting bagi mengenalpasti status kesihatan ekosistem hutan paya bakau.

Kata kunci: hutan paya bakau, fitoplankton, kelimpahan alga berbahaya, pencemaran, indeks kesihatan

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%	percentages
&	and
ºC	degree celsius
µg	microgram
µm	micrometre
ANOSIM	analysis of similarity
ASP	amnesic shellfish poisoning
Bio-ENV (BEST)	best linking of biotic patterns to suites environmental variables
CCA	canonical correspondence analysis
CFP	ciguatera fish poisoning
Chl-a	chlorophyll a
Cd	cadmium
cm	centimetre
CO_2	carbon dioxide
DA	domoic acid
DSP	paralytic shellfish poisoning
H'	diversity index
Ha	hectares
HABs	Harmful Algal Blooms
HCl	hydrochloric acid
HDPE	high density polyethylene
$KMnO_4$	potassium permanganate
L	litre
LM	light microscope

m^3	metre square
MEAN	average
Mg	milligrams
$MgCO_3$	magnesium carbonate
mg	milligrams
ml	millilitres
mm	millimetres
mS	millisiemens
MMFR	Matang Mangrove Forest Reserve
NaOH	sodium hydroxide
NTU	nephelometric turbidity unit
OA	okadaic acid
PCA	principle component analysis
PRIMER	Plymouth Routines in Multivariate Ecological Research
PSP	paralytic shellfish poisoning
pSc	proportion of variables
PSU	practical salinity unit
RELATE	similarity matrices
SE	standard error
SEM	scanning electron microscope
SIMPER	similarity percentages
SRP	soluble reactive phosphorus
TAN	total ammonium nitrogen
TDS	total dissolved solids
TN	total nitrogen
TP	total phosphorus

TS	total solids
TSS	total suspended solids
TRIX	the trophic index
VSP	venerupin shellfish poisoning
wi	weight
wT	sum of proportion of variables

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Mangrove forests are mainly found along the coastlines of tropical and subtropical region, providing valuable socioeconomic benefits. As a productive ecosystem, mangrove forest is a vital component of coastal blue carbon with natural buffer reaction in adapting to climate change through carbon sequestration by absorbing anthropogenic CO_2 emission from the atmosphere (Ghazaly et al., 2019). Additionally, organic matters within productive mangrove forest as well as those produced by tidal inundation, will regulates CO_2 through the decomposition of mangrove litters and trap carbon in mangrove sediments (Jessen et al., 2021; Bouillon, 2011). Mangrove forest is capable of absorbing CO_2 from the atmosphere at approximately 40% higher than rate of other forms of rainforest (Alongi, 2014).

Despite its beneficial role in absorbing atmospheric CO_2 excess, mangrove ecosystems can also be affected by the impact of climate change through extreme weather such as shift of normal rainfall patterns, high air temperature and heat, sea level rise, strong hurricanes and ocean acidification (Duke et al., 2017). Strong tidal currents and high temperature concurrently become the main factors initiating the emission of CO_2 from mangrove sediments (Hien et al., 2018; Collins et al., 2017). Moreover, Sippo et al. (2020) indicated that the emission of CO_2 from mangrove sediments are major in deforested mangrove area, compared to the productive mangrove forest in which the major carbon losses are released to the oceans (83% of total carbon losses) as dissolved inorganic materials that supporting aquatic food web.

Mangroves ecosystem also capable to improve water quality of their ecosystem due to its complex mangrove root systems by filtering contaminants and pollution from runoff, as well as holding onto sediments to reduce erosion and leads to a better water clarity. Restoring and sustaining mangrove ecosystems is substantial to support the ecological link of aquatic food web since mangrove ecosystems are well known to support high biomass of secondary consumers which also provide the economic opportunities in terms of fishery yields (Muro-Torres et al., 2020). According to Sheaves (2017), 9.6 % of fish species (3,161 taxa) were recorded to use mangrove ecosystem. Most of them are juvenile fishes that support over 50% of annual fisheries landing in Malaysian (Wong and Yong, 2020; Piah et al., 2018). However, the main biological components of the mangrove ecosystem are consisting mangrove trees and phytoplankton communities which substantial for mangrove productivity. Phytoplankton are drifting microalgae, whose community structure is greatly influenced by various environmental factors.

Phytoplankton communities are essential in controlling whole mangrove ecosystem since they are capable to sustain energy of the entire ecological system by producing organic compounds from inorganic materials to support mangrove biota (Tičina et al., 2020; Cloern et al., 2014). They form the initial biological component for carbon fixation in the autotrophic marine food chain that allows energy to be transferred to the higher trophic levels (Saifullah et al., 2016). They remove carbon from the surface water through fixed organic matter that sink onto water sediment (Boeuf et al., 2019; Turner, 2015). As eloquently stated by Alongi and Mukhopadhyay (2015), approximately 70% of the total carbon in mangrove sediment is shifted to the coastal waters as dissolved inorganic carbon that support aquatic food web. Thus, changes of phytoplankton community can be used as reliable indicators of water quality and environmental health status (Frau et al., 2019; Challouf et al., 2017; Armstrong, 2003).

The economic viability of many existing and proposed activities on and around Malaysian coasts are dependent on the regulatory measures by the government of Malaysia (Coastal Engineering Division of the Department of Irrigation and Drainage). Recently, tropical and subtropical estuarine ecosystems were found to be seriously affected by climate change and anthropogenic activities (Hallett et al., 2018), but these impacts have yet to be assessed. In addition, rapidly expanding urbanization and development in Malaysian coastal area possess serious threats to mangrove ecosystems since these activities have potential to cause mangrove depletion. Loss of mangrove trees have impacted habitat changes, loss of food, increase of water turbidity, water quality deterioration and decline the fisheries production (Wilkinson et al, 2018).

There are a lot of governmental and non-governmental agencies that have raised concerns on maintaining mangrove ecosystem health and have addressed long-term sustainable development of the ecosystem in the face of rising challenges from unsustainable economic practices. Consequently, the management on mangrove ecosystem is important to ensure each of different components of mangrove ecosystem are connected and well function in order to support high biodiversity and productivity of the mangrove-marine food web. More importantly, the health status of mangroves should always be seriously considered since it has high contribution to local livelihoods. Long-term benefits can be provided for future generations if appropriate conservation and management practices are implemented.

Over the last decades, there has been increasing awareness of mangroves health deterioration which could destroy our natural resources as well as seriously affected the quality of human life due to the occurrence of harmful phytoplankton species. Moreover, recent cases related to HABs of the coastal tropical waters are increased. The magnitude, frequency, and duration of HABs are increasing worldwide primarily due to climate change and anthropogenic activities in the coastal waters (Glibert, 2020; Wells et al., 2015). Shifts in phytoplankton species and the occurrence of HABs can be deleterious to aquaculture and fisheries sectors. According to Paerl et al. (2016), HABs can be induced by high loads of nutrients. The harmful phytoplankton blooms can cause shellfish poisoning to human and lead to massive fish kills, as well as generating economic losses (Moore et al., 2019; Lim et al., 2014). The relationship between phytoplankton community structure in coastal areas with the physicochemical parameters should be well understood to identify factors controlling the mangrove ecosystem health, thereby protecting and improving mangrove ecosystems (Inyang and Wang, 2020; Choudhury et al., 2015).

1.2 Problem statements

Prolonged anthropogenic activities and tropical climate change have caused massive impacts on the coastal ecosystem along Malaysian waters including mangrove habitat. Despite mangrove's critical important, the imminent threats due to anthropogenic pressures can kill them and therefore more sustainable solution to preserve mangrove's water quality is very much needed. To achieve the sustainable use of mangrove resources, the influence of physicochemical processes and functional variations of phytoplankton community structure due to anthropogenic activities need to be determined. Basically, knowledge on phytoplankton biodiversity, productivity and abundance in relation to nutrients and environmental parameters are essential to assess the overall status of the mangrove ecosystem. Research in examining the structure of phytoplankton communities in relation to water quality of Malaysian mangrove estuaries has been elucidated but the overall structure remains unclear. Wan Maznah et al. (2016) had reported spring and neap phytoplankton variability at the Merbok River Estuary in Peninsular Malaysia. Unfortunately, the information on seasonal phytoplankton variability of mangrove estuaries is still needed. Furthermore, no supporting physicochemical conditions data associated on the levels of mangrove disturbance and elaboration of the role of nutrients and other water parameters in starting harmful algal blooms are still lacking. The interrelation between phytoplankton distribution and physicochemical parameters should be well understood in order to identify the factors that controlling mangrove ecosystem health to improve mangrove protection.

As an initial biological component in mangrove ecosystem, data on species composition, abundance, and distribution of phytoplankton, particularly that contributed to HABs event are essential since they undergo spatial-temporal changes in their distribution based on different hydrographical and environmental pressure. There is limited study on harmful phytoplankton as indicators for measuring the impact of mangrove logging activities. An understanding of the species dynamic of phytoplankton will be able to provide a clear view of the ecosystem health since phytoplankton are known as an effective bioindicator of water quality as its growth has higher tolerant to contaminant and pollution. Environmental distress may favour tolerant species over more sensitive ones and subsequently reducing species richness. Although sensitive species were reduced or even disappeared, the displacement of the species surely contributed to sustain phytoplankton species diversity. Some phytoplankton, especially blue-green algae and dinoflagellates can form blooms that could be harmful to others communities along the food chain including humans (Furuya et al., 2018). The shift in phytoplankton community structure of mangrove-estuarine ecosystem should be well understood in order to identify the occurrence of harmful species that can give negative impact to other biological communities as well to the economic sector.

Essentially, the health status of mangroves should always be seriously considered since they are highly contributing to local livelihoods. Detailed studies on phytoplankton dynamics in tropical Malaysian mangrove ecosystem may give valuables information on environmental condition to avoid serious economic loses to local livelihoods. Long-term monitoring of water quality is not a simple task because it is time consuming and involving large number of chemicals used. Essentially, an effective solution to assess water quality in mangrove ecosystem should be cost-effective, time-saving and capable to yield data accuracy. As an initial biological components of water ecosystem that shift accordingly to environmental changes, phytoplankton are a well-known indicator of water quality that capable to be use as index in order to measure ecosystem health and pollution levels. Phytoplankton indices in identifying the status mangrove estuarine-ecosystem are very crucial. More importantly, specific phytoplankton-based index for the assessment of mangrove health status are at limiting number and still needed. The identification of quantitative expressions based on phytoplankton species composition as metrics that could indicate ecosystem health status are crucial and can be useful tool to increase the efficiency in identifying water quality status and provide solution to reduce environmental losses.

1.3 Research objectives

1.4 Main objective

The main objective of this study was to analyze phytoplankton community structure and indicator species of different areas with different disturbance levels within mangrove ecosystem that are reliable to assess the status of mangrove ecosystem health.

1.4.1 Specific objective

This study was undertaken with the following objectives:

1. To examine the dynamic changes of phytoplankton species composition, abundance, distribution, and diversity in different areas with different disturbance levels within mangrove ecosystem.

2. To analyze the relationship between phytoplankton community structure, especially harmful species and the environmental parameters based on areas with different mangrove disturbance and pollution levels;

3. To establish phytoplankton-based index as a reliable indicator of mangrove ecosystem health.

1.5 Hypothesis

In this study, two mangrove ecosystems along coastal waters of Peninsular Malaysia (Matang mangrove of Perak and Pendas mangrove of Johor) with different mangrove disturbance and pollution levels were selected to assess the following hypotheses:

1. Null hypothesis (H0):
   There is no difference in phytoplankton community structure (species composition, distribution and diversity) with different levels of mangrove disturbance and pollution.

   Alternate hypothesis (Ha):
   There is a difference in phytoplankton community structure (species composition, distribution and diversity) with different levels of mangrove disturbance and pollution.

2. Null hypothesis (H0):
   Harmful phytoplankton is not an indicator for measuring the impact of mangrove logging activities and water pollution.

   Alternate hypothesis (Ha):
   Harmful phytoplankton is an indicator for measuring the impact of mangrove logging activities and water pollution.

3. Null hypothesis (H0):
   Phytoplankton-based index is not a reliable indicator to assess mangrove ecosystem health.

   Alternate hypothesis (Ha):
   Phytoplankton-based index is a reliable indicator to assess mangrove ecosystem health.

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