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berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.

@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).



FACTORS CONTRIBUTING TO COCHLODINIUM POLYKRIKOIDES BLOOM IN SEPANGGAR BAY

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THIS DISSERTATION IS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE BACHELOR OF SCIENCE WITH HONOUR IN MARINE SCIENCE.

PERPUSTAKAAN UMIMERSITI MALAYSIA SABAH

MARINE SCIENCE PROGRAMME SCHOOL OF SCIENCE AND TECHNOLOGY UNIVERSITI MALAYSIA SABAH

MAC 2007



I affirm that this dissertation is of my own effort, except for the materials referred to as cited in the reference section.

15th MARCH 2007

LEE SIOW LING

HS2004-4642



CERTIFICATION

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- 2. EXAMINER 1 (Dr. Sitti Raehanah Muhd. Shaleh)
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4. DEAN (Supt./Ks. Prof. Madya Dr. Shariff AK Omang)



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ABSTRAK

Objektif kajian ini adalah untuk menentukan factor-faktor yang mencetuskan keadaan peningkatan populasi Cochlodinium polykrikoides. Cochlodinium polykrikoides pertama dilaporkan di perairan Sabah pada akhir tahun 2003. Semenjak itu, spesis ini ditemui di perairan kita berulang kali. C. polykrikoides tidak membebaskan toxin tetapi ia telah menyebabkan kerugian pada aktiviti marikultur di Jepun dan Korea dengan membunuh ikan melalui kelemasan. Laut berwarna merah yang disebabkan oleh C. polykrikoides ditemui sekali lagi pada Mei-Jun,2006, di kawasan perairan cetek Sepanggar Bay. Dalam kajian ini, penukaran warna air laut diperhatikan dua hari selepas dua hari hujan yang berterusan. Dengan suhu 29-31°C. Kemasinan laut dalam 30.53-33.11 psu. Purata DO dan pH adalah 5.9 mg/L and 8.07. Air yang mempunyai turbiditi rendah(20-40psu) merangsang peningkatan populasi C. polykrikoides. Kepekatan fosfat adalah 0.0051-0.0085 µM/L, manakala nitrat adalah 0.0185-0.0328 µM/l . Peralihan komuniti phytoplankton juga merupakan salah satu faktor. Bagaimanuapun, faktor-faktor ini biasanya saling berinteraksi dan memberi kesan bersama-sama. Bilangan C. polykrikoides dalam lingkungan 7 hingga 1893/cell ml⁻¹ Dalam kajian ini, perbandingan dibuat antara stesen 1 di sangkar ikan dan stesen 2 di kawasan laut terbuka menunjukkan bahawa nutrien adalah faktor utama di stesen 1, manakala pH dan DO merupakan faktor penting di stesen 2. Aktiviti antropogenik seperti eutrofikasi daripada air limpahan ke kawasan ini, pengayaan nutrient dari sangkar ikan; cahaya matahari sepanjang tahun; tambah dengan faktor hujan dan angin; serta sifat air laut yang baik, memyumbang kepada peningkatan populasi dinoflagellate ini.



V

ABSTRACT

The objective of this dissertation is to study the factors controlling Cochlodinium polykrikoides bloom. Cochlodinium polykrikoides was first reported in Sabah water end of 2003. The species is since recurring in our waters. C. polykrikoides do not release toxin but it had caused losses in mariculture activities in Japan and Korea through killing fishes by suffocation. The red discolouration is observed again during May -June, 2006 in a shallow coastal waters of Sepanggar Bay was also caused by C. polykrikoides. In this study, red water discolouration were observed two days after two rainy days with a seawater temperature of 29 to 31°C. Salinity is of 30.53-33.11 psu. Average DO and pH in this study is 5.9 mg/L and 8.07. Waters of low turbidity(20-40 psu) favoured the bloom of C. polykrikoides according to this finding. Nutrient concentration range during the bloom were 0.0051-0.0085 µM/L phosphate , and 0.0185-0.0328 µM/L nitrate. The shift in the phytoplankton community structure also contribute to the bloom. However, these factors sometimes work jointly to give an interaction effect. Abundance of C. polykrikoides ranged from 7 to 1893 /cells ml⁻¹. In this study, comparison of C. polykrikoides bloom between station 1 at cage culture and station 2 without cage culture shows that nutrient is a dominant factor in station 1, whereas pH and DO is the main factors in station 2. Anthropogenic activities such as eutrophication caused by water discharge in this shallow coastal area, and nutrient enrichment in the cage culture area ; year-long sunlight, as well as effects from precipitation and wind stress, coupled with the ideal seawater properties, could have favored the outbreak of this dinoflagellate.



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CHAPTER 1

INTRODUCTION

1.1 Harmful Algal Bloom

Legendre (1990) defined phytoplankton blooms as rapid increases in algal biomass caused by transient imbalances between the rate of primary production and the rate of loss of photosynthetically fixed carbon due to respiration, grazing, and advection. Although the use of the terms "bloom" or "red tide" conjure up an image of algal populations so dense as to be visible, this is not always the case with HABs. Concentrations of only a few cells per liter of some microalgae may produce harmful toxic effects.

Red tide or harmful algal bloom (HAB) is an occasional natural phenomenon in Sabah. During late 2003, caused by *Cochlodinium polykrikoides*, was noticed off Northern Borneo. In Japan and Korea, bloom of *Cochlodinium polykrikoides* has caused severe damage to fisheries during summer and fall(Kim *et al.*,1999). It causes the suffocating of fishes as *Cochlodinium polykrikoides* depletes the oxygen in the



water causing fish kills. Millions ringgits worth of cultured marine fish in cages were killed and lost in Japan and Korea due to these species(Kim et al., 1999).

Studies in eastern Asia found that the optimum conditions for *Cochlodinium* bloom to be of salinity 32-34 psu, temperature 25-28°C, and sufficient sunlight (NOWPAP CEARAC, 2006), which are very similar to the oceanographic conditions in Borneo. However, the study on *Cochlodinium polykrikoides* in Southeast Asia has just started and not much information was available for this species. The *Cochlodinium polykrikoides* bloom was suspected to be originated from ship ballasting activities, however, latest DNA analysis showed the species bloom in Northern Borneo is independent from the species in Korean or Japanese waters (Iwataki *et al.*, 2005).

Thus, my study concentrate on factors that are contributing to Cochlodinium polykrikoides blooms.

1.2 Objectives

- 1. To study the factors controlling the Cochlodinium polykrikoides bloom.
- To compare the factors contributing to *Cochlodinium polykrikoides* bloom in station near cage culture and with no cage culture influence.
- 3. To accumulate data for future study, as mitigation measures can be carried out.



1.3 The Significance of Study

Although HABs occurred long before human activities began to transform coastal ecosystems, a survey of affected regions and of economic losses and human poisonings throughout the world demonstrates very well that there has been a dramatic increase in the impacts of HABs over the last few decades and that the HAB problem is now widespread, and serious. It must be remembered, however, that the harmful effects of HABs extend well beyond direct economic losses and impacts on human health. When HABs contaminate or destroy coastal resources, the livelihoods of local residents are threatened.

This study is conducted following the increased incidence of water discoloration and report of losses to mariculture activities. It is found out that the causative species is *Cochlodinium polykrikoides*. Therefore, the factors regulating the bloom of cochlodinium must be study in order to instill an effective mitigation measure in the future. Besides, there is not much study regarding the effects of water properties on the HAB dynamics in the Malaysia waters. Thus, this research is carried out in the hope of finding an effective solutions towards the outbreaks of Harmful Algal Bloom.



CHAPTER 2

LITERATURE REVIEW

2.1 Cochlodinium polykrikoides

Cochlodinium polykrikoides is an unarmoured, marine, planktonic dinoflagellate species with a distinctive spiral-shaped cingulum. It is a common red tide phenomenon associated with fish kills in Japan and Korea. *Cochlodinium polykrikoides* cells are small, oval and slightly flattened dorso-ventrally. It forms chain under optimum condition, usually from four to eight cells (Figure 2.1). An individual cell has an apical groove is originating from the anterior end of the cingular and sulcal juncture and extending to the dorsal side of the epitheca (Figure 2.2). Cells range in size from 30-40 um in length to 20-30 um in width (Yuki and Yoshimatsu 1989; Fukuyo *et al.* 1990; Taylor *et al.* 1995; Steidinger and Tangen 1996).

Cochlodinium polykrikoides is a known red tide species associated with extensive fish kills and great economic loss in Japanese and Korean waters (Fukuyo et al., 1990; Kim, 1998). Cochlodinium polykrikoides is a cosmopolitan species found in warm temperate and tropical waters (Steidinger and Tangen, 1996). This species was first



reported from the Caribbean Sea along the southern coast of Puerto Rico (Margelef, 1961). It has since been reported in northern Atlantic waters along the American east coast: Barnegat Bay, New Jersey (Silva, 1967), and the York River, Virginia (Zubkoff *et al.*, 1979). It is widely distributed in northwestern Pacific waters along the coasts of Japan and Korea (Fukuyo *et al.* 1990; Kim, 1998).



Figure 2.1 : Chain of 4 Cochlodinium polykrikoides

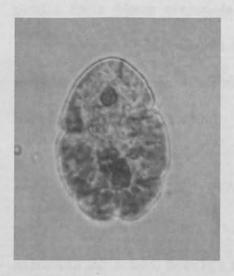


Figure 2.2 : Cochlodinium polykrikoides



2.2 Possible Factors Contributing to Cochlodinium polykrikoides Bloom

2.2.1 Temperature

Temperature limits distribution of ocean phytoplankton and other organisms by affecting density, salinity, and concentration of dissolved gas in the oceans, as well as influencing metabolic rates and reproductive cycles of marine organisms. There can be large differences within one area in phytoplankton species abundance and diversity due to thermoclines. It is formed when surface of ocean is heated by sunlight and warmer surface water cannot displace the dense, colder deep water, causing waters to be stratified. Bloom usually develop on stratified water. However, water over the continental shelf is usually well-mixed and a large thermocline doesn't exist since it is not deep enough. Phytoplankton bloom seldom occurs in well-mixed waters. Temperature can enhance phytoplankton growth rate (Eppley, 1972). Sometimes, a threshold temperature value is needed for a bloom events. In some regions, major spring blooms can occur at temperatures $\leq 0^{\circ}$ C (Townsend *et al.*, 1994). Therefore, it is unlikely that temperature plays a critical role in algal bloom especially in the tropical region where annual temperature variation is small.

2.2.2 Salinity

In most of the oceans, there is a marked difference in salinity between the surface zone and the deep zone. Salinity varies slightly from place to place around the world, and also



varies somewhat with the seasons, affected by temperature and precipitation. The salinity of sea water ranges from about 30 to 40 psu (Castro and Huber, 2003). Phytoplankton native to environment with fairly constant salinity through space and time may not tolerate any change in salinity, as they may be adapted to that specific salinity (Valiela, 1999). Moreover, salinity effects are often associated with the nutrient levels (nitrate, phosphate). Salinity is known to be an important abiotic factor affecting phytoplankton growth. However, the lowest salinity allowing growth is not well documented.

2.2.3 Dissolved oxygen

Oxygen is vital to the survival of marine organisms and it enter ocean by diffusing from atmosphere into surface waters; transported to lower depths by vertical mixing. Phytoplankton generates oxygen by photosynthetic activity. Oxygen is continuously being produced by algae and other aquatic plants and by wind and wave action. It is removed from the system through respiration of aquatic animal organisms, by the biological oxygen demand (BOD) of organisms such as bacteria that break down nonliving organic material, and even by a chemical oxygen demand (COD) caused by decay of dead plants and animals (Redfield *et al.*, 1958). Thermocline can also decrease the level of oxygen due to the high level of bacterial respiration at this depth, but below the thermocline, the amount of dissolved oxygen may increase again since oxygen solubility is increased by colder waters and there are fewer organisms living in deep waters.



2.2.4 Turbidity

Turbidity is influenced by tidal range which affect sediment resuspension rates, and therefore turbidity, which affects water column irradiance. Light availability is the critical source that triggers the spring bloom in the marine environment (Townsend *et al.*, 1994). As in our countries, sunlights are available all year round. However, turbid water will inhibit light penetration to the sea. Surface irradiance varies seasonally and is further modulated by weather condition which are responsible for marked inter-annual variations within each season. Furthermore, the amount of light available for phytoplankton also varies as a function of water transparency and surface mixed layer depth (Townsend *et al.*, 1994). Townsend *et al.* (1994) ran a regression analysis with the pooled data of 5 years combined. It showed that of the meteorological and hydrographical factors, mean water irradiance is the variable that best correlated with chlorophyll a concentration.

2.2.5 pH

The pH of seawaters has a significant effect on the toxicity of total ammonia. Total ammonia is composed of toxic unionized ammonia (NH₃) and nontoxic ionized ammonia (NH4). At high pH levels, total ammonia is shifted more towards its toxic unionized component. Conversely, when pH levels drop, the trend is reversed and total ammonia is shifted back toward its nontoxic ionized form. Phytoplankton utilize nitrogenous waste products in the form of ammonia and nitrite as nutrient sources for growth. Phytoplankton were found to grow over a wide range of pH with some species being



more tolerant of pH shifts while others were sensitive to changes of just 0.1 units. This suggests that shifting pH may affect not only calcifying phytoplankton species but others as well. This has the potential to change the overall distribution of species at the base of the food web. The acidification of the oceans as a result of CO_2 uptake is predicted to have deleterious effects on calcifying organisms such as corals and some phytoplankton species, for example the cocolithophorids (Orr *et al.*, 2005). Yoshimura (2003) also proposed that pH exerts effect to phytoplankton growth by influencing Ferum dissolution in seawater and thus, Ferum uptake by phytoplankton.

2.2.6 Other phytoplankton

Some phytoplankton shows in-between species association. Some species depends on other species for food. Competition also cause the shift in species assemblages if the environmental condition change(Cynthia and Jorge, 2005). It is not known whether *C*. polykrikoides display such characteristic.

2.2.7 Nutrients availability

Phytoplankton growth is influenced by the presence of nutrients, derived either from river-water discharge, precipitation proceedings or from marine upwelling processes. If sufficient nutrients are available, blooming of phytoplankton may occur, either of a single species, or of many species at the same time. They need a wide variety of chemical elements but the two critical ones are nitrogen and phosphorous since they are needed in



quite large amounts but are present in low concentrations in seawater. Phytoplankton need nutrients in well defined ratios. For every 106 atoms of carbon they make into organic matter, they need 16 atoms of nitrogen and 1 atom of phosphorous(Castro and Huber, 2003) Most organisms can't use atmospheric nitrogen gas (N_2) directly but need chemically reactive forms of nitrogen such as nitrate (NO₃) or ammonium (NH₄⁺). There is always plenty of carbon dioxide so phytoplankton keep growing until they have used up all of the useable nitrogen or all of the phosphorous, which ever runs out first. In most of the ocean, nitrogen runs out first and growth is said to be nitrogen limited. Characterization of phytoplankton communities is multidimensional; interferences between nutrient and salinity dependencies lead to combined changes in phytoplankton composition and abundance(Iriarte and Duncan, 2004). Furuya et al., (1986) had proposed that where thermal stratification induce an apparent deficiency of macronutrients near the surface, phytoplankton < 4µm, such as flagellates, monads, and unicellular cyanobacteria made up the major part of phytoplankton biomass. However, under conditions of upwelling which bottom water replenish nutrient rich water to the surface, diatoms grew twice as fast as the smaller form. This results in a shift from a dominance of the small forms to a preponderance of diatoms (Furuya et al., 1986). This observation suggest that growth of diatoms depends on the availability of nutrients.



CHAPTER 3

METHODOLOGY

3.1 Study Area

Sepanggar Bay is located in Kota Kinabalu, Northern Sabah (6° 02' N, 116° 06') (Figure 3.1). It is shallow , with the average depth within the area is approximately 16meters. It is located about 10 km north of Kota Kinabalu city and port, between latitudes 6° 04' N and 6° 05' N and longitudes 116° 07' E and 116° 08' E. The southeast side of the bay is characterised by a sheltered littoral beach immediately south of Menggatal River. The eastern side of the bay is characterized by heavy industry and port facilities while the northern side of the bay is characterised by small and medium scale industry. The western side of the bay is fronted by several water villages. The South west side of the bay is characterised by the islands of Pulau Sepanggar , Pulau Udar Besar and Pulau Udar Kecil. The Menggatal river flow into the Sepanggar bay. This river carry massive amount of treated or untreated sewage from the coastal villages and the urban residential area nearby, which resulted in severe eutrophication, cyanobacteria dominance and declining water quality. Besides, rapid development for tourism purposes across the coastal area of Kota Kinabalu has contributed to the



outbreaks of the blooms, with the local weather condition, poses a threat to the people who relies on the sea in their daily lives, as well as their source of food and income.

Uniform temperatures for Sabah state averaging from 23° C to 33° C with a mean humidity of about 90% for town and plain areas. Rainfalls are copious, and generally two seasons are distinguished, the rainy or wet season; and the dry season. Rainfall is common throughout the year and varies from about 150 cm to over 450 cm per year. In most parts of Sabah the wetter period occurs during the north-east monsoon from October to February and the drier season during the south-west monsoon from March to September. The waters in Sepanggar bay tend to get stratified during long periods of dry spell. Blooms event occurred during the period when successive rainfalls followed by sunny hot days that prevail.



Figure 3.1: Sepangar bay



3.2 Sampling Location

Two stations in the area surrounding UMS jetty situated in Sepanggar Bay have been identified using GPS. Station 1 situated near the cage culture area and station 2 which is located near the open sea. A map of the sampling location is shown in Figure 3.2 and Table 3.1 show both station by Global Positioning System(GPS).

Table 3.1 GPS Location of the sampling location.

GPS Location
N 06°02'558"
E 116°06'397"
N 06°02'062"
E 116°06'328"



Figure 3.2 : Sampling location.



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