EFFECTS OF SALINITY, TEMPERATURE AND IRRADIANCE ON THE GROWTH OF *Cochlodinium polykrikoides*

JOANNA W. DOINSING

BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITY MALAYSIA SABAH

2010

EFFECTS OF SALINITY, TEMPERATURE AND IRRADIANCE ON THE GROWTH OF *Cochlodinium polykrikoides*

JOANNA W. DOINSING

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITY MALAYSIA SABAH
2010

PUMS99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL:____

____

IJAZAH:____

____

SAYA_______________________________ SESI PENGAJIAN:____________

(HURUF BESAR)

Mengaku membenarkan tesis (LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

☐ SULIT (mengandungi maklumat berdarjah keselamatan atau Kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)

☐ TERHAD (mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

☐ TIDAK TERHAD

Disahkan Oleh

_____________________ _____________________

(TANDATANGAN PENULIS) (TANDATANGAN PUSTAKAWAN)

Alamat Tetap:_______________

_____________________ _____________________

_____________________ Nama Penyelia

Tarikh:________ Tarikh:________

CATATAN:-*Potong yang tidak berkenaan.

**Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa /organisasi 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 disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

Date:

Joanna W Doinsing
PS05-004-010

CERTIFICATION

NAME : **JOANNA W. DOINSING**

MATRIC NO. : **PS05-004-010**

TITLE : **EFFECTS OF SALINITY, TEMPERATURE AND IRRADIANCE ON THE GROWTH OF *C. polykrikoides***

DEGREE : **MASTER OF SCIENCE**

VIVA DATE : **19 JANUARY 2010**

DECLARED BY

1. **SUPERVISOR**
   Dr. Sitti Raehanah Muhd. Shaleh ____________________

2. **CO-SUPERVISOR**
   Prof. Dr. Datin Ann Anton ____________________

ACKNOWLEDGEMENTS

First of all, I would like to convey my deepest gratitude to my supervisor, Dr. Sitti Raehanah Muhd. Shaleh and my co-supervisor Prof. Datin Dr. Ann Anton for giving me this opportunity to work on this interesting project and for providing their invaluable help, guidance, encouragement and understanding throughout this study. To Prof. Saleem for giving me the opportunity to further my study in Borneo Marine Research Institute and for his priceless advices during the whole course of my study. Not forgetting Mr. Julian and Mr. Kennedy, for their assistance and suggestions that have encouraged me to take up this master degree study. In addition, I would also like to express my sincere thanks to Dr. Ejria, Dr. Norma, Dr. Sujjat, Sensei Prof. Senoo and Dr. Hoque for their help and constructive suggestions. Not forgetting Dr. Than, heartfelt thanks for his guidance and advices.

My sincere appreciation to my seniors and my labmates, To Mdm. Ching Fui Fui, Miss Nguang Siew Ing, Miss Mok Wen Jye, Miss Madihah Jaafar Sidek, Miss Grace Joy Chin, Mdm. Chelven Lim Ai Chen, Mdm. Asmiza, Miss Marlena, Miss Aimi, Miss Farah and Miss Laura, who have contributed in numerous ways by sharing productive discussions and suggestions, and by being an essential and lively part of an environment in which teaching, learning and research can thrive. I am also deeply grateful to all staffs of BMRI especially, Mr. Asri, Mr. Yusdi, Mdm. Effa and Miss Lucia for their kind assistance and patience in helping me go through my research. To other lecturers, friends and staffs that I have not mentioned here, thank you so much for their help and suggestions.

A special thanks to my parents, Mr. Wong Len Sing @ Doin sing and Mdm. Lim Geok Lui @ Lim Geok Yen for always being there praying for me, encouraging me and understands me. Not forgetting my brothers, Jonathan Wong Tze Chung and Lester Wong Tze Vui, a big thank you to you both for your faith and encouragement. To Adrian Tham Tuck Man, thank you so much for listening to me and instilling in me the positive thoughts and attitude during my difficult times. Their continuous supports and prayers throughout these years have kept me going on. Last but not least, I would like to express my deepest thanks to God for His gracious blessings and for giving me the strength and wisdom each and everyday to go through the hardships in completing this study.

Thank you once again and God bless you all.

Joanna W. Doinsing
04 April 2010

ABSTRACT

EFFECTS OF TEMPERATURE, SALINITY AND IRRADIANCE ON THE GROWTH OF *Cochlodinium polykrikoides*

Harmful algal blooms (HABs) or "red tides" in Sabah have significantly increased in scale, duration and frequency. The most recent causative species is *the Cochlodinium polykrikoides*. Since the first occurrence in January 2005, there have been recurring blooms of the species in the coastal waters of western Sabah. To understand the blooming mechanism of the algae, field and laboratory studies were both carried out. Field studies were carried out at University Malaysia Sabah Jetty off Sepanggar Bay, Kota Kinabalu, Sabah during the period of June 2006 to January 2007, with emphasis on temperature, salinity and transparency factors. The range of temperature, salinity and transparency recorded during bloom periods were 30.4-32.1°C (31.1±0.7°C), 31.1-32.8 ppt (32.2±0.7 ppt) and 1.5-3.5 m (2.5±0.9 m) respectively while the range of temperature, salinity and transparency recorded during non bloom periods were 29.2-31.1°C (30.2±0.7°C), 29.8-31.5 ppt (30.4±0.5 ppt) and 3.5-5.6 m (5.1±0.7 m). A low, negative relationship was observed between transparency and *C. polykrikoides* cell densities ($r^2=0.3325$; $p<0.05$) while no significant relationships was observed with temperature and salinity ($p>0.05$). In the laboratory experiments, batch cultures were grown at 4 sets of temperatures (20°C, 25°C, 30°C and 35°C), 8 different salinities (5, 10, 15, 20, 25, 30, 35 and 40 ppt) and 4 levels of irradiances (50, 100, 150 and 200 μmolm^{-2}.s^{-1}). It was observed that *Cochlodinium polykrikoides* grew well at higher temperatures (25-30°C) and salinities (15-40 ppt) with maximum growth at temperature 30°C and salinity of 30 ppt (0.66 Div. day^{-1}). The optimum growth rate of >0.3 Div. day^{-1} was observed at temperature of 25-30°C and salinities of 20-35 ppt. At all irradiances tested, higher growth rate was detected at irradiance 50 μmolm^{-2}.s^{-1} with exponential growth rates of 0.78±0.03 Div. day^{-1}. Based on the field and laboratory studies, optimum growth parameters for *Cochlodnium polykrikoides* are high temperatures and salinities (30-32°C, 30-33 ppt). These results provide vital information for *Cochlodinium polykrikoides* blooms monitoring and mitigation programs to lessen the negative impacts from these species.

ABSTRAK

Kejadian ledakan populasi alga-bahaya ataupun "red tides" di Sabah telah meningkat secara mendadak dari segi skala, tempoh dan kekerapan. Ledakan populasi spesis Cochlodinium polykrikoides adalah kejadian air pasang merah yang terbaru berlaku di Sabah. Sejak kejadian yang pertama pada Januari 2005, kejadian tersebut telah berlaku berulang kali di perairan pantai barat Sabah. Untuk memahami mekanisma pertumbuhan alga Cochlodinium polykrikoides, kajian lapangan dan makmal telah dijalankan. Kajian lapangan telah dijalankan di jeti Universiti Malaysia Sabah, Teluk Sepanggar, Kota Kinabalu, Sabah dengan member keutamaan kepada faktor suhu, saliniti dan transparensi air dari Jun 2006 sehingga Januari 2007. Julat suhu, saliniti dan transparensi air yang dicatatkan semasa kejadian air pasang merah adalah 30.4-32.1°C (31.1±0.7°C), 31.1-32.8 ppt (32.2±0.7 ppt) dan 1.5-3.5 m (2.5±0.9 m) masing-masing. Julat suhu, saliniiy dan transparensi air yang dicatatkan semasa ketidakhardiran kejadian "red tides" adalah 29.2-31.1°C (30.2±0.7°C), 29.8-31.5 ppt (30.4±0.5 ppt) dan 3.5-5.6 m (5.1±0.7 m). Terdapat hubung kait positif yang lemah antara transparensi air dan kepadatan sel ($p<0.05$; $r^2= 0.3325$) dan tiada hubung kait yang signifikan didapati bagi faktor suhu dan saliniti. Dalam makmal, Cochlodinium polykrikoides dikultur pada 4 set suhu berbeza (20, 25, 30 dan 35°C), 8 paras kemasinan (5, 10, 15, 20, 25, 30, 35 dan 40 ppt) dan 4 paras intensiti cahaya (50, 100, 150 dan 200 $\mu molm^{-2}.s^{-1}$). Cochlodinium polykrikoides didapati tumbuh dengan baik pada suhu (25-30°C) dan saliniti yang tinggi (15-40 ppt) dengan kadar pertumbuhan maksima pada suhu 30°C dan salinity 35 ppt (0.66±0.01 pembahagian sehari). Kadar pertumbuhan yang optima (>0.3 pembahagian sehari) diperhatikan pada suhu 25-30°C dan saliniti 20-35 ppt. Pada kesemua paras cahaya yang dikaji, didapati bahawa pertumbuhan Cochlodinium polykrikoides yang tertinggi adalah pada paras intensiti cahaya 50 $\mu molm^{-2}.s^{-1}$ dengan kadar pertumbuhan sebanyak 0.78±0.03 pembahagian sehari. Berdasarkan kajian lapangan dan makmal, Cochlodinium polykrikoides didapati tumbuh dengan kadar optima pada parameter-parameter suhu dan saliniti yang tinggi (30-32°C, 30-33 ppt). Keputusan kajian ini ,menyumbangkan maklumat yang amat penting kepada program pemantauan dan program mitigasi ledakan populasi Cochlodinium polykrikoides bagi mengurangkan kesan negatif spesis tersebut.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

HABs	harmful algal blooms
%	percent
^{o}C	degree celsius
ppt	part per thousand
$\mu molm^{-2}.s^{-1}$	micro mol per meter squared per second
CFP	ciguatera fish poisoning
PSP	paralytic shellfish poisoning
DSP	diarrhetic shellfish poisoning
NSP	neurotoxic shellfish poisoning
ASP	amnesic shellfish poisoning
cells L^{-1}	cells per liter
µg	microgram
km	kilometer
µm	micrometer
CH_4	methane
NE	northeast
SW	southwest
ms^{-1}	meter per second
NO_3-	nitrate
NH_4+	ammonium
NO_2^-	nitrite
N_2	dinitrogen
E_k	half saturation value
m	meter
mL	milliliter
h	hour
$Div.day^{-1}$	division per day
K^{-1}	specific growth rate
Gen.time	generation time
L	liter
CDOM	chromophoric dissolved organic matter
Mm	millimeter
dH_2O	distilled water
g	gram
g/L	gram per liter

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

The microscopic planktonic algae of the world oceans are critical food for filter-feeding bivalve shellfish (oyster, mussels, scallops, clams) as well as for the larvae of commercially vital crustaceans and finfish. In most cases, planktonic algae are beneficial for aquaculture and wild fishes industries. However, in some situations, the proliferation of planktonic algae (so-called 'algae blooms'; up to millions of cells per liter) can have negative effects, causing severe economic losses to aquaculture, fisheries and tourism operations as well as having major environmental and human health impacts (Hallegraeff, 2004). These events are referred as "Harmful Algal Blooms" (HABs).

HABs are natural phenomena that occurred throughout recorded history. The first recorded references to what were most likely outbreaks of HABs are documented in Captain James Cook's journal, in 1770, upon arrival off the Great Barrier Reef, Australia, and also in documents from Captain George Vancouver's voyage to British Columbia in 1793 in an area known as Poison Cove. In fact, Vancouver noted that it was taboo to eat shellfish for local native tribes when the seawater became bioluminescent due to dinoflagellate blooms (Dale and Yentsch, 1978). Paleoecological records have also revealed the presence of cyanobacterial blooms more than 8000 years ago in the Baltic Sea (Bianchi *et al.,* 2000).

While harmful algal blooms are completely recurrence natural phenomenon in the past two decades, such events appear to have increased in frequency, intensity and geographical distribution (Figure 1.1). Besides affecting almost every coastal area in the United States, harmful algal blooms events had been observed to expand its area and frequency of occurrences in South China Sea particularly in the southern China, northern and southern coast of Vietnam, west of Philippines, western Malaysia, Brunei and Palawan Island during the period of 1980 to 2003

(Wang *et al.,* 2007). Here are a few of many examples of toxic blooms causing negative impacts and economical losses in all parts of the world:

In Japan, harmful blooms of dinoflagellates and raphidophytes kill finfish and shellfish in aquaculture sites. The average economic losses associated with these blooms are about one billion yen per year. Efforts to reduce nitrogen and phosphorus in the water and sediment have led to a decrease of incidents, but an increase in the number of causative species. Besides, coastal shell fish aquaculture in northern and western Japan is seriously affected by PSP and DSP toxins produced by several dinoflagellates species (GEOHAB, 2001).

In Mexico, 45% of environmental emergencies recorded in 1996 were associated with toxic algal blooms. Most of these cases occurred on the Pacific coast, with some human poisoning cases related mainly to the consumption of oysters. Toxin analyses revealed levels above the standards of the World Health Organisation. Total economical losses due to confiscations of mollusks from the market and from hospital treatments were estimated roughly to be several million US$ (GEOHAB, 2001).

In natural eutrophic upwelling systems on the west coast of South Africa, high biomass dinoflagellate blooms are often associated with anoxic events and in some instances the production of hydrogen sulphide. For many years these events have been responsible for large faunal mortalities. In the southern Benguela, a single events have been responsible for the stranding of an estimated 2000 tons of rock lobster with a value of 50 million US$ (GEOHAB, 2001).

In Philippines, since the first record of a toxic dinoflagellate bloom in 1983, over 2000 PSP poisoning cases, leading a total of 115 deaths, have been associated with toxic blooms. The economic losses are estimated as high as 10 million PHP for each PSP event (GEOHAB, 2001).

The Scandinavian countries bordering the Baltic Sea are affected by massive blooms of hepatotoic cyanobacteria. Kills of domestic animals and human skin irritations are associated with these phenomena. Fish-farming in the Scandivian coastal regions of the North Sea and the Atlantic Ocean suffer from occasional toxic blooms of haptophyte flagellates, which may cause death to a wide range of marine organisms and cause extensive economic damage to commercial fisheries (GEOHAB, 2001).

The first report of HABs and shellfish in Malaysia was in 1976 when the marine dinoflagellate *Pyrodinium bahamense* var. *compressum* bloomed in Brunei Bay on the west coast of Sabah (Roy, 1977). The bloomed eventually spread to other parts of the Sabah west coast and have continued to occur almost annually in the state (Usup and Azanza, 1998). Over the years, many poisoning cases have been reported, including several fatalities. These also resulted in significant losses to fishermen as the public are afraid to consume all types of seafood during a bloom event, which normally last two to three weeks. For many years HABs and shellfish toxicity in Malaysia were considered problems unique to the west coast of Sabah only. However, subsequent HABs events show the probability of HABs and shellfish toxicity occurring in more locations in Malaysia (Usup *et al.,* 2002).

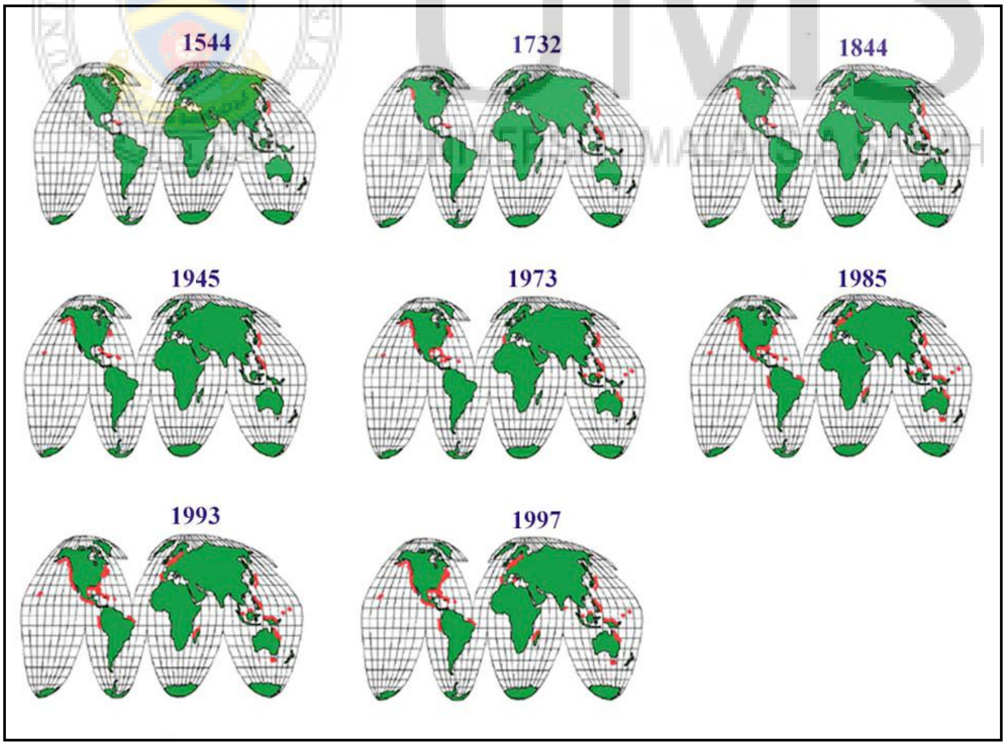

Figure 1.1: Worldwide distribution of HABs.
Source: Bushaw-Newton & Sellner (1999)

1.2 STATEMENT OF PROBLEMS

Since the first report of *Pyrodinium bahamense* var. *compressum* red tides in 1976, HABs in Malaysia have occurred occasionally over three decades in the coastal waters of western Sabah (Anton *et al.,* 2000; Roy, 1977;). Recently in January 2005, the first evidence of harmful algal blooms resulting in red discolorations of coastal waters was observed in Sepanggar Bay, off Kota Kinabalu in East Malaysia (0.600°N, 114.04°E). These blooms extended as far north as Tuaran (6.18°N, 116.23°E), a coastal town with extensive aquacgfulture activities (Figure 1.2). The species responsible for the blooms was identified as *Cochlodinium polykrikoides* which has caused fish mortalities in cage-cultures (Anton *et al.,* 2008) (Figure 1.3).

Cochlodinium polykrikoides appears to be one of the most notorious causative species of HABs occurring recently and regularly in coastal waters of western Sabah. This microalgal species bloomed in Brunei in December 2004, reached Sabah, Malaysia, by January 2005 and eventually to Palawan, Philippines in March 2005 (Azanza, 2005). Harmful blooms of this species since then have been an annual feature in the Western coast of Sabah, causing serious effects to aquaculture, fisheries and tourism operations.

Blooms of *C. polykrikoides* were reported off the western coast of the island Kyushu, Japan, and the southern coast of Korea (Kim *et al.,* 2004). They were also reports in other parts of the world; Phosphorescent Bay in Puerto Rico (Margalef, 1961), in Puerto Quetzal in Guatemala (Rosales-Loessener *et al.,* 1996), in Quanshou Bay in China (Du *et al.,* 1993), in Manzanillo Bay (Morales-Blake and Hernández-Becerrill, 2001) and the Gulf of California in Mexico (Gárate-Lizárraga *et al.,* 2000).

HABs dominated by *C. polykrikoides* had caused economic losses of US $95.5 million in 1995 (Kim *et al.,* 1997) and US $10 to 20 million per year in 2000-2003 in Korean aquaculture industries (Jeong *et al.,* 2004). They had also caused losses of CAN $2 million in Canada in 1999 (Whyte *et al.,* 2001) and US $36 million in Japan in 2000 (Kim *et al.,* 2004). In Southern China Sea, event of *C. polykrikoides* during March and April 1998 was reported to have caused tremendous damage to the coastal aquaculture industry, wiping out 1500 tonnes of farmed fish, which was equivalent to half of the entire Hong Kong aquaculture production of 1997 (Anderson, 1998; Tang *et al.,* 2003).

The initiation of a bloom requires an inoculums of cells, which can be from several sources and may involve different life stages, e.g., cysts (Steidinger and Garcés, 2006), as well as favourable environmental conditions of temperature, light, nutrients and water salinity (Zingone and Enevoldsen, 2000; Daranas *et al.,* 2001). Studies on the influence of environmental factors on HABs as well as *C. polykrikoides* are well documented in other countries. For instance, in western Pacific, *Cochlodinium* is associated with warm (18 to 30°C), euryhaline (30 to 34 ppt) conditions in but exhibits a cooler range of temperatures (11 to 31°C) in the eastern Pacific, with similar salinities (29.6 to 35.8 ppt). In Malaysia however, the study is still non-existent. This current lack of appropriate data prevents the resolution of *C. polykrikoides* blooms effects in Malaysia.

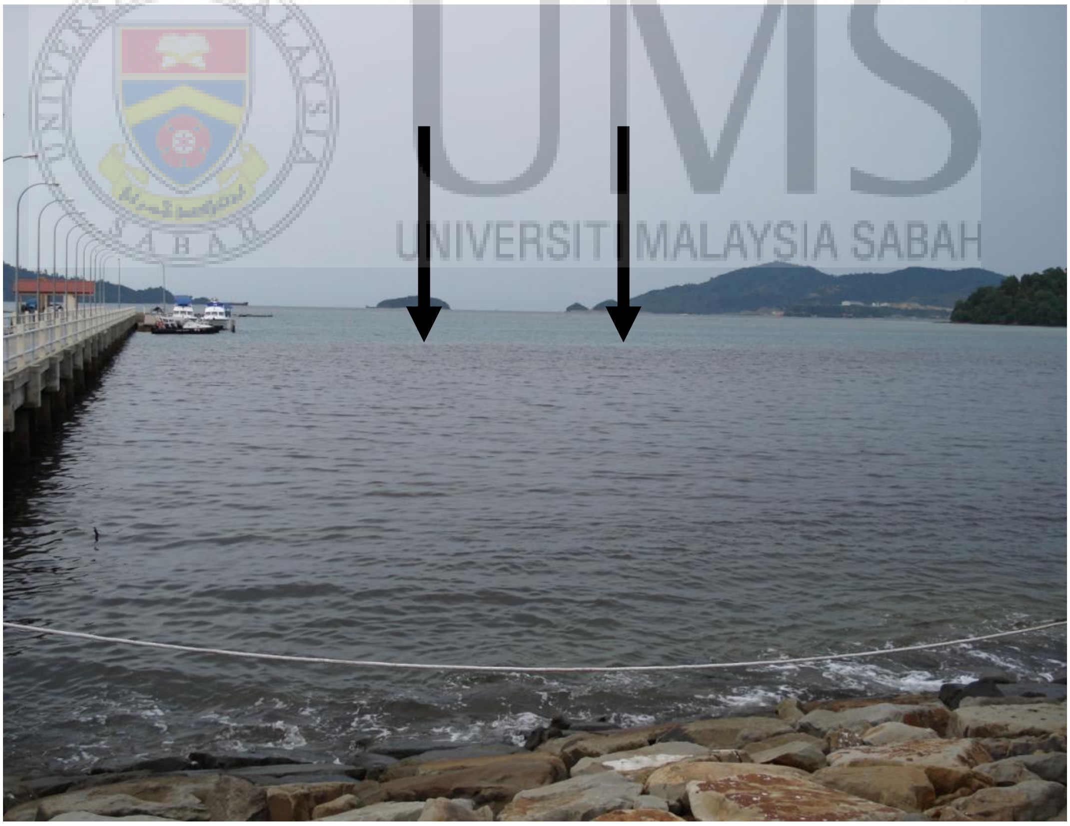

Figure 1.2: Red water discolouration caused by *C. polykrikoides* bloom in Sepanggar Bay, off Kota Kinabalu, Sabah in June 2005 (arrows)

Figure 1.3: Dead fish seen floating during *C. polykrikoides* bloom in June 2006 (Arrows).

1.3 SIGNIFICANCE OF STUDY

Sabah, located on Borneo Island, is a tropical situate surrounded with coastal waters. It has a growing aquaculture and mariculture industry that contributes to the country's export of high valued finfish, tiger shrimp, and seaweeds. Marine aquaculture has been estimated to contribute more than 43% by volume and 73% by value of total aquaculture production in Sabah, equating to 11,846 metric tones valued at ~US$50 million. In Sabah overall, aquaculture contributes 6% by volume and 21% by value to the total national fisheries (Liaw and Fung, 2000).

The damage caused by *C. polykrikoides* blooms on the fisheries and aquaculture industries has posed a serious threat to the economy of the state of Sabah due to its dependence on marine and agricultural resources. Therefore, there is an urgent need to clarify the outbreak mechanism of the blooms (Anton *et al.,* 2008). In this pioneering work, besides understanding the basic characteristic of *C. polykrikoides* by observing environmental conditions during blooms in field, we examined the effects of varying temperature, salinity and irradiance conditions on the growth of *C. polykrikoides* isolated from Sepanggar Bay, Sabah under laboratory conditions. This is important in order to understand the physiology of the organism and in predicting harmful algal bloom occurrences. Besides, this could also be used as an important biochemical marker to distinguish between geographical isolates as an identification tool in the study of interrelationships between different and among *C. polykrikoides* populations.

1.4 RESEARCH OBJECTIVES

This pioneering work aims to determine the effects of varying temperature, salinity and irradiance conditions on the growth of *C. polykrikoides* isolated from Sabah waters. Results on field studies and laboratory studies on the effects of varying temperature, salinity and irradiance on the growth of *C. polykrikoides* are presented in this study. This is important to understand the tolerance of the organism and in predicting harmful algal bloom occurrences. The specific objectives of this research are;

1. To determine the abundance of *C. polykrikoides* blooms at UMS Jetty, off Sepanggar Bay.
2. To determine the individual effects of temperature, salinity and irradiance on the growth of *C. polykrikoides* under controlled conditions.
3. To determine the optimum temperature, salinity and irradiance on the growth of *C. polykrikoides*.

1.5 HYPOTHESIS

The hypotheses of this research are;

1. *C. polykrikoides* is associated with high temperature (30 to 31°C) and high salinity (31 to 34 ppt) in the field.
2. *C. polykrikoides* grows best in high temperature, high salinity and high light environment.

CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW OF HARMFUL ALGAL BLOOMS (HABs)

2.1.1 Definition, Types and Pattern of HABs

Harmful Algal Blooms (HABs) refers to events whereby the proliferations of planktonic dinoflagellates in marine or freshwaters lead to massive fish kills, contaminate seafood with toxins and alter ecosystems in harmful ways (GEOHAB, 2001). As summarized in Table 2.1, there are three different types of HAB. The first type of the species are those that produce basically harmless water discolorations; however, under exceptional conditions in sheltered bays, the dense growing of blooms lead to indiscriminate kills of fish and invertebrates through oxygen depletion (Hallegraeff, 2004).

The second type of HABs are species that produce potent toxins that can find their way through the food chain to humans, causing a variety of gastrointestinal and neurological illness, such as paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), ciguatera fish poisoning (CFP), neurotoxic shellfish poisoning (NSP), cyanobacterial toxin poisoning and estuarine associated syndrome. Figure 2.1 shows routing through which toxins effect many different tropic compartments in which algae are the primary producer. The third type of the HABs species are those that are non-toxic to human but harmful to fish and invertebrates by damaging and clogging their gills (Hallegraeff, 2004).

Table 2.1: Examples of the different types of HABs.

Source: Sze (1993)

No.	Types of HABs
1.	Species that produce basically harmless water discolorations; however, under exceptional conditions in sheltered bays, the dense growing of blooms lead to indiscriminate kills of fish and invertebrates through oxygen depletion. Examples: Dinoflagellates *Akashiwo sanguinea*, *Gonyaulax polygramma*, *Noctiluca scintillans*, *Scrippsiella trochoidea*; cyanobacterium *Trichodesmium erythraeum*
2.	Species that produce potent toxins that can find their way through the food chain to humans, causing a variety of gastrointestinal and neurological illness, such as paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), ciguatera fish poisoning (CFP), and neurotoxic shellfish poisoning (NSP), cyanobacterial toxin poisoning and estuarine associated syndrome. Examples: **PSP**- dinoflagellates *Alexandrium catenella, A. cohorticula*, *A. fundyense*, *A. fraterculus*, *A. leei*, *A. minutum*, *A. tamarense*, *Gymnodinium catenatum*, *Pyrodinium bahamense* var. *compressum* **DSP**- dinoflagellates *Dinophysis acuta*, *D. acuminata*, *D. caudata*, *D. fortii*, *D. norvegica, D. mitra*, *D. rotundata*, *D. sacculus*, *Prorocentrum lima* **ASP**- diatoms *Pseudo-nitzschia australis*, *P. delicatissima*, *P. multiseries*, *P. pseudodelicatissima, P. pungens* (some strains), *P. seriata* **CFP**- dinoflagellate *Gambierdiscus toxicus*, *? Coolia* spp., ? *Ostreopsis* spp., ? *Prorocentrum* spp. **NSP**- dinoflagellate *Karenia brevis* (Florida), *K. papilionacea*, *K. selliformis*, *K. bicuneiformis* (New Zealand) **Cyanobacterial toxin poisoning**- cyanobacteria *Anabaena circinalis* (freshwater), *Microcystis aeruginosa* (freshwater), *Nodularia spumigena* **Estuarine associated syndrome**- through aerosols from dinoflagellates *Pfiesteria piscicida*, *P. shumwayae*
3.	Species that are non-toxic to human but harmful to fish and invertebrates by damaging and clogging their gills. Examples: Diatoms *Chaetoceros concavicorne*, *C. convolutes*; dinoflagellates *Karenia mikimotoi*, *K. brevisulcata*, *Karlodinium micrum*; prymesiophytes *Chrysochromulina polylepis, Prymesium parvum*, *P. patelliferum*; raphidophytes *Heterosigma akashiwo, Chattonella antique*, *C. marina*, *C. verruculosa*