THE EFFECTS OF LIGHT INTENSITY AND PHOTOPERIOD ON THE GROWTH OF *Cochlodinium polykrikoides.*

CORNELIA PETER AHMAN

THESIS IS SUBMITTED IN PARTIAL FULLFILLMENT IN REQUIREMENT FOR BACHELOR SCIENCE

MARINE SCIENCE PROGRAM
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITY OF MALAYSIA SABAH

2008

PUMS99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: THE EFFECTS OF LIGHT INTENSITY AND PHOTOPERIOD ON THE GROWTH OF *Cochlodinium polykrikoides*

IJAZAH: IJAZAH SARJANA MUDA SAINS

SAYA CORNELIA PETER AHMAN (HURUF BESAR) SESI PENGAJIAN: 2005 / 2006

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 institutsi pengajian tinggi.
4. Sila tandakan (/)

[ ]	SULIT	(Mengandungi maklumat yang 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

Cornelia
(TANDATANGAN PENULIS)

NURULAIN BINTI ISMAIL
LIBRARIAN
UNIVERSITI MALAYSIA SABAH
(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: Kg. Inobong, Penampang Sabah (W.D.T No 88, 89509 Penampang, Sabah)

Tarikh: 22/05/08

Nama Penyelia

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 declare that this is my own work with the exception that all short forms and quotes are stated their resources for each one of them.

31 March 2008

Cornelia

CORNELIA PETER AHMAN

HS2005-3842

CERTIFIED BY

Signatures

1. **SUPERVISOR**

   (Dr. Sitti Raehanah Shaleh)

2. **EXAMINER 1**

   (Prof. Datin Dr. Hjh Ann Anton.)

3. **EXAMINER 2**

   (Mr. Kennedy Aaron Aguol)

4. **DEAN OF SCHOOL**

   (SUPT/KS Prof. Madya Dr. Shariff.

   A.K Omang)

ACKNOWLEDGEMENT

First of all, I want to thank God for His blessings and wills which allowed me to complete my thesis on time.

A heartfelt gratefulness is also directed towards my supervisor, Dr. Sitti Raehanah Shaleh whose guidance, advices, financial support as well as perseverance had help me through the completion of my thesis.

I also want to express my gratitude to Ruseli Kien and my family for without their understanding and support, the task of completing my thesis would be very stressful.

Last but not least, a bunch of thanks to my friends, lab assistants, Mr. Yusdi, Ms. Lusia and Mrs Hanifah for their helps and advices that fill up any inadequacies during my experiment.

ABSTRAK

Fenomena 'Harmful algal bloom' (HAB) di perairan Sabah baru-baru ini adalah disebabkan oleh dinoflagellate marin *Cochlodinium polykrikoides* yang sebelum ini biasanya kerap berlaku di perairan Korea dan Jepun. Kejadian HAB yang disebabkan oleh *Cochlodinium polykrikoides* telah menyebabkan kematian ikan dalam sangkar secara besar-besaran dan kerugian ekonomi. Kejadian HAB *Cochlodinium polykrikoides* berlaku di kawasan tropika dan juga kawasan bermusim. Satu eksperimen telah dijalankan untuk mengkaji kesan keamatan cahaya dan fotoperiod terhadap pertumbuhan *Cochlodinium polykrikoides* di dalam makmal. Eksperimen tersebut telah dijalankan selama tiga minggu dalam bilik kultur pada suhu tetap sebanyak 25±2°C dan saliniti tetap sebanyak 30±2ppt. Sebanyak tiga fotoperiod berbeza (24H:0, 16H L: 8H D, 12H L: 12H D) dan empat keamatan cahaya(50μmol $m^{-2}s^{-1}$, 100 μmol $m^{-2}s^{-1}$, 150μmol $m^{-2}s^{-1}$, 200μmol $m^{-2}s^{-1}$) telah digunakan dalam eksperimen ini. Keputusan eksperimen menunjukkan bahawa bilangan sel *Cochlodinium polykrikoides* yang tinggi dihasilkan pada 16H L: 8H D untuk semua keamatan cahaya. Bilangan sel *Cochlodinium polykrikoides* dihasilkan adalah rendah pada keamatan cahaya lebih daripada 100 μmol $m^{-2}s^{-1}$. Kandungan klorofil a pada kultur *Cochlodinium polykrikoides* adalah tinggi pada fasa eksponen kultur iaitu pada minggu kedua. Keamatan cahaya dan fotoperiod mempengaruhi pertumbuhan *Cochlodinium polykrikoides* dan telah dibuktikan melalui analisis statistik yang menunjukkan perbezaan bererti.

ABSTRACT

The marine planktonic dinoflagellate *Cochlodinium polykrikoides* have been responsible for the recent occurrences of harmful algal blooms in Sabah's coastal waters which previously occur commonly in Korea and Japan coastal water. The bloom of *Cochlodinium polykrikoides* had caused major aquaculture fish kills and economic losses. *Cochlodinium polykrikoides* blooms occur in both temperate and tropical regions. A laboratory experiment was carried out to determine the effects of light intensity and photoperiod on the growth of *Cochlodinium polykrikoides*. The experiment was carried out for three weeks in a constant temperature growth room of 25±2°C and constant salinity of 30±2 ppt. There were three different photoperiods (24H L: 0, 16H L: 8H D, 12H L: 12H D) and four light intensities (50µmol $m^{-2}s^{-1}$, 100 µmol $m^{-2}s^{-1}$, 150µmol $m^{-2}s^{-1}$, 200µmol $m^{-2}s^{-1}$) used to examine the effects of the two factors on the growth of *Cochlodinium polykrikoides*. The results of the experiment indicated that higher cell density was achieved at 16H L: 8H D condition at all light intensity. Lower cell density was observed in light intensity higher than 100µmol $m^{-2}s^{-1}$. The chlorophyll *a* concentration was higher during the exponential phase of cultures which is during the second week. Light intensity and photoperiod influenced the growth of *Cochlodinium polykrikoides* as significant effects were evidenced from the statistical analysis done.

LIST OF CONTENT

LIST OF TABLE

LIST OF FIGURE

LIST OF PHOTO

LIST OF ABBREVIATION

°C	degree celcius
E	Absorbance
kg	kilogram
Lux	lux
l	liter
M	Molarity
mg/m^3	microgram per meter cubic
ml	milliliter
ms^{-1}	meter per second
rpm	round per minute
ppt	part per thousand
µg/l	microgram per liter
µm	micrometer
$\mu mol\ m^{-2}s^{-1}$	micro mol per meter square per second
W/m^{-2}	watt per meter square

CHAPTER 1

INTRODUCTION

1.1 Harmful Algal Blooms (HABs)

Harmful algal bloom (HAB) are naturally occurring phenomenon where the microscopic species of algae or larger multicellular algae accumulated in the aquatic or marine environment to the extent that they can cause negative devastating impacts to the environment, marine organisms and humans as well as to the coastal economies.

In general, most harmful algal blooms are caused by plants (photosynthetic organisms) that form the 'base' in the food chain (Bushaw-Newton & Sellner, 1999). Phytoplankton is held responsible for the majority events of harmful algal blooms occurred throughout the world. However, the blooms can also be caused by non-chlorophyll containing species that is the heterotroph species but they have similar forms to the phytoplankton species. In the Intergovernmental Oceanographic Commision (IOC) (2007) website, it stated that there are about 300 species of microscopic algae that are reported at times to form mass occurrences (blooms). Dinoflagellates, diatoms, microflagellates, cyanobacteria and chrysophytes are included in the 300 species of microalgae that cause the algal bloom.

There are two distinguish classes of organism that cause the harmful algal bloom phenomena according to Intergovermental Oceanographic Commission (IOC) website content

(2007). The first one is the toxin producer class. The organisms that belong to this group are very harmful as they produce toxin which can affect humans' health. When a bloom cause by species that belongs to this group, the toxin release by the species will contaminate seafood and affects human when the contaminated seafood is consumed. Others marine organisms such as fish may also be killed by the toxin. The toxin can be accumulated in the food chain and affects human.

The other class of organisms that cause harmful algal bloom is the high-biomass producers. Contrary to the toxin producer class, the organisms in this class do no produce toxin. Instead, they occur in high cell densities as they multiply rapidly in the marine environment. The number of cells can reach million per litre of seawater. Although the organisms do not produce toxin, they can kill fish or other marine organisms by causing anoxia (lack of oxygen) or hypoxia (low oxygen) condition as they decay in masses (Bushaw-Newton & Sellner, 1999). They also can cause a disruption of the fish and shellfish habitat because of their dense population leaving no room for respiration activity of the other marine organisms. Unpleasant smells, scums and also algal mats can be produced by this class of organisms (IOC, 2007).

The harmful algal bloom phenomenon was once commonly referred as the 'red tide' phenomena. The name 'red tide' is derived by the colour imparted by the suspended algae in the water, but this is a misnomer because not all harmful algal bloom events are red, some of the colour imparted by suspended algae can be brown, green or even colourless (Bushaw-Newton & Sellner, 1999).

1.2 Bloom mechanism

Generally, the organisms that cause harmful algal bloom are plants or in this case phytoplankton. The bloom occur when there is rapidly increasing number of algae to the extent that it dominates the local planktonic or benthic community (Bushaw-Newton & Sellner, 1999). According to Bushaw-Newton and Sellner (1999), the explosive growth of algae or phytoplankton is caused by metabolic response to a particular stimulus such as nutrients enrichment or environmental condition like a change in water temperature or by physical concentration of certain species due to the local patterns in water circulation.

Under ideal condition, phytoplankton may grow or divide. For phytoplankton to grow, light, inorganic nutrients such as phosphate (PO_4), nitrate (NO_3), ammonium (NH_4) and carbon dioxide (CO_2) are essential for the growth to occur. The suitable combination of light, nutrients, temperature and salinity may promote the bloom of algae to occur. Phytoplankton can reproduce asexually by dividing the cell. In the ocean, the generation time of phytoplankton can range from hours to few days. According to Northwest Fisheries Science Center (2007), the noticeable algal bloom is between 100,000 to 1,000,000 cells per litre.

1.3 Effects of Harmful Algal Bloom (HAB)

The occurrences of harmful algal bloom events had caused devastating negative impacts to the local community where it occurred. Both the toxin producer and high-biomass producer cause major loss to the local community especially the coastal area where it occurred.

The toxin produced by the harmful algal bloom species can affect the health of human through the consumption of contaminated seafood especially bivalves and fishes. The most well known poisoning caused by harmful algal bloom events are the paralytic shellfish poisoning (PSP), ciguatera fish poisoning (CFP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP) and diarrheic shellfish poisoning(NOAA Magazine Online, 2003). The term 'fish' and 'shellfish' are used because the toxin is concentrated in the fish and shellfish that ingested the harmful algae. People who are exposed to the toxin produce by the harmful algae will show symptoms such as gastrointestinal, neurological, cardiovascular and hepatological symptoms (Bushaw-Newton & Sellner, 1999). The symptoms can cause fatalities in human.

Harmful algal bloom events also affected living resources mainly the seafood resources. Both natural and aquaculture resources will be damaged. Toxin produced by harmful algal bloom species can cause mass mortality of marine organisms. This will reduce the marine resources in that area as there will be less fish or crustaceans to capture. Predation by the harmful algae species is also another way that that can reduce the living resources as they can accumulate and ingest the fish tissue (Bushaw-Newton & Sellner, 1999). The accumulation of harmful algae species will also results in local anoxic conditions as the oxygen in that area of accumulation will be used up as the species decay in mass. Other marine organisms will be difficult to do respiration as the oxygen is depleted in that area. Thus, marine organisms will be dead and this can diminish living resources in that area (Sellner *et al.*, 2003).

The most significant harmful effect the harmful algal bloom event has to the local area, second after the effect on human health is major losses in economic. Due to the blooms, tourism and recreation activities as well as seafood related industry will experience great loss (Sellner *et al.*, 2003). The bloom events will sometimes produce an unpleasant smell and mucus in the water; this will affect the tourism and recreation activities. Swimming and snorkeling as well as diving activities are avoided because of harmful algal bloom. In the case of seafood related industry, many mariculture farms and cages will be closed due to mass mortality of fishes caused by the harmful algal bloom events.

The harmful effects of the harmful algal blooms had cause it to become a public concern so that preventive measures has to be taken to avoid or lessen the negative impacts of the harmful algal bloom on the local area.

1.4 Significance of study

The algal species *Cochlodinium polykrikoides* causes many harmful algal bloom events throughout the world mostly in Japan and Korea coastal waters and recently in the South China Sea area particularly the western coast of Sabah, Malaysia. Little studies have been done on this harmful alga species in Sabah compared to other harmful algae species such as *Karenia brevis* and *Heterosigma akashiwo*. This study of the effect of light intensity and photoperiod on the growth of *Cochlodinium polykrikoides* can aid in other future research as a reference for further study on the characteristics of this species.

The bloom of *Cochlodinium polykrikoides* has caused many economic losses especially to the aquaculture industry as it kill fishes in mass. A study on the physical parameters such as light intensity and photoperiod can helps in better understanding of the physiological responses of *Cochlodinium polykrikoides* to the environmental conditions. Thus, aiding in prediction of the bloom and monitoring activities. Good monitoring and prediction technique can help in lessening the negative impacts of the *Cochlodinium polykrikoides* blooms.

1.5 Objectives of the study

The study focuses on the light intensity and photoperiod as parameter affecting growth of *Cochlodinium polykrikoides*. The main objectives of this study are:-

a) To determine the effect of light intensity and photoperiod on the growth of *Cochlodinium polykrikoides*.

b) To determine the effect of light intensity and photoperiod on the chlorophyll a content of *Cochlodinium polykrikoides*

c) To determine the optimum light intensity and photoperiod for the growth of *Cochlodinium polykrikoides*.

CHAPTER 2

LITERATURE REVIEW

2.1 Harmful algal bloom occurrences in the world

The harmful algal bloom phenomenon is not a new one. It has occurred for millions of years. There are written references of harmful algal bloom dating back to bibilical times and the presence of dinoflagellates fossils dated million years ago (Bushaw-Newton & Sellner, 1999).

Harmful algal bloom is a naturally occurring phenomenon. However, there is an increasing frequency of reported harmful algal blooms throughout the world. The harmful algal bloom has been spreading widely across the globes (Figure 2.1) causing enormous economic losses and threats to human health. The harmful algal bloom has affected almost every coastal area in the United States according to Bushaw-Newton and Sellner (1999). During the period of 1980-2003, the harmful algal bloom events had been observed to expand its area of occurrences and the frequency of occurrence has increased and varied in South China Sea particularly in the southern China, northern and southern coast of Vietnam, west of Philippines, western Malaysia, Brunei and Palawan Island (Wang *et al.*, 2007).

Harmful algal blooms are also reported in other countries such as Japan, Korea and South Africa. According to the article written by Bushaw-Newton and Sellner (1999), the same species of harmful algal may recur in the same geographic area. It can become episodic in that area it has occurred. They also wrote that same species of harmful algae may affect different kind of organisms depending on the types of organisms existed in the area of harmful algal bloom occurred.

The increasing frequency and the expanded area of the occurrence of harmful algal bloom events are believed to be induced by anthropogenic loading activities (Sellner *et al.*, 2003). The harmful algal bloom phenomena continues to pose a major threat to human health, environmental degradation and economic losses with increasing occurrences and wide spread area of occurrence.

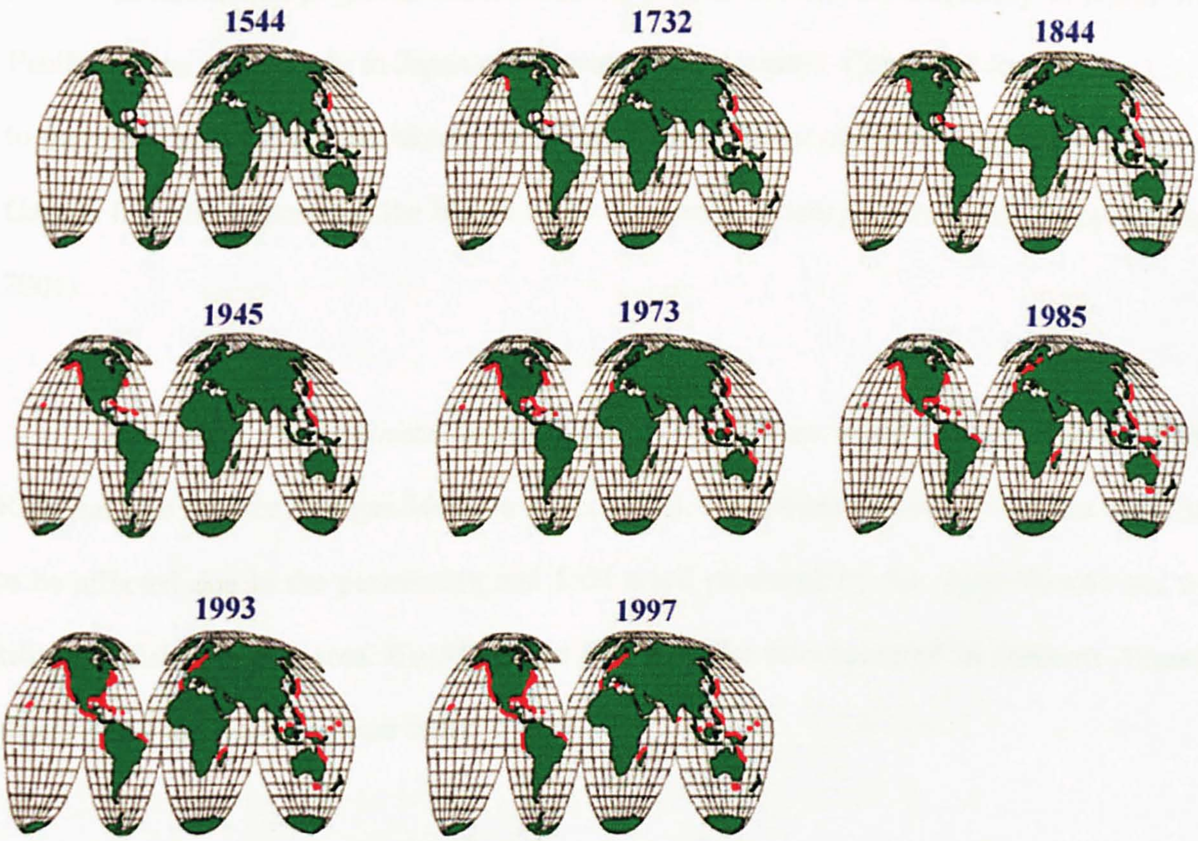

Figure 2.1 Worldwide distribution of HABs.

(Source from Bushaw-Newton and Sellner, 1999)

2.2 Occurrence of *Cochlodinium polykrikoides* bloom

Cochlodinium polykrikoides is known to cause mass mortalities of fishes when it blooms and resulting in major economic losses regarding the aquaculture industry. *Cochlodinium polykrikoides* occurred in temperate and tropical waters of northern hemisphere (NOWPAPCEARAC, 2006). The first ever reported case was in the Caribbean Sea along the southern coast of Puerto Rico.

Cochlodinium polykrikoides has been known to occur most frequently in North West Pacific region particularly in Japan and Korean coastal waters. China and Russia also known to had been affected by the bloom caused by *Cochlodinium polykrikoides*. Hong Kong and Canada had also experience the bloom cause by *Cochlodinium polykrikoides* (Daily Express, 2004).

Blooms of *Cochlodinium polykrikoides* also occurred in the Pacific coast of Costa Rica and also Mexico (Vargas-Montero *et al.*, 2004). The bloom caused the tourism activities to be affected due to the penetrating and fetid smell produced by the algae blooms and the killing of fishes in that area. *Cochlodinium polykrikoides* also occurred in northern Atlantic waters along the American east coast.

During the late 2003, *Cochlodinium polykrikoides* had started to bloom in Northern Borneo (Chun, 2007). The first reported bloom case of this species occurred on the year 2004 along the coastal water areas of Kota Kinabalu and Tuaran. In the recent case, on January

2007 the *Cochlodinium polykrikoides* bloom have caused massive kills of caged marine fishes in Pulau Gaya (Daily Express, 2007).

The area where *Cochlodinium polykrikoides* blooms have occurred left devastating impacts to the ecosystems of the marine environment and the local economic losses.

2.3 *Cochlodinium polykrikoides*

The *Cochlodinium* genus was established by Schutt (1894) with a description of circumscribing unarmoured dinoflagellates possessing the cingulum encircling the cell more than 1.5 times (Matsuoka, 2006).*Cochlodinium polykrikoides* is a marine dinoflagellate that lack thecal plates. This scientific name was given by Margalef 1961 who found it in the Carribean Sea, Puerto Rico. *Cochlodinium polykrikoides* is senior synonym of *Cochlodinium Heterolobatum* Silva 1967 (Matsuoka, 2006).

A solitary cell of *Cochlodinium polykrikoides* is ellipsoidal and slightly flattened dorso-ventrally in shape. The size of *Cochlodinium polykrikoides* ranges from 30-40μm in length and 20-30μm width. This species has distinct spiral-shaped cingulum. This cingulum is deep and located transversely on the cell. It makes a descending left spiral of 1.8-1.9 turns on the cell (NOWPAPCEARAC, 2006). The sulcus is narrow and shallow which is nearly run parallel to the cingulum. *Cochlodinium polykrikoides* is commonly can be found in chain form (Photo 2.1). They form chain under optimum condition. A single chain contains less than 8 cells. Dinoflagellates species that use the chain forming strategy is believed to use the strategy as an adaptation to favor bloom formation by increasing cell motility, thus allowing chainformers to exploit effectively stratified water columns (Margalef, 1978). *Cochlodinium polykrikoides* possessed numerous chloroplasts that are yellow-brown in colour and rod or ellipsoidal in shape.

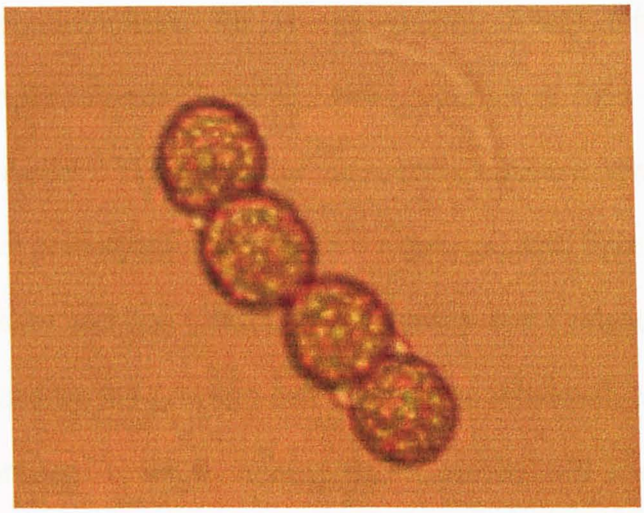

Photo 2.1: Chain form of *Cochlodinium polykrikoides* (4 cells) under 100x magnification.

(Source from Cornelia, 2007)

The harmful algae species, *Cochlodinium polykrikoides* do not produce toxin. It is considered as 'ichthyotoxic' to fish as the species responsible for the massive killing of fishes (Kim *et al.*, 2002). Recently in western coast of Sabah, on January 2007, another case of massive fish killing was reported in Pulau Gaya where the *Cochlodinium polykrikoides* bloom had caused massive killed of 40kg caged giant grouper with a concentration of 4500 *Cochlodinium polykrikoides* cells per litre (Daily Newspaper, 2007).

The studies in Japan and Korea had found that the suitable bloom conditions for *Cochlodinium polykrikoides* are to be around 32-34 ppt salinity, 25-28°C in temperature and a good amount of sunlight – these conditions similar to the oceanographic conditions in Sabah (North Borneo) (Chun , 2007).

REFERENCES

Baek, S.H., Shimode, S & Kikuchi, T. 2007. Growth of Dinoflagellates, *Ceratium furca* and *Ceratium fusus* in Sagami Bay, Japan: The Role of Temperature, Light Intensity and Photoperiod, *Harmful Algae*.

Brand,L.E. & Guillard, R.R.L. 1981. The Effects of Continuous Light and Light Intensity on the Reproduction Rates of Twenty-Two Different Species of Marine Phytoplankton. *Journal of Experimental Biology and Ecology* **50**, 122-132

Bushaw-Newton, K.L. and Sellner, K.G. 1999 (on-line). Harmful Algal Blooms. In: NOAA's State of the Coast Report. Silver Spring, MD: National Oceanic and Atmospheric Administration. http://state-of-coast.noaa.gov/ bulletins/html/hab_14/hab.html

Chun, K.T. 2007. Harmful Algal Bloom Monitoring Via Satellite in Northern Borneo. *UNU e-Newsletter* **20**.

Daily Express. 2004. Red Tide Threat Stays. http://www.dailyexpress.com.my/news.cfm?NewsID=24363

Daily Express.2007.Red Tide Strikes Caged Fish .http://www.dailyexpress.com.my/news.cfm?NewsID=46758

Dixon, G. K. & Syrett, P. J. 1988. The Growth of Dinoflagellate in Laboratory Cultures. *Journal of Phycology* **109**, 297-302.

Fehling, J, Davidson, K & Bates, S.S. 2005. Growth Dynamics of Non-toxic *Pseudo-nitzschia delicatissima* and Toxic *P. seriata* (Bacillariophycae) Under Simulated Spring and Summer Photoperiods. *Harmful Algae* **4**, 763-769.

Gilstad, M. and Sakshaug, E. 1990. Growth rates of 10 Diatom Species from the Barents Sea at Different Irradiances and Day Length. *Marine Ecology Progress Series* **64**, 169-173.

Guillard, R. R. L. (1973) Division rates. *In* Stein, J. R. (ed.) *Handbook of phycological methods. Culture Methods and Growth Measurements.* Cambridge University Press, Cambridge, pp. 289-311.

Kim, D.K, Oda, T, Muramatsu, T, Kim, D.I, Matsuyama, Y and Honjo, T. 2002. Possible Factors Responsible for the Toxicity of *Cochlodinium polykrikoides*, a Red Tide Phytoplankton. *Competitive Biochemistry and Physiology Part C* **132**, 415-423.

Kim, D. I., Matsuyama, Y., Nagasome, S., Yamaguchi, M., Yoon, Y. H., Oshima, Y., Imada, N., Honjo, T. 2004. Effects of Temperature, Salinity and Irradiance on the Growth of the Harmful Red Tide Dinoflagellates *Cochlodinium polykrikoides* Margalef (Dinophyceae). *Journal of Plankton Research* **26**, 61-66.

Kim, N.G. & Lee, C.G. 2001. A Theoretical Consideration on Oxygen Production Rate in Microalgal Cultures. *Biotechnology and Bioprocess Engineering* **6**, 352-358.

Kirk, J.T.O. 1983. Light and Photosynthesis in Aquatic systems. Cambridge University Press, Cambridge. Pp 219-253.

Kromkamp, J. C. and Claquin P., 2005. Role of Cell Cycle in the Metabolism of Marine Microalgae. In: D.V. Subba Rao (ed). 2006. *Algal cultures, Analogues of Blooms and Applications*. Science Publishers Enfield (NH) USA.

Hu, Q. 2004. Environmental Effects on Cell Composition. In: Richmond, A.(ed.).*Handbook of Microalgal Culture: Biotechnology and Applied Phycology*. Blackwell Science Ltd, UK. Pp 83-84.

Intergovermental Oceanographic Commision. 2007. The IOC Harmful Algal Bloom Programme. http:// ioc.unesco.org/hab/intro.htm.

Lee, R.E.. 1999. *Phycology (3^{rd} edition)*. Cambrige University of Press, UK.

Magana, H.A. and Villareal, T.A. 2006. The Effects of Environmental Factors on the Growth Rate of *Karenia brevis* (Davis) G.Hansen and Moestrup. *Harmful Algae* **5**, 192-198.

Margalef, R. (1978) Life forms of phytoplankton as survival alternatives in an unstable environment. *Oceanologica Acta*, **1**, 493-509.

Masodíjek, J., Koblízěk, M & Giuseppe, T. 2004. Photosynthesis in Microalgae. In: Richmond, A.(ed). *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*. Blackwell Science Ltd, UK. Pp 22-33.

Matsubara, T, Nagasoe, S, Yamachi, Y, Shikata, T, Shimasaki, Y, Oshima, Y and Honjo, T. 2007. Effects of Temperature, Salinity and Irradiance on the Growth of the Dinoflagellate *Akashiwa sanguinea*. *Journal of Experimental Marine Biology and Ecology* **342**, 226-230.

Matsuoka, K. 2006. Recent Progress on Cochlodinium polykrikoides (Dinophyceae). Institute for East China Sea Research, Nagasaki University.

Nagasoe, S, Kim, D.I, Shimasaki, Y, Oshima, Y, Yamaguchi, M and Honjo, T. 2006. Effects of Temperature, Salinity and Irradiance on the Growth of the Red Tide Dinoflagellate *Gyrodinium instriatum* Freudenhal et Lee. *Harmful Algae* **5**, 20-25.

National Oceanographic and Atmospheric Administration Magazine Online.2003. NOAA Marine Biotoxins Programs. http://www.magazine.noaa.gov/magarchive.html#mag2003

Northwest Fisheries Science Center.2007. Algal Bloom Dynamics. http:// www.nwfsc.noaa.gov/hab/habs_toxins/phytoplankton/algal_dynamics.html

NOWPAPCEARAC,2006.CochlodiniumHomepage.http://www.cearacproject.org/wg3/cochlo -entrance

Parsons, T.R., Maita, Y. and Lalli, C.M. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergammon Press Ltd. U.K.

Prezelin, B.B.1982. Effects of Light Intensity on Aging of the Dinoflagellate *Gonyaulax polyedra*. *Marine Biology* **69**, 129-135.

Sakshaug, E.and Holm-Hansen, O., 1986. Photoadaptation in Antarctic Phytoplankton: Variations in Growth Rate, Chemical Composition and P vs I Curves. *Journal of Phytoplankton Research*. **8**, 459–473.

Sellner, K.G, Doucette, G. J and Kirkpatrick, G. J. 2003. Harmful Algal Blooms: Causes, Impacts and Detection. *Journal of Industrial Microbiology Biotechnology* **30**, 383-406.

Vargas-Montero, M., Freer, E., Jiménez-Montealegre, R. & Guzmán, J.C. 2004. Extensive Blooms Due to *Cochlodinium polykrikoides*. *Harmful Algae News* **26**, 1-7.

Wang, S.F., Tang, D. L., He, F.L, Fukuyo, Y. and Azanza, R.V. 2007. Occurrences of Harmful Algal Blooms (HABs) Associated with Ocean Environments in the South China Sea.