UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND DISTRIBUTION OF MARINE DINOFLAGELIATES AT SEBATU AND SUNGAI RAMBAI, MALACCA, MALAYSIA

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BY

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UPM

DEDICATIONS

To my beloved family and husband.....

without your support this thesis would not have been possible

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In the name of ALLAH the most merciful and honourable

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

IDENTIFICATION AND DISTRIBUTION OF MARINE DINOFLAGELLATES AT SEBATU AND SUNGAI RAMBAI, MALACCA, MALAYSIA

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Faculty : Science and Environmental Studies

Phytoplankton is an important organism in the aquatic ecosystem as a primary producer. However, some species of phytoplankton also causes red tides or harmful algal blooms (HAB) which lead to negative impacts on human health, economics, aquaculture, fisheries and tourism. In this study, dinoflagellates, the main causative organism of red tides, were identified and enumerated from samples taken at Sebatu and Sungai Rambai, Malacca. In the identification of dinoflagellates, thecal plate tabulation and morphological characters of the cells were observed by light and electron microscopy. Thirty four species of dinoflagellates were identified, which consists of 10 potential HAB species viz. *Prorocentrum micans, Dinophysis caudata,*

D. rotundata, Ceratium furca, Alexandrium tamiyavanichii, Gonyaulax spinifera, Gymnodinium catenatum, G. sanguineum, P. shwartzii and *Noctiluca scintillans.* Among these species, *A. tamiyavanichii* and *G. catenatum* are the potential paralytic shellfish poisoning (PSP) species. In identifying *A. tamiyavanichii*, the most important characters are the apical pore plate (aap), sulcal posterior plate with a posterior attachment pore (pap) and the presence of the ventral pore (vp) on the first apical plate (1'). This species showed a constant size, is cingular in shape and the pap is central in position. *Alexandrium tamiyavanichii* occurred throughout the year in low numbers. The highest cell density of *Alexandrium tamiyavanichii* was only observed in July at station A with 27.00 ± 6.00 cells/L which corresponded with high nutrient concentrations viz. nitrate and phosphate concentration (3.91 ± 0.02 mg/L and 0.36 ± 0.06 mg/L respectively) and low silicate concentration (0.02 ± 0.00 mg/L). In identifying *G. catenatum*, the most important characters are the cingulum displacement and the extended sulcus into the epicone and hypocone. A high density of *G. catenatum* was also recorded in July at station A (389.00 ± 30.50 cells/L) which coincided with high nitrate and phosphate concentrations (3.91 ± 0.00 mg/L, 0.36 ± 0.06 mg/L). The high number of cells of *G. catenatum* in July also coincided with the high toxin level, 325 MU as recorded by the Fisheries Department. As such, *G. catenatum* may be suspected as the PSP producer responsible for the 1993 and 1994 incidences. However, until futher studies are conducted, *A. tamiyavanichii* and *G. catenatum* remain as potential PSP species. In horizontal and vertical distribution studies of dinoflagellates along the transect, the densities of dinoflagellates were high at 2-3 km offshore and well mixed through the water column. The data obtained

however, are not enough for understanding in detail the horizontal and vertical distributions of dinoflagellates at the sampling stations and therefore, futher studies should be conducted.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains.

IDENTIFIKASI DAN TABURAN DINOFLAGELATA MARIN DI SEBATU DAN SUNGAI RAMBAI, MELAKA,MALAYSIA

Oleh

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Fitoplankton merupakan organisma yang penting di dalam ekosistem sebagai pengeluar primer. Namun begitu, sebahagian daripada fitoplankton boleh menjadi kembangan alga beracun yang mendatangkan kesan negatif ke atas kesihatan manusia, ekonomi, akuakultur, perikanan dan pelancongan. Dalam kajian ini, dinoflagelata iaitu organisma yang sering dihubungkaitkan dengan kembangan alga beracun telah dikenalpasti dan populasinya dikira dari contoh sampel yang dikutip dari Sebatu dan Sungai Rambai, Melaka. Untuk mengenalpasti spesies dinoflagelata, pemerhatian dibawah mikroskop cahaya dan mikroskop elektron dilakukan untuk melihat susunan plat dan morfologi sel. Hasil dari pemerhatian, 34 spesies dinoflagelata telah

dikenalpasti dan 10 spesies daripadanya adalah spesies yang berpotensi menyebabkan kembangan alga beracun iaitu *Prorocentrum micans*, *Dinophysis caudata*, *Ceratium furca*, *Alexandrium tamiyavanichii*, *Gonyaulax spinifera*, *Gymnodinium catenatum*, *G. sanguineum*, *P. shwartzii* dan *Noctiluca scintillans*. *Alexandrium tamiyavanichii* dan *G. catenatum* merupakan spesies yang berpotensi menyebabkan keracunan paralitik. Ciri-ciri penting yang digunakan untuk mengenalpasti *A. tamiyavanichii* adalah kehadiran liang pada plat apikal (aap), liang sambungan (pap) pada plat sulkal di bahagian posterior dan liang di bahagian ventral (vp) plat apikal (1'). Spesies ini juga mempunyai saiz sel yang tetap, berbentuk singular dan pap terletak di tengah-tengah plat posterior. Bilangan sel *A. tamiyavanichii* adalah rendah sepanjang tahun. Bilangan sel *A. tamiyavanichii* yang tinggi hanya didapati pada bulan Julai di stesen A dengan nilainya 27.00 ± 6.00 sel/L. Pada bulan ini, kepekatan nutrien iaitu nitrat dan fosfat juga tinggi (3.91 ± 0.02 mg/L dan 0.36 ± 0.06 mg/L). Untuk identifikasi *G. catenatum*, ciri-ciri penting yang dilihat adalah nisbah singulum dan sulkus yang memanjang dari epiteka ke hipoteka. Bilangan sel *G. catenatum* juga didapati tinggi pada bulan Julai di stesen A (389.00 ± 30.50 sel/L). Kepekatan nitrat dan fosfat pada bulan tersebut iaitu 3.91 ± 0.00 mg/L dan 0.36 ± 0.00 mg/L. Tahap toksin juga tinggi pada bulan ini iaitu 325 MU seperti yang dilaporkan oleh Jabatan Perikanan. Oleh itu, *G. catenatum* mungkin merupakan spesies yang menyebabkan keracunan paralitik pada kejadian keracunan yang berlaku pada tahun 1993 dan 1994. Walaubagaimanapun, kedua-dua spesies, *A. tamiyavanichii* dan *G. catenatum* hanya

diramalkan berpotensi menyebabkan keracunan paralitik sehingga ujian pengesahan penghasilan toksin dijalankan.Untuk taburan menegak dan mendatar di sepanjang transek, didapati dinoflagelata banyak terkumpul pada jarak 2-3 km dari pantai tetapi populasinya tidak terkumpul pada kedalaman tertentu. Walaubagaimanapun data ini tidak mengcukupi untuk menerangkan dengan terperinci taburan dinoflagelata secara menegak dan mendatar, maka kajian selanjutnya perlu dilakukan.

CHAPTER I

INTRODUCTION

Red tides or harmful algal blooms (HAB) are well known problems around the world. This is because the occurrence of HAB brings negative effects on human health, economic losses in aquaculture, fisheries and tourism (Hallegraeff, 1993). In Malaysia, HAB cases caused by *Pyrodinium bahamense* var. *compressum* and a few cases by *Chattonella marina* and *Noctiluca scintillans* have been reported in Sabah and the Johore Straits (Maclean, 1989). Their occurences caused illnesses, death, shellfish farm closure, reduction in fisherman's catch and economic losses (Ming and Wong, 1989 ; Jothy, 1984).

Dinoflagellates are the main causative organisms for most red tides. The algae produce a potent toxin which affects humans through the food chain and causes fish mortality leading to great economic losses and coastal pollution. Identification of the causative species is important as the first step in managing the red tides, followed by a knowledge of its biology, ecology, life cycle, including its cystic stage, mechanism of toxin production and composition, which will help to understand the red tides.

In identification of dinoflagellates, thecal plate tabulations for thecate species and morphological characteristics such as shape, size of the cells i.e. cell outline, position of girdle and sulcus groove and girdle dispalcement for athecate species are used (Fukuyo and Taylor, 1989). To date, numerous dinoflagellate species have been identified and classified but changes in their groupings have been made recently or new groups are created due to undetail descriptions by previous taxonomists, differences in opinions among taxonomists, inconsistancy in morphological features of some cells (Zonneveld and Dale, 1994), a few references on its taxonomy plus there are not many advanced devices available for detail and accurate observations. By using instruments such as the epifluorenscence microscope (Mackenzie et al., 1996), light microscope with differential interference contrast and both scanning and transmission electron microscopes, identification of dinoflagellates is much easier and interesting. The use of staining agents such as lugol-iodine and fluorochrome calcofluor white (Hansen, 1995) allows detailed observations of the cells. With the advancement in technology, species that were previously identified as unarmoured or simple are actually more diverse morphologically (Faust and Balech, 1993) and allows the identification of most species (Horriguchi, 1995).

Nevertheless, identification using morphological characteristics has disadvantage, especially when there occurs morphological variation within a species. Futhermore, morphological characteristics cannot determine toxin compositions and concentrations (Anderson et al., 1994) and needs a lot of skill and time. As a result, taxonomists have suggested other means of identification of dinoflagellates i.e. using polyclonal antisera

and blocking antibodies (Mendoza et al.,1995) and using toxin profiles (Oshima et al., 1993). The methods suggested however, are still not able to overcome the above problems and have their own disadvantages.

Apart from taxonomy, the ecology of dinoflagellates is an important aspect, in understanding the factors which can determine the growth and the interaction of dinoflagellates with the environment. By understanding the ecology of dinoflagellates, red tide outbreaks may be predicted and managed. Therefore, a monitoring programme is one of the mechanisms suggested in understanding the ecology (Watson et al., 1992). The monitoring programme includes monitoring the plankton, the physical and chemical enviromental parameters and nutrient levels (Taylor, 1992). By monitoring, sites where harmful algal species are most abundant can be detected. Monitoring must be accessible or important for fisheries, aquaculture and the ecosystem around it.

Paralytic shellfish poisoning (PSP) is a well known phenomenon in Malaysia and in the world. Its impact on human health and economic losses have long been documented (Hallegraeff, 1993). A number of species were implicated to PSP but in the Indo West Pacific, *Pyrodinium bahamense* var. *compressa* is known to be the causative organism. This species was first described in 1906 in the tropical Atlantic ocean, but the first harmful incidence was in 1972 in Papua New Guinea (Maclean, 1989). The incident caused food poisoning in three children. Since then, toxic *Pyrodinium bahamense* var. *compressa* has spread to Brunei Darulssalam and Sabah