Faculty of Resource Science and Technology

Phytoplankton Composition in Muara Tebas Estuary

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DECLARATION

I hereby certify that this final year project report submitted for the Aquatic Resource Science and Management degree at University Malaysia Sarawak is based on my original work and no portion of this work has been submitted for the award of any other degree of qualification in any other university or other institution.

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List of Abbreviations

ASP	Amnesic shellfish poisoning
BWD	Ballast water discharge
CFP	Ciguatera fish poisoning
dH_2O	Distilled water
DSP	Diarrhetic shellfish poisoning
H_2SO_4	Sulphuric acid
HAB	Harmful algae bloom
$KMnO_4$	Potassium permanganate
NEM	Northeast monsoon
NH_3	Ammonia
NH_4^+	Ammonium
NO_3-N	Nitrate nitrogen
NSP	Neurotoxic shellfish poisoning
pH	Potential of Hydrogen
PO_4^{3-}	Orthophosphate
PSP	Paralytic shellfish poisoning
SEM	Scanning electron microscope
SiO_2	Silicate
SWM	Southwest monsoon

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ABSTRACT

Harmful Algal Blooms (HABs) is not negligible as their occurrence poses threat to human health, ecosystem and environmental deterioration, as well as negative implications to the aquaculture and fisheries economy. Phytoplankton dynamic in tropical countries, especially in conjunction with physicochemical influences to the production and growth of phytoplankton species are still poorly understood. In this study, qualitative and quantitative samples of phytoplankton were obtained from October 2012 to March 2013. Macronutrients especially silicate showed to have direct impact on cell density trend, while *in-situ* parameters showed weak relation to cell density with exception to Secchi depth. Cell density was also affected by fluctuations of nutrients with northeast monsoon (NEM) that influence precipitation and upwelling in the west coast of Sarawak. A total of 61 taxa of phytoplankton was identified to the generic level, with 41 diatoms (18 pennate and 23 centric), 17 dinoflagellates, 1 silicoflagellates and 2 cyanobacteria.

Keywords: Muara Tebas; estuary; phytoplankton; composition; *ex-situ*; *in-situ*

ABSTRAK

Kejadian ledakan alga berbahaya (HAB) tidak boleh diabaikan kerana kejadian tersebut membawa ancaman kepada kesihatan manusia, ekosistem dan kemerosotan alam sekitar, di samping implikasi negatif kepada akuakultur dan ekonomi perikanan. Dinamik fitoplankton di kawasan muara sungai di negara tropikal, terutamanya di bawah pengaruh fizikokimia kepada penghasilan dan pertumbuhan spesies fitoplankton masih kurang difahami. Dalam kajian ini, kualitatif dan kuantitatif sampel diperolehi dari Oktober 2012 ke Mac 2013. Makronutrien terutama silikat menunjukkan kesan langsung kepada trend kepadatan sel, manakala parameter *in-situ* menunjukkan hubungan yang lemah dengan kepadatan sel, kecuali kedalaman Secchi. Kepadatan sel juga dipengaruhi oleh fluktuasi nutrient dengan monsun timur laut yang memberi kesan kepada taburan hujan dan upwelling di kawasan pantai barat Sarawak. Fitoplankton telah dikenalpasti ke tahap genus, yang mana 61 taxa telah ditemui. Terdapat 41 diatom (18 pennate dan 23 centric), 17 dinoflagellate, 1 silicoflagellate dan 2 cyanobakteria.

Kata kunci: Muara Tebas; muara; fitoplankton; komposisi; *ex-situ*; *in-situ*

1.0 Introduction

Phytoplanktons are among the most important marine organism that account for the world's primary production. The trophic role of phytoplankton serves as a support to the grazing food chain, which are mostly prey to zooplankton communities. However, Harmful Algae Blooms (HABs) related toxicity has also brought implications on human health and ecosystem. Human intoxification outbreak that had caused severe health issues and eventual fatality due to consumption of contaminated bivalves, which had accumulated phytoplankton toxins from feeding (Amzil *et al*., 2001; Wright *et al*., 1989). Bioaccumulation and biomagnification throughout the entire ecosystem's food web not only imposed threats to human health and environmental deterioration, it had also brought detrimental effects to the aquaculture and fisheries economy.

The estuaries and coastal waters are important ecosystems to the phytoplankton community. These areas are where the mixing of freshwater and marine water happens, in which the physicochemical properties and biological processes characterized the phytoplankton composition. Usual high fluctuations in water parameters in estuarine areas, such as the salinity, temperature and light are among the factors influencing growth, nitrate uptake and toxin production of HABs (Lim & Ogata, 2005; Lim *et al*., 2006; Lim *et al*., 2007).

Moreover, due to the fact that there is usually a great number of human population living within the vicinity, it is undeniable that these waters are affected by anthropogenic activities. HAB species in the past few years had been known to be transferred and delivered by human assisted expatriation via international ship ballast water (Boalch, 1994; Hallegraeff & Fraga, 1998; Edwards *et al*., 2001; Lilly, Kulis *et al.,* 2002; Popels & Hutchins, 2002; Doblin *et al.,* 2004). These introduced HABs had resulted in several implications that threatened the native species (Cosper *et al*., 1987; Tracey, 1988) and affected local fisheries (Boalch & Harbour, 1977; Hallegraeff *et al*., 1988; Hallegraeff, 1992; Lilly *et al*., 2002). One example of HAB species found in coastal waters of Malaysia is *Alexandrium minutum*, a dinoflagellate capable of causing paralytic shellfish poisoning (PSP) in humans, and from which was originally described in the red tide of Alexandria harbor.

In this study, Muara Tebas estuary was selected as the study site. It is located at the river mouth of Sungai Sarawak, connecting to the South China Sea. This area is potentially susceptible to algal blooms as the upper stream of the river is compacted with domestic and industrial areas; moreover it is also a potential site of ballast water discharge (BWD) as it is one of the main and most crossed route for shipping and cruises. Hence, this study was done to attain a better understanding of phytoplankton community in brackish water, where the specific objectives were:

(i) to determine the phytoplankton composition in the estuary;
(ii) to determine the factors affecting species composition in the estuary;
(iii) to determine the possible occurrence of HAB species in Muara Tebas.

The fluctuations of water parameters and its effect on phytoplankton composition would be helpful in aiding future predicaments or monitoring of certain phytoplankton species, especially those related to HABs that might impose threat to economy, ecosystem and health. Besides, any result of discovery of introduced HABs would indicate needs for implementation of stricter regional BWD and aquaculture activity management and regulations.

2.0 Literature Review

2.1 Toxic phytoplankton species in Malaysian waters

In Malaysia, the very first case of intoxification by shellfish poisoning and HABs was by the dinoflagellate *Pyrodinium bahamense* var. *compressum* in Brunei Bay on the west coast of Sabah in the year 1976 (Roy, 1977) (Table 2.1). According to Usup and Azanza (1998), the bloom had eventually spread to the other parts of the Sabah west coast. Other HAB species usually occurred in Sabah were *Cochlodinium polykrikoides* and *Gymnodinium catenatum* (Adam *et al*., 2011). It seemed that HABs cases had only been constricted to this region until the year 1991, where three people were diagnosed with intoxication after consumption of mussels from a culture farm in Sebatu Melaka, and the causative agent was identified as dinoflagellate *Alexandrium tamiyavanichi* (Usup *et al*., 2002). Consequently, in September 2001 another case of PSP was documented following the consumption of contaminated toxic bivalve 'Lokan', *Polymesoda* sp. in Tumpat, Kelantan. The responsible organism for the incident was *Alexandrium minutum* (Lim *et al*., 2004). Other species of *Alexandrium* recorded in Malaysian waters were *Alexandrium taylori* and *Alexandrium peruvianum* (Lim *et al*., 2005).

Other potentially toxic dinoflagellates, *Dinophysis* and *Prorocentrum* are also common and occur in high densities in Malaysia. These organisms are capable of causing diarrhetic shellfish poisoning (DSP) in which symptoms of intoxication includes gastrointestinal symptoms. However, it is not lethal and can be recovered in three days' time. As for ciguatera fish poisoning (CFP), where symptoms could be of gastrointestinal, neurological and cardiovascular problems, it might also lead to paralysis and death but usually it is less severe. *Gambierdiscus toxicus, Ostreopsis ovata, O. lenticularis* and *Coolia* sp. were among the benthic dinoflagellates involved (Lim *et al*., 2005).

The amnesic shellfish poisoning (ASP) cases in Malaysia is not much known although presence of its causative species, the diatom *Pseudo-nitzschia* species is among the most abundant plankton in Malaysian coastal water.

Table 2.1: List of HABs species found in Malaysian water.

Location	**HABs species**	**Literature source**
Sabah	▪ *Pyrodinium bahamense* var. *compressum*	(Roy, 1977)
	▪ *Cochlodinium polykrikoides*	(Adam *et al*., 2011)
	▪ *Gymnodinium catenatum*	(Adam *et al*., 2011)
Melaka	▪ *Alexandrium tamiyavanichii*	(Usup *et al*., 2002)
Kelantan	▪ *Alexandrium minutum*	(Lim *et al*., 2004)
Others	▪ *Alexandrium taylori*	(Lim *et al*., 2005)
	▪ *Alexandrium peruvianum*	(Lim *et al*., 2005)
	▪ *Gambierdiscus toxicus*	(Leaw *et al*., 2011)
	▪ *Ostreopsis ovata*	(Leaw *et al*., 2001)
	▪ *Ostreopsis lenticularis*	(Leaw *et al*., 2001)
	▪ *Coolia malayensis*	(Leaw *et al*., 2010)
	▪ *Pseudo-nitszchia* spp.	(Leaw *et al*., 2011)

2.2 Ballast water: Introduced HAB species

Introduction of non-native species into new ecological niche or environment has always led to shifting of species composition, diversity and abundance of the local population. Implications regarding introduction of species via BWD such as alteration of species composition, destroying the ecosystem's food web and to a certain extend might cause depletion of local population.

Presence of cyst (Williamson, 1996) and non-cyst forming (Doblin *et al.*, 2007) estuarine or marine HABs were documented in ship's ballast water. In a few case studies done throughout the voyages in North American Great Lakes, potentially toxic freshwater HABs *Microcystis* and *Anabaena* had survived ballast water transits and were later delivered to locations beyond their original locality into the Great Lakes (Doblin *et al.*, 2007) or other coastal ports (McCarthy & Crowder, 2000; Ruiz & Smith, 2005).

Presence of HABs in the ballast water tanks which provided them with dark environment, in contrast with exposure to sufficient light intensity, degradation of extracellular toxins could have persist longer (Mitrovic *et al.,* 2005).

2.3 Ecology, distribution and dynamics

Blooms of phytoplankton occur relatively frequent in the world, some regions seasonally and at various locations with different conditions. In coastal waters of Sabah, HABs occured almost all the time. The most obvious factors controlling phytoplankton biodiversity and production were nutrient concentration, composition and availability (Adams *et al.,* 2001; Ferguson *et al.*, 2004; Jacquet *et al.*, 2006).

Phytoplankton dynamics were known to be associated with monsoon and related coastal environment (Tang, 2006). During the case study in Sabah, HABs were affected by seasonal variation, wind speed and total amount of rainfall in relation with its unique topography. Heavy rainfall during the southwest monsoon (SWM) brought by the typhoon season (June to November) in the Philippines loaded vast amount of nutrients from river run-offs (Adams *et al*., 2001). Furthermore, the suitable wind speed at certain depth of waters aided in well-mixed nutrients in the water column, stimulating upwelling activities (Trainer *et al*., 2000; Trainer *et al.,* 2002) as well as minimizing differences of other water parameters, such as the temperature, pH and salinity (Adams *et al*., 2001). Parameters such as temperature, pH, salinity and light, were known to affect the phytoplankton dynamics (Borkman & Smayda,1998; Hansen, 2002; Mutshinda *et al*., 2013; Ndebele-Murisa *et al*., 2010).

Between both HABs dinoflagellates and diatoms, according to Smayda (1997), flagellates had more obvious ecophysiological differences when compared with diatom. These differences included vulnerability to turbulence, blooming which depends on water gradients or stratifications, and also in terms of nutritional diversity.

Moreover, the understudied tropical estuary is made up of brackish water, a mixture of water from terrestrial origin and marine leading to complex gradient of physicochemical components, which were also affected by sewage water and other effluents from urban and industrial activities (Rochelle-Newall, 2011). Subsequently, these properties influenced the structure and function of phytoplankton (Rochelle-Newall, 2011).

Regarding the worldwide increase in HABs phenomenon, understanding dynamics of phytoplankton composition is becoming more crucial. Therefore, continuous documentation and research on phytoplankton composition and dynamics can serve as an insight to water quality besides detecting drastic changes present in the aquatic ecosystem, as well as to manifest cause of changes (Patil & Anil, 2011).

3.0 Materials and methods

3.1 Study site

This study was carried out in Muara Tebas estuary, positioned at N 01°39' E 110°29' (Figure3.1). Samples were collected from a jetty at Muara Tebas. The estuary is an inlet of the sea reaching into the Sarawak River valley, influenced by thermal and salinity fluctuations. Tourism and industrial activities are active in this area and this causes delivery of anthropogenic nutrients to estuary water in a dynamic state. This area is also the main routes for ships and cruises, which increase the influences of BWD in phytoplankton species community. Besides, small scale aquaculture activities could also be found in the vicinity.

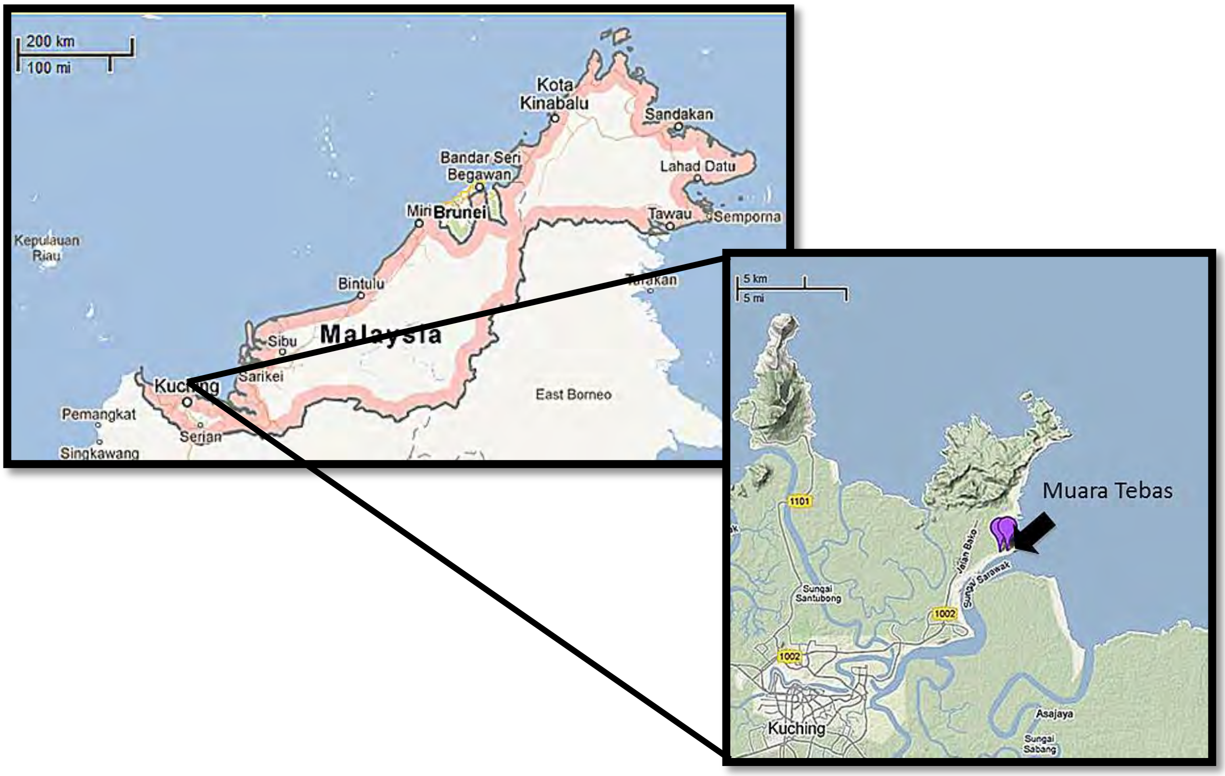

Figure3.1: The location of Kuching on the map of East Malaysia, where arrow on the right figure showing the study site, Muara Tebas.

3.2 Collection of Phytoplankton and Water Samples

Water samples were collected fortnightly from October 2012 to March 2013 at a jetty of Muara Tebas estuary (Figure 3.1). Both qualitative and quantitative samples were taken for phytoplankton and nutrient analysis. Qualitative samples were collected by filtering brackish water through a 20µm mesh net, whereas quantitative samples were collected using Van-Dorn water sampler and were kept in 1 litre bottles. The *in-situ* parameters, such as salinity was measured using the HANNA Digital Refractometer. Temperature and pH readings were both obtained using HANNA pHep 4 Meter. Water transparency was measured with Secchi disk and measuring tape.

3.4 Physical and Chemical Analysis

3.4.1 Water filtration and nutrient analysis

Water samples were collected and filtered through Whatman GF/C filters using filter water system. The filtered samples were then kept frozen under -20 ˚C before being analyzed. *Ex-situ* parameters such as nitrate-nitrogen (NO_3-N), reactive phosphorus (PO_4^{3-}-P) and silicate (SiO_2) were determined in the laboratory using colorimetric method (Hach, 2005). PO_4^{3-}-P was analyzed using PhosVer 3 (Ascorbic Acid) Method whereby PhosVer 3 was added to 10 ml of the sample at wavelength of 880 nm. NO_3-N was analyzed using Cadmium Reduction Method at wavelength of 507 nm. In this method, NitriVer 3 Nitrate powder pillow was added to 25 ml of the sample. SiO_2 was determined at wavelength of 651 nm using Heteropoly Blue Method after Molybdate 3 Reagent and Citric Acid Reagent powder pillows were added into 10 ml of the samples. Concentrations of each parameter were determined by using spectrophotometer HACH kit DR2800.

3.4.2 Chlorophyll *a* Analysis

A total of 500 mL water sample collected were filtered through a 47 mm GF/C glass fibre filter. After filtration, the filtered water samples were preceded to nutrients analysis while the filter paper was used for Chl-*a* analysis. The filter paper was grinded with mortar and pestle in 5-6 ml 90% aqueous acetone, followed by centrifugation process. The supernatant were extracted into a 1 cm path length quartz cuvette, and the extraction was measured at the following wavelengths: 750, 664, 647, and 630 nm which are the maximum absorption wavelength of Chlorophyll *a*, *b*, and *c*. The Chl-*a* concentration will be calculated using formulae:

$$\boldsymbol{CHL\ (\mu g\ L^{-1}) = C_a \times \frac{v}{VL}}$$

$$Where, C^a = \ C^a\ concentration\ present\ in\ \mu g\ mL^{-1}$$

$$v = volume\ of\ acetone\ in\ mL$$

$$V = volume\ of\ seawater\ in\ L$$

3.4.3 Rainfall distribution

Data of daily rainfall distribution of the area was collected from Rampangi Station of Malaysian Meteorology Department.

3.5 Phytoplankton Analysis

3.5.1 Cell enumeration and identification

All concentrated samples were fixed with acidic Lugol's iodine solution and were kept at room temperature for cell enumeration. Cell densities of each phytoplankton were identified under light microscope to genus level and were counted using Sedgwick-Rafter counting cell slide in triplicates for each sample.

3.5.2 Morphological observation using SEM

To study the morphology of phytoplankton species, the organic matter of samples were removed by oxidation (Lundholm *et al.*, 2002). Samples were acidified by addition of sulphuric acid (H_2SO_4), potassium permanganate ($KMnO_4$) and oxalic acid. Samples went through centrifugation at 3000rpm for 10 minutes and finally were rinsed with distilled water (dH_2O) to remove acid and salt contents. Samples were filtered on 0.2µm pore size membrane filter paper by using vacuum-manifold, dried and coated by auto fine coater before observation under JEOL JSM-6390LA Analytical Scanning Electron Microscope (JEOL, Japan).

4.0 Results

4.1 Qualitative data: Relative abundance of phytoplankton composition

A total of 61 phytoplankton taxa were identified to the genus level (Appendix A). There were 17 genera of dinoflagellates identified, namely *Alexandrium*, *Ceratium*, *Ceratocorys*, *Cochlodinim*, *Dinophysis*, *Gonyaulax*, *Gymnodinium*, *Metadinophysis*, *Noctiluca*, *Peridinium*, *Prorocentrum*, *Protoperidinium*, *Pyrocystis*, *Pyrophacus*, *Scripsiella* and *Zygabikodinium*. The remainings were 41 diatoms (18 pennate and 23 centric), 1 silicoflagellate (*Dictyocha*), and 2 cyanobacteria (*Anabaena* and *Lyngbya*).

The centric diatom *Skeletonema* had the highest abundance throughout the 9 sampling dates, particularly during year end of 2012 and first month of 2013 where its abundance dominated more than three quarters of the total phytoplankton species, particularly in 28 Nov, 19 Dec and 8 Jan where the species abundance reached 83.62%, 89.39% and 82.39% (Figure 4.1.1). The second most abundant phytoplankton was *Chaetoceros* spp., especially where its relative abundance was the highest in 30 Oct (Figure 4.1.1). The subsequent most abundant phytoplankton was from the group Cyanobacteria, mainly made up of *Anabaena* and *Lyngbya*, followed by diatom Nitzschia spp., centric diatoms *Cyclotella* and *Coscinodiscus* (Figure 4.1.1).

Overall, there were 12 taxa that could be found throughout the sampling periods. There were ten diatoms, which were pennate diatoms *Navicula*, *Nitzschia*, *Surirella* and *Thalassionema*, as well as centric diatoms *Chaetoceros*, *Cyclotella*, *Guinardia*, *Odontella*, *Rhizosolenia* and *Skeletonema*, although with rather inconsistent abundance. Dinoflagellates that were quite prevalent were *Prorocentrum* and *Protoperidinium*.

There was altogether nine uncommon phytoplankton found with very low (0.11%) frequency of occurrence (Table 4.1). The uncommon pennate diatoms were *Diatoma,* *Licmophora, Lioloma,* and *Meuniera*, whereas the centric one was *Dactyliosolen* only. There were four rare dinoflagellates found, which were *Ceratocorys, Cochlodinium, Metadinophysis* and *Pyrocystis.* There were 28 potentially harmful phytoplanktons found, consisting of five from pennate diatoms, nine centric diatoms, 11 dinoflagellates, one silicoflagellates and two cyanobacteria (Table 4.1).

Micrographs of some phytoplankton were also taken from light microscope (Figure 4.1.2, 4.1.3 & 4.1.4) and scanning electron microscope (Figure 4.1.5).

4.2 Quantitative data: Total cell density

The total cell density ranged from 10,550 cells L^{-1} to 196,850 cells L^{-1}. The total cell density from 9 sampling dates showed was very inconsistent, with obvious trend resulted by the amount of diatoms (Figure 4.2.1). The trend showed a rapid decrease as much as 87.85% in 30 Oct from 10 Oct and slightly increased in the subsequent sampling period, then drastically increased to the highest peak in 28 Nov, followed by irregular decrease and increase in cell density towards the end of the sampling periods.

The phytoplankton was grouped into four major groups, the diatom group, cyanobacteria, dinoflagellates and silicoflagellate. It could be observed that the total cell density was dominated by diatom, approximately 85% of all cells, followed by cyanobacteria (12.33%) which were more prominent than other groups. Similar trends between groups could be observed in 10 Oct to 30 Oct and 8 Jan to 6 Mar periods. However, trends of other sampling periods were not as obvious.

The 10 most abundance phytoplankton in Muara Tebas, according to total cell density in descending order was: *Skeletonema*, *Chaetoceros,* Cyanobacteria*, Nitzschia, Cyclotella, Thalassionema, Coscinodiscus, Thalassiosira, Navicula,* and *Rhizosolenia* (Figure 4.2.2).

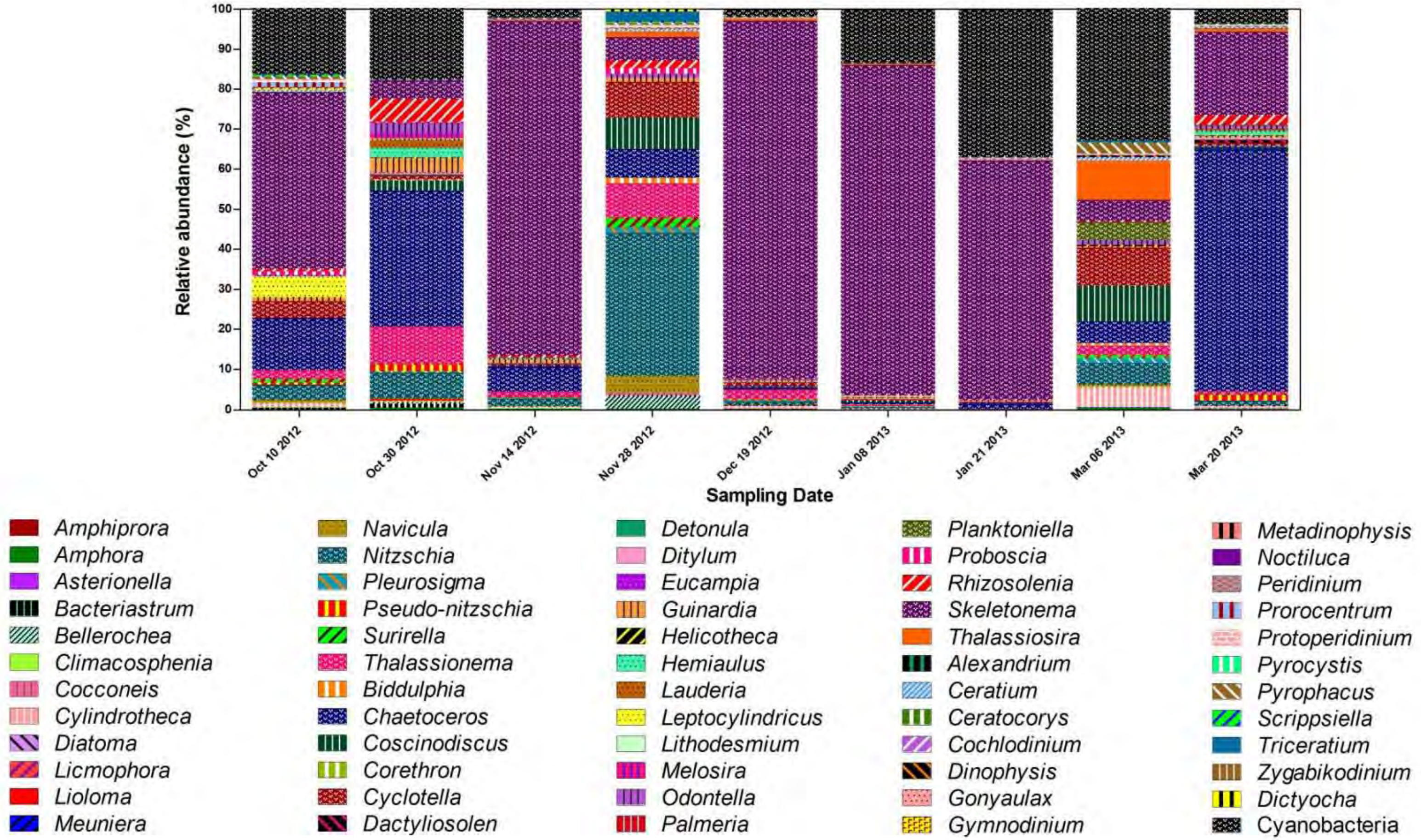

Figure 4.1.1: Relative abundance of various phytoplankton taxa for 9 sampling dates ranging from October 2012 to March 2013.