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Harmful algal bloom causative collected from Hong Kong waters

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Abstract

Harmful algal blooms (HABs) have increased globally in recent years. In Hong Kong, a record algal bloom, caused by *Gymnodinium mikimotoi* and *Gyrodinium* sp. HK'98 (subsequently described as *Karenia digitata*) occurred in March and April 1998. Almost all fishes died in the affected cages, and the estimated economic loss caused by the HAB was HK$315 000 000 (equivalent to US $40 000 000). Most of the known toxic or harmful algal species are dinoflagellates. Some common dinoflagellate species such as *Ceratium furca, Gonyaulax polygramma, Noctiluca scintillans, Heterocapsa triquetra, Prorocentrum minimum, Prorocentrum sigmoides*, and *Prorocentrum triestinum* frequently bloom in Hong Kong waters. Others, such as *Alexandrium catenella, Alexandrium tamarense, Gymnodinium mikimotoi, Gymnodinium* cf. *breve, Gymnodinium catenatum, Dinophysis caudata, Dinophysis acuminata,* and *Gambierdiscus toxicus* bloom only occasionally, but their toxic effects or potentially toxic and harmful effects are very significant. Some important toxic and harmful, or potentially toxic and harmful dinoflagellate species are described. Among them, *Gambierdiscus toxicus*, a potential ciguatera fish poison producing species, and *Gymnodinium* cf. *breve*, a neurological shellfish poison producing species were new records from Hong Kong waters.

Introduction

Sournia (1995) summarized that out of a total number of between 3365 and 4024 marine phytoplankton species, about 184-267 species (~6%) are responsible for algal blooms (or red tides), including diatoms, dinoflagellates, raphidophytes, prymnesiophytes, and silicoflagellates, and about 60–78 species (~2%) are toxic. Among these 60–78 toxic species, 45–57 are dinoflagellates. It is thus clear that most of the toxic species (73–75%) are dinoflagellates.

Dinoflagellates are the most important group of marine phytoplankton producing both toxic and harmful algal blooms (Steidinger, 1983, 1993; Anderson, 1989; Hallegraeff, 1993). The negative effects of dinoflagellates include the biotoxins, physical damage, and the anoxia or hypoxia they produce. So far, five major groups of toxins produced by marine phytoplankton and their effects have been identified. They are amnesic shellfish poisoning (ASP) (Bates et al., 1989); diarrhetic shellfish poisoning (DSP) (Yasumoto et al., 1980); neurological shellfish poisoning (NSP) (Baden & Mende, 1982); paralytic shellfish poisoning (PSP) (Shimizu, 1979); and ciguatera fish poisoning (CFP) (Yasumoto et al., 1977). Of these five groups of toxins, four (DSP, NSP, PSP, CFP) are mainly produced by dinoflagellates (Shumway, 1990; Yasumoto, 1990). Beside toxins, mass accumulation of dinoflagellate cells can also cause physical damage or clog fish and invertebrate gills, for example *Noctiluca scintillans, Gymnodinium mikimotoi, Gymnodinium sanguineum*, and *Cochlodinium polykrikoides*; or cause depletion of dissolved oxygen, such as *Gonyaulax polygramma*, and *Scrippsiella trochoidea* (Hallegraeff, 1993).

In Hong Kong, as elsewhere, dinoflagellate blooms are the most harmful. The first harmful algal bloom (HAB) caused by a dinoflagellate (*Noctiluca scintillans*) was recorded in the south bays of Hong Kong (Morton & Twentyman, 1971). In 1988, a continuous bloom of *Gonyaulax polygramma* lasted for three and

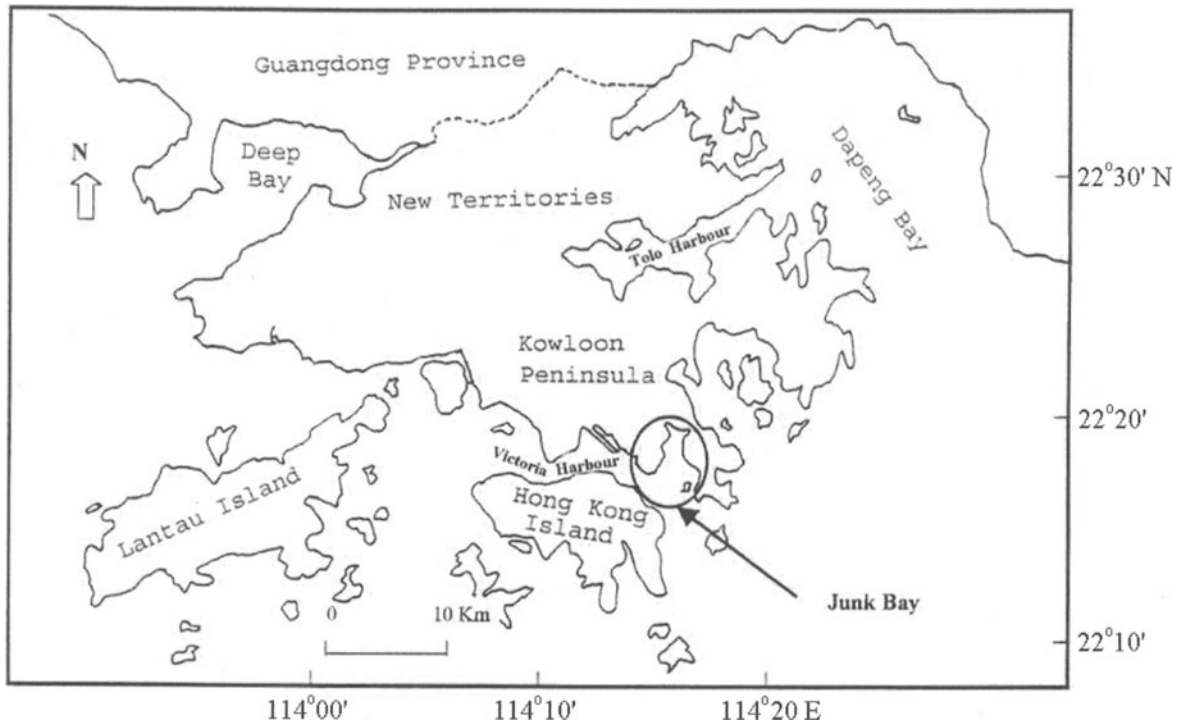


Figure 1. Map of Hong Kong and location of Junk Bay.

a half months from early February to May in Tolo Harbour. The collapse of the bloom in May caused the whole of the Tolo Harbour waterbody to become anoxic, resulting in a massive fish kill in the fish culture zone (Lam & Yip, 1990). The first toxin-related HAB caused by *Alexandrium catenella* was recorded in inner Junk Bay in 1989 (Ho & Hodgkiss, 1993). The toxicity of PSP in green-lipped mussel increased from 2280 MU kg^{-1} meat when the bloom was first detected to 13500 MU kg^{-1} meat within a week (EPDHK, 1990).

A review of HAB occurrences in Hong Kong from 1975 to 1986 (Wong, 1989) showed that of a total of 26 identified HAB causative species, 17 were dinoflagellates (65%). In a total of 133 HAB incidents, 102 were caused by dinoflagellates (77%), and almost all of the fish kills were caused by dinoflagellates.

In Spring 1998, a record bloom of *Gymnodinium/Karenia* hit Hong Kong waters. About two thirds of the mariculture farms (estimates are 1000 out of 1500) were affected. Almost all fish died in the affected cages, and the estimated economic loss was HK $315 000 000 (equivalent to US $40 000 000). The causative species were *Gymnodinium mikimotoi* and a new dinoflagellate species, *Karenia digitata* (Yang et al., 2000) and, since then, more and more harmful algal species have been found (Lu & Hodgkiss, 1999b).

Hong Kong is one of the worst HAB affected areas in the world, with a high diversity of harmful and toxic algal species, especially dinoflagellates. The objective of this study was to investigate the morphology and diversity of toxic and harmful, or potentially toxic and harmful species in Hong Kong waters, with attention focused on Junk Bay, where the first PSP event happened.

Materials and methods

Hong Kong is located in the north-eastern part of the subtropical South China Sea. It is surrounded by sea to the south, east, and west. Junk Bay is located on the south of the Kowloon Peninsula, facing Hong Kong Island (Fig. 1). Phytoplankton samples were taken regularly (three times a month from 1997 to 1998) from Junk Bay, and occasionally from other sites in Hong Kong waters.

Net and water samples were taken at each sampling. Net samples were collected using a 10 μm

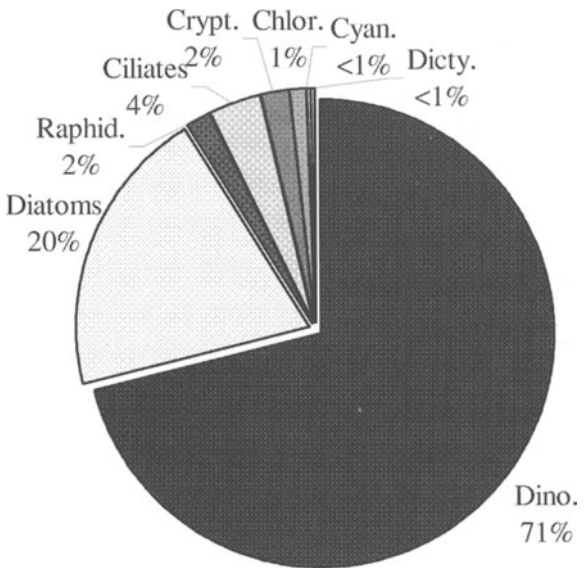


Figure 2. Percentage contributions of different taxonomic groups to algal blooms recorded in Hong Kong waters from 1975 to 1998 (from the red tide database, Agriculture, Fisheries and Conservation Department, Government of the Hong Kong SAR).

mesh size phytoplankton net. The samples were preserved using Lugol's solution. Some live samples were collected and taken back to the laboratory immediately without preservation after each sampling, and the live samples were used for isolation and cultivation of some important species. Preserved samples were stored for subsequent quantitative studies.

For most of the armored dinoflagellates, preserved net samples were good for observation under both light and electron microscopes. For unarmored and some fragile armored dinoflagellates, live water samples were observed under the light microscope, and then single cells of each species were picked out and cultivated.

'K' (Keller et al., 1987) and 'f/2' (Guillard, 1975) media were applied for algal culture. The light dark cycle was 12L:12D. Culture temperature was different for different species. *Gambierdiscus toxicus*, a tropical species, was kept at 25 °C. For all other species, the culture temperature was 20 °C.

Preparation of dinoflagellates for scanning electron microscopy (SEM) followed methods recommended by Takayama (1985) and Truby (1997). The combined fixation technique was applied to unarmored dinoflagellates. Fixed dinoflagellate specimens were washed and dehydrated, followed by critical-point-drying and sputter-coating with gold-palladium. They were finally examined on a Leica S-440 Scanning Electron Microscope.

Table 1. Dinoflagellate species recorded in Hong Kong which have had the harmful impacts indicated elsewhere

Species	Impacts
Alexandrium catenella (Whedon et Kofoid) Balech *	Toxic
Alexandrium tamarense (Labour) Balech*	Toxic
Amphidinium carterae Hulburt	Toxic
Ceratium furca Ehrenberg (Claparède *et* Lachmann)*	Fish kills
Ceratium fusus (Ehrenberg) Dujardin	Fish kills
Cochlodinium polykrikoides Margelef*	Fish kills
Dinophysis acuminata Claparède *et* Lachmann	Toxic
Dinophysis acuta Ehrenberg	Toxic
Dinophysis caudata Saviller-Kent	Toxic
Dinophysis fortii Pavillard	Toxic
Dinophysis tripos Gourret	Toxic
Gambierdiscus toxicus Adachi *et* Fukuyo	Toxic
Gonyaulax polygramma Stein*	Fish kills
Gymnodinium cf. *breve* Davis	Toxic
Gymnodinium catenatum Graham	Toxic
Gymnodinium mikimotoi Miyake *et* Kominami *ex* Oda*	Fish kills
Gymnodinium sanguineum Hirasaka*	Fish kills
Gyrodinium instriatum Freudenthal *et* Lee*	Fish kills
Heterocapsa triquetra (Ehrenberg) Balech*	Fish kills
Karenia digitata Yang, Takayama, Matsuoka *et* Hodgkiss*	Fish kills
Noctiluca scintillans (Macartney) Ehrenberg*	Fish kills
Prorocentrum dentatum Stein*	Fish kills
Prorocentrum micans Ehrenberg*	Fish kills
Prorocentrum minimum (Pavilard) Schiller*	Fish kills
Prorocentrum sigmoides Böhm*	Fish kills
Prorocentrum triestinum Schiller*	Fish kills
Scrippsiella trochoidea (Stein) Loeblich*	Fish kills

* Those species which have caused blooms (with or without negative impacts) in Hong Kong waters.

Results

A total of 27 harmful, or potentially harmful dinoflagellate species were identified during the study (Table 1). Of these, 17 species were harmful and caused toxicity or fish kill events in Hong Kong waters before or during the study period. Ten species, which previously had not caused HABs in Hong Kong, are potentially harmful since they have been proven to be toxic or harmful elsewhere in the world.

Newly recorded harmful dinoflagellate species

Gymnodinium cf. *breve* (Plate 1), a potentially NSP producing species, and *Gambierdiscus toxicus* (Plate 2), a potentially CFP producing species (Lu & Hodgkiss, 1999a) were new records of harmful algal species for Hong Kong waters. Both species were collected from plankton samples in Junk Bay.

Harmful dinoflagellate species

The harmful event caused by *Gymnodinium mikimotoi* and *Karenia digitata* during the study period was the biggest in the historical record of HABs in Hong Kong, both in terms of the area it affected and the economic losses which resulted. The most common harmful dinoflagellate species which were frequently observed in relatively higher density in Hong Kong waters were: *Noctiluca scintillans, Prorocentrum micans, Prorocentrum minimum, Ceratium furca, Gonyaulax polygramma, Scrippsiella trochoidea*, and *Gymnodinium mikimotoi*.

Potentially harmful dinoflagellate species

So far in Hong Kong only PSP toxins have been confirmed in shellfish from local waters. The causative species was believed to be *Alexandrium catenella* (Ho & Hodgkiss, 1993). However, other potentially important PSP producing species like *Alexandrium tamarense* and *Gymnodinium catenatum* were frequently observed during the present study.

In addition, DSP producing species such as *Dinophysis acuminata, D. acuta, D. caudata* and *D. fortii*; the NSP producing species *Gymnodinium* cf. *breve*, and the CFP producing species *Gambierdiscus toxicus* were also observed during this study.

Discussion

Dinoflagellates and HABs in Hong Kong

Hong Kong is believed to be one of the most severely harmful algal bloom affected areas in the world (Smayda, 1990). It is true that there are all kinds of potentially harmful algal species present, which have been linked to DSP, NSP, DSP, CFP, and ASP (produced by diatoms), as well as fish kill species. Amongst the HAB causative species in Hong Kong waters, dinoflagellates play a very important role. A red tide database from the Agriculture, Fisheries and Conservation Department of the Hong Kong Government showed that a total of 534 identified algal blooms have been recorded from 1975 to July 1998. Of these, 377 (71%) were caused by dinoflagellates (Fig. 2). Twenty six (42%) of the total 62 species responsible for the blooms were dinoflagellates (Fig. 3). Most importantly, out of a total of 50 fish kill incidents during this time, 48 were caused by dinoflagellates, and another two were caused by combinations of diatoms and dinoflagellates. So dinoflagellates caused 98% of the fish kill incidents.

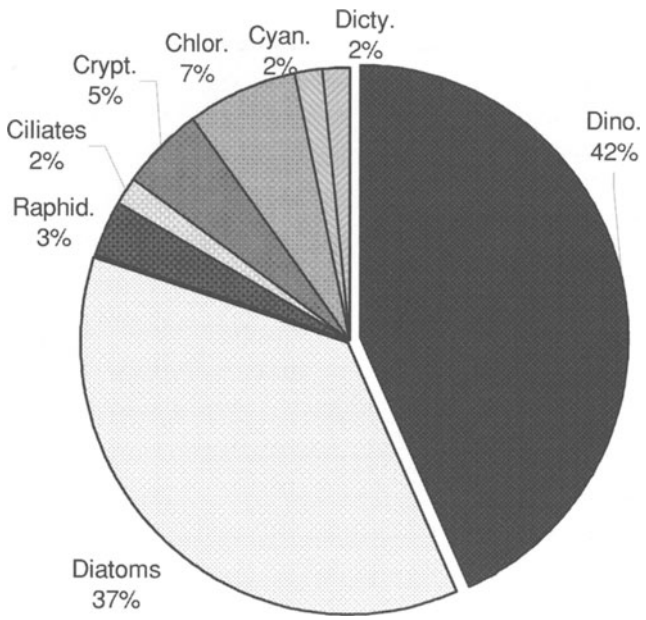
Potential harmful dinoflagellate species and harmful algal blooms

In Hong Kong, only the negative effects of PSP and CFP, and fish kills, have been reported. It has been suggested that the CFP was from imported reef fishes. Dinoflagellates containing the toxins responsible for NSP and DSP have not been reported previously.

Ciguatera toxins originate in benthic, epiphytic dinoflagellates, mainly *Gambierdiscus toxicus* (Yasumoto et al., 1977), which are grazed by herbivorous reef fish, the toxins then being transferred to carnivorous reef fish. Humans become intoxicated by eating these toxic reef fish. Ciguatera poisoning occurs throughout the Caribbean and tropical Pacific regions. There are about 50 000 people poisoned each year throughout the world (Steidinger, 1993). *Gambierdiscus toxicus* lives epiphytically on red, brown and green seaweeds, and also lives free in sediments and coral rubble, occasionally appearing in plankton suspended by current.

Gambierdiscus toxicus was first observed in planktonic samples from Junk Bay in 1997 (Lu & Hodgkiss, 1999b). Fortunately the cell number was quiet low. The toxicity of the species has not been tested. In Hong Kong, the occurrence of fish contaminated with CFP is common, and many people have been affected (Lu & Hodgkiss, 1999a). Though ciguatera is not linked in any way to local harmful algal blooms, and it has been proven that it was a result of the consumption of imported tropical coral reef fish, the occurrence of *Gambierdiscus toxicus* is still a potential source for ciguatera. Therefore, more attention should be paid to this species, and more studies should be carried out.

Gymnodinium breve originated NSP incidents have only been reported in the Gulf of Mexico (Steidinger, 1993), and more recently in New Zealand (Chang et al., 1995). The impacts of NSP are extremely severe,



Legend:

Chlor.=Chlorophyceae	Cryp.=Cryptophyceae	Cyan.=Cyanophyceae
Dicty.=Dictyochophyceae	Dino.=dinoflagellates	Raphid.=Raphidophyceae

Figure 3. Percentage contributions of different causative species to the algal blooms recorded in Hong Kong waters from 1975–1998 (from the red tide database, Agriculture, Fisheries and Conservation Department, Government of the Hong Kong SAR).

causing massive fish kills as well as mortalities of marine animals such as whales, porpoises, manatees, dolphins, sea turtles, and sea birds through food chains (Anderson & White, 1989; Landsberg & Steidinger, 1998).

The distribution of *Gymnodinium breve* is very restricted. It has only been confirmed in the Gulf of Mexico, the southeast coast of the United States and the West Indies. However, *G. breve*-like cells have been recorded from Japanese, European, Australian, and New Zealand waters (Steidinger & Tangen, 1997). The *Gymnodinium breve*-like species found in Hong Kong waters will undoubtedly spread and so, the toxicity and population dynamics of the species should be studied.

In addition to *Alexandrium catenella*, several other *Alexandrium* species as well as *Gymnodinium catenatum,* which are potential PSP producing species, have been observed in the present study. The chain-forming species of *Gymnodinium catenatum* associated with PSP was first found in 1976 in northwest Spain (Anderson et al., 1989), and the first death (of three people from eating toxic oysters and coquina contaminated by *Gymnodinium catenatum*) was reported in 1979 in Mexico (Cortes-Altamirano, 1987). This species is the most important source of PSP on the Iberian coast, causing severe problems to shellfish fisheries (Anderson, 1989). The species and incidents have also been reported in the Gulf of Mexico, Gulf of California, South America, Italy, Japan, the Philippines and Tasmania. Though this species is present in Hong Kong waters, it has never bloomed, and there is no evidence that it has caused problems in local waters.

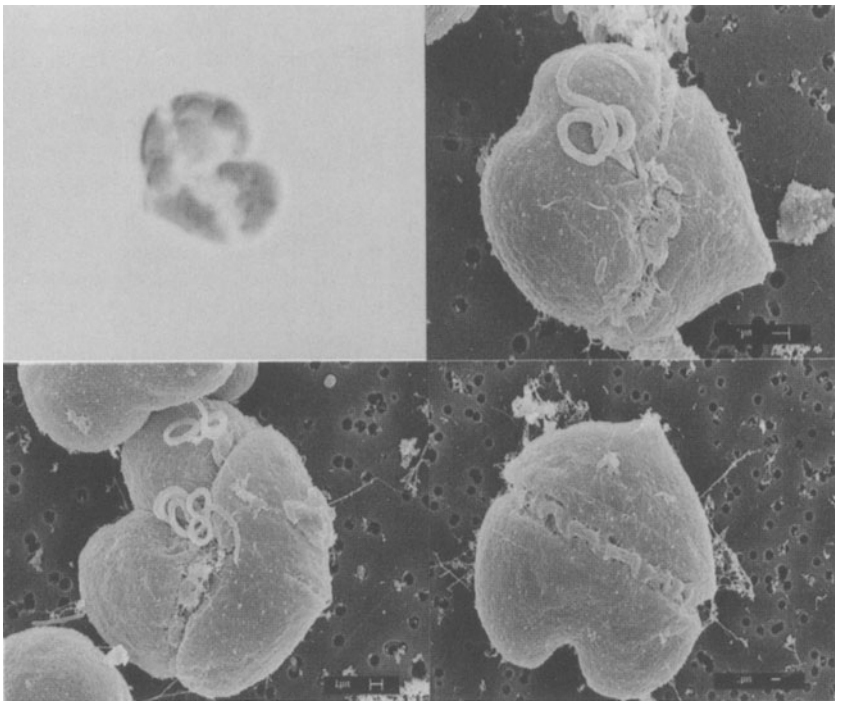


Plate 1. Gymnodinium cf. *breve.*

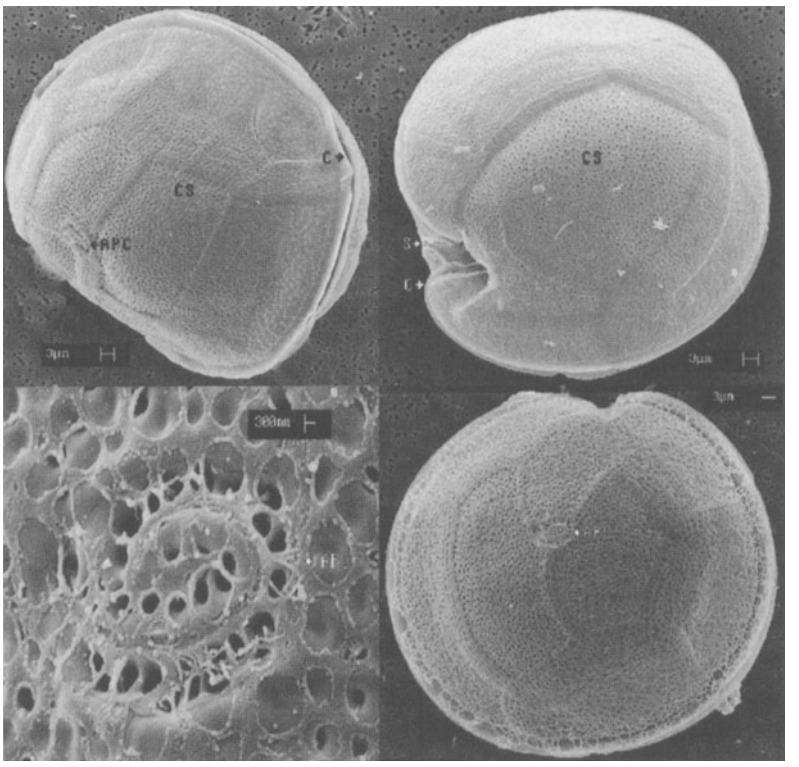


Plate 2. Gambierdiscus toxicus.

Fish kill dinoflagellates

According to historical HAB records of fish kill events, three groups of dinoflagellates have been identified as responsible for the incidents: the *Gymnodinium/Karenia* group, the *Prorocentrum* group and the *Noctiluca/Gonyaulax* group. These group were separated on the basis of the fish kill (or potential fish kill) capabilities of the species, and the frequency with which they bloomed in Hong Kong waters.

Gymnodinium/Karenia *group*

This group of species is the most harmful in Hong Kong waters and it has caused the majority of fish kill incidents. The species involved are *Gymnodinium mikimotoi, Karenia digitata,* and some other small unidentified *Gymnodinium* species. *Gymnodinium sanguineum,* and *Gyrodinium instriatum* might also be included in this group because of their high density during the present study, though they have never caused fish kills in Hong Kong waters. This group of species was abundant in spring, and most of the fish kill blooms happened in spring.

Prorocentrum *group*

This group of species includes *Prorocentrum dentatum, P. micans, P. minimun, P. sigmoides,* and *P. triestinum.* It is the second largest group responsible for fish kills in Hong Kong. The fish kills caused by this group of species were due to hypoxia or anoxia. The *Prorocentrum* species can be observed year round with highest cell density in spring.

Noctiluca/Gonyaulax *group*

The species in this group are mainly *Noctiluca scintillans* and *Gonyaulax polygramma. Noctiluca scintillans* is the most common bloom causative species in Hong Kong waters (Wong, 1989). Although some toxic substance has been reported to be produced by *Noctiluca scintillans* (Okaichi & Nishio, 1976), so far only one fish kill incident has been reported. *Gonyaulax polygramma* is another major causative species in Hong Kong. In 1988, a HAB caused by this species lasted as long as three months (Lam & Yip, 1990), which is the longest lasting bloom in Hong Kong. So far two fish kill incidents were caused by this species. The characteristics of this group of species are high frequency of blooms and low ability to kill fishes. *Ceratium furca* and *Scrippsiella trochoidea* should also be included in this group.

Acknowledgements

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