Chapter 3

Ecology and oceanography of harmful marine microalgae (Project-2)

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Introduction

In the last decade many HAB scientists noticed that HAB phenomena in Southeast Asian region were changing its nature; occurrence of new HAB species, their increased frequency, widen affected geographical area, and prolonged duration of the occurrences. Moreover variety of phenomena, i.e. new types of toxins and mortality of marine organisms, also increased. In this chapter features of the problems cause by several endemic and newly invaded HAB species are described in details, together with biological and chemical characters of the organisms. Each section is prepared by leader(s) of 4–10 scientists who work on each topic from member countries, i.e. Pyrodinium bahamense by Furio and Cayme, Alexandrium and Gymnodinium catenatum by Po Teen, benthic dinoflagellates by Omura and Fukuyo, taxonomy and distribution of Pseudo-nitzschia by Po Teen, domoic acid production of Pseudo-nitzschia by Dao, Nitzschia by Kotaki, Cochlodinium by Matsuoka, Heterocapsa by Iwataki, and Noctiluca by Sriwoon, Lirdwitayaprasit, and Furuya. Fukuyo and Kodama made an editorial arrangement.
Toxin Producing Microalgae

PSP toxin producing microalgae

Pyrodinium bahamense

The toxic dinoflagellate Pyrodinium bahamense var. compressum (Fig. 1) has caused adverse socio-economic problems in the Southeast Asian region for more than 3 decades now. This organism produces saxitoxin and other toxin derivatives that cause Paralytic Shellfish Poisoning (PSP), resulting from human ingestion of shellfish, commonly the filter-feeding bivalves that accumulate toxins as they feed on this organism. The organism is therefore responsible for human illnesses and deaths due to PSP, and repeated closures of harvesting of both wild and farmed shellfish and small pelagic fish from affected areas and marketing/trading seafood ban to consuming public.

The occurrence of P. bahamense var. compressum blooms and PSP episodes seemed to have limited geographical distribution in the Southeast Asian region mainly confined in Brunei Darussalam, west coast of Sabah, Malaysia, Indonesia and the Philippines (Fig. 2). Among them, the Philippines has the greatest number of bloom outbreaks and affected areas with highest number of PSP cases recorded (Azanza and Taylor 2001, Relox and Bajarias 2006).

Indonesia: PSP phenomenon in Kao Bay, Halmahera, East Indonesia was first reported in 1977, but only in 1993 PSP problem became obvious when the causative organism was first identified as Pyrodinium bahamense var. Compressum (Praseno and Wiadnyana 1996). In 1994, harmful algal bloom occurred in Ambon Bay and Wiadnyana and Sidabutar (1997) also identified the same causative organism, with a cell count of 1.6 × 10^6 cells/l. Since then, P. bahamense blooms have been sporadically reported in 13 coastal areas in Indonesia (Fig. 2) such as those described in various coastal waters in Lampung Bay, Jakarta Bay, Udjing Pandang, East Flores waters, off Seabitik Island, and Hurun Bay Ambon Bay (Thoha and Pangabean, pers. comm.). There have been a total of 427 PSP cases with 17 deaths reported in the country from 1983 to 1987 (Azanza and Taylor 2001). A record on PSP cases caused by P. bahamense blooms in the country has not been updated.

Malaysia: Blooms of P. bahamense associated with PSP events have been reported in the entire west coast of Sabah, Malaysia since 1976 when it first bloomed in Brunei Bay (Roy 1977). During this outbreak which lasted for 4 months, 202 cases of PSP with 7 fatalities were recorded (Roy 1977). Since then it has been reported that a frequently recurring event confined only in the west coast of Sabah (Fig. 2), particularly in Kimanis Bay and Kota Kinabalu Bay (Usup et al. 2002). The remainder of 407 PSP cases with 37 deaths (Fig. 3) has been reported countrywide including those accounted for other PSP-causing organisms such as Alexandrium species (Azanza and Taylor 2001). Additional PSP incidents in Sebatu, Melaka and Tumpat Kelantan have been documented in 1997 and September 2001, respectively, but the toxicity in shellfish were attributed to the occurrence of various species of Alexandrium (A. tamiyavanichii, A.
minutum, A. lusitanicum and A. Tropicale) (Usup et al. 2002). No occurrence of P. bahamense bloom has been ever documented in Sarawak.

Philippines: The first PSP outbreak associated with P. bahamense bloom in Western Samar Bays, Central Philippines in 1983 has been inferred as gradual HAB dispersal event within the region. Evidence of the increasing frequency and intensity of P. bahamense bloom in the country has been observed since the late eighties and early nineties. Blooms of P. bahamense and PSP have been widely affected the Philippine coastline causing extensive losses to the shellfish industry and human health problems (Estudillo and Gonzales 1984, Corrales and Gomez 1990, Azanza and Taylor 2001, Furio and Gonzales 2002, Relox and Bajarias 2006). The country has experienced more than 40 outbreaks of P. bahamense blooms in 27 coastal areas since 1983. There have been 2,465 reported PSP cases with 146 deaths from 1983 to date (Relox et al. pers. comm.). Figure 2 shows the various coastal areas in the Philippines where P. bahamense blooms occurred. Outbreaks in 1983, 1987 and 1988 resulted in direct losses to the mussel industry of $5 million each year, with equivalent indirect losses due to lack of consumer confidence in seafood products (Gonzales 1989a, b).

Dinoflagellate cysts play an important role in the initiation, recurrence and geographical expansion of HABs. The geographical distribution and abundance of cysts in marine sediment has become very essential information in giving early warnings of the presence of toxic species and the continuing recurrence of HABs in a given area.

Cysts of P. bahamense var. compressum are widespread in relatively high concentrations in the sediments of most coastal waters in the region where the species is endemic. Cysts of other toxic dinoflagellates are present at significantly low counts as follows: A. cf. minutum in mariculture areas in Pangasinan (Baula et al. 2008), and in Subic Bay (Furio et al. unpublished) all in NW Philippines; A. cf.
tamiyavanichii and Protoceratium reticulatum in Masinloc Bay, NW Philippines and A. cf. minutum and P. reticulatum in Western Samar Bays, Central Philippines (Furio et al. unpublished). Protoceratium reticulatum cyst is also present in the surface sediments of coastal waters of Sabah, Malaysia (Furio et al. 2006) and in other several basins in the Philippines (Reotita et al. 2008). The presence of cysts as enumerated above could well be a useful tool for explaining the population dynamics of these toxic species within the region as they could also pose potential risks for future bloom events.

Alexandrium

The genus Alexandrium Halim comprised of more than 30 known species. One third of the species have been reported globally to cause shellfish poisonings by their production of saxitoxin (STX) and its analogues (Anderson et al. 1990). The human intoxication incidence was due to the consumption of contaminated shellfish mollusks by the toxins which is commonly known as paralytic shellfish poisoning (PSP). In Southeast Asia, Pyrodinium bahamense var. compressum remained the main causative organism in the region, with more than two thousand cases of poisoning reported up to date (Furio and Gonzales 2002). In recent years, PSP events become more and more complicated with the presence of other species of toxin producers, Alexandrium species and Gymnodinium catenatum.

There are two species of Alexandrium, A. tamiyavanichii and A. minutum are the two progeners of PSP in Southeast Asian regions. A. tamiyavanichii was first described as Protogonyaulax cohorticula (Kodama et al. 1988) from the Gulf of Thailand and subsequently designated as A. cohorticula (Ogata et al. 1990). However, Balech (1994, 1995) re-examined the Thai specimens and based on some morphological differences described it as a new species, A. tamiyavanichii.

The thecal morphology of A. tamiyavanichii differs slightly from its morphological very closely related species, A. cohorticula. The two species can be distinguished by their anterior sulcal plate (s.a.), in conjunction to the posterior margin of the first apical plate (1’S) (Balech 1995). Detailed thecal morphology of the Malaysian strains was investigated and documented (Lim et al. 2007a) (Fig. 4A). A. tamiyavanichii generally contains toxins GTX1-5, C1-2, neoSTX and STX. However the toxin composition of Southeast Asia strains differed slightly compared
Ecology and oceanography of harmful marine microalgae (Project-2)

5

to the temperate strains from Japan (Ogata et al. 1990, Nagai et al. 2005). The toxin composition of Malaysian and Thai strains is dominated by GTX4+1 with more than 80% mole toxin (Lim et al. 2007a). However, it is interesting to note that the Malaysian strain and Japanese strains are closely related genetically with no genetic divergence observed in the LSU ribosomal gene sequences (Leaw et al. 2005). Phylogenetic analysis revealed that the species is closely related to the A. tamarense/fundyense/catenella species complex (Leaw et al. 2005).

A. minutum is among the smallest cell of Alexandrium, the species was first described by Halim in 1960 from Alexandria harbor, Egypt. The species shares the feature of short posterior sulcal plates with its closely related taxa with some distinguishable characters (e.g. Balech 1995, MacKenzie and Todd 2002, Hansen et al. 2003, Montresor et al. 2004, Lim et al. 2007a) (Fig. 4B). This small round dinoflagellate showed unique growth physiology to other Alexandrium. A. minutum in the Southeast Asia region is a euryhaline species which is commonly found in low salinity waters (Lim et al. 2004, 2007b). This is consistent with the experimental studies of cultures from Malaysia and Vietnam (Lim et al. 2005, 2007b). The high salinity tolerance of A. minutum was also reported in other regions (Hwang and Lu 2000). The species can also adapt to low light intensity with optimal growth (Lim et al. 2006). Ultrastructures examination of thylakoid peripheral arrangement in the species provided further evidence for the shade adaptation strategy of the species. In contrast, several other Alexandrium species have been shown to be light-adapted (Lim unpublished).

Toxin profiles of A. minutum from Southeast Asia regions possess unique toxin composition compared to its temperate counterparts (Hansen et al. 2003, Lim et al. 2007a). GTX4 and GTX1 remained the predominant toxin congeners regardless of environmental changes (Lim et al. 2005, 2007b). Recently, a new toxin congener was discovered from A. minutum from northern Vietnam (Lim et al. 2007b). Toxin profile cluster analysis as well as genetic analysis of ribosomal genes of the Southeast Asia strains indicated low divergence among the strains found in the regions (Lim et al. 2007a).

Alexandrium tamiyavanichii have been reported to co-exist with Pyrodinium blooms in Manila Bay, Philippine (Furio...
and Gonzales, 2002) and Kota Kinabalu (Mohammad-Noor et al. 2000). In Peninsular Malaysia, bloom of *A. tamiyavanichii* in the early 1990s had caused three person hospitalized. The blooms occurred in the Straits of Malacca where country national mussel cultivation program was implemented. The species showed unique toxin composition with GTX1-5, STX and dcSTX and C1-2 (Lim et al. 2006), in comparison to toxin profile reported from Japanese strains (Nagai et al. 2005) and South Africa (Ruiz Sebastian et al. 2005). This toxic dinoflagellate species was confined to tropical and subtropical waters. In the Southeast Asia regions, the species has been reported to occur in the Gulf of Thailand (Fukuyo et al. 1989, Kodama et al. 1990), Manila Bay of Philippines (Montojo et al. 2003), Sebatu, Malaysia in the Strait of Malacca (Lim et al. 2004), Kota Kinabalu, Malaysia (Fig. 5). Recently, the species was also reported from the tropical waters of Brazil (Menezes et al. 2008).

In the Southeast Asia region, *A. minutum* had been reported in Thailand (Matsuoka et al. 1997) and Vietnam (Yoshida and Fukuyo 2000), however, with no PSP incident reported. In 2001, bloom of the species in the northeastern of Peninsula Malaysia has resulted in hospitalization of six persons with one casualty (Lim et al. 2004). Toxicity analyses of contaminated shellfish contained mainly GTX1, 2, 3 and 4 (Lim et al. 2004). Recently, the species was also been found in several aquaculture locations in Port Dickson and Kota Belud, Malaysia (Lim unpublished). Bloom of *A. minutum* was also reported in the northern Luzon, Philippines (Bajarias et al. 2003). The distribution of the species is shown as in Fig. 5.

Other species of *Alexandrium* were also distributed widely in the Southeast Asia.
The potentially toxic *A. cf. tamarense* was found to occur in the waters of Thailand and Malaysia (Piumsomboon et al. 2000, Usup et al. 2002, Lim et al. 2003), while *A. taylori* and *A. peruvianum* were found distributed in the Kuching waters of Malaysia (Lim and Ogata 2005). In Vietnamese waters, more than 16 species of *Alexandrium* have been reported (Nguyen-Ngoc 2004).

*Gymnodinium catenatum* is the only naked dinoflagellate that is associated with paralytic shellfish poisoning (PSP). The organism forms chain in the natural environment, with moderate cell size (Fig. 6). Blooms of this species have been resulted in shellfish contamination worldwide. In the temperate Pacific region, *G. catenatum* has been reported from Australia (Hallegraeff et al. 1988), Japan (Matsuoka and Fukuyo 1994), and Korea (Park et al. 2004). In the Southeast Asia regions, the species is only found in certain locations with very few reports of occurrences, such as in the Manila Bay of Philippines (Fukuyo et al. 1993) and Kota Kinabalu, Malaysia (Mohammad-Noor et al. 2002) which co-existed with the *Pyrodinium* blooms. The species was also found to be present in the phytoplankton assemblage in Singaporean waters (Holmes et al. 2002), Lombok, Indonesia (Sidharta and Ahyadi 2007) and most recently in Thailand (Lirdwitayaprasit et al. 2008). The Thai strain was reported with n-sulfocarbomoyl C1-2 as the major congeners and GTX1-4 as minor components in the toxin composition (Lirdwitayaprasit et al. 2008). The Singapore strains of *G. catenatum* on the other hand were reported with unique toxin profile which dominated by GTX1+4, with small amount of GTX2+3, neoSTX and STX (Holmes et al. 2002).

**Benthic dinoflagellates potentially causing Ciguatera Fish Poisoning**

Ciguatera fish poisoning (CFP) is a common food-borne disease related to the consumption of subtropical and tropical marine finfish. CFP is the most common and widespread seafood poisoning afflicting approximately 50,000 victims/year in the world (Fleming et al. 2000). The concerned ciguatoxic-fish are either feeding on small algae species known as dinoflagellates or feeding on toxic herbivore fish. The main toxic dinoflagellate is *Gambierdiscus toxicus* (Adachi and Fukuyo 1979) which is found primarily in sub- and tropical areas where it lives in association with macroalgae, sand and rock. *Ostreopsis* and *Prorocentrum* are known as species occurring with *Gambierdiscus toxicus* (Fukuyo 1981), and may have implication in some symptoms of CFP.

In Japan CFP has been known for a long time only in Okinawa, the most southern islands having subtropical climate. However, several food poisoning cases suspected as CFP occurred in recent years after eating spotted knifefish (*Oplegnathus punctatus*) caught by surf fishing at several cities facing the Pacific Ocean in the temperate area of Japan. CFP causative species *Gambierdiscus toxicus* seems to have expanded the distribution area to the temperate region (Omura and Fukuyo un-
Recent reports of potentially CFP causative species, together with some associating ones, in Southeast Asia and Japan were summarized as follows.

**Gambierdiscus**


**Ostreopsis and Prorocentrum**

Nine species of *Ostreopsis* has been known in the world (*Ostreopsis belizeanus, O. caribbenaus, O. heptagona, O. labens, O. lenticularis, O. marinus, O.
mascarenensis, O. ovate, O. siamensis). They usually occur associating with Gambierdiscus and other benthic microalgae. Among them, Ostreopsis labens, O. lenticularis and O. ovata were reported from Malaysia (Leaw et al. 2001, Mohammad-Noor et al. 2006), O. lenticularis, O. marinus and O. ovata from Vietnam (Larsen and Nguyen 2004), O. ovate, O. siamensis from the Ryukyu Island, Japan (Fukuyo 1981, Koike et al. 1991). ASP toxin producing microalgae

**Pseudo-nitzschia**

Pseudo-nitzschia is a genus of marine pennate diatom comprised of thirty four species (Table 1) known up to date, with almost half of the known species are reported to produce naturally neurotoxins, domoic acid (DA) and its derivatives (Bates 2000). Contamination of DA in shellfish mollusks are the cause of human intoxication in Canada in the late 1980s, as well death of marine bird and mammals in the coasts of the United States (Scholin et al. 2000).

Under light microscope, Pseudo-

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**Table 1. Occurrence of Pseudo-nitzschia species in the Southeast Asia.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Country</th>
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<tbody>
<tr>
<td></td>
<td>Malaysia</td>
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<tr>
<td>Pseudo-nitzschia americana</td>
<td>—</td>
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<tr>
<td>Pseudo-nitzschia brasiliana</td>
<td>—</td>
</tr>
<tr>
<td>Pseudo-nitzschia caciantha</td>
<td>—</td>
</tr>
<tr>
<td>Pseudo-nitzschia calliantha**</td>
<td>+b</td>
</tr>
<tr>
<td>Pseudo-nitzschia cuspidata</td>
<td>—</td>
</tr>
<tr>
<td>Pseudo-nitzschia delicatissima**</td>
<td>+b</td>
</tr>
<tr>
<td>Pseudo-nitzschia dolorosa</td>
<td>+d</td>
</tr>
<tr>
<td>Pseudo-nitzschia fraudulenta**</td>
<td>—</td>
</tr>
<tr>
<td>Pseudo-nitzschia cf. grani</td>
<td>—</td>
</tr>
<tr>
<td>Pseudo-nitzschia heimi</td>
<td>—</td>
</tr>
<tr>
<td>Pseudo-nitzschia inflata</td>
<td>—</td>
</tr>
<tr>
<td>Pseudo-nitzschia micropora</td>
<td>+b</td>
</tr>
<tr>
<td>Pseudo-nitzschia multistriata**</td>
<td>+b</td>
</tr>
<tr>
<td>Pseudo-nitzschia pungens</td>
<td>+db</td>
</tr>
<tr>
<td>Pseudo-nitzschia pseudodelicatissima**</td>
<td>—</td>
</tr>
<tr>
<td>Pseudo-nitzschia cf. sinica</td>
<td>—</td>
</tr>
<tr>
<td>Pseudo-nitzschia subpacifica</td>
<td>—</td>
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</tbody>
</table>

Pseudo-nitzschia can be differentiated from other genera of diatoms based on the characteristic of colonies or chains formed with overlapping cells. Pseudo-nitzschia is divided into two subgroups, seriata-group with valve width larger than 3 μm and delicatissima-group with valve width smaller than 3 μm (Hasle and Syvertsen 1997). The ultrastructural observation of frustules of the cell is important in species identification. Identification to species is only possible with the aid of advanced electron microscopy.

In Southeast Asia, a total of seventeen species of Pseudo-nitzschia have been recorded so far, with five species known to be toxic (Lundholm et al. 2002, Prisholm et al. 2002, Larsen and Nguyen 2004, Bajarias et al. 2006, Yap-Dejeto 2010, Su 2010). No serious ASP case was reported from the region, however, contamination of DA in shellfish was reported in Philippines (Bajarias et al. 2006), Vietnam (Dao et al. 2009) and some tropical Asian countries (Takata et al. 2009) with no records of human poisoning.

In Malaysian waters, Skov noted the presence of five species in Sabah waters, namely, P. brasiliiana, P. calliantha, P. delicatissima, P. micropora and P. multistriata (Skov pers. comm. in Larsen and Nguyen 2004). In the recent studies, four species of Pseudo-nitzschia were documented and identified based on cultures and natural samples. They are P. pungens, P. brasiliiana, P. dorolosa and P. calliantha (Lim unpublished data). Interestingly, P. pungens and P. brasiliiana are widely distributed in Malaysian waters and were observed in samples collected from both the Straits of Malacca and South China Sea; occurrence of P. dorolosa and P. calliantha on the other hand is only confined to limited locations (Lim unpublished data).

Very few studies have been carried out to understand the bloom mechanism of Pseudo-nitzschia especially in the tropical waters. In Malaysian waters, a study was conducted to investigate the seasonal occurrence of Pseudo-nitzschia and its related environmental conditions at two estuarine waters of Sarawak, Malaysia. Cell density of Pseudo-nitzschia peaked during the months of April–May over the sampling period of 2007–2010 (Su 2010). Increases of Pseudo-nitzschia cell density was likely corresponding to low precipitation, high salinity and pH, with no clear trend with changes of macronutrient condition of the waters (Su 2010). The Pseudo-nitzschia cells were postulated to be brought into the estuaries by the semidiurnal tidal cycle (Su, 2010). Higher cell density of Pseudo-nitzschia was also observed during the dry season from December to May in Philippines (Yap-Dejeto et al. 2008) and Thailand (Udomratana et al. 2008). Presence of potentially toxic species might pose potential threats of ASP in the areas with shellfish farming farm if no monitoring effort was implemented.

Domoic acid (DA) is well known as a causative toxin of amnesic shellfish poisoning (ASP) which was first found in Canada (Wright et al. 1989). Since a pennate diatom Pseudo-nitzschia multiseries was identified as the causative plankton species in Canadian case, several species of Pseudo-nitzschia have been reported to produce a significant level of domoic acid (Bates et al. 1989). However, these studies have been limited in temperate and cold waters (Bates 2000, Trainer et al. 2008). Little is known about the DA-producing diatom in tropical waters. No information on the accumulation of DA has been obtained for tropical bivalves. Under these circumstances, Takata et al. (2009) screened the occurrence of DA in South East Asian countries and reported that bivalve belonging to a genus Spondylus accumulate a significant level of DA while other bivalve species do not. These findings indicate the occurrence of DA-producing plankton such as Pseudo-nitzschia.
spp. in these areas. As a considerable level of DA was also detected in *S. versicolor* in Nha Phu Bay, Vietnam, studies on DA in Nha Phu Bay, have been conducted in collaboration with Japanese members under the current project (Dao et al. 2009a, 2009b).

In the monitoring study on DA level of *S. versicolor* in association with that of plankton net samples, clear correlation was observed between them, indicating the presence of DA-producing plankton in the bay. Light microscopic observation of the plankton net samples showed the occurrence of *Pseudo-nitzschia* spp. but their cell number was not dominant. In a trial in which plankton cells in the samples were fractionated by successive filtration through sieves with different pore sizes, most of DA in the plankton sample was found to be concentrated in the fraction with small size particles (0.6–10 µm fraction). Light microscopic observation showed that *Pseudo-nitzschia* spp. and *Nitzschia* spp. were dominant in the fraction (Dao et al. 2009b). These results suggest that *Pseudo-nitzschia* species in the fraction is the causative species of DA. Thus, unicellular cultures were made from *Pseudo-nitzschia* species observed in 0.6–10 µm fraction. DA was detected by LC-MS/MS analysis in more than half the strains established, though the level was significantly low (unpublished data). Interestingly, all the DA-producing strains were identified as *Pseudo-nitzschia caciantha*, indicating that this species is at least one of the causative organisms for DA in *S. versicolor* in Nha Phu Bay, Vietnam.

Although DA production is not analyzed, more than 10 species of *Pseudo-nitzschia* including *P. caciantha* are listed in Vietnamese (Larsen and Nguyen 2004) and Japanese water (Yap-Dejeto et al. 2010). These include potentially toxic species such as *P. calliantha*, *P. delicatissima*, *P. multistriata*, and *P. pungens* (IOC, http://www.marinespecies.org/hab/index.php). DA production of *Pseudo-nitzschia* is reported to be influenced by various factors. Further studies on DA production of *P. caciantha* is under progress.

*Nitzschia navis-varingica*

In the field survey on harmful algal species in Vietnam, plankton net samples collected from a resting shrimp culture pond in Do Son, Vietnam were brought back to Japan. Cells of algal species suspected to produce DA were isolated for unialgal cultures, and tested for DA production by HPLC with fluorescence detection (Pocklington et al. 1990, Kotaki et al. 1999). Significant level of DA production was detected in some of the culture strains (maximum 1.7 pg/cell) (Kotaki et al. 2000). Cells of all the strains positive for DA production were morphologically the same as follows.

LM observation revealed that the cells possess two chloroplasts at each end of the cell and are lanceolate in valve view. Cells are 38–110 µm long and 9–11 µm wide. In girdle view, the cells are rectangular and slightly indented at the middle. Most cells make ribbon-shaped colonies while growing (Fig. 9). In TEM, characteristic silica ridges are observed in the wall of the raphe canal and on the mantle. The girdle bands are ornamented by silica warts and...
In the screening of *N. navis-varingica* in the Philippines, some strains of the species isolated from Bulacan Estuary, Manila Bay, were found to produce only isodomoic acids A (IA) and B (IB). No DA was detected in these strains (Kotaki et al. 2005). Isodomoic acid C was reported together with DA in shellfish and in the causative diatom *P. australis* (Rhodes et al. 2003, Holland et al. 2005). Trace amount of isodomoic acids A, D, E, F and 5′-epi-DA were detected together with DA in the tropical bivalves *Spondylus* spp. (Takata et al. 2009). Although it is uncertain whether these isomers are the artifacts of DA (Wright et al. 1989, Quilliam 2003) or bio-synthesized one by diatom, trace amount of isomers were detected together with high level of DA in *Pseudo-nitzschia multiseries* (Takata et al. 2009). However, the level of isomers in *P. multiseries* is usually vanishingly low. The finding of the *N. navis-varingica* strains which produce only isomers of DA seems to be interest-
ing from the standpoint of metabolism including biosynthesis of DA.

The toxicity of IA, IB and IC was reported to be significantly lower than that of DA (Munday et al. 2008), suggesting that these toxins pose a lower risk to humans. However, the possible risk due to bioconversion of isomers to DA in the shellfish could not be ruled out. Metabolic transformation of isomers to DA is currently under investigation.

After finding N. navis-varingica strains that produce IA and IB instead of DA, screening of toxin producing-N. navis-varingica was performed not only for DA but also for IA and IB. As a result, 5 types of toxin composition namely DA, DA-IB, IA-IB, IB, DA-IA-IB were confirmed among the N. navis-varingica strains tested (Fig. 10). Comparison of the toxin composition between the sub-strains and the parental strain showed that the toxin composition is stable in a strain. DA and DA-IB types were the major toxin composition types, because these types were seen in all isolates obtained from Vietnam (near Haiphong), southern Philippines (southern part of Manila Bay and some areas near Tacloban), Japan (Tohoku and Okinawa districts) (Kotaki et al. 2005, 2008), Thailand (near Bangkok) (Bajarias et al. 2006) and Indonesia (south Sulawesi) (unpublished data). Only the isolates obtained from limited areas in Luzon Island, the Philippines showed the rest toxin composition types. The isolates obtained from Bulacan Estuary, Manila Bay and Iba Estuary, Zambales showed an IA-IB toxin composition (Kotaki et al. 2005, Bajarias et al. 2006). The isolates from Alaminos, Pangasinan showed the toxin composition of only IB. One isolate from Cavite Estuary, Manila Bay showed the DA-IA-IB toxin composition (unpublished data). All of the diatoms were morphologically the same species N. navis-varingica.

When axenic cultures were made from some uni-algal culture of DA-IB type, the toxin composition changed its toxin composition to IA-IB type, suggesting the bacterial effect on controlling the toxin composition (Kotaki et al. 2008). Possible factors affecting the toxin composition such as bacteria, salinity, pH and/or genetic difference among strains are important to solve the toxin production mechanism of the diatom. Efforts to solve these factors are currently under way.

Red Tide Causative Species

Cochlodinium: Red tide causative species with fish kills

Taxonomy of Cochlodinium polykrikoides and morphologically similar species

In Asian waters, five species tentatively attributable to the genus Cochlodinium
Y. FUKUYO et al.

Table 2. Morphological features of four HAB species attributable to the genus Cochlodinium.

<table>
<thead>
<tr>
<th>C. convolutum</th>
<th>C. cf. geminatum</th>
<th>C. polykrikoides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td>ca. 60–70 µm</td>
<td>ca. 40–40 µm</td>
</tr>
<tr>
<td>Eye spot</td>
<td>non</td>
<td>non</td>
</tr>
<tr>
<td>Cingulum</td>
<td>ca. 1.5</td>
<td>ca. 1.5</td>
</tr>
<tr>
<td>Sulcus</td>
<td>deep</td>
<td>Shallow, just below the cingulum</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Spherical, center</td>
<td>Spherical, epicone</td>
</tr>
<tr>
<td>Chloroplast</td>
<td>Rod-like, longitudinally granulate</td>
<td>Rod-like, longitudinally granulate</td>
</tr>
<tr>
<td>Other</td>
<td>Four cells chain</td>
<td>16 cells chain</td>
</tr>
</tbody>
</table>

have been observed up to date (Fig. 11). These are all photosynthetic with morphologically variable chloroplasts, and have more or less made blooms; Cochlodinium catenatum Okamura, Cochlodinium convolutum Kofoid and Swezy, Cochlodinium fulvescens Iwataki, Kawami and Matsuoka, Cochlodinium cf. geminatum (Schütt), and Cochlodinium polykrikoides Margalef (Table 2). C. catenatum described from Tokyo Bay in 1916 by Okamura was morphologically similar to C. polyrkikoides (Matsuoka et al. 2008), however, the taxonomical relationship of these species is still unclear.

Cochlodinium polykrikoides Margalef has been well known to form large-scale blooms (e.g. Matsuoka et al. 2008, 2010). In Asian waters, this species was first observed in the Yatsushiro Sea, Japan in 1976 accompanied with huge economic damage (Kagoshima Prefectural Fisheries Station 1995). Thereafter, this species has occurred almost every summer in Japan and Korea during the last thirty years. In Southeast Asia, C. polykrikoides was first detected in the Philippines in 2002 (Relox Jr. and Bajarias 2003) and then in coastal waters of Hong Kong, Philippines and Malaysian Sabah (Iwataki et al. 2008, Matsuoka et al. 2008). Recent progress on a molecular phylogenetic study on C. polykrikoides, at least four ribo-types (American-Malaysia type, Philippines type, East Asian type including the so-called Cochlodinium sp. Kasasa type) were recognized (Iwataki et al. 2008, Matsuoka et al. 2010).

Cochlodinium fulvescens, morphologically similar to C. polykrikoides, was first described in the Asian coastal waters as well as the east coast of the North America by Iwataki et al. (2007). Cochlodinium cf. geminatum was initially found in the coast of Hong Kong accompanied with fish-kill. This species can produce a resting cyst preservative in sediments. According to this nature, the cyst of Cochlodinium cf. geminatum has been recorded from tropi-
Ecology and oceanography of harmful marine microalgae (Project-2)

Fig. 12. Geographical distribution of harmful *Cochlodinium* in East and Southeast Asia.

C. polykrikoides has been reported from Sabah, Malaysia (Anton et al. 2008), Palawan, the Philippines (Azanza et al. 2008), Balayan Bay of Luzon Island, the Philippines (Relox Jr. and Bajarias 2003) up to date. According to Iwataki et al. (2008), *C. polykrikoides* occurring from Sabah was assignable to the American-Malaysian ribo-type and *C. polykrikoides* from Luzon Island to the Philippines ribo-type. Around the East China Sea, most of *C. polykrikoides* were designated into the East Asian ribo-type (Iwataki et al. 2008, Matsuoka et al. 2010).

*C. fulvescens* occurred in Hurun Bay of Sumatra, Indonesia (Matsuoka et al. 2008). *C. convolutum* has been recorded from Sebatu in south of Malacca Strait, Malaysia (Lim pers. comm.). These occurrences of *C. fulvescens* and *C. convolutum* never caused any serious damages for aquaculture. Other occurrences of *Cochlodinium* were recorded in the inner Gulf of Thailand in 1991 and 1992 with discolorations (Lirdwitayaprasit 2003), but unfortunately species of these *Cochlodinium* were unknown.

Bloom with fish kill in Southeast Asia (Fig. 13)

In Southeast Asia, only few cases of fish-kill events caused by *C. polykrikoides* have been reported from Luzon and off Palawan of the Philippines and Saba of Malaysia. As previously mentioned, other photosynthetic species of *Cochlodinium*, *C. fulvescens* and *C. cf. geminatum* are
harmful, but no incidents given by these species were reported so far.

In the Philippines, Relox Jr. and Bajarias (2003) reported the event from Balayan Bay of Luzon Island with large mass mortality of reef fishes. According to Azanza et al. (2008), kills of reef fishes including *Plectropomus leopardus*, *Scarus* sp., *Lethrinus haematopterus*, *Gymnothorax undulates*, and *Naso brevirostris* happened with a *Cochlodinium* bloom along the western coast of Palawan. In Sabah of Malaysia, a bloom of *C. polykrikoides* killed various finfish cultured in cages. For considering the toxicity of *C. polykrikoides*, it is notable that not only finfish but also other benthic organisms were also killed (Miyahara et al. 2004, Furuya et al. 2010).

Other characteristics of *Cochlodinium polykrikoides* blooms

There is satellite evidence for the advection of *C. polykrikoides* blooms from the Korean Peninsula to western Japan (Miyahara et al. 2005, Onitsuka et al. 2010). Also, the large bloom occurred along Palawan Island, Philippines was resulted by moving from Brunei across Malaysian waters to Philippines (Azanza et al. 2008). Thus, *C. polykrikoides* can be transported even in less nutrient of off-shore area for so long distances, over 500 km in the case of Sabah to Palawan (Azanza et al. 2008).

**Heterocapsa: Red tide causative species with shellfish kills**

The genus *Heterocapsa* is composed of relatively small armored dinoflagellates, including a species responsible for harmful red tides, *H. circularisquama* (Horiguchi 1995). Although some *Heterocapsa* species such as *H. pygmaea*, *H. rotundata* and *H. triquetra* have been recognized as red tide-forming flagellates, they have not caused harmful effects. Since the first occurrence of *H. circularisquama* in Kochi Prefecture, Japan in 1988, its harmful red tides have spread in the coastal waters of western Japan involving mass mortalities of shellfishes. Since the red tides have sporadically occurred in shellfish culturing bay areas of the red tide un-reported, its migration in Japanese coasts has been supposed to be artificial, e.g. surface transportation of shellfishes (Honjo et al. 1998). However, the distinctive red tides involving harmful effects only for shellfishes have not been reported, and distribution of *H. circularisquama* was unclear before the first occurrence in Japan. A possibility of its original distribu-
tion was assumed to the tropical or subtropical areas because the optimum temperature and salinity for growth were relatively high compared to other red tide flagellates occurred in Japan (Yamaguchi et al. 1997). This presumption was supported by the evidence for *H. circularisquama* red tides in Hong Kong during 1986–1987, immediately before the first occurrence in Japan (Iwataki et al. 2002b). It also suggested that *H. circularisquama* might distribute in Southeast Asian coast with similar environmental condition to Hong Kong.

For understanding of the *H. circularisquama* red tides we carried out taxonomic study on the genus *Heterocapsa* to distinguish the harmful species, and distribution survey including other *Heterocapsa* species.

### Taxonomy

After the species description of *H. circularisquama* in 1995, eight *Heterocapsa* species (*H. arctica*, *H. horiguchii*, *H. huensis*, *H. lanceolata*, *H. orientalis*, *H. ovata*, *H. psammophila* and *H. pseudotriquetra*) were described mainly for the purpose of discrimination from the harmful species *H. circularisquama* (Horiguchi 1997, Iwataki et al. 2002a, 2003, 2004, 2009, Tamura et al. 2005). Most recently a subspecies, *H. arctica* subsp. *frigida* was reported from the Baltic Sea (Rintala et al. 2010), and consequently 16 species have so far been assigned to the genus. Since *Heterocapsa* species share similar morphological characters, light microscopic identification is difficult for some species. Testal plate arrangements of these species have been recognized to be identical, Po, cp, 5′, 3a, 7′, 6c, 5s, 5″, 2″″, even though the variation are often found in culture condition. Using light microscopy, the position of the nucleus and pyrenoid, i.e. located in the epitheca or hypotheca, and the cell shape such as the larger epitheca or presence of antapical horn, are also available for provisional species identification, however, these combination are inadequate for unambiguous identification for many *Heterocapsa* species (Iwataki 2008). On the other hand, ultrastructure of body scale is a reliable morphological character for species identification, and species recently described have been established based mainly on this diagnostic character. The body scale is tiny organic cell covering situated on the cell surface, composed of a reticulated basal plate ca. 200–500 nm in diameter and a three-dimensional framework on the plate. Since the size, shape of basal plate, numbers of vertical and horizontal bars are congruous in each species and apparently different from other species, the scale structure is available for species discrimination. For example, scale of *H. circularisquama* consists of a circular basal plate with a central and six marginal uprights connected by horizontal bars one another, by which species can be distinguished from others. Ultrastructure of *Heterocapsa* body scales was summarized in Iwataki et al. (2004) and almost all species can be identified based on the structure. Moreover, the body scale structure is kept in preserved phytoplankton specimen and therefore presence of *H. circularisquama* in Hong Kong was demonstrated after 15 years from the blooming (Iwataki et al. 2002b).

### Distribution of *H. circularisquama*

Since it was strongly suggested that the harmful species *H. circularisquama* distributes not only in western Japanese coasts but also in the coasts of Southeast Asia, we surveyed presence of this species in Vietnamese coasts, Hai Phong, Hue, Nha Trang and Phu Quoc Island during in 2006 and 2007. In the samples collected from these coasts, a *Heterocapsa* was found at two locations in Hue. The cell is relatively small and resembling *H. pygmaea* due to having plural pyrenoids located above the nucleus in the hypotheca, while the scale shape is similar to that of *H. ildefina* with a difference in number of marginal up-
rights on basal plate. This species was revealed to be an undescribed *Heterocapsa* species on the basis of body scale structure and the sequencing of ITS region as a molecular marker, consequently it was described as a new species *H. huensis* under collaboration with Vietnamese scientists (Iwataki *et al.* 2009). The harmful species, *H. circularisquama*, has not been detected from Vietnamese coasts, therefore the occurrence has so far been reported only from Hong Kong in Asia, except for the continuous reports from Japanese coasts involving economic losses of shellfish cultures. Recently, the presence of *H. circularisquama* was unexpectedly reported from Cuba, as a preliminary identification without body scale structure (Moreira González 2010). This implies that habitation expansion of *H. circularisquama* due to artificial transfer or unveiling its cryptic flora is now unveiling, and supports the importance to monitor the occurrence of *H. circularisquama* in Southeast Asia to understand its distribution and mechanism of globalization.

**Noctiluca:** Red tide species associated with eutrophication

*Noctiluca scintillans* is a common red tide species which is world wide distributed in the coastal area. *Noctiluca* red tides appear as pinkish red in various temperate and subtropical waters but causes greenish discoloration in tropical waters of the western Pacific and the Indian Ocean (Elbrächter and Qi 1998, Harrison *et al.* pers. comm.). This difference in color is due to the presence of a tiny green flagellate endosymbiont, *Pedinomonas noctilucae*, in *N. scintillans* cells (Sweeney 1971). Therefore, *N. scintillans* harbouring the symbiotic green algae is referred to as green *Noctiluca* and red *Noctiluca* means those lack of the symbionts. The outbreak of green *Noctiluca* red tide is also the common phenomena in the eutrophicated waters such as Jakarta Bay (Adnan 1984), Manila Bay (Jacinto *et al.* 2006) and the upper Gulf of Thailand (Suvapeepun 1989). In Manila Bay, since 1998 up to the present, green *Noctiluca* has dominated other phytoplankton species during the period that *Pyrodinium* used to bloom (Jacinto *et al.* 2006) and the bloom of green *Noctiluca* formed occasionally almost whole area of the bay since 2001 (Furuya *et al.* 2006a). In the Gulf of Thailand, green *Noctiluca* is a main causative red tide species. The bloom of green *Noctiluca* was first reported in 1957 (Charernphol 1958), since then the blooms occurred more frequently in the eastern part of the upper Gulf and the study on seawater discoloration has been focused on its impact on fisheries. The dense blooms of green *Noctiluca* occasionally cause the mass mortality of both coastal fishes and shrimp culture (Adnan 1989, Suvapeepun 1989, Lirdwitayaprasit *et al.* 1995, Pollution Control Department 2003). Green *Noctiluca* is selected as one of targeted species for cooperative international research in GEOHAB Asia (Furuya *et al.* 2010).

Red *Noctiluca* is characterized as a voracious predator with a diverse diet ranging from phytoplankton to copepods and fish eggs (Hattori 1962, Schaumann *et al.* 1988, Nakamura 1998). A linkage of increasing blooming events of red *Noctiluca* with progressive eutrophication of coastal waters is suggested from increasing prey availability due to eutrophication (Porumb 1992). In contrast, the presence of photosynthetic endosymbiont in green *Noctiluca* implies different dependence on environmental conditions from that of red *Noctiluca*. The symbiont ensures the survival of green *Noctiluca* during the shortage of food particles (Saito *et al.* 2006). Thus, it is highly probable that nutrient and light intensities affect growth of green *Noctiluca* directly and indirectly through the growth of both phytoplankton as prey and the symbiont.
Therefore, the research group of *Noctiluca* in the JSPS-ORI multilateral project on coastal oceanography (the research group hereafter) aims to understand the mechanism of the apparent expansion of bloom of green *Noctiluca* in the Southeast Asian waters by ecophysiological research of this species.

**Life history and physiology**

The life cycle of red *Noctiluca* comprises both asexual binary fission and sexual reproduction (Zigmark 1970). The complete sexual process in red *Noctiluca* was reported by Fukuda and Endoh, 2006 while green *Noctiluca* performs sexual reproduction in the same manner as the red one (Lirdwitayaprasit 2002). Gametocytes of green *Noctiluca* are frequently observed in the upper Gulf of Thailand during the SW monsoon (Sriwoon et al. 2008). In cultures of green *Noctiluca*, gametocytes are constantly observed during the exponential growth phase (T. Lirdwitayaprasit and K. Furuya unpublished data). Therefore, the marked occurrence of gametocytes in the upper gulf was indicative of active growth of green *Noctiluca*.

Since green *Noctiluca* harbors *P. noctilucae*, photosynthesis of *P. noctilucae* is of a particular concern of the research group. Saito et al. (2006) showed that cultures of non-feeding strains, isolated from the upper Gulf of Thailand, grow photoautotrophically for generations, but they also feed on *D. tertiolecta*, indicating phagotrophy is facultative. Net photosynthesis was significantly higher in the non-feeding strains than the feeding ones. The difference is due to high respiration activity in the feeding strains. This is consistent with a observation in a natural population of Manila Bay, where net photosynthesis was significantly higher in cells lacking food vacuoles than those with food vacuoles (Saito et al. unpublished data). The relationship of photosynthesis with irradiance is characterized by saturation at low light intensity and absence or weak photoinhibition, showing efficient utilization of a wide range of light intensities.

The vegetative growth of green *Noctiluca* was also investigated using cultures and in natural populations. The research group found that under optimal growth conditions there is no significant difference in growth rate between green *Noctiluca* (0.33 day$^{-1}$) and red *Noctiluca* (0.28 day$^{-1}$) isolated from Gulf of Thailand and the Seto Inland Sea, respectively (Furuya et al. 2006b). The role of phagotrophy on population growth of green *Noctiluca* in the upper Gulf of Thailand clearly showed that the higher abundance of *N. scintillans* in the SW monsoon than in the NE monsoon was consequence of active growth supported by phagotrophy (Sriwoon et al. 2008). This was consistent with laboratory evidence that feeding strains grow faster than non-feeding strains (Furuya et al. 2006b).

A cell cycle analysis conducted in a natural population in Manila Bay revealed a diurnal rhythm in cell division, peaking during early morning. The in situ specific growth rate of 0.16 d$^{-1}$ as determined from a diurnal rhythm of nuclear DNA content is within a range reported for the heterotrophic *N. scintillans* in temperate waters (Furuya et al. 2006b).

**Ecology**

Population dynamics of green *Noctiluca* associated with the monsoon cycle in the upper Gulf of Thailand was investigated and found that there is a distinct association between the abundance of *N. scintillans* and the monsoon cycle, with its blooms occurring during the southwest (SW) monsoon from May to September, and low abundance during the northeast (NE) monsoon from November to February. The higher nutrient in SW monsoon rather than NE monsoon is the favor condition for algal growth and the higher abundance of *N. scintillans* in the SW monsoon is manifested primarily by higher
growth of *Noctiluca* through both sexual and asexual reproduction supported by phagotrophy (Sriwoon *et al.* 2008). Hansen *et al.* (2004) revealed that phagotrophy on *Pyrodinium bahamense* var. *compressum* contributed significantly (30%) to the direct growth of green *Noctiluca*, suggesting that this species may play an important role as the grazer to control other phytoplankton population. Sriwoon *et al.* (2008) concluded that growth of phytoplankton as prey of green *Noctiluca* during the SW monsoon is a key factor of the bottom-up control of the population dynamics of *N. scintillans*, and that the seasonal shift in the circulation pattern associated with the monsoon cycle plays a crucial role in blooming of *N. scintillans* by producing favorable food conditions.

**Bloom and eutrophication**

As consequences of progressive eutrophication in the Southeast Asia coastal water, in particular near megacities, increased phytoplankton standing stock and zooplankton production persist for months. Thus, the elevated food availability likely favors phagotrophy of green *Noctiluca* and enhances its active growth. The endosymbiosis apparently provides advantage to green *Noctiluca* over phytoplankton by increased chance to survive or maintain its population under unflavored conditions such as during the NE monsoon when nutrient concentrations decrease. Although green *Noctiluca* is harmless, negative impacts of its dense bloom on fisheries are well recognized particularly during a decay process of blooms due to massive slime production which can clog the gills, oxygen depletion, high ammonia concentration released from green *Noctiluca* cells (Subrahmanian 1985, Schaumann *et al.* 1988, Xie *et al.* 1993). Therefore, the prevention of its dense bloom is of a public concern. Our understanding of ecophysiology of green *Noctiluca* has much advanced during the last decade. However, our knowledge on field phenomena is still much limited in the upper Gulf of Thailand and Manila Bay. Therefore, we are not sure how much applicable the existing knowledge to a certain area. For example, Jakarata Bay is known for its highly eutrophic conditions, and occurrence of green *Noctiluca* blooms, but little is known for bloom formation mechanisms. This is the case in various Southeast Asian waters. After the JSPS-ORI programme on coastal oceanography, the establishment of a new framework for international cooperative studies on eutrophication and expansion of green blooming is requisite not only to advance our knowledge, but also to obtain better governance of coastal waters.

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Ecology and oceanography of harmful marine microalgae (Project-2)


