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Biology, ecology and bloom dynamics of the toxic marine dinoflagellate *Pyrodinium bahamense*

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ABSTRACT

It has been 40 years since the first recorded toxic bloom of *Pyrodinium bahamense* occurred in Papua New Guinea in 1972. Subsequently this species has increased in importance as a paralytic shellfish poisoning toxin (PSTs) producer in several regions of the world, especially in the Indo-west Pacific. *P. bahamense* is a thecate tropical/subtropical euryhaline dinoflagellate. Available data indicate that it forms blooms only in waters of 20 psu or higher salinity and at temperatures above 20 °C. It is monospecies with two varieties, namely var. *compressum* and var. *bahamense*. For many years it was widely accepted that only var. *compressum* is toxic and is limited to the tropical Pacific while var. *bahamense* is nontoxic and is limited to the tropical Atlantic. It is now known, however, that there are at least two locations where the varieties co-occur and it has also been proven that var. *bahamense* in Florida waters also produce PST. *P. bahamense* has a life cycle typical of many dinoflagellates. It has a heterothallic sexual cycle that produces a large spiny spherical resting cyst. The toxicity profile of *P. bahamense* is also very simple with most isolates producing only dc-STX, STX, neoSTX, B1 and B2 toxins. Further studies are needed in order to resolve the varietal status of the species and also to understand the environmental factors that determine its toxicity and bloom dynamics.

1. Introduction

The thecate *Pyrodinium bahamense* is a very important member of paralytic shellfish toxin (PST)-producing marine dinoflagellates especially in tropical waters. This species have caused more human illnesses and fatalities than any other PST producing dinoflagellates. *P. bahamense* gained prominence from the early 1970s with a spate of toxic blooms in the Indo-Pacific and the Pacific coast of central America. The first confirmed toxic bloom of *P. bahamense* occurred in Papua New Guinea in 1972 (Maclean, 1989). Subsequently first incidences of toxic blooms of the species occurred in Brunei and Sabah, Malaysia in 1976, Manila Bay, the Philippines in 1983, Mindanao, the Philippines in 1983, Ambon, Indonesia in 1994, Palawan Island, the Philippines in 1998 and the Pacific coast of Guatemala in 1987 (Roy, 1977; Maclean, 1989; Rosales-Loessener, 1989; Wiadnyana et al., 1996; Sombrito et al., 2004). At present, *P. bahamense* continues to be a significant cause of seafood toxicity in Southeast Asia and has also emerged as a potentially important source of toxicity on both the Pacific and Atlantic coasts of central America, including Florida (Landsberg et al., 2006; Martinez-Lopez et al., 2007; Garate-Lizarraga and Gonzalez-Armas, 2011).

Early on *P. bahamense* had a reputation as a very difficult species to culture in the laboratory. It was only since the early 1990s that cultures became routinely available in some laboratories and this opened opportunities for studies on the life cycle, physiology, toxicity and genetics of the species. The first comprehensive review of the species was published in 1998 (Usup and Azanza, 1998) and since then more new knowledge have been generated on this important species. The most profound change in our knowledge of the species arguably has to do with the debunking of the long-standing belief in the non-toxicity of the variety *bahamense* and the absence of toxic population in the tropical Atlantic.

2. Taxonomy, history and paleobiology of *P. bahamense*

2.1. Taxonomic history

In 1906, Plate first described *P. bahamense* from the New Providence Island in the Bahamas. Thereafter, Bohm (1931)

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observed *P. bahamense* samples from the Persian Gulf and noted slight morphological differences such as shorter apical horn and antapical spine, and anterioposteriolly more compressed body and gave a new taxonomic status as forma, and named *P. bahamense* forma *compressa*. Later, Matzenauer (1933) described independently *Gonyaulax schilleri* from the Red Sea that is similar to the present *P. bahamese* var. *compressum*, and observed four apical plates in the epitheca of this dinoflagellate. However, Schiller (1937) realized that *G. schilleri* is a junior synonym to *P. bahamese* f. *compressa*, and proposed a new combination for this species, *Pyrodinium schilleri* (Matzenauer) Schiller.

In 1939, Woloszynskia and Conrad reported *Pyrodinium phoneus* from toxic blooms that occurred on the Belgian coast of the North Sea. However, this species is now considered to belong to the genus *Alexandrium* rather than *Pyrodinium* (Taylor and Fukuyo, 1989) on the basis of its smooth theca, round cell shape and plate pattern. In 1942, Tafall found two different species of *Pyrodinium* from the Mexican Pacific coast and described them as *P. bahamense* and *P. schilleri*, the latter being the same as *P. bahamense* f. *compressa*.

Steidinger et al. (1980) made a morphological comparison of both *P. bahamense* from the Caribbean Sea and *P. bahamense* f. *compressa* from the west Pacific, and proposed a new taxonomic status for the latter as *P. bahamense* var. *compressa*. They also suggested that only *P. bahamense* var. *compressa* (=var. *compressum*) is toxic, while *P. bahamense* var. *bahamense* (autonym) is not toxic. However, it has since been proven that var. *bahamense* isolates could produce PSTs in culture (Landsberg et al., 2006).

Questions remained as to whether the species should even be split into varieties. Balech (1985) made detailed observations on the external morphology and plate patterns of both var. *compressum* collected from the Philippines and var. *bahamense* from the Caribbean Sea and concluded that *P. bahamense* is monospecific with insufficient morphological difference between these two to be separated even as varieties. Matsuoka et al. (1989) also reported that the cyst of *P. bahamense* var. *compressum* in Southeast Asia has mostly the same morphology as the cyst of var. *bahamense*. However Badylak et al. (2004) concluded that the variety *bahamense* from the Indian River Lagoon, Florida was significantly different and should be separated from the variety *compressum* based on key morphological and dimensional characteristics. Later Martinez-Lopez et al. (2007) reported an occurrence of cells morphologically similar to var. *bahamense* from the Gulf of California in the eastern Pacific. This was one of the first report on the occurrence of the var. *bahamense* in the Pacific. Based on these evidence Morquecho (2008) re-examined the morphology of *P. bahamense* collected from the Gulf of California and concluded that the two varieties are not taxonomically distinct. These views notwithstanding, the genus *Pyrodinium* is now widely accepted as monospecies with two varieties namely var. *bahamense* and var. *compressum* under the concept proposed by Steidinger et al. (1980). This concept consists of the following points pertaining to the var. *compressum*: (1) broader apical horn without prominent apical spine, (2) anterior-posteriorly compressed body, (3) formation of long chains, (4) sometimes apparently having five apical plates, (5) different surface markings, and (6) producing neurotoxins.

2.2. Morphological description of P. bahamense

The Kofoidian plate formula of *P. bahamense* is Po, pc, 4′, 0a, 6″, 6c, 5 + ?s, 5‴, 2⁗ (Fig. 1). Cells are usually subspherical to laterally ellipsoidal, covered with thecal plates and ornamented with apical projection or node (Fig. 2(1)) and anterior projection (Fig. 2(2)). Major thecal plates are thick with many tiny knobs (Fig. 2(3)) that are evenly distributed on the plates. Trichocyst pores are also numerous and clearly visible and sometimes distributed along the sutures. Cingular lists are well developed without ribs. Sulcal fins are prominent on both sides of the sulcal region and sometimes cover most parts of the sulcus. Tiny knobs are present on cingular lists and sulcal fins (Fig. 2(4)). An apical projection is formed from the extension of sutures around the apical pore plates although the

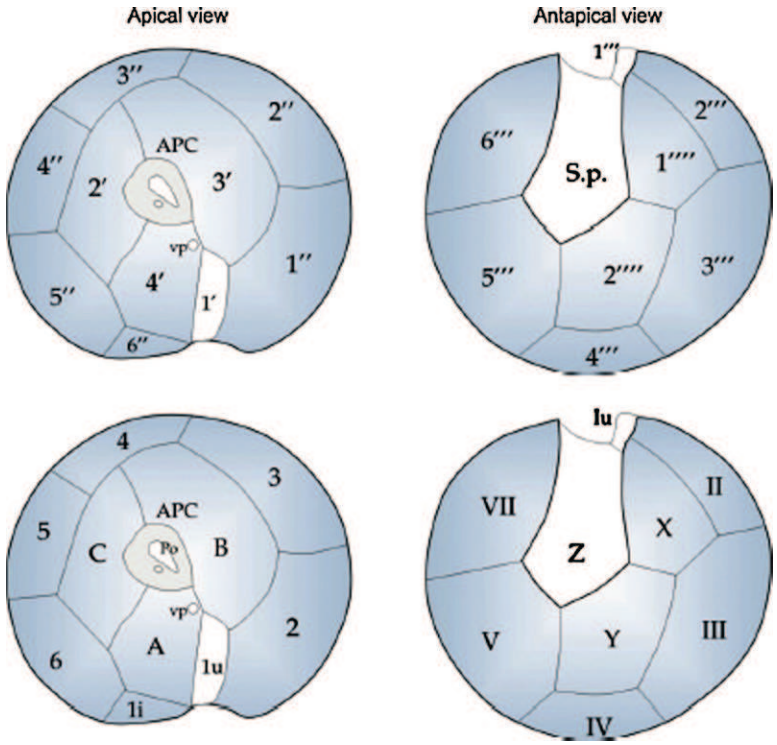

Fig. 1. *Pyrodinium bahamense* thecal plate patterns according to the Kofoid scheme (top) and Taylor–Evitt scheme (bottom).

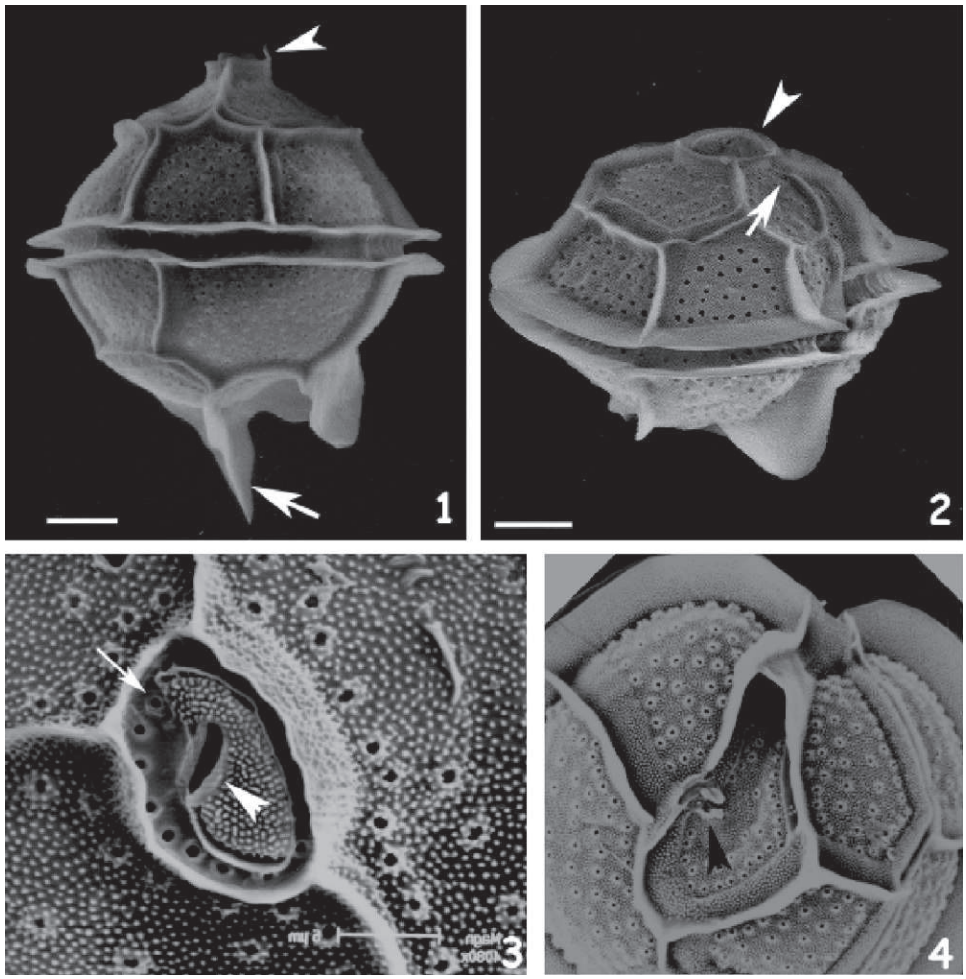

Fig. 2. Scanning electron micrographs of two varieties of *Pyrodinium bahamense*. (1) The variety *bahamense*. Arrows indicate the apical horn and antapical horn. (2) The variety *compressum*. Arrows indicate the apical node and the ventral pore. (3) Closeup of the apical pore plate. Arrows indicate the comma-shaped canopy and trichocyst pore. (4) The sulcal posterior plate. Arrow indicates the posterior attachment pore. All specimens collected from Qatar of Persial Gulf. Scale bar: 10 μm.

extension of the suture can be very variable from almost no extension (just a node) to five times the height of the suture. The apical pore plate is circular-triangular and contacts with plates 2′, 3′ and 4″, and comma-shaped dorsio-ventrally accompanied by a small pore the shape of which is variable (Fig. 2(3)). The 1′ is irregularly rhomboidal, smallest among the apical plates and not directly in contact with the apical pore plate. The suture between the 1′ and 4′ is sometimes incompletely developed near the apical pore plate. A small ventral pore is present near the left anterior part of the 4′ plate (Fig. 2(3)).

Around the apical pore plate, sutures extend to the anterior, forming a structure like a short and wide cylinder or a shallow and wide hole. At the posterior end of the vegetative cell, two projections develop (Fig. 2(1)). The left projection is always larger and taller than the right, and sometimes ornamented with a membrane rising up from sutures. However, the length and width of these projections can be variable with chain formation (Fig. 2(2)). The large posterior sulcal plate (S.p.) is irregularly quadrangular in shape and sometimes has small posterior connecting pores on the left side (Fig. 2(4)). The sulcal sinisteral anterior plate (S.s.a.) and the first postcingular plate are clearly separated by a well-developed suture, similar to those seen in other plate boundaries.

2.3. Phylogeny of P. bahamense

Pyrodinium is classified into a recognizable taxon under the subfamily Pyrodinioideae by Fensome et al. (1993) based on the fossilizable dinosporin cysts. The classification of *P. bahamense* is generally phenetic (Steidinger et al., 1980; Balech, 1985), but in some cases a phylogenetic hypothesis is implied (Fensome et al., 1993; Leaw et al., 2005). The molecular phylogenetic inference of *P. bahamense* constructed based on nuclear encoded SSU ribosomal RNA genes (Usup et al., 2002; Zhang et al., 2005), LSU rRNA gene (Ellegaard et al., 2003; Leaw et al., 2005), and mitochondrial cytochrome b (Zhang et al., 2005) generally place *Pyrodinium* in the Gonyaulacales (Fig. 3). Leaw et al. (2005) revealed that *Pyrodinium* is paraphyletic based on LSU rDNA phylogeny and morphological data of specimens identical to *P. bahamense* var. *compressum* collected from Sabah, Malaysia. The analysis resulted in a grouping of *P. bahamense* with some species of *Alexandrium* in the subgenus *Gessnerium* Balech, supporting the monophyly of *Pyrodinium* and *Alexandrium* (Leaw et al., 2005).

P. bahamense has always been considered as a sister taxon of *Alexandrium* based on plate tabulation and structural homology (Steidinger et al., 1980; Balech, 1985). Morphology-based phylogenetic analysis of Leaw et al. (2005) also supported the sister clade of *P. bahamense* with *Alexandrium taylori* Balech and *Alexandrium foedum* Balech (Fig. 3). However, they were distinguished at the generic level by Balech (1985) based on the strongly developed theca with apical and antapical spines in *P. bahamense* compared to the thinly thecated *Alexandrium* species; and by Fensome et al. (1993) based on the metasert first apical homologue (1u) and the position of the right sulcal plate and the first postcingular homologue in the sulcus (Fig. 1). The molecular phylogenetic

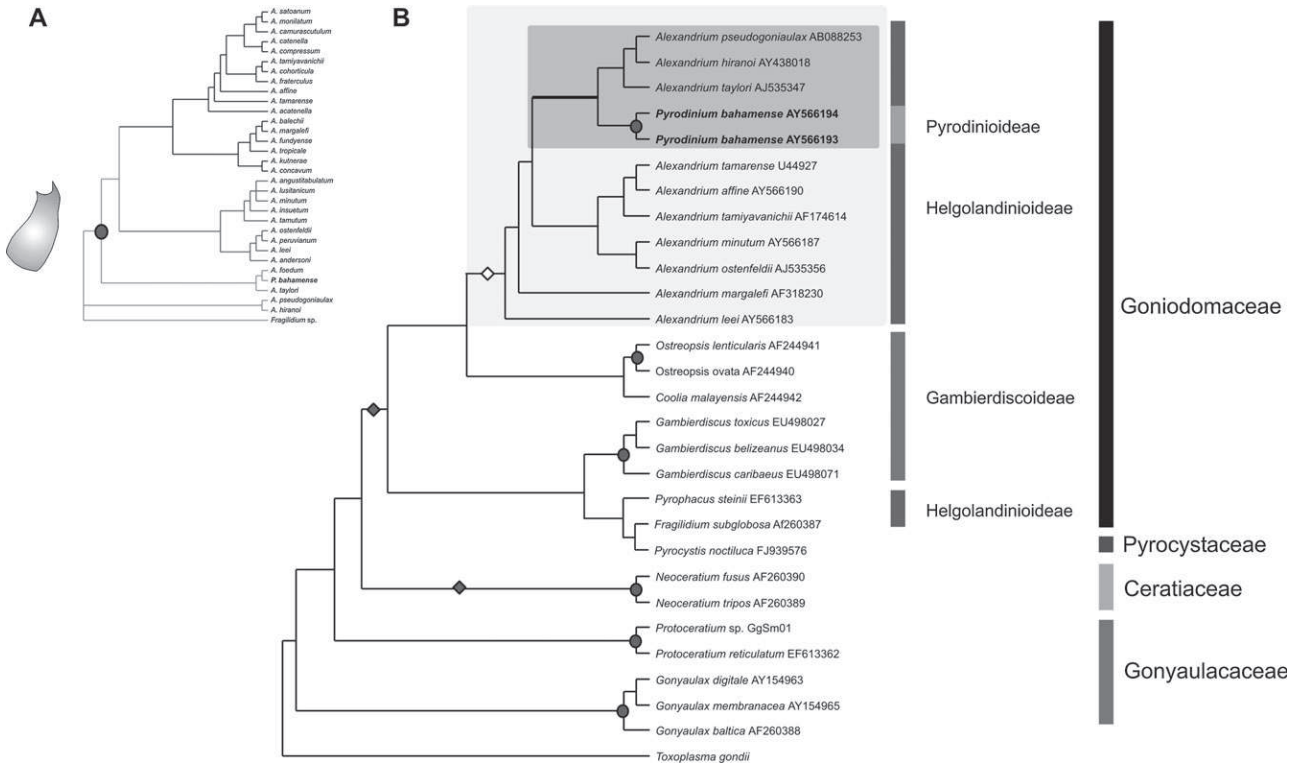

Fig. 3. (A) Morphology-based phylogeny of *Pyrodinium bahamense* in relation to *Alexandrium* species. Circle indicates transition node supported by the character of elongated oblique posterior sulcal plate. (B) Relationship of the 28S rRNA gene phylogeny and the classification of Gonyaulacales erected by Fensome et al. (1993). Filled diamonds indicate monophyletic families; hollow diamonds indicate paraphyletic families/subfamilies. Filled circles show monophyletic genera.
Adapted from Leaw et al. (2005).

analysis by Leaw et al. (2005) consistently grouped *P. bahamense* with *Alexandrium pseudogoniaulax* and *A. taylori*. Morphologically they share the same pattern of posterior sulcal plate (S.p. in Kofoidian or Z plate in Taylor–Evitt homology), whereby the S.p. is relatively long and twisted to the left (Balech, 1985). Furthermore, the first apical plate of the two *Alexandrium* species is trapezoid, metasert as described by Fensome et al. (1993), which is similar in *Pyrodinium*. If the clade is evolutionarily valid, *Pyrodinium* may not be monospecific, although at this stage further analysis and data are needed to support any change in the nomenclature.

Currently it is still not possible to resolve the issue of varietal separation of *P. bahamense* based on molecular analysis. Molecular information such as the second internal transcribed spacer (ITS2) transcript could be of taxonomic value since the marker has been proven useful in distinguishing biological species (Coleman, 2007, 2009). A model of the ITS2 transcript of *P. bahamense* var. *compressum* from Sabah, Malaysia revealed a typical four-helix structure of ITS with universal motif (Fig. 4). However, no comparative study between the var. *compressum* and var. *bahamense* has been performed.

2.4. Cyst morphology and taxonomy of P. bahamense

The resting cyst of *P. bahamense* is mostly spherical (Fig. 5(1)) and composed of two layers tightly appressed except for the proximal base of processes. The surface of the cyst is coarsely granular (Fig. 5(2)). Processes are many and intratabular (Fig. 5(2)). The stalks are hollow, slender, and cylindrical to tubular, rarely bifurcate, with open and patulate distal ends (Fig. 5(2)). Length of processes is very variable, and sometimes can be very short to nodular. The archeopyle is basically saphopylic and epicystal, although epicystal paraplates rarely remain on the epicyst (Fig. 5(2) and (3)).

Rossignol (1962) was the first to describe a cyst variety characterized by short processes from the Pleistocene sediments of Israel. This variety was called *Hystrichosphaeridium zoharyi* var. *ktana*. Wall (1967) then moved *H. zoharyi* into the newly erected genus *Hemicystodinium*. Based on the result derived from cyst incubation experiments, Wall and Dale (1969) concluded that *H. zoharyi* is a resting cyst of *P. bahamense*. Bujak et al. (1980) revised the genus *Hemicystodinium*, established a new genus *Polysphaeridium* and transferred *H. zoharyi* to this new genus. Thus at present the resting cyst of *P. bahamense* is referred to as *Polysphaeridium zoharyi* (Rossignol) Bujak, Downie, Eaton and Williams in the paleontological classification system. These different types are also observed in modern cysts of *P. bahamense* collected from the Atlantic and Pacific regions. Therefore, these cysts with short or nodular processes do not correspond to a particular variety of the motile forms. There is also no significant size difference between the var. *bahamense* and var. *compressum* cysts. In an incubation experiment carried by Wall and Dale (1968), an organic resting spore (=resting cyst) ornamented with numerous spines was found in *P. bahamense* collected from Phosphorescence Bay. A vegetative cell identical to *P. bahamense* developed from the athecate *Gymnodinium*-like cell that germinated from the cysts incubated.

3. Distribution of *P. bahamense*

3.1. Geographical distribution of vegetative cells

A long-standing belief in the ecology of *P. bahamense* is that populations are nicely segregated with the var. *compressum* exclusive to the Pacific and var. *bahamense* exclusive to the Atlantic. It is now known however that there are at least two locations where the two varieties co-occur, namely in the Arabian Gulf (Glibert et al., 2002) and the Pacific coast of Mexico (Garate-

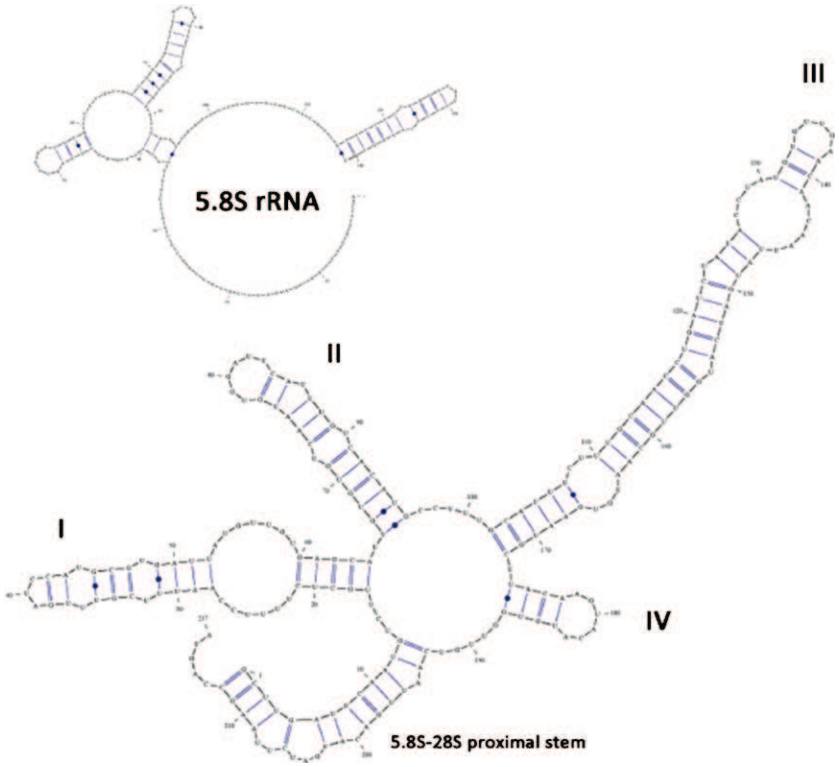

Fig. 4. ITS2 transcript of *Pyrodinium bahamense* var. *compressum* from Sabah, Malaysia. I–IV denote common ITS2 helices.

Lizarraga and Gonzalez-Armas, 2011). Locations at which motile cells of *P. bahamense* have been reported are shown in Fig. 6(1). These locations are grouped into three regions, the Caribbean Sea and Central America, Persian Gulf and the Red Sea, and the western Pacific. Around the Caribbean Sea, this species was originally described from the New Providence Island, and currently can be found in Florida, the Bahamas, eastern coast of Mexico, Belize, Guatemala and the Pacific coast from Mexico to Panama. In the western Pacific, this species has been found in waters of the Philippines, Malaysia, Brunei, Indonesia, and in several oceanic islands such as Fiji, Palau and possibly Solomon Islands. In the Persian Gulf, cysts of *P. bahamense* were recorded from surface sediments (Bradford and Wall, 1984), and more recently plankton forms of *P. bahamense* var. *compressum* have also been confirmed from Kuwait Bay (Glibert et al., 2002). From the Luanda coast of Angola in the east of the south Atlantic, occurrence of *P. bahamense* has been reported (Rangel and Silva, 2006). It could be of the variety *compressum* because cells were found in chains of more than seven cells. PSTs were also detected in shellfish from the same location (Vale et al., 2009). An interesting observation is that the distribution pattern of *P. bahamense*, especially in the northern hemisphere mostly coincides with areas where mangrove forests are, or have been, well developed. Whether or not this coincidence has any significance is not known.

3.2. Geographical distribution of cysts

The occurrence of *P. bahamense* cysts from modern surface sediments was first reported by Wall and Dale (1969) in samples from Bermuda. Since then the cysts have been found in many locations including those beyond the distribution areas of vegetative cells (Fig. 6(2)). These locations are primarily tropical to subtropical coastal areas. There have been only few records of occurrences of *P. bahamense* cysts from off-shore pelagic areas. *P.*

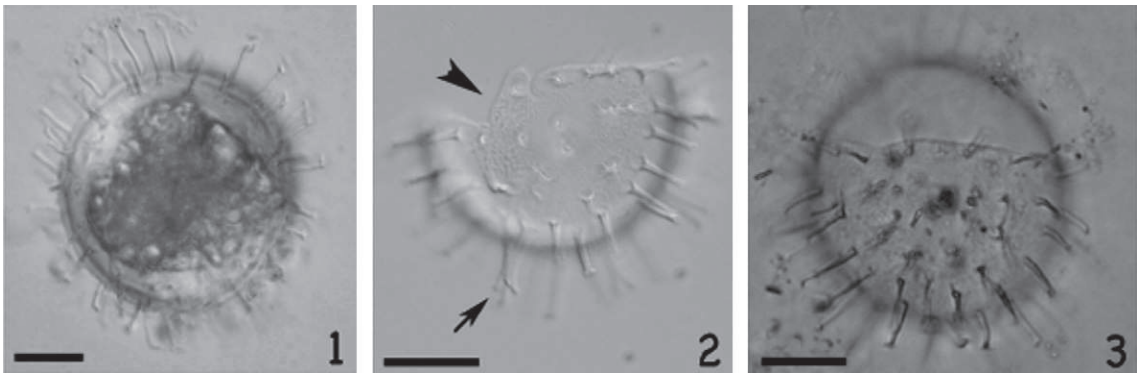

Fig. 5. Morphology of *Pyrodinium bahamense* resting cyst. (1) Living cysts from Masinloc Bay, Phillipine. (2) Hypocyst showing epicystal archeopyle with sulcal noth (arrow) and cylindrical tube process with bified distal extremities (arrow) from Bermuda. (3). Empty cyst from Masinloc Bay, Philippine. Scale bar: 10 μm.

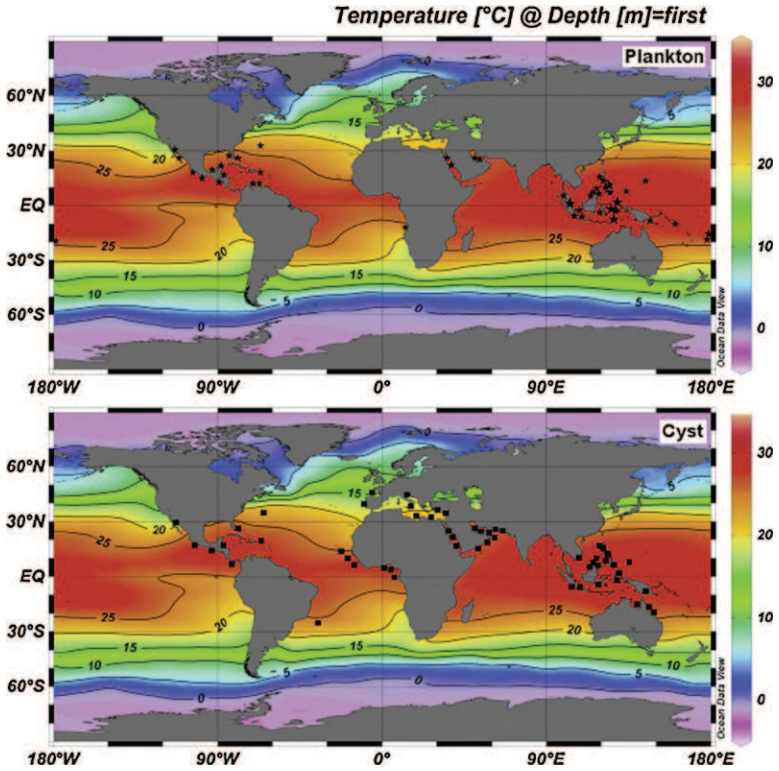

Fig. 6. Geographical distributions of plankton form (upper panel) and cyst form (lower panel) of *Pyrodinium bahamense* in relation to surface water temperature in summer. Temperature was gridded using the gridded 1-degree World Ocean Atlas 2005 (Locarnini et al., 2006) and the Ocean Data View software (Schlitzer, 2010).

bahamense cysts occur from both the Atlantic and Pacific regions, and the Caribbean Sea where the variety *bahamense* occurs. Cysts are also found from the Baha California in the eastern Pacific and in the Persian Gulf, and in these two areas motile forms of both varieties coexist. The geographical distribution of the modern cyst of *P. bahamense* is wider than that of the motile form. Since the motile form of *P. bahamense* does not occur throughout the year, in some cases the cells are not detected in plankton samples. The cysts could also have been advected to and deposited at locations far from where they were formed.

3.3. Vertical distribution of cysts in sediments

The oldest occurrence of the fossil cysts identical to *P. bahamense* (=*P. zoharyi*) was recorded from Late Paleocene–Early Eocene coastal plain sediments of Maryland, the United States (McLean, 1976) and around 51 Ma (the early Eocene) in the Atlantic (Williams et al., 1993). In Southeast and East Asia, *P. zoharyi* was found in Pleistocene sediments collected from the South China Sea (Mao and Harland, 1993) and from central Japan (Matsuoka, 1976).

The pattern of initial *P. bahamense* var. *compressum* blooms in Southeast Asia in the late 1970s to mid-1980s suggested that the cells or cysts were transported from place to place. In order to gain more understanding on the history of *P. bahamense* in the region a series of sediment core samples were collected for cyst analysis and dating. In the case of Manila Bay, Furio et al. (1996) concluded that *P. bahamense* might have inhabited the bay since the 1950s. In later studies Sombrito et al. (2004) and Siringan et al. (2008) concluded that *P. bahamense* cysts were present in Malampaya Sound, Palawan since the 1970s and Manila Bay since the 1920s (Fig. 7). In Indonesia, Mizushima et al. (2007) reported that *P. bahamense* cysts were probably present in Hurun Bay since the 1860s and Ambon Bay since the 1850s. In Sabah, Malaysia the oldest occurrence of *P. bahamense* cysts was probably around 1966, about 10 years earlier than the first recorded bloom (Furio et al., 2006). These results suggested that the explosion of *P. bahamense* blooms in Southeast Asia in the early 1980s might have been more the result of significant environmental changes in the region rather than the result the species being newly introduced to the area.

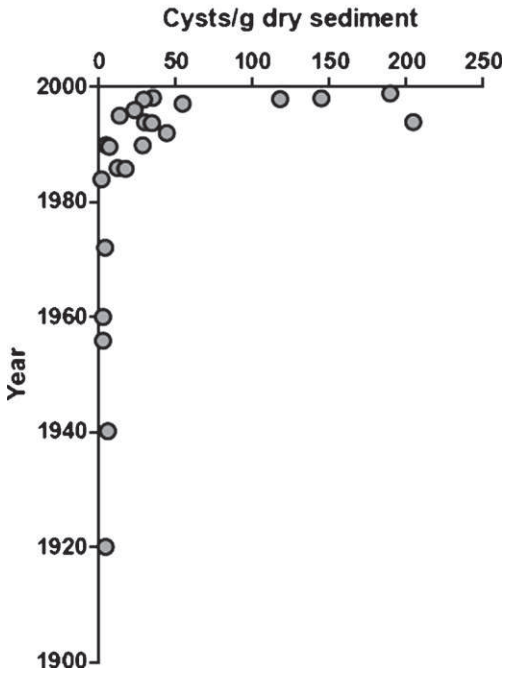

Fig. 7. Vertical distribution of *Pyrodinium bahamense* cysts at several locations in Manila Bay, the Philippines.
Modified from Siringan et al. (2008).

4. The physiology of *P. bahamense*

In this day and age whenever deleterious environmental events, including HABs, occur it is quite common practice to put the blame on climate change and eutrophication. However, the validity of these assumptions is difficult to prove in the absence of comprehensive physiological knowledge on a particular HAB species especially under in situ conditions. Surprisingly not many laboratory and field studies have been conducted on the physiology of *P. bahamense* especially when compared to other PST producing species. This could be largely due to inaccessibility of specimens to most researchers. For many years *P. bahamense* was considered as a very difficult species to culture in the laboratory and it was only since the early 1990s that cultures became regularly available for physiological and toxicity studies. As it turns out the species can grow readily in several of the commonly used seawater based culture media such as ES-DK (Kokinos and Anderson, 1995) and f/2. However cell densities in culture, typically less than 10,000 cells mL^{-1}, are still lower than those normally obtained for *Alexandrium*. To date physiological studies have focused on *P. bahamense* var. *compressum* isolates from Malaysia and the Philippines. Nonetheless, with recent evidence that the var. *bahamense* is also toxic it is expected that more physiological data will be available in the near future and in fact there are already significant information on the ecology of this variety.

4.1. Salinity, temperature and light requirements

Most of the available field and laboratory data indicate that *P. bahamense* is a tropical euryhaline species (Table 1). In Malaysia waters, blooms of *P. bahamense* var. *compressum* have typically been in waters of salinities 30 psu or higher (Usup et al., 1989). Similarly in Papua New Guinea blooms occurred in water of salinities 28 psu or higher (McLean, 1976) while in the Philippines the blooms are usually in water of 31 psu or higher salinity (Azanza-Corrales and Hall, 1993). Wall and Dale (1969) reported that the optimum salinity for *P. bahamense* var. *bahamense* was 35 psu. The high salinity requirement of *P. bahamense* was also evident from earlier efforts to culture the species. Oshima et al. (1985) and McLaughlin and Zahl (1961) found that diluting seawater by 10% resulted in poor growth of their cultures. In laboratory experiments a *P. bahamense* var. *compressum* clone from Malaysia did not survive at 16 psu (Usup, 1995). Salinity tolerance however might differ by geographical location. For example *P. bahamense* var. *bahamense* in Florida has a salinity range of 10–45 psu, although blooms only occurred at 20 psu or higher (Phlips et al., 2006).

Seawater temperature in the natural habitat of *P. bahamense* var. *compressum* in the west Pacific ranges from 25 °C to 31 °C. Laboratory studies on an isolate from Malaysia (Usup, 1995) showed that the temperature limits for growth are 22–34 °C, with optimum growth at 28 °C. In one study on an isolate of var. *compressum* from the Philippines the growth temperature range was 23–36 °C (Gedaria et al., 2007). In the case of var. *bahamense* in Florida, cells only appeared in the water column when the water temperature reached 20 °C and blooms formed only when the temperature was 25 °C or higher (Phlips et al., 2006). In the coastal waters of the Baja California Peninsula where both var. *compressum* and var. *bahamense* exist, the water temperature during their occurrences was 24–31 °C (Garate-Lizarraga and Gonzalez-Armas, 2011). All these data support the tropical and subtropical origin of *P. bahamense* although its range could expand if seawater warming happens.

Not much data is currently available on effects of irradiance on the physiology of *P. bahamense*. During previous red tides in Sabah, Malaysia living cells of *P. bahamense* were found in high density ($\sim 10^5$ cells L^{-1}) at depths of 20 m and more, deeper than the euphotic zone (Usup et al., 1989). Downwelling irradiance in a bloom patch would also be significantly reduced as a result of self-shading. At present the length of time in which *P. bahamense* could remain viable under low light conditions is still not known. In laboratory cultures, *P. bahamense* was able to growth well at irradiance value of 50 $\mu E\ m^{-2}\ s^{-1}$. Compensation for low light conditions as evidenced by an increase in cellular chlorophyll *a* content, however, occurred at 90 $\mu E\ m^{-2}\ s^{-1}$ (Usup, 1995). Walsh et al. (2011) surmised that *P. bahamense* on the east coast of Florida are characteristic of dark-adapted species. There is also evidence that *P. bahamense* can grow well under continuous illumination, so apparently a dark phase is not required in its cell cycle (Usup, 1995).

4.2. Nutrient requirements

It has been suggested that *P. bahamense* may have fastidious nutrient requirements, judging by the difficulty in establishing laboratory cultures of the species (Blackburn and Oshima, 1989). However since then much progress has been made and currently *P. bahamense* cultures are available in several laboratories worldwide. The best culture media are based on enriched natural seawater such as the ES-DK medium (Kokinos and Anderson, 1995). A common pattern that emerged from instances where culturing were successful was the requirement for soil extract supplement, regardless of the medium employed. This was true for cultures of isolates from Palau (Oshima et al., 1985), Malaysia (Usup, 1995) and the Carribean (McLaughlin and Zahl, 1961). In the study of Usup (1995) it was found that soil extract supplement did not significantly affect division rates, but resulted in prolongation of the exponential phase of growth. As a result densities achieved in batch cultures increased from 1600 cells mL^{-1} without soil extract supplement to 4700 cells mL^{-1} when 10 mL L^{-1} soil extract was added to the medium. Usup (1995) provided strong evidence

Table 1
Salinity and temperature ranges for occurrence and growth of *P. bahamense*.

Variety	Salinity (psu)	Temperature (°C)	Location	Study
compressum	35[b]			Wall and Dale (1969)
	24.7–36.8[a]	26.2–30.7[a]	Papua New Guinea	Maclean (1977)
	30[a]		Sabah, Malaysia	Usup et al. (1989)
	32–38.5[a]	27.5–33[a]	Banban Bay, Philippines	Azanza-Corrales and Hall (1993)
	>30 (20–36)[b]	28–30 (20–38)[b]	Sabah, Malaysia	Usup et al. (1994)
	35[a]	31–32[a]	Chiapas, Mexico	
	36 (26–36)[b]	25 (23–36)[b]	Philippines	Gedaria et al. (2007)
	–	24.5–31[a]	Baja California Peninsula	Garate-Lizarraga and Gonzalez-Armas (2011)
bahamense	>25 (10–45)[a]	30[a]	Indian River Lagoon, Florida	Phlips et al. (2004, 2006)

[a] Field observations.
[b] Laboratory experiments.

that the soil extract served as a source of selenium. The highest yield obtained in Se-supplemented cultures (ca. 6000 cells mL^{-1}) was comparable to or even better than yields obtained in soil extract supplemented cultures. Results from the same study also indicated that *P. bahamense* could utilize selenite (Se-IV) and organic selenide but not selenate. Landsberg et al. (2006) successfully cultured *P. bahamense* from Florida in ES-DK medium supplemented with sodium selenite. The relevance of these findings to *P. bahamense* growth in its natural habitat remains to be tested but nevertheless the data indicate the potential importance of land-derived nutrients in promoting blooms of the species in coastal waters.

In most laboratory cultures of *P. bahamense* nitrate is used as the nitrogen source. There is also evidence that the species is able to utilize urea, while its tolerance to ammonia seems to be low. Its ability to utilize organic nitrogen may be very limited since clones could not grow on alanine, arginine or histidine in cultures. It is also evident that *P. bahamense* is able to utilize both inorganic and organic phosphorus (Usup, 1995).

In ES-DK medium, cultures only showed evidence of nitrogen limitation effects in terms of reduced growth rate, lower cell density and reduced chlorophyll *a* content at nitrate concentration of less than 100 μM (Usup and Anderson, 1996). Cell toxin content was relatively constant over the range of added nitrate from 60 to 500 μM. The fact that toxin content was maintained even at nitrate concentrations that were limiting to growth and chlorophyll-*a* content suggested that a significant portion of the cell nitrogen was diverted to toxin biosynthesis which in turn suggested an important role of the toxins to the cells. There was no evidence for the utilization of toxins as a source of nitrogen even under prolonged nitrogen limitation.

There are some evidence to suggest that *P. bahamense* is not competitive and will not become dominant under high nitrogen and phosphorus conditions. However the relationship between *P. bahamense* abundance and phosphorus and nitrogen concentrations is inconclusive in the absence of experimental data. In Florida, peak abundance of *P. bahamense* seemed to occur at total phosphorus concentrations of >100 μg L^{-1}, while the threshold total nitrogen concentration for the occurrence of *P. bahamense* could be about 600 μg L^{-1} (Phlips et al., 2006). In Sabah, Malaysia it was observed that *P. bahamense* abundance declined during peak N and P concentrations when the phytoplankton was dominated by two other bloom-forming species *Cochlodinium polykrikoides* and *Gymnodinium catenatum* (Adam et al., 2011). The relatively slow growth rates of *P. bahamense* may preclude it from competing successfully with faster growing phytoplankton in environments with short water residence times and/or persistent high nutrient loading rates. Conversely, large size and motility may provide *P. bahamense* with an ability to search for and store nutrients in more stable water columns, when the supply of new inorganic nutrients is more restricted or episodic (Phlips et al., 2006).

5. *P. bahamense* bloom dynamics

The ecophysiology and bloom dynamics of *P. bahamense* are still relatively not well-understood. Perhaps the most studied are blooms of the var. *bahamense* in the Indian River Lagoon in Florida. Long-term monitoring in this area has enabled hypotheses to be made regarding factors that affect *P. bahamense* survival, standing crop, bloom formation, and persistence. Some of the factors that are important in this area are most likely also influential in other systems where blooms of *P. bahamense* occur. There have also been efforts to model the development and dynamics of *P. bahamense* blooms in Manila Bay, the Philippines especially with regard to how the blooms would spread in the bay in relation to origin of initiation (Villanoy et al., 2006). In Sabah, Malaysia a year-long monitoring exercise provided some indication on the dynamics of *P. bahamense* var. *compressum* in relation to environmental factors and competing bloom species (Adam et al., 2011).

5.1. Bloom initiation

Resting cysts have been proven as important sources of seed populations in *Alexandrium* and *Gymnodinium* blooms, particularly in temperate regions (Anderson, 1984, 1989; Hallegraeff, 1993; Villanoy et al., 1996; McGillicuddy et al., 2003). In the case of *P. bahamense* the relative importance of resting cysts as opposed to vegetative cells (background population) in initiating blooms may vary by location. Azanza-Corrales and Crisostomo (1996) conducted detailed mapping of resting cysts distribution in Manila Bay, the Philippines and found densities to be highest in Bataan and Cavite. These were also the two areas where blooms normally appeared first and were most persistent in the bay. Villanoy et al. (1996) found that in Manila Bay the highest density of *P. bahamense* cysts in the water column occurred during the northeast monsoon, when vertical mixing was most intense. They proposed that in a relatively shallow, semi-enclosed area like Manila Bay, the appearance of *P. bahamense* blooms is related to the resuspension of resting cysts by turbulence. It was also evident that the supply of viable cysts in the bay was replenished from blooms that occurred periodically in the bay, for example over the years 1988–1998 (Siringan et al., 2008). Previously it was reported that *P. bahamense* cysts were very rare in coastal sediments of Sabah, Malaysia (Usup and Azanza, 1998). More recently another series of sediment core samples were obtained and in some of the cores from Brunei Bay *P. bahamense* cysts reached more than 50 cysts/g dry sediment (Furio et al., 2006).

5.2. Bloom development

Regardless of the source of seed population, the factors responsible for the development of *P. bahamense* blooms remain poorly understood. Typical peak cell density in a *P. bahamense* bloom patch in Sabah Malaysia was on the order of 10^6 cells L^{-1} (Usup et al., 1989). At the present it is uncertain if such high cell densities are due to enhanced in situ growth, physical accumulation, or both. Observations that *P. bahamense* blooms tended to be patchy in both the horizontal and vertical dimensions could not discount either mechanism. Maclean (1977) estimated that the growth rate of *P. bahamense* populations during blooms in Papua New Guinea after the bloom reached its peak was ca. 0.3 divisions d^{-1}, comparable to the maximum of ca. 0.4 divisions d^{-1} obtained in laboratory batch cultures (Usup, 1995).

Marked horizontal and vertical patchiness of *P. bahamense* cells in a bloom suggest that seed populations were delivered into a water mass containing sufficient concentrations of a limiting nutrient, which could be of terrestrial origin. This may help explain the observations that *P. bahamense* blooms tend to occur after periods of heavy rain (Usup and Lung, 1991; Phlips et al., 2006). Over the 9 years of sampling in the Indian River Lagoon in Florida from 1997 to 2005, *P. bahamense* var. *bahamense* abundance was low during the prolonged drought period from the fall of 1998 through the early summer of 2001. By contrast, *P. bahamense* var. *bahamense* densities were dramatically elevated during the high rainfall period from the summer of 2001 to 2004 (Phlips et al., 2006). More research is clearly needed to elucidate the relative importance of biological and physical factors in the establishment and maintenance of these blooms.

Phlips et al. (2006) posited that the single unifying theme in the success of *P. bahamense* var. *bahamense* based from observations in Florida was that mixed salinities, shallow depths, and long water

residence times were characteristic of all of the environments where *P. bahamense* var. *bahamense* was found in significant numbers. They further proposed 3 hypotheses that tie these observations to the issue of competitive strategies: (1) the euryhaline character of *P. bahamense* var. *bahamense* allows it to out compete more stenohaline taxa in ecosystems with temporally variable salinity regimes, (2) the toxin-producing capability of *P. bahamense* var. *bahamense* reduces top-down control of standing crop potential, and (3) the relatively slow growth rates of *P. bahamense* var. *bahamense* may preclude it from competing successfully with faster growing phytoplankton in environments with short water residence times and/or persistent high nutrient loading rates. Conversely, large size and motility may provide *P. bahamense* var. *bahamense* with an ability to search for and store nutrients in more stable water columns, when the supply of new inorganic nutrients is more restricted or episodic.

5.3. Bloom decline

The disappearance of *P. bahamense* blooms is just as sudden as its appearance. In Sabah, Malaysia, patches of *P. bahamense* blooms typically remained intact for periods of 10–14 d (Usup et al., 1989). The primary factor that leads to the decline of these blooms is still unknown. It has been observed from field samples that at least one species of tintinnid, *Flavella* sp. is able to feed on *P. bahamense* (Usup et al., 1989). In the Philippines there is evidence that *Noctiluca scintillans* is an important grazer on *P. bahamense* (Azanza et al., 2010) and tends to succeed *P. bahamense* in dominance. The relative importance of grazing, nutrient limitation and dissipation by physical forces in the termination of *P. bahamense* is another aspect where more studies are needed.

Studies by Villanoy et al. (1996), Azanza-Corrales and Crisostomo (1996) and Sombrito et al. (2004) in Manila Bay indicate that sexual reproduction and production of resting cysts are important events at the end of *P. bahamense* blooms. Production of new cysts is a contributing factor to the perpetuation of *P. bahamense* blooms especially in semi-enclosed bays and lagoons. Factors that promote the sexual cycle are still unknown, although work by Corrales et al. (1995) suggest that nutrient limitation is an important factor.

5.4. Periodicity of P. bahamense blooms

Blooms of *P. bahamense* are generally aperiodic and unpredictable. Attempts have been made to correlate these events with periodic cycles of weather and meteorology. On a large temporal scale, there is at least some circumstantial evidence that major *P. bahamense* blooms coincide with peaks of El Nino and La Nina cycles, both in the western Pacific and Florida (Maclean, 1989; Usup and Azanza, 1998; Phlips et al., 2006). The exact nature of this relationship is not clear although it might have to do with enhanced delivery of nutrients into the coastal waters.

At a smaller, local scale blooms are more stochastic although long term observation data do reveal some degree of predictability (Azanza and Taylor, 2001) (Table 2). In Sabah, Malaysia from 1976 to 1986 *P. bahamense* blooms coincided with the inter-monsoon period, with most outbreaks occurring in July and Dec-Jan (Usup and Lung, 1991). More recently, from a 12 month study in the waters of Kota Kinabalu Sabah throughout 2007, it was found that *P. bahamense* density peaked in March, August and November (Adam et al., 2011). In Ambon Bay, Indonesia the peak of *P. bahamense* blooms also tend to be during March–August. In the Philippines, for the period of 1987–1997, *P. bahamense* blooms were primarily during January–July throughout the country. Interestingly starting in 1998 the period of the blooms extended until November, although the reasons for this is not yet known

Table 2
Occurrence of *P. bahamense*.

Year	72	73	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92
Philippines										7–8	–	–	–	4–9	4–9, 12	2–7	1–7	1–7, 4–10	1–7, 11–12
Malaysia			2–4	–	–	9	5	–	–	12	1–3, 10–11	1–5, 8–11	2–6, 12	2–5, 7–12	1–5, 8–12	2–4, 6, 11–12	6–11	1, 5–12	1–5, 7–12
Brunei			3–5				4–7, 9–10	1–3, 7		1–9	1–9	1–9	1–9	1–9	1–9				
Indonesia																			
Papau New Geinea	3–7	3, 5																	
Florida																			
Costa Rica																			
El Salvador																			
Guatemala												12		7–8					
Mexico																12		1	

Year	93	94	95	96	97	98	99	00	01	02	03	04	05	06	07	08	09	10
Philippines	1–10	1–7,11–12	1–7, 12	1–7	1–8, 8–10	1–11	1–11				2			6–8	3–12	1–12	9–10	2–11
Malaysia	1–5, 7–12	1–4, 7–12	1–6, 8–12	3, 8–12	1–3, 8–12	1–8					12	1–3	1–3	–	–	–	2	–
Brunei																		
Indonesia		7, 8	3, 8	8–9	10–12	7–8												
Papau New Geinea																		
Florida					9[a]	8[a]	7[a]	7[a]	5–8, 9–10[a]	7–8[a], 8–10[a]	9[a]	8[a]						
Costa Rica													11–12					
El Salvador									8–12	1			11–12	1–4				
Guatemala				11					8									
Mexico				11					11				5[a]	1–3, 6[a]		6[a]		8

1–12 denote month of January to December.
[a] Var. *bahamense*.

(Azanza and Taylor, 2001). On the west coast of Mexico most blooms of *P. bahamense* were in November–March (Garate-Lizarraga and Gonzalez-Armas, 2011). In a subtropical region like Florida, blooms of *P. bahamense* would be more subjected to favourable water temperature. Thus in the tropical/subtropical environment of Florida Bay, *P. bahamense* var. *bahamense* was observed nearly year round whereas in the subtropical/warm temperate environments of the Indian River Lagoon and Tampa Bay, *P. bahamense* var. *bahamense* was generally restricted to the warm season, i.e. April through October (Phlips et al., 2006).

6. Toxicity of *P. bahamense*

6.1. Capability of PSP-toxin production in relation to taxonomy

The var. *compressun* is well known to cause PSP in Southeast Asia coastal waters and the Pacific coast of central America. Until 2002, the var. *bahamense* in the Atlantic was assumed to be nontoxic. However, Landsberg et al. (2002, 2006) confirmed that *P. bahamense* occurring in the Indian River Lagoon, Florida, USA can produce saxitoxin, although it has never been known to cause any PSP incident. However, from 2002 to 2004 there were at least 28 cases of saxitoxic puffer fish poisoning due to toxins of *P. bahamense* origin (Walsh et al., 2011). It has been suggested from paleoecological studies that as far back as the lower Eocene to the Holocene blooms of *P. bahamense* in Florida coastal ecosystems have coincided with animal mortalities (Emslie et al., 1996). Ammons et al. (2001) reported on the presence of PSTs in mussels from Trinidad but the source of the toxins was not identified. It remains a mystery as to why so far no PSP cases have been recorded from areas where *P. bahamense* var. *bahamense* is found.

6.2. P. bahamense toxin profile

P. bahamense has a relatively simple toxin profile since it produces only a small subset of the known PSTs (Wiese et al., 2010). Isolates from the Indo-Pacific produce dc-STX, STX, neo-STX, B1 and B2. This toxin profile is typical for both cultured and natural samples (Usup et al., 1994; Hummert et al., 1997; Montojo et al., 2006; Gedaria et al., 2007). Isolates from other regions were reported to have slightly different toxin profiles. Isolates from Guatemala contained STX, neoSTX, GTX2, GTX3 and GTX4 (Rosales-Loessener, 1989). In the Indian River Lagoon in Florida, *P. bahamense* most likely produced STX, dc-STX and B1 as detected in toxic puffer fish and bloom population of var. *bahamense* (Landsberg et al., 2006). Studies on shellfish contaminated by *P. bahamense* PSTs in Malaysia and the Philippines showed that there were very minimal differences in toxin profiles of the dinoflagellate and the shellfish (Montojo et al., 2006; Usup et al., 2006).

6.3. Effects of growth conditions on PST production

In batch culture most of the toxin production occurred during early to mid-exponential phase, so as the population grows older the cell toxin content decreases but the total toxin in the population remains constant (Fig. 8A). Peak toxin content in a cell is typically 300–400 fmol (Usup et al., 1994; Gedaria et al., 2007). Different growth conditions did not significantly affect toxin content but could have marked effects on toxin profile, particularly in the ratios of the different PSTs. However, lower cell division rates could result in increased cellular toxin content because the toxin has not been divided into daughter cells (e.g. Fig. 8B). Details of the effects of growth conditions on toxin content and toxin profile of a *P. bahamense* isolate from Malaysia can be found in Usup et al. (1994) and results from a similar experiment on a Philippine isolate can be found in Gedaria et al. (2007).

The recent evidence on the toxicity of var. *bahamense* from Florida begs the question as to why there has been no report of PSP in areas where the variety is known to occur. Blooms of the var. *compressum* in the Pacific have always been toxic and to date no nontoxic clone of var. *compressum* has been reported. Balech (1985) argued that the difference in toxicity of the two varieties could be a manifestation of the effects of environmental factors on the organism. There has been suggestion that the toxicity of var. *bahamense* may have fluctuated over time although this is quite difficult to prove. Emslie et al. (1996) suggested for example that massive deaths of sea birds about 54 million years ago in coastal waters off Sarasota, Florida coincided with accumulation of *P. bahamense* cysts. Walsh et al. (2011) also argued for the importance of environmental factors, specifically removal of top-down predators and a change from eutrophic to oligotrophic conditions, as critical in increasing the dominance and toxicity of *P. bahamense* in Florida waters. The recent elucidation of PST biosynthetic pathways and cluster of genes involved in cyanobacteria (Mihali and Neilan, 2009; Mihali et al., 2011), coupled with advances in sequencing technology, will lead to similar

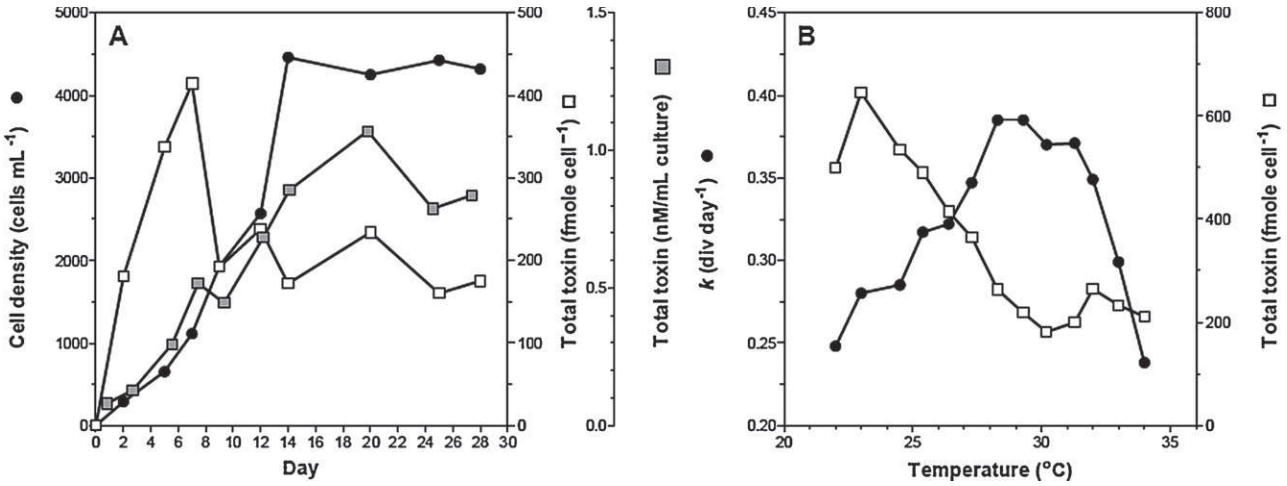

Fig. 8. (A) Patterns of culture density, cellular toxin content and total amount of toxin in a batch culture of a *Pyrodinium bahamense* var. *compressum* clone from Sabah, Malaysia in ES-DK medium. (B) Effects of temperature on growth rate and cellular toxin content of a *Pyrodinium bahamense* var. *compressum* clone from Sabah, Malaysia in ES-DK medium.
Modified from Usup et al. (1994).

developments for PST producing dinoflagellate species. It will then be possible to determine if both var. *compressum* and var. *bahamense* possess these genes and how environmental conditions affect the expression of these genes. It would also be useful to determine the toxicity of more var. *bahamense* clones using highly sensitive techniques. It could be that var. *bahamense* is weakly toxic and the toxic effects only become apparent after magnification along the food chain, for example the one that involves puffer fish in Florida.

7. Summary and future perspective

It has been 40 years since the first toxic bloom of *P. bahamense* was reported in Papua New Guinea. Since then significant progress has been achieved in certain aspects of the biology and ecology of the species. Its life cycle is now well understood, as is its toxicity. Nonetheless important questions remain. One of the most important is the varietal status of the species, especially since the supposedly nontoxic variety *bahamense* has now been proven capable of PST production. Perhaps detailed molecular comparison of the two varieties could resolved their status.

The second very important aspect is to better understand the factors that play important roles in the bloom dynamics and toxicity of the species. For example, does the nature of the watershed that borders a coastline influence the expression of toxicity? Similarly, how does land derived nutrients affect the development of *P. bahamense* blooms? With the apparent increase of importance of *P. bahamense* in certain tropical and subtropical regions of the Atlantic coastal waters, there might be more incentive to carry out comparative studies between the west Pacific and tropical Atlantic populations. For example, Phlips et al. (2006) noted that the single unifying theme in the success of *P. bahamense* var. *bahamense* that arises from observations in Florida is that mixed salinities, shallow depths, and long water residence times are characteristic of all of the environments where *P. bahamense* var. *bahamense* is found in significant numbers. However, in Malaysian waters blooms of the variety *compressum* are more common in full strength seawater and in open coastlines where water residence times are likely to be short. It is thus proposed here that comparative studies along the guidelines of the GEOHAB Science Plan would be very beneficial to better understand the bloom dynamics of this important species.

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