NATURAL TOXINS 2:254–262 (1994)

Growth and Toxin Production of the Toxic Dinoflagellate *Pyrodinium bahamense* Var. *compressum* in Laboratory Cultures

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ABSTRACT Toxin production of a Malaysian isolate of the toxic red tide dinoflagellate *Pyrodinium bahamense* var. *compressum* was investigated at various stages of the batch culture growth cycle and under growth conditions affected by temperature, salinity, and light intensity variations. In all the experiments conducted, only 5 toxins were ever detected. Neosaxitoxin (NEO) and gonyautoxin V (GTX5) made up 80 mole percent or more of the cellular toxin content and saxitoxin (STX), GTX6 and decarbamoylsaxitoxin (dcSTX) made up the remainder. No gonyautoxins I–IV or C toxins were ever detected. In nutrient-replete batch cultures, toxin content rapidly peaked during early exponential phase and just as rapidly declined prior to the onset of plateau phase. Temperature had a marked effect on toxin content, which increased 3-fold as the temperature decreased from the optimum of 28°C to 22°C. Toxin content was constant at salinities of 24‰ or higher, but increased 3-fold at 20‰. Toxin content decreased 2-fold and chlorophyll content increased 3-fold when light intensity was reduced from 90 to 15 μE m-2 s-1. This accompanied a 30% decrease in growth rate. Toxin composition (mole % individual toxin cell-^{1}) remained constant throughout the course of the nutrient-replete culture and during growth at various salinities, but varied significantly with temperature and light intensity changes. At 22°C, GTX5 was 25 mole % and NEO was 65 mole %, while at 34°C, GTX5 increased to 55 mole % and NEO decreased proportionally to 40 mole %. When light intensity was reduced from 90 to 15 μE m-2 s-1, NEO decreased from 55 to 38 mole %, while GTX5 increased from 40 to 58 mole %. These data suggest that low light and high temperature both somehow enhance sulfo-transferase activity. In general, toxin content and composition in *P. bahamense* were both influenced by factors that alter the growth rate of the species. The data show, however, that toxin content was not determined solely by growth rate but, more likely, by the manner in which the cells allocate their nitrogen and carbon to various cellular components and processes under various growth conditions. Several of the toxin production patterns observed in this *P. bahamense* isolate were similar to those of PSP toxin-producing *Alexandrium* and *Gymnodinium* species, but there were also significant differences.

Key Words: *Pyrodinium bahamense,* Toxin production, Growth, Malaysia, Paralytic shellfish poisoning, PSP

INTRODUCTION

The thecate, chain-forming dinoflagellate *Pyrodinium bahamense* Plate var. *compressum* Böhm [Steidinger et al., 1980] is the species primarily responsible for outbreaks of paralytic shellfish poisoning (PSP) in the tropical Pacific. This species has caused toxic red tide blooms in coastal waters of several countries in Southeast Asia [Beales, 1976; Estudillo and Gonzales, 1984; Harada et al., 1982a; Maclean, 1975, 1979, 1993; Roy, 1977; Usup et al., 1989] and on the Pacific coast of Guatemala in Central America [Maclean, 1993; Rosales-Loessener et al., 1989]. In Southeast Asia there have been approximately 200 fatal cases of PSP due to consumption of bivalves contaminated by toxins produced by this dinoflagellate.

Despite the obvious public health and economic importance of *P. bahamense,* it has been the subject of few studies other than anecdotal field observations of bloom events [e.g., Maclean, 1977; Usup et al., 1989]. This is due in large part of failure to establish laboratory cultures of the species [Blackburn and Oshima, 1989]. The only documented success in this respect was by Harada et al. [1982a,b] who maintained a clone from Palau for a few years. Using high-performance liquid chromatography (HPLC) analysis, the Palau isolate was found to produce saxitoxin (STX), neosaxitoxin (NEO), decarbamoylsaxitoxin (dcSTX), gonyautoxin 4 (GTX4), and gonyautoxin 5 (GTX5) [Harada et al., 1982a,b, 1983]. Subsequently, bivalves and planktivorous

Received June 6, 1994; accepted for publication June 24, 1994.

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fish from Palau, Borneo, and the Philippines contaminated by the dinoflagellate were also analyzed using HPLC and found to contain the same suite of toxins present in the cultured cells from Palau [Oshima, 1989]. The toxins detected in cells collected during a red tide outbreak on the Pacific coast of Guatemala in 1987 were somewhat different from those detected in the Palau isolate. The Guatemalan cells STX, NEO, GTX2, GTX3, and GTX4 [Rosales-Loessener et al., 1989]; no dcSTX was detected.

No studies have yet been published on the effects of environmental conditions on the toxin content and composition of *P. bahamense*. Most investigations of paraytic shellfish toxin production have focused on toxic species in the genus *Alexandrium*, as well as the nonthecate *Gymnodinium catenatum*. In cultures of *Alexandrium* spp, it has generally been found that the total amount of toxin in a cell (toxin content) is highest during the midexponential phase of growth, and decreases as the cultures age [Anderson et al., 1990b; Boczar et al., 1988; Hall, 1982; Kim et al., 1993; Proctor et al., 1975]. White [1978] studies the effects of salinity on toxin production and found that the toxin content of *Alexandrium excavatum* (= *Gonyaulax excavata*) increased as the salinity of the culture medium increased. A later investigation by Anderson et al. [1990b] indicated, however, that the toxin content of *Alexandrium* did not vary with salinity. The effects of temperature on toxin production were studied by Hall [1982], Ogata et al. [1987], Boyer et al. [1987], and Anderson et al. [1990b], all of whom found that toxin content per cell increased as culture temperature decreased. Ogata et al. [1987] demonstrated that lower light intensity led to increased toxin production. Studies have also been carried out on effects of N- and P-limitation on toxin production in *Alexandrium* spp. In batch cultures of *A. tamarense*, N-limitation led to a decrease in toxin content per cell, while P-limitation caused dramatic increase in toxin content at the start of the stationary phase [Anderson et al., 1990b; Boyer et al., 1987; Hall, 1982]. Similar results were obtained by Anderson et al. [1990b] for *A. tamarense* and *A. fundyense* grown in semicontinuous cultures.

The advent of reliable HPLC methods for the analysis of PSP toxins [Oshima et al., 1989; Sullivan and Wekell, 1984] has enabled detailed comparisons of toxin composition (that is, the relative proportions of the individuals toxins produced) among isolates. One aspect that has remained uncertain is the constancy of the toxin composition of a particular isolate. Studies by Boyer et al. [1987], Cembella et al. [1987], Hall [1982], Ogata et al. [1987], and Kim et al. [1993] on *Alexandrium* species indicated that while the toxin content of an isolate may change due to culture conditions, the toxin composition remained constant. Others have shown, however, that significant composition changes occur during prolonged senescence in batch cultures [Boczar et al., 1988] or under steady state nutrient limitation [Anderson et al., 1990a]. Isolated of *G. catenatum* from Japan, Spain, and Australia have been found to have a characteristic and stable toxin composition under various culture conditions [Oshima et al., 1993]. In that particular study, it was also found that isolates from Australia produced a different suite of toxins than those from Japan and Spain. It was suggested that toxin composition could be used as a biochemical marker to distinguish between geographical isolates of *G. catenatum*, an idea explored previously for *Alexandrium* species by Hall [1982], Cembella et al. [1987], and Anderson et al. (Marine Biology, in press).

We present results from an investigation of toxin production by a laboratory culture of the toxic red dinoflagellate *Pyrodinium bahamense* var. *compressum* clone from Sabah, Malaysia. Toxin production and growth were investigated over a typical batch culture cycle, and under the influence of variations in temperature, salinity, and light intensity. This is a signficant development in studies of the toxicity of this important species, adding growth stage and environemntal effects to the toxin composition data reported by Harada et al. [1982a,b; 1983] on an isolate from Palau.

MATERIALS AND METHODS

Pyrodinium bahamense var. *compressum* clone PbSA01 was isolated from plankton samples collected during a red tide outbreak on the west coast of Sabah, Malaysia in December, 1991. Cultures of the clone were routinely maintained in modified ES-I medium of Provasoli [1968] at 29°C under at 14:10 h light:dark (L:D) cycle. Details of this medium are to be published elsewhere [Usup et al., in preparation]. The same medium was used in all experiments reported here. Filtered and autoclaved Vineyard Sound seawater (30‰ salinity) was used as the base for the medium.

Toxin production over the growth cycle was studied in 25 mL batch cultures at 20°C on a 14:10 h L:D cycle. Exponential phase cultures were transferred into fresh culture tubes, and growth was monitored through in vivo fluorescence measurements on a Turner Designs Model 10 fluorometer. Groups of 4 tubes were harvested at various stages of the culture for toxin analysis. Two subsamples were withdrawn from each harvested tube and preserved in Lugol's iodine for cell counts. The contents of 2 tubes were then pooled together and centrifuged at moderate speed on a clinical bench top centrifuge for 10 min. The supernatant was aspirated away and the pellet was resuspended in fresh, sterile medium. The suspension was then transferred into 1.5 mL Eppendorf tubes and centrifuged at 3,500 rpm for 5 min on a Savant microcentrifuge. The supernatant was aspirated away, and 200–250 μL of 0.05 N acetic acid was added to the pellet. The suspension was then sonicated on ice for 1 min using a fine-tip probe (Branson Model 975, Danbury, CT), and the extract was stored frozen until analysis. In all experiments where the cells were cultured under a 14:10 h L:D cycle, harvests were made 6–8 hr into the light phase, well after cell division was completed.

The effects of temperature on growth and toxin production were studied using a temperature-gradient bar [Watras et al., 1982], in which a range from 20°C to 40°C was established. Four 25-mL tube cultures were acclimated to each temperature over 3 transfer cycles. Growth was monitored through in vivo fluorescence measurements on the Turner Designs fluorometer. Cultures were grown under continuous illumination and were harvested in late exponential growth phase. Methods for the harvesting of the cultures and toxin extraction were similar to those used in the growth stage experiment.

The effects of salinity on growth and toxin production were studied in batch cultures using Sargasso Sea water (salinity 36‰) as the medium base. A range of salinities in the medium was established by dilution with Milli-Q water (Millipore Corp., Medford, MA). Four culture tubes were grown at each salinity. The cells were allowed to acclimate to each salinity over 3 transfers at 29°C under a 14:10 h L:D cycle. Growth was monitored through in vivo fluorescence measurements, and cultures were harvested for toxin analysis at midexponential growth phase using methods already described.

Light intensity effects on growth and toxin production were studied in the temperature gradient bar at 29°C under continuous illumination. The amount of light reaching each culture tube was adjusted using window screen material as filters. The light intensity in each tube was measured using a light meter (Model QSL-100P, Biospherical Instruments Inc., San Diego, CA). Six tubes at each light intensity were allowed to acclimatize through 3 transfer cycles. Four tubes were harvested for toxin analysis in late-exponential phase using methods already described. Two tubes were harvested separately for chlorophyll extraction in 95% acetone, and the amount of chlorophyll in each extract was calculated based on absorbances according to the equations given in Parsons et al. [1984].

Samples were analyzed by the 3-step isocratic elution method of Oshima et al. [1989] for the HPLC determination of the PSP toxins, with the following modifications. For the C1–C4 toxins, column temperature was maintained at 30°C and the postcolumn reaction temperature at 45°C to aid in separation of the toxins and to achieve greater sensitivity for toxins C1 and C2. The mobile phase used for the elution of toxins C1–C4 was adjusted to pH 5.8 with 0.05 M acetic acid. Gonyautoxins were analyzed with a column temperature of 23°C and a postcolumn reaction temperature of 35°C. By lowering the postcolumn reaction temperature, the fluorescence of GTX1, GTX4, and GTX6 are enhanced. Column and postcolumn reaction temperatures were maintained at 24°C and 50°C, respectively, for STX, NEO, and dcSTX. Neosaxitoxin fluorescence response was increased by lowering the postcolumn temperature from the recommended 60°C to 50°C. The column eluate was mixed with 7 mM periodic acid in 80 mM sodium phosphate buffer. The HPLC system consisted of a Waters 600E multisolvent delivery system connected to a Waters Wisp 700 autosampler and a Shimadzu RF-535 fluorescence detector. A Beckman C8 Ultrasphere Octyl 5μm analytical column (4.6 mm × 25 cm) and a Brownlee MPLC New Guard Column were used for the separation of the toxins. External standard solutions generously supplied by Y. Oshima (Tohoku University, Sendai, Japan) were run prior to sample analysis and after every fourth sample. Toxin composition profiles were determined from replicate analyses (two injections) from each of 2 separate samples. Abbreviations used throughout this text are: STX, saxitoxin; NEO, neosaxitoxin; GTX1, 4, gonyautoxins 1 and 4; GTX2, 3, gonyautoxins 2 and 3; GTX5, gonyautoxin 5 (or B1) [Hall, 1982]; GTX6, gonyautoxin 6 (or B2); C1, 2, toxins C1 and C2; C3, C4, toxins C3 and C4; and dc, decarbamoyl.

Cell counts were made in a Sedgewick-Rafter slide at 200× magnification. Culture growth rates were calculated from cell densities at the beginning and end of the exponential phase.

RESULTS

A typical growth profile of isolate PbSA01 in nutrient-replete batch cultures is shown in Figure 1a. Under normal growth in modified ES medium, the growth rate achieved was 0.3–0.4 div d^{-1}. Total toxin content (fmol $cell^{-1}$) increased dramatically during early exponential phase and reached a peak of ca. 400 fmol $cell^{-1}$ during midexponential phase (Fig. 1b). Toxin content then decreased just as rapidly as the cultures approached stationary phase and remained steady at ca. 200 fmol $cell^{-1}$. Toxins produced by this isolate of *Pyrodinium bahamense* var. *compressum* are GTX6, GTX5, NEO, dcSTX, and STX. No C toxins or GTX1–GTX4 were detected under any of the culture conditions studied. Even though there was a marked variation in total toxin content, toxin composition remained constant through the culture cycle. There is indication, however, of an increase in NEO and a decrease in STX in stationary phase (Fig. 1c). Gonyautoxin V and NEO made up ca. 90 mole % of the total toxin content throughout the course of batch culture growth.

This *P. bahamense* isolate was able to grow at temperatures between 20–38°C, with optimum growth between 28–30°C under continuous illumination (Fig. 2a). Even though the isolate grew at temperatures higher than 30°C, those cells showed aberrant morphology. Temperature had marked effects on toxin content as there was an approximately 3-fold increase in toxin content with a 7°C decrease under continuous illumination, and every 2° change in temperature significantly altered the toxin content (Fig. 2b). Temperature also had marked effects on toxin composition: the mole percent of GTX5 increased from 25% to 55% as temperature increased from 22°C to 34°C, while NEO decreased from 70% to 40% (Fig. 2c).

This *P. bahamense* isolate was able to grow at salinities of 20‰ and higher (Fig. 3a), with optimum growth at 30‰ or

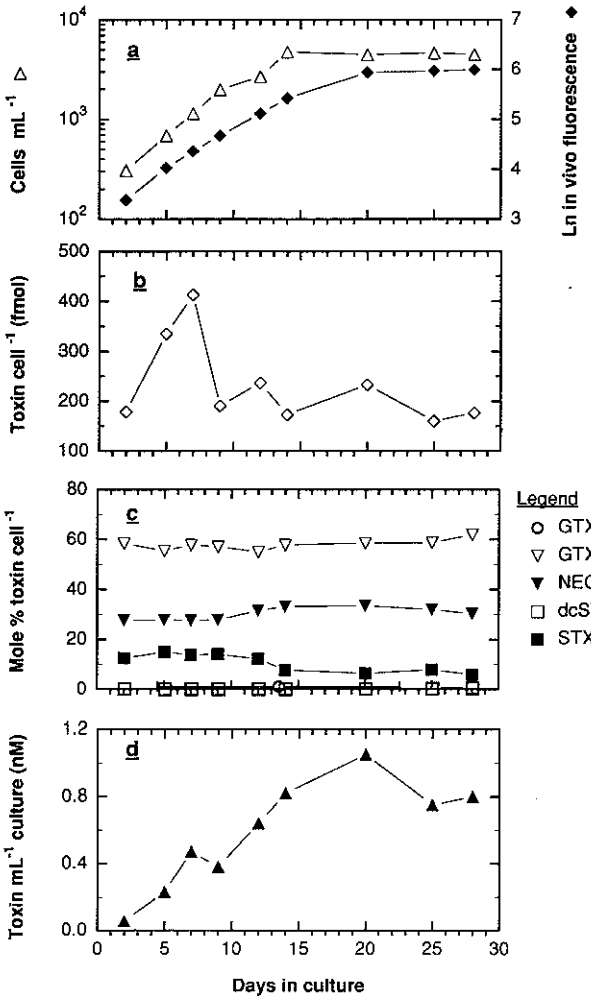

Fig. 1. Growth and toxin production in nutrient-replete batch cultures in modified ES medium. a: Cell density and in vivo fluorescence profiles. b: Cellular total toxin content over time. c: Cellular toxin composition profile over time. d: Toxin content in 1 mL of culture over time. Toxin data represent means from duplicate samples; each ran twice on the HPLC. Error bars are small.

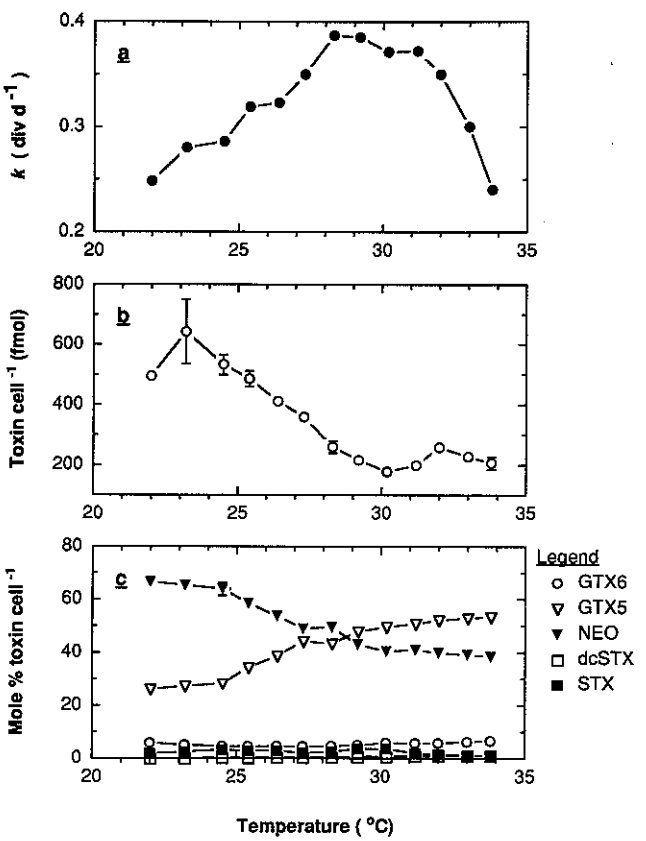

Fig. 2. Growth and toxin production as functions of culture temperature. a: Division rates. b: Cellular total toxin content. c: Cellular toxin composition.

higher. Total toxin content was relatively constant over the salinity range of 24–36‰, at ca. 400 fmol cell^{-1}. At 20‰ salinity, however, the toxin content tripled to almost 1,200 fmol cell^{-1} (Fig. 3b). This increase in toxin content coincided with a 30% decrease in growth rate. Salinity variations had less effect on toxin composition than temperature variations. Over the salinity range studied, the mole percents of GTX5 and NEO each varied by less than 10% (Fig. 3c).

Light intensity effects on growth rates are shown in Figure 4a. The clone was able to grow well at low light intensity; growth rate was constant at intensities from 50–150 μE m^{-2} s^{-1}. There was a doubling in chlorophyll content per cell at 50 μE m^{-2} s^{-1} over that at 100 μE m^{-2} s^{-1} (Fig. 4b). Toxin content per unit chlorophyll increased 4-fold as light intensity increased. Light intensity variations also affected toxin composition. NEO decreased by ca. 15% from 55% at 20 μE m^{-2} s^{-1} as light intensity increased (Fig. 4c). GTX5 increased by ca. 15% from 40% over the same light range.

DISCUSSION

The manner in which toxin content and toxin composition vary with environmental conditions and growth stage in culture has been the subject of much study in the *Alexandrium* species [Anderson et al., 1990b; Boczar et al., 1988; Boyer et al., 1987; Cembella et al., 1987; Hall, 1982, Kim et al., 1993; Prakash 1967; Proctor et al., 1975, White and Maranda, 1978]. Here we present the results of similar studies in another PSP toxin-producing species, *P. bahamense*. These data are highly significant not only because this is a different genus, but also because they represent only the second study of the toxicity of *P. bahamense*, which has stubbornly refused most culturing attempts [Blackburn and Oshima, 1989].

Our results, discussed in more detail below, indicate that *P. bahamense* toxicity varies in some ways that are similar to the *Alexandrium* species, but there are significant differences as well. Among the most obvious differences are the

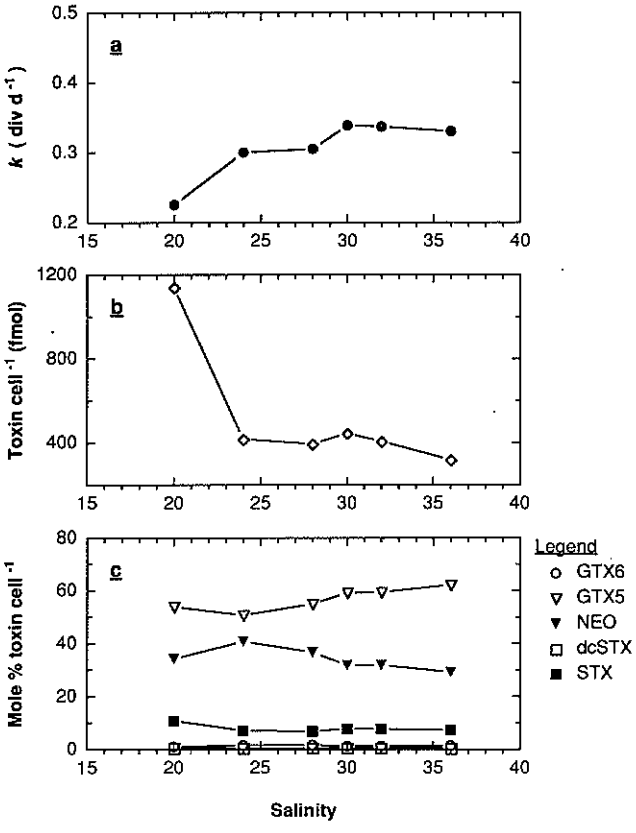

Fig. 3. Growth and toxin production as functions of culture medium salinity. a: Division rates. b: Cellular total toxin content. c: Cellular toxin composition.

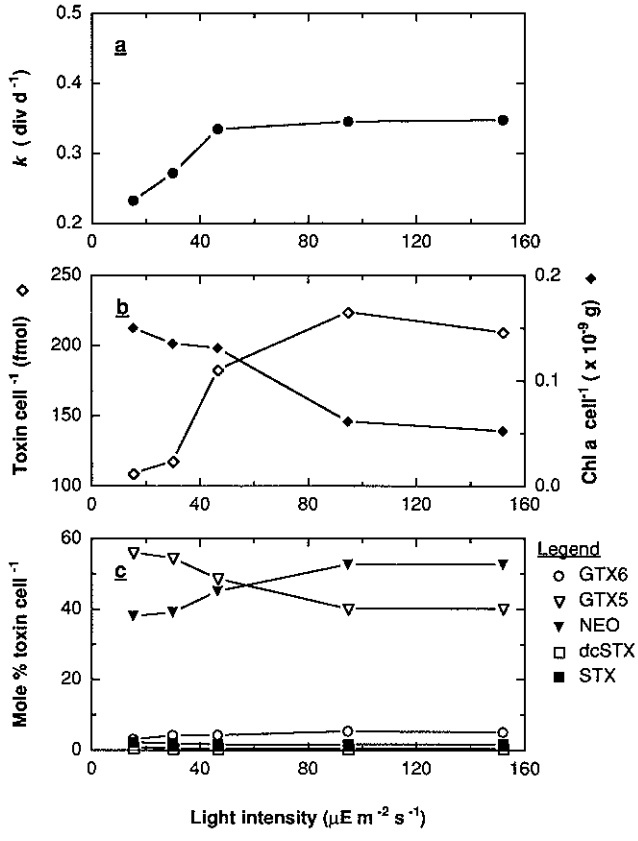

Fig. 4. Growth and toxin production as functions of light intensity. a: Division rates. b: Cellular total toxin and chlorophyll *a* contents. c: Cellular toxin composition.

limited number of toxins produced compared to most toxigenic *Alexandrium* and *Gymnodinium* species, the dramatic enhancement of toxin content at low salinities, and the pronounced toxin composition changes that occur with temperatures and light intensity variations. Now that *P. bahamense* is in culture, and studies like these are possible, a new challenge arises—namely, interpreting these physiological responses in the context of natural environmental variability encountered by *P. bahamense* populations in natural waters.

Toxin Composition

There are currently at least 24 PSP toxins known [Oshima et al., 1993]. Of these, *P. bahamense* clone PbSA01 produces only STX, NEO, dcSTX, GTX5, and GTX6, a profile which is similar to those produced by the *P. bahamense* isolate from Palau [Harada et al., 1982a,b, 1983] and those detected in several species in PSP toxin-contaminated shellfish and finfish from Southeast Asia [Oshima, 1989]. In contrast, *P. bahamense* cells collected during the 1987 red tide in Guatemala were found to produce GTX2 and GTX3, but not dcSTX or C-toxins [Rosales-Loessener et al., 1989]. Though the number of analyzed clones is limited, it is possible that *P. bahamense* may be genetically incapable of producing C toxins. In this respect *P. bahamense* differs from *Alexandrium tamarense, A. fundyense,* and *Gymnodinium catenatum,* but may be similar to *A. minutum* (unpublished data) and *A. cohorticula* [Kodama et al., 1988].

In the *P. bahamense* isolate from Palau, Oshima et al. [1987] found that STX and GTX5 were the major toxins (28.2 and 47.1 mole percent, respectively) while NEO was only 15.3% of the total toxin per cell. With our Sabah isolate, NEO and GTX 5 were always the major toxins (>80 mole % combined) under all the conditions studied. There is thus a possibility that toxin composition in *P. bahamense* may show regional differences, as has been found in *Alexandrium* spp [Anderson et al., Marine Biology, in press; Cembella et al., 1987; Hall, 1982] and *Gymnodinium catenatum* [Oshima et al., 1993]. Clearly, toxin production in additional *P. bahamense* isolates need to be investigated.

Our data on *P. bahamense* indicate that the relative proportion of each toxin in a cell (toxin composition) is not a fixed characteristics, but can vary with growth conditions. In nutrient-replete batch cultures, toxin composition was constant over time. It should be noted, however, that the study was terminated before the cultures went into prolonged stationary phase, so changes of the type reported by Boczar et al. [1988] cannot be ruled out. Toxin composition

changes were evident, however, when *P. bahamense* was subjected to temperature stress or light intensity variations. When toxin composition changes in *Pyrodinium* occurred, they took place in a systematic manner over the environmental factor continuum (i.e., there were no discontinuities or sudden changes). In this respect, toxin composition behaves in a manner similar to other cellular constituents such as chlorophyll.

Direct comparisons to other species are difficult to make, since *Pyrodinium* produces fewer toxins than do most toxigenic *Alexandrium* or *Gymnodinium* species. Oshima et al. [1993] reported the absence of toxin composition change in *G. catenatum* cultured at 25, 17, and 12.5%°C. The toxin content of that isolate, however, was composed of 90 mole % C1–C4 toxins, with only trace amounts of GTX5 and NEO, whereas *Pyrodinium* has 90 mole % or more GTX5 + NEO and no C toxins. Cembella et al. [1987] also did not see toxin composition changes in *Alexandrium* isolates that produce predominantly C toxins.

Toxin Content Variability

The toxin content of a cell will be a net result of the balance between toxin synthesis and losses due to catabolism and leakage (true losses) and allocation into daughter cells (division losses) [Anderson et al., 1990b]. It would thus be reasonable to expect an inverse proportionality between toxin content and culture growth rates. Such a relationship has indeed been demonstrated in several species of *Alexandrium* subjected to temperature and light stresses in batch cultures [Anderson et al., 1990b; Ogata et al., 1987; Proctor et al., 1975]. It has also been shown, however, that this inverse relationship did not apply to all culture conditions [Anderson et al., 1990b].

Virtually all studies on toxin production by the *Alexandrium* species in batch cultures have shown that toxin content is highest in early exponential phase and lowest in stationary phase [Anderson et al., 1990b; Boczar et al., 1988; Boyer et al., 1987; Cembella et al., 1987; Hall, 1982; Kim et al., 1993; Prakash, 1967; Proctor et al., 1975; White and Maranda, 1978]. We observed a similar pattern in toxin content in nutrient-replete batch cultures of *P. bahamense* clone PBSA01 (Fig. 1b). Toxin content at midexponential phase was twice that at late-exponential and stationary phases of the cultures. Only speculations have been offered to explain these patterns. One suggestion is that batch cultures might not be in nutritionally-balanced growth during early exponential phase [Flynn and Flynn, Proc. 6th Int. Conf. Toxin Marine Phytoplankton, in press; Smith et al., 1992] as a result of nitrogen "upshock" when cells are transferred into fresh, nutrient-replete medium. During this phase, nitrogen uptake exceeds the amount of carbon available for protein synthesis, resulting in the accumulation of glutamate, and possibly other amino acids in the intracellular free amino acid pool [Flynn and Flynn, Proc. 6th Int. Conf. Toxin Marine Phytoplankton, in press]. Since arginine is proposed to be the most likely precursor in PSP toxin biosynthesis [Shimizu et al., 1984], the elevation of toxin content during the early exponential phase of batch cultures could be a result of the conversion of excess free arginine into PSP toxins following the initial surge in uptake of nitrogen. Utilization of N for the synthesis of cellular components was most likely responsible for the reduction and eventual cessation of toxin synthesis after early exponential growth.

Studies on *Alexandrium* spp. have invariably shown that toxin content increased as the growth temperature decreased [Anderson et al., 1990b; Hall, 1982; Ogata et al., 1987]. This was also clearly the case with this clone of *P. bahamense* (Fig. 2b). The Q10 for growth rate for the temperature range of 22–32°C was 1.46, less than the average value of 1.8 obtained for several other phytoplankton species in batch cultures [Eppley, 1972; Raven and Geider, 1988]. In contrast, the Q10 for toxin content over the same temperature range was 2.67. Thus the rate of increase of toxin content due to a decrease in culture temperature was higher than the rate at which growth rate decreased over the same temperature range. This indicates that the elevation in toxin content at low temperatures is not due simply to low division rates, but is related to other factors such as the turnover rates of cellular components. For example, Anderson et al. [1990a] reported that in nutrient-replete batch cultures of *A. fundyense* grown at 15°C, toxin content and protein content followed similar patterns, peaking in midexponential growth. In cultures grown at 8°C, however, protein content remained low and constant while toxin content peaked. Furthermore, when toxin content was expressed on the basis of cellular nitrogen content, the values for cells grown at 8°C were at least 2–3 times higher than those for cells grown at 15°C [Table 1 in Anderson et al.,1990a]. The implication is that elevation in toxin content at low temperatures results from allocation of more cellular nitrogen to toxin synthesis and less to protein synthesis. The maximum change in toxin content induced by temperature variations in *P. bahamense* was 3-fold (from 200 to 600 fmol cell^{-1}), comparable to the maximum change found in *A. fundyense* (from 125 to 400 fmol cell^{-1}) by Anderson et al. [1990a].

Effects of salinity on toxin content have been relatively little-studied in any of the PSP toxin-producing dinoflagellates, and what results there are remain confusing. White [1978] found a 2-fold increase in toxin content of *A. excavatum* (= *A. fundyense*) at 37‰ compared to control cultures at 31‰ salinity. However, Anderson et al. [1990b] found no effects of salinity on the toxin content of *A. fundyense* in acclimated cultures or those subjected to short-term changes in salinity. With *P. bahamense* there was no elevation of toxin content with increasing salinity, but there was a significant increase at low salinity (Fig. 3b). As the division rate decreased by approximately 27% from 0.3 div d^{-1} at 24‰ to 0.22 div d^{-1} at 20‰ salinity, over the same salinity range the toxin content increased 300% from 400 to 1,200

fmol cell^{-1}. This huge discrepancy between the rates of change in division rate and toxin content indicates that the elevation of toxin content at low salinity involves metabolic differences, and is not simply due to accumulation of toxin in the slowly dividing cells.

While growth stage and temperature effects on toxin content are quite similar among *P. bahamense* and other PSP toxin-producing species, the response to salinity changes is not consistent. Part of the problem may relate to the different experimental designs that have been used. In one investigation, White [1978] acclimated *A. fundyense* to normal salinity before subjecting the cultures to short-term exposures to higher salinities, while Anderson et al. [1990a] exposed *A. fundyense* to low and high salinity medium over a period of 10 days. In our study on *P. bahamense,* cultures were acclimated to each particular salinity before being harvested for toxins. The contrasting results obtained from these studies may also be a reflection of the different responses associated with short- and long-term changes in salinity.

Considering that light is of primary importance in the growth and physiology of these dinoflagellates, it is somewhat surprising that its effects on toxin prodution have not been well studied. In the one study reported thus far [Ogata et al., 1987], the results suggested that toxin content in *A. tamarense* increased when light intensity decreased, although the trend was not strong. Our results (Fig. 4b) clearly show that toxin content decreased as light intensity decreased. The decrease in toxin content coincided with a decrease in growth rate, although the effect on toxin content was evident at a higher light intensity (ca. 95 μE m^{-2} s^{-1}) than the level that affected growth rate (ca. 45 μE m^{-2} s^{-1}). It was also clear from Figure 4b that the decrease in toxin content coincided with an increase in the amount of chlorophyll *a* in the cell. The allocation of N for chlorophyll synthesis to offset the effect of low light may have resulted in less being available for toxin synthesis. It is also well known that nitrate uptake is suppressed under low light conditions in several species of phytoplankton [Syrett, 1981].

Results from this and other studies on the PSP toxin-producing dinoflagellates suggest that the key to understanding the effects of environmental factors on toxin content would be to resolve how those factors affect the allocation of N and C to various cellular components and processes. Suboptimal environment conditions can lead to stresses which can translate into slow growth rates or into altered metabolism. It is evident, however, that reduction in growth rate does not necessarily result in high toxin content. As shown in Figure 5, a clear relationship between growth rate and toxin content was only evident when the *P. bahamense* cultures were subjected to temperature or light stress.

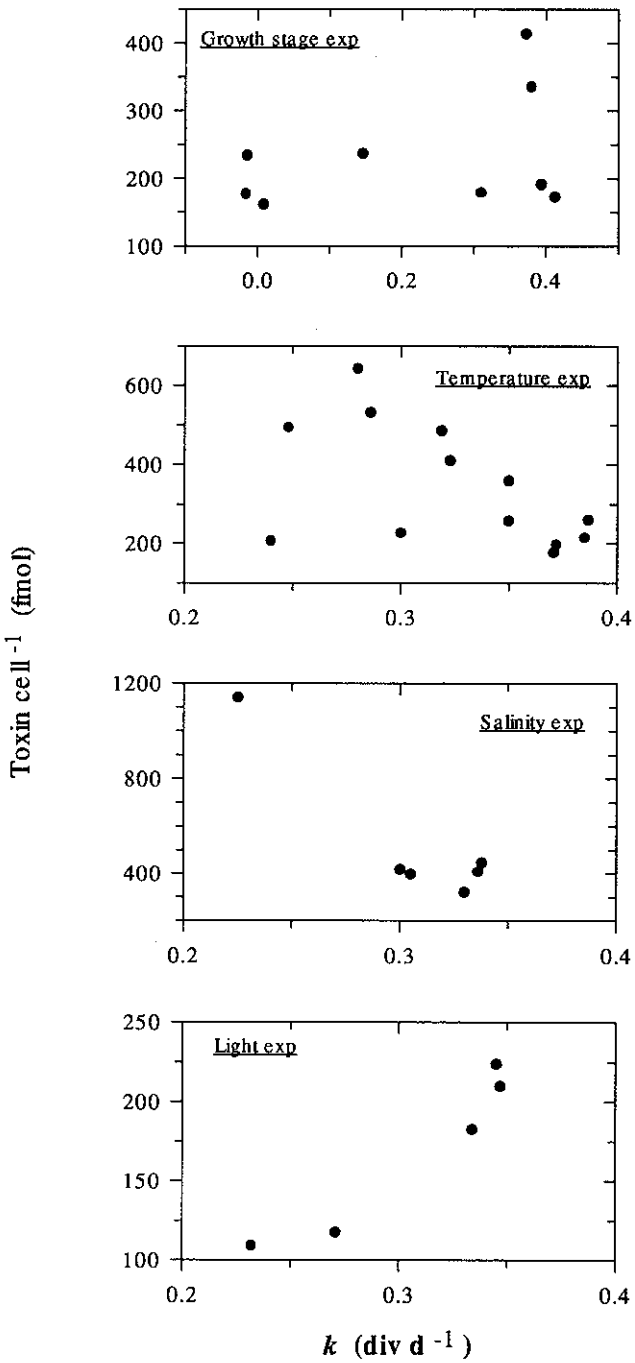

Fig. 5. Relationship between cellular total toxin content and division rate under various culture conditions.

Biochemical Considerations

In *Pyrodinium* and, possibly, *Alexandrium* and *Gymnodinium,* it seems likely that the first toxin synthesized is STX (Oshima, personal communication). STX would then be rapidly converted enzymatically to either GTX5 or NEO, and NEO could be further converted into GTX6. Environmental factors could thus affect toxin production at 2 levels. Factors that affect the biosynthetic process prior to the production of STX will ultimately determine the toxin content of the cell, while effects that occur after the production of STX will affect toxin composition. If environmental factors

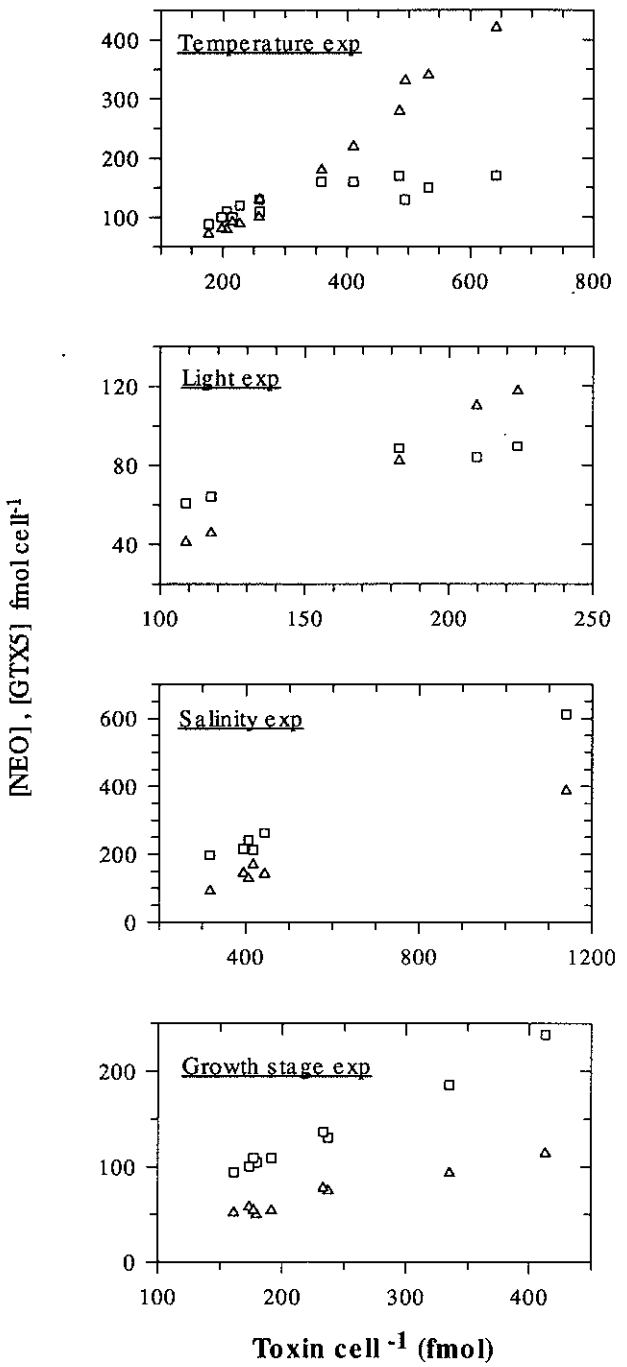

Fig. 6. Relationship between GTX5 (triangles) and NEO (squares) concentrations with cellular total toxin content under various culture conditions.

do not alter cell metabolism and the conversion of STX to the other toxins, then the concentrations of individual toxins would be directly proportional to total toxin content, and toxin composition would remain constant.

Evidence presented here showed that in this *P. bahamense* clone, temperature and light intensity variations affected the conversion of STX to NEO and/or GTX5, resulting in toxin composition changes. Since NEO and GTX5 are the major toxins produced by the species, it is to be expected that a change in the mole percentage of one would result in a reciprocal change in the mole percentage of the other. As summarized in Figure 6, however, the relative rates of change in the actual cellular concentrations of the 2 toxins were not always equal, indicating that the conversion process may not be strictly linear. In the temperature experiment, where toxin composition change was most pronounced, the concentration of NEO increased linearly with total toxin content, whereas GTX5 did not. GTX5 concentration was maximal at temperatures of 26°C or lower, and at 33°C or higher. Within these 2 temperature ranges, either the conversion of STX to GTX5 was suppressed or the conversion of STX to NEO was enhanced. This could have occurred through the modulation of enzyme activity (e.g., of N-sulfotransferases) by temperature.

In the light intensity experiment in which only moderate toxin composition changes were observed, it was again the concentration of NEO that increased significantly by a factor of 3 as the total toxin content increased 2-fold, whereas the concentration of GTX5 increased by only a factor of 1.5. In the salinity experiment, both NEO and GTX5 concentrations increased linearly with total toxin content, although the slope of the curves suggested that the concentration of NEO increased at a higher rate than that of GTX5.

In summary, if the toxin profile of a particular isolate is a result of the expression of enzymes involved in toxin biosynthesis [Oshima et al., 1993], then our data on toxin content and composition illustrate the complex manner in which environmental factors can modulate the action of those enzymes.

ACKNOWLEDGMENTS

Our research was supported in part by the National Science Foundation through grants OCE-8911226 and INT-9224474. G.U. was supported by a fellowship from the Malaysian government. The authors also thank Dr. Y. Oshima for generously providing toxin standards used in the HPLC analysis, and for fruitful discussions. Mr. Sahrol Hamzah and Mr. Martubat Jamlan assisted with the field work in Sabah in 1991. Contribution number 8790 from the Woods Hole Oceanographic Institution.

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