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# Effect of salinity on growth and toxin production of *Alexandrium minutum* isolated from a shrimp culture pond in northern Vietnam

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**Abstract** A clonal culture of a Vietnamese strain of *Alexandrium minutum*, AlexSp17, was subjected to different salinity treatments to determine the growth and toxin production of this strain that produces a novel toxin analogue, deoxy GTX4-12ol. The experiment was carried out in batch cultures without pre-acclimatization at seven salinity treatments from 5 to 35 psu, under constant temperature of 25°C, illumination of 140 μmol photon $m^{-2}$ $s^{-1}$, and 12:12 light/dark photoperiod. The strain grew in all salinity treatments, with optimum growth at 10–15 psu. However, the specific growth rate (0.2 $day^{-1}$) was lower than those reported in Malaysian strains and other strains from different geographical areas. The optimum range of salinity for the growth of this species agreed with field observations of the locality of origin. No significant change in toxin profiles was observed at different salinities. The cellular toxin quota, Qt, was not affected by the salinity-dependent growth rate. The toxin GTX4-12ol is presumed to be a transformation product of GTX4 from specific cellular reductase enzymes. Further investigation at the molecular level of toxin biosynthesis and subcellular enzyme activities is needed to provide insight in the production of this unique toxin analogue.

**Keywords** *Alexandrium minutum* · Salinity · Toxin production · GTX4-12ol · Vietnam

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## Introduction

In the tropics, dry–wet seasonal precipitation with freshwater influence results in salinity fluctuations in estuarine waters. This, in turn, affects the growth physiology of organisms living in estuarine environments, particularly the phytoplankton. Many studies have shown that seawater salinity is the important exogenous factor that affects the bloom dynamics of *Alexandrium* species (e.g., Cembella et al. 1988; Cembella and Therriault 1989). For instance, a study of *Alexandrium tamarense* in the Gulf of Maine showed that low-salinity coastal currents promoted the sudden increase in cell density (Anderson 1998). Giacobbe et al. (1996) found that spring blooms of *Alexandrium minutum* in the Mediterranean Sea coincided with the increase in rainfall and freshwater runoff. Our current understanding on the growth physiology and toxin production of *Alexandrium* is mainly derived from studies on the species from temperate estuaries, which may not truly reflect the growth physiology and toxin production of tropical counterparts.

In Southeast Asian countries, the occurrence of *A. minutum* has been frequently reported in estuarine waters (Lim et al. 2004; Lim and Ogata 2005), aquaculture ponds (Matsuoka et al. 1997; Yoshida et al. 2000; Lim et al.

2007a), and semi-enclosed bays. All *A. minutum* strains found in this region produce paralytic shellfish toxins (PSTs), with only minor differences in the toxin profiles among strains (Hwang and Lu 2000; Lim et al. 2004). However, distinct toxin profiles were reported in strains reported from higher geographical regions, notably for temperate strains. A New Zealand strain of *A. minutum* contained predominantly neosaxitoxin (Chang et al. 1997) while Danish strains produced sulfocarbamoyl toxins (C toxins) as the principal toxin congeners (Hansen et al. 2003).

Recently, we documented a new STX analogue, deoxy GTX4-12ol, in Vietnamese strains of *A. minutum* (Lim et al. 2007a). In our HPLC analysis, the peak appeared consistently between the peaks of GTX4 and GTX1. In addition, LC-MS/MS analysis showed that the analogue with parental ion $[M+H]^+$ at *m/z* 396 was identical to the *m/z* values of GTX3 and GTX2, but differed from GTX4/GTX1. NMR data finally confirmed the identity of the analogue as deoxyGTX4-12ol. This finding, together with the six new analogues (Onodera et al. 1997; Llewellyn et al. 2004), increases the number of saxitoxin derivatives to more than 30. Our understanding of saxitoxin biosynthesis is mainly based on work on toxic cyanobacteria (Shimizu et al. 1985; Kellmann et al. 2008) and several enzymes involved in the toxin transformation of shellfish or microalgae (Sako et al. 2001). Little is known about the biosynthesis in dinoflagellates. It has been postulated that the derivative, GTX4, is most likely the first end product of saxitoxin biosynthesis (Shigeru Sato, personal communication), while others suggested this to be C toxin (Wang and Hsieh 2001).

Physiological studies in relation to toxin production of *Alexandrium* species, particularly of those originating from tropical waters, are lacking. Various studies have shown that toxin profiles of *Alexandrium* species can be affected by environmental conditions such as salinity (Parkhill and Cembella 1999), light (Ogata et al. 1989; Lim et al. 2006), and macronutrients (Flynn et al. 1996). In this study, the growth physiology and toxin production of a Vietnamese *A. minutum* strain, AlexSp17, were investigated at different salinity regimes. Cell toxin quota and toxin production of the strain were analyzed with HPLC and the relationship of GTX4 and GTX4-12ol at various salinity regimes was determined.

## Materials and methods

*Alexandrium minutum* was first discovered in May 2004 in shrimp aquaculture ponds at Do Son, Hai Phong, Northern Vietnam. Subsequently, field observations on the abundance of *A. minutum* in the shrimp ponds and its related environmental conditions (pH, dissolved oxygen, salinity, and temperature) were carried out fortnightly from May to October 2004. The physico-chemical parameters were analyzed by a YSI multi-parameter probe (YSI, USA). For qualitative samples, a 20-μm mesh net was towed horizontally within the first few meters of the surface. Once samples had been collected, they were fixed with formalin and viewed under a microscope for qualitative assessment. Quantitative samples were taken by a 2-L Niskin sampler. One-liter samples were concentrated through a 20-μm mesh to 20 mL samples. Concentrated samples were then fixed with Lugol's solution. Cells were counted and identified to species level using an inverted microscope with a magnification of ×400. A total of 1 mL of samples was placed into a Sedgwick-Rafter counting slide, allowed to settle for 15 min, and cells were counted.

Several clonal cultures of *A. minutum* were established from the ponds. The cultures of *A. minutum* strain AlexSp17 used in this study were initially established in Daigo's IMK medium for Marine Microalgae (Daigo, Tokyo, Japan) and later transferred and maintained in ES-DK medium (Kokinos and Anderson 1995) at 25°C under a 14:10-h light/dark cycle at 140 μmol photons $m^{-2}$ $s^{-1}$ of cool white fluorescent light in a temperature-controlled growth chamber. Salinity of the medium was adjusted to 15.0±1.0 psu using natural filtered seawater as the medium base. The culture was clonal but not axenic.

For the salinity experiment, cells of *A. minutum* AlexSp17 was inoculated into seven salinity treatments (5–35 psu). Lower salinity medium was prepared by diluting filtered seawater with distilled water. The experiment started with the inoculation of late exponential phase culture at 20 psu with an initial cell density of approximately 350 cells $mL^{-1}$ into each 250-mL flask containing 200 mL of medium at the desired salinity without any pre-acclimatization.

Growth was monitored daily by microscopic cell counting. Cell counts were carried out by subsampling 0.1 mL of samples, fixed with Lugol's solution, and placed into a Sedgwick-Rafter counting slide. The sample was allowed to settle for 15 min and counted under an Olympus BX51 microscope. Specific growth rate ($\mu$, $day^{-1}$) was calculated over the exponential growth phase using the following equation:

$$\mu = \frac{\ln N_1 - \ln N_0}{t_1 - t_0}$$

where $N_0$ and $N_1$ are the cell density at time $t_0$ and $t_1$, respectively.

Salinity experiments were performed in duplicate, with at least duplicate cell counts for each salinity treatment flask. Each $\mu$ value of a single set of salinity treatments corresponds to the mean of a minimum of four determinations ± standard deviation.

### HPLC toxin analysis

Paralytic shellfish toxins were determined by subsampling 10 mL media from each salinity treatment flask in 2- to 3-day intervals. Cells were collected by centrifuging at 3,000×*g* for 10 min. Toxins were then extracted by adding acetic acid (0.5 N) and the cells were disrupted by ultrasonication for 3 min in an ice bath. The slurry was spun at 12,000×*g* for 10 min and the supernatant was then transferred to new tubes and kept at −20°C until further analysis. HPLC analysis was carried out using the isocratic, post-column derivatization method of Oshima (1995) with minor modification. The samples were run through a Wakosil C18 column (4.6 mm i.d.×15 cm, 120 Å, 4 μm). Chromatographic conditions were as follows. For the STXs, the mobile phase was 2 mM heptanesulfonate in 30 mM ammonium phosphate buffer and 6% acetonitrile (*v*/*v*), pH 7.1. For the GTXs, the mobile phase was 2 mM heptanesulfonate in 30 mM ammonium phosphate buffer, pH 7.1, and for the C toxins, the mobile phase was 2 mM tetra-butyl ammonium in acetate buffer, pH 5.8. The post-column oxidizing reagent was 7 mM periodic acid in 80 mM sodium phosphate buffer, pH 9.0, while the acidifier was 0.5 M acetic acid. Sample injection volume was 10–20 μL. Flow rates for the mobile phases were 0.8 and 0.4 mL $min^{-1}$ for each post-column reagent. The reaction coil temperature was kept at 70°C water bath for all runs. Detection wavelengths were set at 330-nm excitation and 390-nm emission. Toxin identification and quantification were determined by comparison with authentic toxin standards. The concentration of GTX4-12ol was determined from the purified GTX4-12ol that was initially quantified by NMR.

The toxin production rate, $R_{tox}$ (fmol toxin $cell^{-1}$ $day^{-1}$), for total toxin, GTX4, and GTX4-12ol was determined using the equations of Anderson et al. (1990).

$$R_{tox} = \frac{(T_1 - T_0)}{\overline{N}\Delta t}$$

$$\overline{N} = \frac{N_1 - N_0}{\ln N_1 - \ln N_0}$$

where $N$ is the cell density at each stage while $T$ is the total toxin concentration at time, by multiplying the toxin content (fmol $cell^{-1}$) with cell density ($N$).

## Results

The occurrence of *A. minutum* was first observed in May 2004 in the shrimp aquaculture ponds at Do Son, Hai Phong, Northern Vietnam. Over the 6-month period, salinity in the pond ranged between 9 and 21 psu (Fig. 1). Surface temperature fluctuated in the range of 28.7–33.5°C (Fig. 1). Cells of *A. minutum* was observed on three occasions at salinities in the range of 11.0–16.4 psu, with the highest cell density of 920 cells $L^{-1}$ observed at 15 psu (Fig. 1).

### Growth responses to various salinity regimes

In all salinity treatments, the cells grew exponentially with a short lag phase (Fig. 2a). Generally, growth was optimal at a salinity of 10–15 psu, with $\mu$ of around 0.2 $day^{-1}$. The maximum cell yield of 22,000 cells $mL^{-1}$ was observed at 10 psu (Fig. 2b). Growth was strongly suppressed at 5 psu (<0.1 $day^{-1}$) and 35 psu (~0.1 $day^{-1}$), with maximum cell densities decreasing around eightfold compared to 10 psu (2,500 cells $mL^{-1}$). At 10–30 psu, $\mu$ decreased significantly with elevated salinity ($P$=0.0031). Transfer of cultures from 5–35 to 10 psu did promote growth of the cultures (data not shown).

### Toxin production at various salinity regimes

Cell toxin quota (Qt) varied over the growth stages as well as the salinity regimes (Fig. 3). Pronounced increment of Qt over the whole period of experiment was observed in all treatments (10–35 psu) except at 5 psu. There was no significant difference in Qt between 5 and 10 psu ($P$= 0.1452) despite the significant differences in specific growth rates between the two treatments. The Qt of cultures at 15–25 psu peaked at around 20 fmol PST $cell^{-1}$. On the other hand, the highest Qt was observed at 30 and 35 psu with the toxin quota of 30 fmol PST $cell^{-1}$ (Fig. 3).

GTX4 and GTX4-12ol remained the major toxin congeners regardless of the growth stage or the salinity regime and were up to 50 and 30 mol% of total toxins, respectively. Other toxins (GTX3, GTX2, STX, neoSTX, dcSTX, and C2) remained as traces throughout the experiment and contributed only to <10 mol% of total toxins (Fig. 4).

At the lower suboptimal salinity of 5 psu, proportions of GTX4 and GTX4-12ol did not show significant increment/decrease throughout the experiment; furthermore, no significant changes were observed for the minor toxins (Fig. 4a). However, above 10 psu, a general trend of gradual changes of the proportion of major and minor toxins was observed, where the proportion of GTX4 decreased gradually when approaching the late exponential phase or stationary phase (Fig. 4b, c). On the other hand, the proportion of GTX4-12ol increased up to 45–50% of total toxin content at the later growth stages. It is interesting to note that at a higher suboptimal salinity of 35 psu, similar patterns in toxin proportions were observed (Fig. 4c).

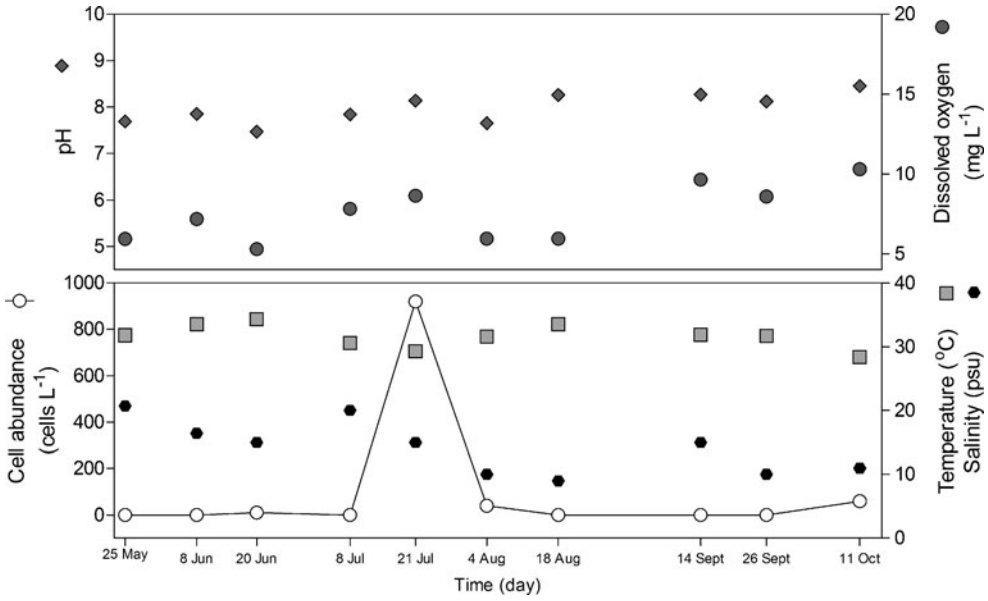


**Fig. 1** *Alexandrium* cell abundance (cells $L^{-1}$) with the physico-chemical parameters (pH, dissolved oxygen, water temperature, and salinity) collected during May and October 2004 in the shrimp ponds

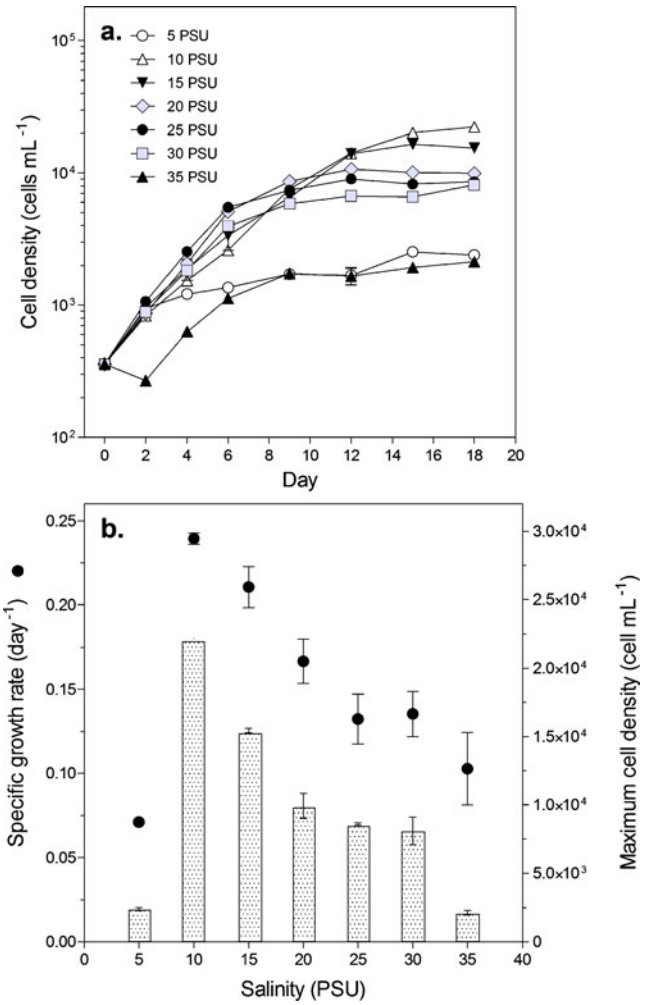


**Fig. 2** Growth (**a**), specific growth rates and maximum cell densities (**b**) of *A. minutum* AlexSp17 at different salinity regimes. *Error bars* represent ± SD ($n$=4)

Total toxin production rate, $R_{tox}$, was found to increase when the salinity was increased from 5 to 20 psu (Fig. 5). However, further elevation of salinity resulted in a decline in the toxin production rates (Fig. 5). Both toxin production rates for GTX4 and GTX4-12ol followed similar trend as $R_{tox}$ of total toxins.

## Discussion

### Growth physiology

In this study, the growth physiology of *A. minutum* originating from tropical estuarine waters in relation to salinity was investigated. Salinity treatments were carried out without pre-acclimatization of the cultures. The intent was to observe cell physiology reacting to the realistic salinity fluctuations in nature. In tropical estuarine waters with a semi-diurnal tidal cycle, salinity fluctuations from the lowest to the highest salinity and vice versa could happen in about a 6-h period. Organisms that reside in estuarine waters will experience drastic change of salinity in a considerably short time frame, particularly when river runoff is abundant due to precipitation.

The Vietnamese *A. minutum* strain exhibited a unique pattern of salinity tolerance with a remarkably narrow optimum salinity range (10–15 psu). This is unique in contrast to other strains reported from Taiwan (Hwang and Lu 2000), Malaysia (Lim and Ogata 2005), and Thailand (Ogata T., unpublished data) which showed wide optimum salinity range from 5 to 30 psu. Optimum growth rate of the strain was significantly lower than the growth rate observed in other strains of *A. minutum* under similar culture conditions (Lim and Ogata 2005) and some other strains

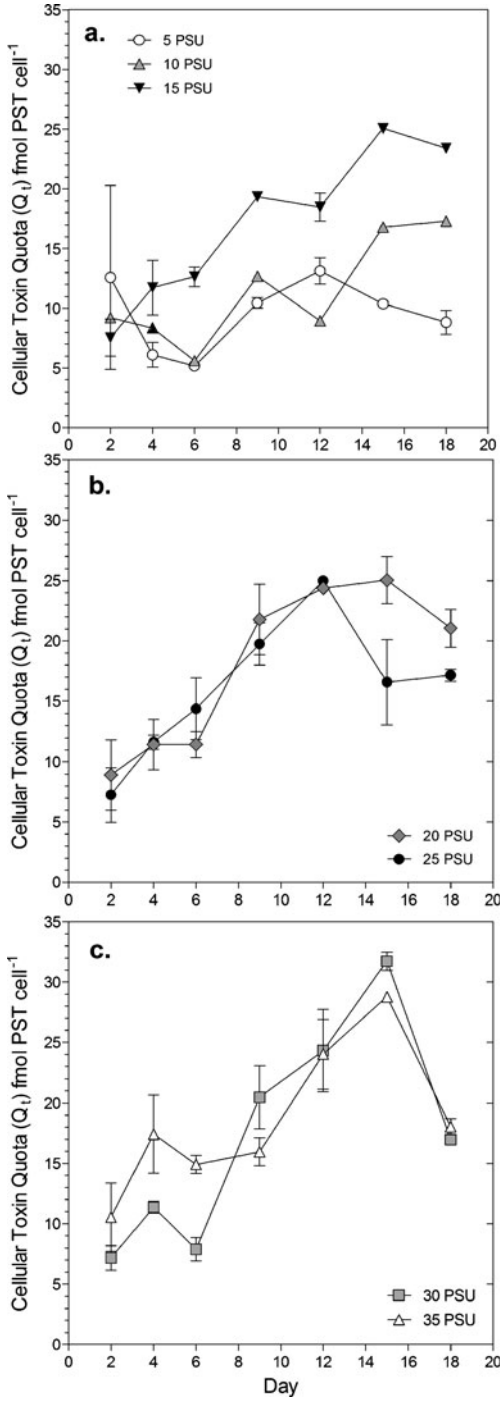


**Fig. 3** Cell toxin quota (Qt) of *A. minutum* AlexSp17 at different salinity regimes. *Error bars* represent ± SD ($n$=2)

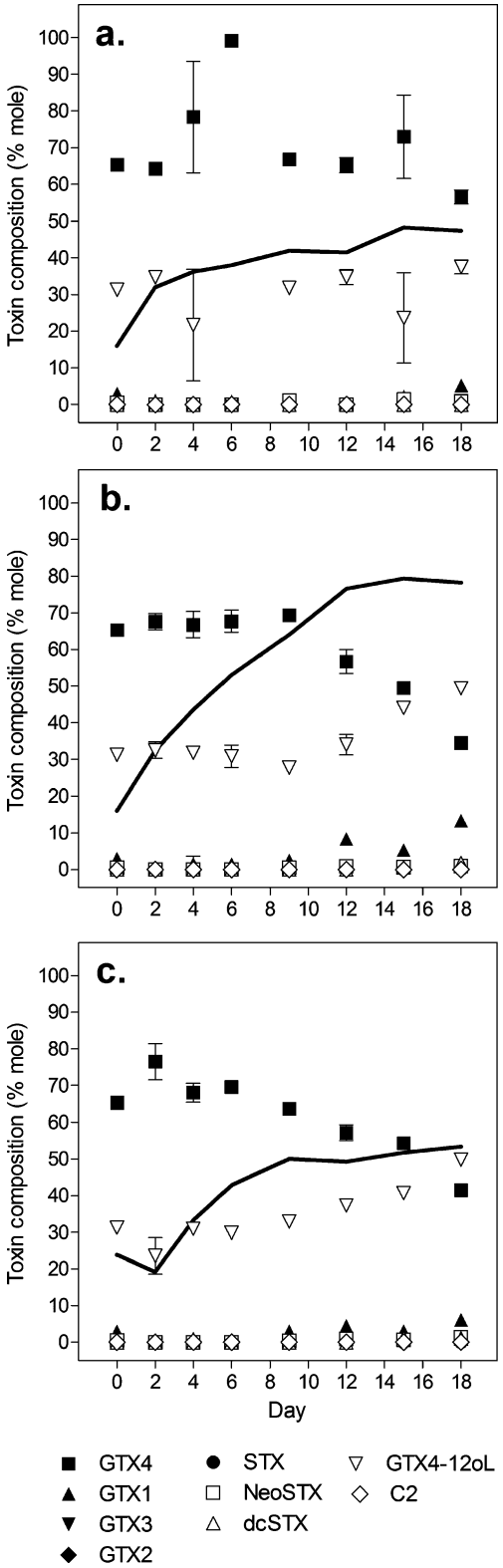


**Fig. 4** Toxin composition (mol%) of *A. minutum* AlexSp17 at 5 psu (**a**), 15 psu (**b**), and 35 psu (**c**). The *solid lines* represent the growth curves shown in Fig. 2a

that possess high growth rates (Chang and McClean 1997; Grzebyk et al. 2003; Table 1). These results clearly indicate the presence of different ecotypes of *A. minutum* under environmental selection pressure. *A. minutum* strains with narrow optimum salinity at the higher salinity range have been observed in strains from different geographical origin, particularly from temperate waters. The French strains

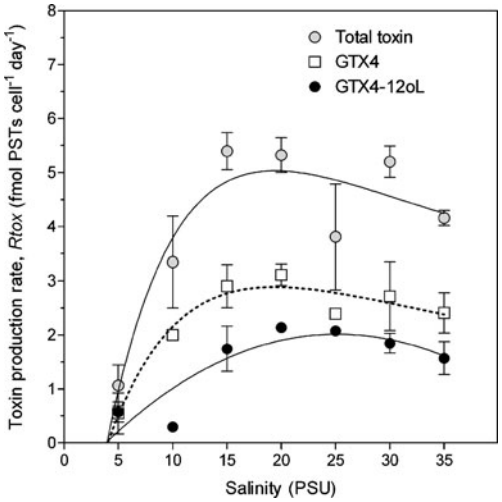


**Fig. 5** Toxin production rates, $R_{tox}$, of *A. minutum* AlexSp17 at different salinity regimes. *Error bars* represent ± SD ($n$=2)

showed an optimum growth at salinity ranges of 20–37 psu (Grzebyk et al. 2003), whereas in Australian strains of *A. minutum*, optimum growth was observed at 26 psu with salinity tolerance between 21 and 35 psu (Cannon 1993; Table 1). The genetic makeup of the European and Pacific strains of *A. minutum* has been proven as two distinct genetic groups (De Salas et al. 2001; Lim et al. 2007a, b). Both the temperate and tropical counterparts might belong to two physiologically distinct groups or ecotypes based on the salinity tolerance, i.e., high-euryhaline species with optimum salinity of >20 psu and low-euryhaline species with optimum salinity of <20 psu (Lim and Ogata 2005). The present results are in agreement with the results of Lim and Ogata (2005). Different degrees of salinity tolerance were also exhibited in other tropical PST-producing *Alexandrium* species (Lim and Ogata 2005). Among the *Alexandrium* species found in Malaysian waters, *Alexandrium tamiyavanichii* showed no growth at salinities below 20 psu which was considered to reflect stenohaline species. This was considered as evidence of the relationship between salinity tolerance and the natural habitats from which the strains originated. This species was isolated from coastal water with relatively less influence from river plumes (Usup et al. 2002; Lim et al. 2006). Adaptation of *Alexandrium* species to estuarine waters will depend on the capability to tolerate salinity fluctuations.

## Toxin quota and profile

The relationship between growth, salinity tolerance, and cell toxin of PST-producing dinoflagellates has been the subject of arguments and studies for decades. One of the well-accepted explanations is that salinity-dependent growth inversely affects the cellular toxin quota, Qt, in PST-producing species (Hamasaki et al. 2001). This explanation failed to clarify the positive correlation of growth and Qt in some species of *Alexandrium* (e.g., White 1978; Parkhill and Cembella 1999). In the present study, growth rates were suppressed at both 5 and 35 psu, with growth rate <0.1 $day^{-1}$. However, Qt was much higher at 35 psu than at 5 psu (Fig. 3). Our study provides further evidence that Qt is not the result of salinity-dependent growth rate, but may rather be a balance of osmoregulation that involves changes in cell biovolume (Table 2 in Lim and Ogata 2005) and cellular amino acid utilization (for osmoregulation, toxin production, and growth).

**Table 1** Growth and toxin production of *A. minutum* under difference salinity regimes

| Species (strain) | Culture conditions | | | Optimal growth rate, $\mu$ ($day^{-1}$) | Cellular toxin quota, Qt (fmol $cell^{-1}$) | Locality |
|---|---|---|---|---|---|---|
| | Irradiance (μmol photons $m^{-2}$ $s^{-1}$) | Temperature (°C) | Salinity (psu) | | | |
| *A. minutum* (AlexSp17) | 140 | 25 | 5–35 | $\mu$: 0.240 ± 0.007 $day^{-1}$ (10 psu) | Qt: 5–30 fmol $cell^{-1}$; Qt decreased with lifted salinity | Vietnam (in this study) |
| *A. minutum* (AmKB06) | 140 | 25 | 2–30 | $\mu$: 0.3–0.5 $day^{-1}$ (5–30 psu) | Qt: 4–12 fmol $cell^{-1}$; Qt decreased with lifted salinity | Malaysia(a) |
| *A. minutum* (AmKB02) | 10–140 | 15–25 | 15 | $\mu$: 0.2–0.5 $day^{-1}$ | Qt: 10–40 fmol $cell^{-1}$ | Malaysia(b) |
| *A. minutum* (AM89BM) | 100 | 18 | 12–37 | $\mu$: ≥0.5 $day^{-1}$ (20–37 psu) | Qt: 10–50 fmol $cell^{-1}$; Qt increased at lower salinities | France(c) |
| *A. minutum* (AMBOP006) | 25–100 | 18 | – | $\mu_{max}$: 0.50 $day^{-1}$ | – | New Zealand(d) |
| *A. minutum* | 0–100 | 12–25 | 21–35 | $\mu$: 2.2–6.7 $day^{-1}$ | – | Australia(e) |

References: (a) Lim and Ogata (2005); (b) Lim et al. (2007a, b); (c) Grzebyk et al. (2003); (d) Chang and McClean (1997); (e) Cannon (1993)

It is also noteworthy that the Vietnamese strain of *A. minutum* possesses a unique toxin profile in comparison to other *A. minutum* from neighboring waters, particularly the Malaysian strain (Lim and Ogata 2005; Lim et al. 2007a, b). The Vietnamese strain produces a novel analogue, dedeoxy-GTX4-12ol (Lim et al. 2007a, b). In this study, the concentration of this compound was accurately determined from the purified compound quantified by NMR data (data not shown). Thus, the actual concentrations reported here show slight differences from our previous study where the initial concentration was determined based on the fluorescent response of GTX4 (Lim et al. 2007a, b).

Toxin composition of the strain remained consistent in the early exponential phase in all the salinity treatments, which is in agreement with the observation on the Malaysian strain. No significant difference was observed in the toxin composition throughout the experiment at low suboptimum salinity (5 psu). However, at optimum and high suboptimum salinities, the toxin compositions showed substantial changes at the later stages of the experiment (late exponential to stationary phases), where the proportion of GTX4 decreased while GTX4-12ol increased. We presume that enzymatic transformation of GTX4 to GTX4-12ol occurred in the cells by the enzymatic reduction at the Carbon-12. This is consistent with the observations on the Malaysian strain, except the transformation occurred at N1 group (Lim and Ogata 2005). GTX4 as the first end product of saxitoxin biosynthesis has been postulated because it is the predominant toxin component under favorable or environmental stress conditions, particularly in *A. minutum* from the Asia Pacific region. Conversion of toxins from GTX4 to other toxin components has been shown to occur chemically and through biological transformation (Asakawa et al. 1987; Oshima 1995; Kodama 2000). Several explanations have been proposed on the roles of saxitoxin production in the cells, either as a defensive mechanism (Turner et al. 1998) or nitrogen storage (Boyer et al. 1987); however, the precise reasons for toxin production are unclear. It is interesting to note that in our previous (Lim and Ogata 2005) and present studies, toxin transformation at the later stages of experiments occurred in the same manner where highly toxic compound (GTX4) were converted to low (GTX2+3) or non-toxic compounds (GTX4-12ol). GTX4-12ol has lack of binding affinity to mammalian sodium channels and thus is considered non-toxic. Based on a dynamic model of growth and toxin production of *Alexandrium fundyense*, John and Flynn (2002) proposed that toxin synthesis could be selection-neutral if cost of synthesis to the cells is not significant. If toxin synthesis is beneficial to the cell, conversion from highly toxic components to low or non-toxic components would not be beneficial (John and Flynn 2002). Studies on toxin biosynthesis at the molecular level may provide a better view of the functional role of the saxitoxin family. Further study on the genetic makeup of the species in comparison with other closely related strains should provide insights into the toxin production of *Alexandrium* species. Apart from that, long-term field investigation of the species are essential to gain better understanding of the ecological responses of the species to salinity as well as other environmental parameters.

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## References

Anderson DM (1998) Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. Springer, Berlin, pp 29–48

Anderson DM, Kulis DM, Sullivan JJ, Hall S, Lee C (1990) Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium* spp. Mar Biol 104:511–524

Asakawa M, Tagaki M, Iida A, Ioshi M (1987) Studies on the conversion of paralytic shellfish poison (PSP) components by biochemical reducing agents. Eisei Kagaku 33:50–55

Boyer GL, Sullivan JJ, Andersen RL, Harrison PJ, Taylor FJR (1987) Effects of nutrient limitation on toxin production and composition in the marine dinoflagellate *Protogonyaulax tamarensis*. Mar Biol 96:123–128

Cannon JE (1993) Growth in culture of the toxic dinoflagellate *Alexandrium minutum* from the Port River, South Australia. In: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea. Elsevier, New York, pp 741–745

Cembella AD, Therriault JC (1989) Population dynamics and toxin composition of *Protogonyaulax tamarensis* from the St. Lawrence Estuary. In: Okaichi T, Anderson DM, Nemoto T (eds) Red tides—biology, environmental science and toxicology. Elsevier, New York, pp 81–85

Cembella AD, Therriault JC, Beland P (1988) Toxicity of cultured isolates and natural populations of *Protogonyaulax tamarensis* from the St. Lawrence Estuary. J Shellfish Res 7:611–621

Chang FH, McClean M (1997) Growth responses of *Alexandrium minutum* (Dinophyceae) as a function of three different nitrogen sources and irradiance. N Z J Mar Freshw Res 31:1–7

Chang FH, Anderson DM, Kulis DM, Till DG (1997) Toxin production of *Alexandrium minutum* (Dinophyceae) from the Bay of Plenty, New Zealand. Toxicon 35:393–409

de Salas MF, van Emmerick MJ, Hallegraeff GM, Negri AP, Vaillancourt RE, Bolch CJ (2001) Toxic Australian *Alexandrium* dinoflagellates: introduced or indigenous? In: Hallegraeff GM, Bolch CJ, Lewis RJ (eds) Harmful algal blooms 2001. IOC of UNESCO, Rome, pp 214–217

Flynn K, Jones KJ, Flynn KJ (1996) Comparisons among species of *Alexandrium* (Dinophyceae) grown in nitrogen- or phosphorus-limiting batch culture. Mar Biol 126:9–18

Giacobbe MG, Oliva FD, Maimone G (1996) Environmental factors and seasonal occurrence of the dinoflagellate *Alexandrium minutum*, a PSP potential producer, a Mediterranean lagoon. Estuar Coast Shelf Sci 42:539–549

Grzebyk D, Bechemin C, Ward CJ, Verite C, Codd GA, Maestrini SY (2003) Effects of salinity and two coastal waters on the growth and toxin content of the dinoflagellate *Alexandrium minutum*. J Plankton Res 25:1185–1199

Hamasaki K, Horie M, Tokimitsu S, Toda T, Taguchi S (2001) Variability in toxicity of the dinoflagellate *Alexandrium tamarense* isolated from Hiroshima Bay, western Japan, as a reflection of changing environmental conditions. J Plankton Res 23:271–278

Hansen G, Daugbjerg N, Franco JM (2003) Morphology, toxin composition and LSU rDNA phylogeny of *Alexandrium minutum* (Dinophyceae) from Denmark, with some morphological observations on other European strains. Harmful Algae 2:317–335

Hwang DF, Lu YH (2000) Influence of environmental and nutritional factors on growth, toxicity, and toxin profile of dinoflagellate *Alexandrium minutum*. Toxicon 38:1491–1503

John EH, Flynn KJ (2002) Modelling changes in paralytic shellfish toxin content of dinoflagellates in response to nitrogen and phosphorus supply. Mar Ecol Prog Ser 225:147–160

Kellmann R, Mihali TK, Jeon YJ, Pickford R, Pomati F, Neilan BA (2008) Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria. Appl Environ Microbiol 74:4044–4053

Kodama M (2000) Ecobiology, classification, and origin. In: Botana LM (ed.) Seafood and freshwater toxins-pharmacology, physiology, and detection. Marcel Dekker, New York, pp 125-149

Kokinos JP, Anderson DM (1995) Morphological development of resting cysts in cultures of the marine dinoflagellate *Lingulodinium polyedrum* (= *L. machaerophorum*). Palynology 19:143–166

Lim PT, Ogata T (2005) Salinity effect on growth and toxin production of four tropical *Alexandrium* species (Dinophyceae). Toxicon 45:699–710

Lim PT, Leaw CP, Usup G (2004) First incidence of paralytic shellfish poisoning on the east coast of Peninsular Malaysia. In: Phang SM, Chong VC, Ho SS, Mokhtar N, Ooi JLS (eds) Marine science into the new millennium: new perspectives and challenges. University of Malaya Maritime Research Centre, Kuala Lumpur, pp 661–667

Lim PT, Leaw CP, Usup G, Kobiyama A, Koike K, Ogata T (2006) Effects of light and temperature on growth, nitrate uptake, and toxin production of two tropical dinoflagellates: *Alexandrium tamiyavanichii* and *Alexandrium minutum* (Dinophyceae). J Phycol 42:786–799

Lim PT, Sato S, Van Thuoc C, Tu PT, Huyen NTM, Takata Y, Yoshida M, Kobiyama A, Koike K, Ogata T (2007a) Toxic *Alexandrium minutum* (Dinophyceae) from Vietnam with new gonyautoxin analogue. Harmful Algae 6:321–331

Lim PT, Leaw CP, Ogata T (2007b) Morphological variation of two *Alexandrium* species responsible for paralytic shellfish poisoning in Southeast Asia. Bot Mar 50:14–21

Llewellyn L, Negri A, Quilliam M (2004) High affinity for the rat brain sodium channel of newly discovered hydroxybenzoate saxitoxin analogues from the dinoflagellate *Gymnodinium catenatum*. Toxicon 43:101–104

Matsuoka K, Fukuyo Y, Yoshida M (1997) *Alexandrium minutum* Halim collected from aquaculture ponds in tropical and subtropical coastal waters. In: Sudara S (ed) Marine conservation and resource rehabilitation. Chulalongkorn University, Chiangrai, pp 85–94

Ogata T, Kodama M, Ishimaru T (1989) Effect of water temperature and light intensity on growth rate and toxin production of toxic dinoflagellates. In: Okaichi T, Anderson DM, Nemoto T (eds) Red tides: biology, environmental science, and toxicology. Elsevier, New York, pp 423–426

Onodera H, Satake M, Oshima Y, Yasumoto T, Carmichael WW (1997) New saxitoxin analogues from the freshwater filamentous cyanobacterium *Lyngbya wollei*. Nat Toxins 5:146–151

Oshima Y (1995) Postcolumn derivatization liquid chromatography method for paralytic shellfish toxins. J AOAC Int 78:528–532

Parkhill JP, Cembella AD (1999) Effects of salinity, light and inorganic nitrogen on growth and toxigenicity of the marine dinoflagellate *Alexandrium tamarense* from northeastern Canada. J Plankton Res 21:939–955

Sako Y, Yoshida T, Uchida A, Arakawa O, Noguchi T, Ishida Y (2001) Purification and characterization of a sulfotransferase specific to N-21 of saxitoxin and gonyautoxin 2+3 from the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). J Phycol 37:1044–1051

Shimizu Y, Gupta S, Norte M, Hori A, Genenah A, Kobayashi M (1985) Biosynthesis of paralytic shellfish toxins. In: Anderson DM, White AW, Baden DG (eds) Toxic dinoflagellates. Elsevier, New York, pp 271–274

Turner JT, Tester PA, Hansen PJ (1998) Interactions between toxic marine phytoplankton and metazoan and protistan grazer. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. Springer, Berlin, pp 453–474

Usup G, Leaw CP, Ahmad A, Lim PT (2002) *Alexandrium* (Dinophyceae) species in Malaysian waters. Harmful Algae 1:265–275

Wang D, Hsieh DPH (2001) Dynamics of C2 toxin and chlorophyll-a formation in the dinoflagellate *Alexandrium tamarense* during large scale cultivation. Toxicon 39:1533–1536

White AW (1978) Salinity effects on growth and toxin content of *Gonyaulax excavata*, a marine dinoflagellate causing paralytic shellfish poisoning. J Phycol 14:475–479

Yoshida M, Ogata T, Thuoc CV, Matsuoka K, Fukuyo Y, Hoi NC, Kodama M (2000) The first finding of toxic dinoflagellate *Alexandrium minutum* in Vietnam. Fish Sci 66:177–179