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GROWTH RESPONSES OF FIVE NON TOXIC *ALEXANDRIUM* SPECIES (DINOPHYCEAE) TO TEMPERATURE AND SALINITY

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ABSTRACT

Growth response of five clonal cultures of *Alexandrium* obtained from tropical and temperate waters were examined. Experiments were carried out in eighteen variable temperature-salinity conditions (temperatures of 15°C, 20°C, and 25°C; salinities between 5 to 30 psu) under constant illumination of 150 ± 10.0 μmol $m^{-2}s^{-1}$ at 15:9 light:dark photo-cycle. Our results showed optimum growth of all *Alexandrium* species at 20 - 25°C. The salinity range for optimum growth however varied among the species. Growth rates of *A. affine, A. insuetum*, and *A. fraterculus* (0.28 – 0.37 day^{-1}) were higher than those of *A. leei* and *A. pseudogoniaulax* under the same culture conditions (0.14 – 0.22 day^{-1}). The three temperate species showed positive growth at suboptimum temperature, 15°C, but the tropical species did not grow and died off. Salinity tolerance of the five species in decreasing order was *A. pseudogoniaulax* > *A. leei* > *A. insuetum* > *A. affine* > *A. fraterculus*. Results of the present study showed vast variations in salinity tolerance among the *Alexandrium* species regardless the geographical origins. Adaptation of the temperate species at higher temperature indicated that the species might proliferate in warm tropical waters.

Keywords: *Alexandrium*, Growth, Salinity, Temperature

INTRODUCTION

The genus *Alexandrium* Halim comprised of more than 30 species. Approximately one-third of these species capable of producing a suit of sodium channel blocking toxins or commonly known as paralytic shellfish toxins (PSTs). Various researches have been carried out to gain better understanding of the eco-physiology of *Alexandrium* species, however these studies were mainly carried out on *A. tamarense* (Lebour) Balech (Ogata *et al.*, 1987), *A. fundyense* Balech (Anderson *et al.*, 1990), *A. minutum* Halim (Flynn *et al.*, 1994; Chang and McClean, 1997), and *A. tamiyavanichii* Balech (Previously as *Protogonyaulax cohorticula*) (Ogata *et al.*, 1989; Lim and Ogata, 2005).

Little is known about the ecology and physiology of non toxic *Alexandrium* species. Some of these non PSTs producing species have been shown to be distributed worldwide and co-occurrence with the toxic counterpact, while some caused negative impact on marine environment or its flora and fauna (Delgado *et al.*, 1997)). In tropical waters, high precipitation coupled with strong freshwater output has been resulted in salinity fluctuation in the estuarine waters. On the other hand, temperature showed significant seasonal variation in higher latitude. It is generally presumed that the species from different geographical regions possess specific ecological adaptation to the natural environments where they are originated. To test the hypothesis that tropical *Alexandrium* species is more euryhaline while the temperate species is more eurythermal, growths of five *Alexandrium* species originated from both tropical and temperate waters were examined in eighteen variable temperature-salinity conditions.

MATERIALS AND METHODS

Cultures

Clonal cultures of tropical *A. affine* and *A. leei* were established from live plankton

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specimens collected from Malaysian waters as described earlier (Usup *et al.*, 2002), while the temperate isolates of *A. insuetum, A. pseudogoniaulax* and *A. fraterculus* were obtained from Sanriku coast, northeastern of Japan (Kaga *et al.*, in press). Cultures were maintained in ES-DK medium (Kokinos and Anderson, 1995) under a light intensity of 150 ± 10.0 μmol m^{-2} s^{-1} and 15:9 hour light:dark photoperiod. Natural seawater (35 psu) from Okkirai Bay, Japan was used as the medium base. Stock cultures of tropical and temperate strains were maintained at 25°C and 20°C respectively.

Salinity and Temperature Experimental Design and Setup

Salinity and temperature experiment was carried out in eighteen variable temperature-salinity treatments, with salinities 5, 10, 15, 20, 25, and 30 psu and temperatures 15, 20 and 25°C. All treatments were in duplicate. Salinity was adjusted by diluting with deionized distilled water to desired salinity. Exponential-phase cells were then used as inoculates to prepare 25 mL batch cultures. Growth was determined daily or alternate day by *in vivo* fluorescence and cell counts using light microscope. Relationship between *in vivo* fluorescence and cell density for each species was established by obtaining cell density at respective *in vivo* fluorescence reading. Specific growth rate (¼, day^{-1}) was then calculated from *in vivo* fluorescence-estimated cell density during the exponential growth phase using the following equation:

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

where N is the estimated cell density at time *t*.

RESULTS

In Vivo Fluorescence Measurement of Cell Growth

Growth of five species was monitored using *in vivo* fluorescence and microscopic cell count. There were strong correlations between *in vivo* fluorescence and microscopy cell counts in all the species examined (*A. affine*, $r^2 = 0.96$, $P<0.0001$; *A. leei*, $r^2 = 0.95$, $P<0.0001$; *A. insuetum*, $r^2 = 0.99$, $P<0.0001$; *A. fraterculus*, $r^2 = 0.94$, $P<0.0001$; *A. pseudogoniaulax*, $r^2 = 0.98$, $P<0.0001$) during the exponential growth phase (Fig. 1). This allowed the estimation of cell density from the *in vivo* fluorescent measurements (Fig. 1).

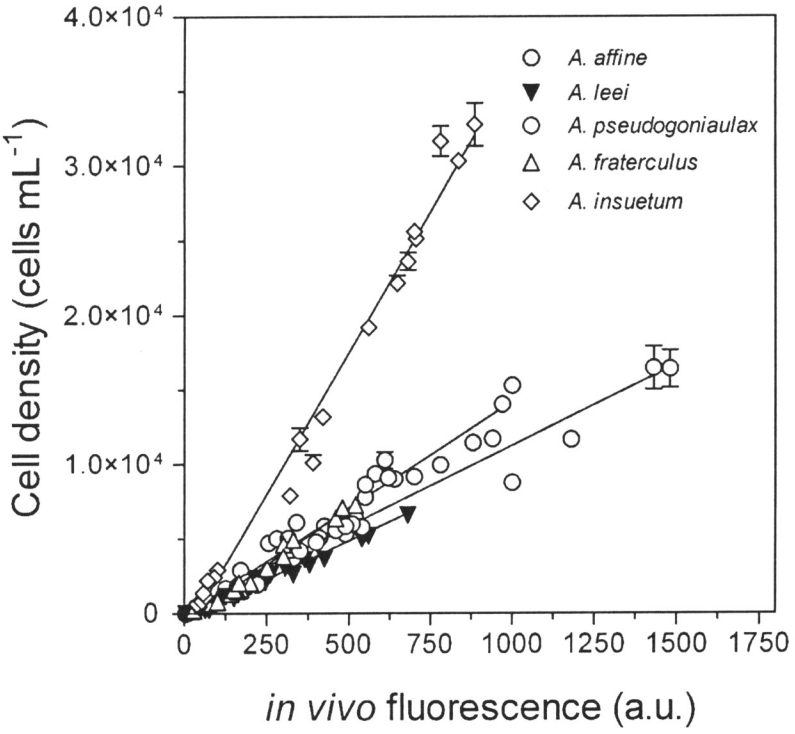

Figure 1. Relationship of cell density and *in vivo* fluorescence of the five *Alexandrium* species.

Effects of Temperature and Salinity on Cell Growth

All *Alexandrium* species in the present study showed optimum growth at 20 - 25°C (Fig. 2). At 15°C, only temperate strains of *A. insuetum*, *A. pseudogoniaulax*, and *A. fraterculus* grew suboptimally with low positive growth rates (>0.02 day^{-1}). The two tropical species of *A. affine* and *A. leei* however did not grow and died off after

The optimum salinity of tropical *A. affine* was observed within the range of 20 to 30 psu (0.19 – 0.37 day^{-1}) with the highest growth rate at 25 psu (Fig. 3). The species did not grow at 10 psu or lower. At low temperature (15°C), no growth was observed in *A. affine*. The optimum salinity regime for *A. insuetum* was observed within 15 to 30 psu (0.29 – 0.34 day^{-1}) with the highest growth rate at 20°C and a salinity of 30 psu. Growth rates did not

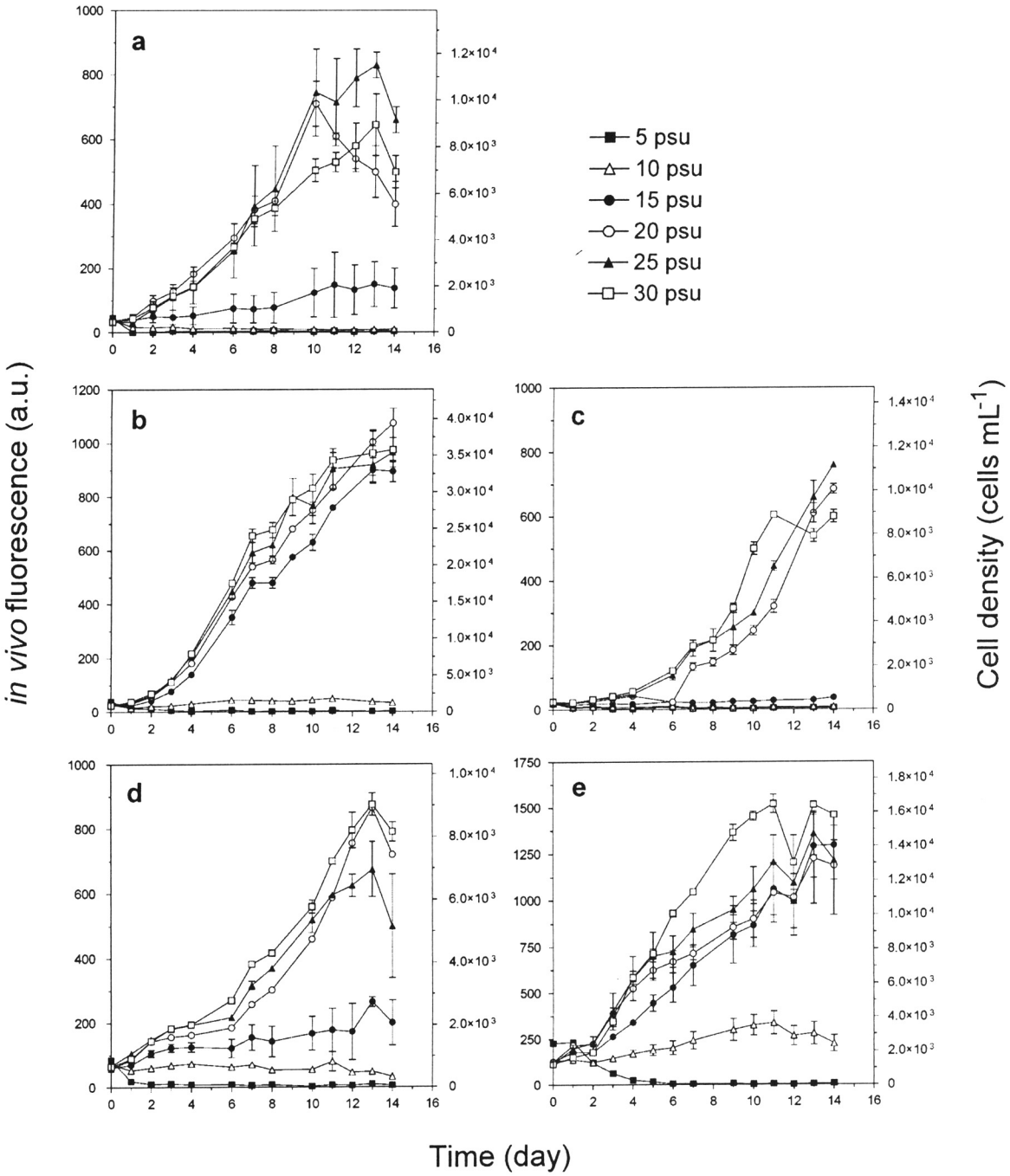

Figure 2. Growths of *Alexandrium* species under various salinities at 25°C by *in vivo* fluorescent measurement. (a) *A. affine*, (b) *A. insuetum*, (c) *A. fraterculus*, (d) *A. leei*, (e) *A. pseudogoniaulax*.

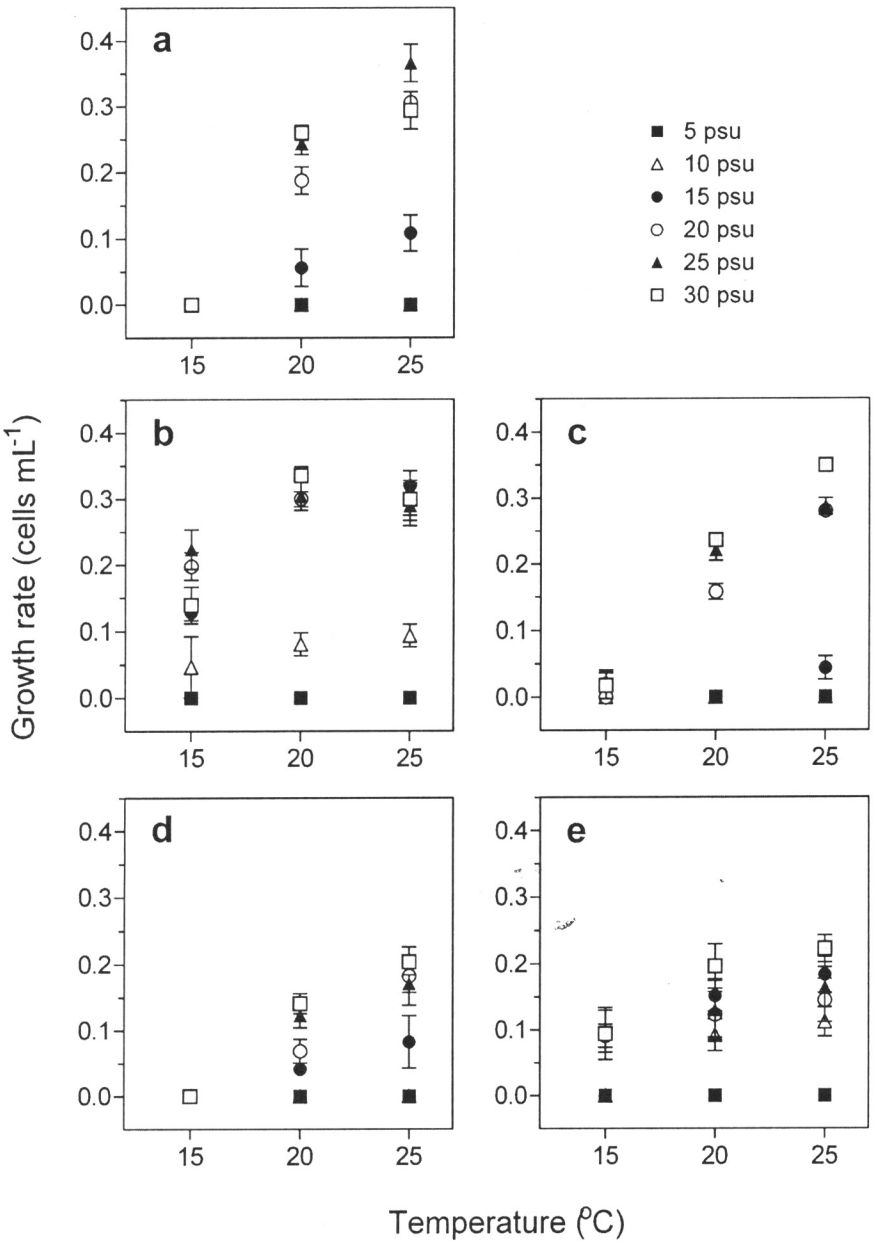

Figure 3. Specific growth rates of *Alexandrium* species under variable salinity and temperature conditions, (a) *A. affine*, (b) *A. insuetum*, (c) *A. fraterculus,* (d) *A. leei,* (e) *A. pseudogoniaulax*.

vary significantly in the optimum temperature regime (20 - 25°C). However, the growth rates decrease significantly at suboptimum temperature ($P<0.01$). The species grew suboptimally at a salinity of 10 psu in the temperature range examined (0.05 – 0.10 day^{-1}) but did not survive at 5 psu.

The salinity range for optimum growth of *A. fraterculus* was 20 – 30 psu (0.16 – 0.35 day^{-1}). The growth rates decreased significantly at lower temperature ($P<0.01$). No growth was observed below 10 psu. In contrast, *A. leei* and *A. pseudogoniaulax* showed lower growth rates compared to the other three species. At optimum temperatures range, *A. pseudogoniaulax* could tolerate a wide salinity range of 10 - 30 psu with growth rates of 0.10 – 0.22 day^{-1}. At suboptimum temperature, the cells could not survive at salinity below 15 psu but grew at salinities between 20 to 30 psu (0.10 day^{-1}). The tropical *A. leei* possessed almost similar optimum growth pattern as the tropical *A. affine* although with lower growth rates. The cells grew optimally at salinities between 20 to 30 psu and cells died off at salinities below 10 psu.

DISCUSSION

Temperature is one of the pivotal environmental factors to understand the ecological and physiological conditions of microlagae and their surrounding habitats, as temperature regulates the key biological processes in organisms, including photosynthesis, cellular enzymatic activities and respiration.

Effect of temperature on these key biological processes would result in changes of growth rates (Raven and Geider, 1988). Temperature effects on survival and growth also useful in explaining the biogeographical distribution of a particular macro and microalgae species (Pakker and Breeman, 1996). In the present study, growths of *Alexandrium* species varied significantly with temperature. The highest growth rate for all the species examined was obtained at the upper temperature range between 20 to 25°C. This temperature range was coincided with the temperature range of subtropical to tropical waters that are consistently more than 20°C, and the water temperature during summer in the temperate waters. However, the temperate species showed an optimum temperature range which was higher than expected from their natural habitats and exhibited a broader tolerance to temperature compared to the tropical strains. At suboptimum temperature (15°C), the temperate species grew suboptimally while both tropical strains of *A. affine* and *A. leei* showed no positive growth. The results obtained in this study supported the hypothesis that the temperate strains can adapt wider temperature ranges. In Sanriku coast of Northeastern Japan, where the temperate strains originated from, the temperature at 5 - 10 m depth varied significantly with the lowest temperature observed in winter (6°C) and the highest at late summer (20°C). However, warm water condition only stay on for a short period of time (less than 3 weeks). Occurrence of these species was reported from warmer month from September to November (Kaga *et al.*, in press). No blooms of these species have been observed thus far over the four-year period (2000-2003) (Kaga et al., in press).

Growth response to temperature observed in the tropical *A. affine* was similar to that with tropical *A. tamiyavanichii* (previously as *P. cohorticula*, Ogata *et al.*, 1989). Both tropical and temperate *A. tamiyavanichii* showed an optimum growth at 25°C but the temperate strains showed better tolerance to lower temperature limit (Ogata *et al.*, 1989). Our results also consistently agreed with the previous findings and supported the hypothesis that temperate strains could tolerate broader temperature ranges.

Interestingly, our result on the effect of temperature was also in agreement with the proposed life form types of Smayda and Reynold (2001). The five species fell within the life form type of IV, V and VI that consisted of raphidophytes and almost all toxic dinoflagellates (Smayda, 2006). The PST-producing *A. tamiyavanichii* and *A. minutum* also fell in this group (Lim *et al.*, 2006). However, the *Alexandrium* species examined in the present study belong to non PST-producing strains. This indicated that the life form type IV, V and VI not only consisted of toxic species but might also include some other non toxic dinoflagellates.

Salinity is important in determining the horizontal distribution of a species in estuaries. Numerous bloom events of toxic *Alexandrium* have been associated with the estuarine waters (Cembella and Therriault, 1989; Lim and Ogata, 2005) Giacobbe *et al.* (1996) had shown that the spring blooms of *A. minutum* in Mediterranean Sea were coincided with the increase in rainfall and freshwater run off. Low salinity coastal current in the Gulf of Maine has also shown to promote cell proliferation of *A. tamarense* in Casco Bay regions (Anderson, 1998).

Salinity tolerance of the five species in decreasing order was *A. pseudogoniaulax* >*A. leei* > *A. insuetum* > *A. affine* > *A. fraterculus*. It is interesting to note that there were vast variations in salinity tolerance observed among the *Alexandrium*. The hypothesis that tropical species showed stronger salinity tolerance is rejected based on the result obtained in present study. Both the tropical *A. affine* and *A. leei* strains showed a high salinity optimum between 20 to 30 psu and could not tolerate salinity below 10 psu.

Difference degree of salinity tolerance was also exhibited in the tropical PST-producing *Alexandrium* species (Lim and Ogata, 2005). Among the *Alexandrium* species found in Malaysia waters, *A. minutum* has been shown to be strong euryhaline with optimum salinity range between 5 to 30 psu, while *A. tamiyavanichii* showed no positive growth at salinity below 20 psu. This was consistent with the habitats of the species where *A. tamiyavanichii*, *A. affine* and

A. leei were isolated from waters with salinity of 28 psu, and with less influence of freshwater plumes (Usup *et al.*, 2002; Lim *et al.*, 2006).

Presence of selective pressure in the environments was probably the reason for ecotypic variation. For example, among the *A. minutum* species, higher salinity optimum strains and wide salinity optimum strains have been observed from different localities. Most of the *A. minutum* strains showed optimum salinity that is higher than 15 psu (Chang and McClean, 1997; Grzebyk *et al.*, 2003). The Malaysian and Taiwanese strains showed wide salinity tolerance from 3 to more than 30 psu (Hwang and Lu, 2000; Lim and Ogata, 2005). Adaptation of *Alexandrium* species in the estuarine waters will depend on the capability to tolerate salinity fluctuation. The allochthonous species might be transported into the estuary by tidal currents from outside the estuary. Ability of the species to tolerate salinity fluctuation will give an advantage in the availability of terrestrial derived nutrients. In estuaries water with long residence time, populations of autochthonous plankton species may grow and develop. Result of the present study showed that at least four species, i.e. *A. affine*, *A. insuetum*, *A. leei* and *A. pseudogoniaulax* were able to maintain growth in brackish water environment. This ability gives an edge over other species that showed narrow salinity tolerance, such as *A. fraterculus* (present study) and *A. tamiyavanichii* (Lim and Ogata, 2005).

In conclusion, the temperate *Alexandrium* examined in this study exhibited a high tolerance to temperature while the tropical species had a weak tolerance to lower temperature. This implied that the chances of the tropical species to proliferate in temperate environments remain insignificant. However, adaptation of the temperate species at warm tropical temperatures indicated that these species might dominate in warmer water if the species was transported by means of natural or human activities.

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