Competition Among Dinoflagellate *Alexandrium Tamarense*, Raphidophyte *Heterosigma Carterae* and Diatom *Skeletonema Costatum* under Combinations of Two Temperatures and Five Salinities

YAN Tian (颜天), ZHOU Mingjiang (周名江)
(Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China)

QIAN Peiyuan (钱培元)
(Biology Department, Hong Kong University of Sciences and Technology, Hong Kong, China)

Received Jan. 9, 2002; revision accepted Oct. 10, 2002

**Abstract**  Competition among HAB (Harmful Algal Bloom) species Dinoflagellate *Alexandrium tamarense*, Raphidophyte *Heterosigma carterae*, and Diatom *Skeletonema costatum* was studied in the laboratory. Experiments with these three major HAB species under combinations of different salinities (10, 18, 25, 30, 35) and temperatures (19°C, 25°C) were carried out. The results showed that *S. costatum* successfully competed with the other two species at salinities of 18, 25, 30, and 35 at temperatures of 19°C and 25°C. However, *H. carterae* showed its advantage at low salinity of 10 and became the single dominant species at salinity 10 and 25°C. *A. tamarense* could not compete successfully with the other two species especially at low salinities. However, it could remain at low density in the presence of higher densities of other algae.

**Key words:** *Alexandrium tamarense*, *Heterosigma carterae*, *Skeletonema costatum*, Harmful Algal Bloom, competition, temperature, salinity

**INTRODUCTION**

Frequent occurrence of HAB (Harmful Algal Bloom) has been observed in China since the 1970's. The great harm to the marine ecosystem and potential threat to human health caused by HAB have become a major concern. However, the mechanism of HAB formation is still poorly understood.

Both physical and chemical factors are considered as main causes for HAB formation, because under optimal temperature, salinity and nutrient conditions, HAB species grow fast and are more likely to form blooms. Changes in these environmental factors may lead to a shift in dominating species within a phytoplankton community. However, biological factors including grazing, competition and interaction among algae are also important for understanding the HAB formation mechanism. Some scientists realized the role of competition among several different species (Cannon, 1996; Davidson et al., 1999). In order to better understand the role of competition in the HAB formation...
mechanism and to establish mathematical models locally, three contrasting HAB species Dinoflagellate *Alexandrium tamarense*, Raphidophyte *Heterosigma carterae* and Diatom *Skeletonema costatum* were chosen for the present study. These three HAB species have different harm mechanisms and distribute in southern to northern Chinese waters (Qian et al., 1983; Wang, 1989; Guo, 1994; Li and Xia, 1995). *A. tamarense* is a species potentially producing PSP (Paralytic Shellfish Poison) toxic to humans; *Heterosigma carterae* is a main fish killing species found all over the world; and *S. costatum* is a species causing most frequent HAB in the world, and is sometimes harmful to marine organisms due to oxygen depletion during the decay process of this non-toxic species. A study on the combined effects of temperature, irradiance and salinity on each of the above species was reported (Qian and Yan, 2000). This paper reports the results of research on the competition among these three species in mixed cultures under different combinations of temperature and salinity.

MATERIALS AND METHODS

*A. tamarense* (Lebour) Balech (ATHK) isolated from Daya Bay, Guangdong Province, China, was provided by Prof. Qi; *H. carterae* (Hulburt) Taylor was collected from Jiaozhou Bay, Shandong Province, China; *S. costatum* (Greville) Cleve was obtained from a culture of CC-MP1332 (Culture Center for Marine Phytoplankton, Maine, USA). The experiment was conducted in the Biology Department, Hong Kong University of Science and Technology. Cultures were grown in enriched seawater (Harrison et al., 1980). One part of natural seawater blended with two parts of artificial seawater (Harrison et al., 1980) was used to provide natural trace substances for algal growth. To avoid precipitation during autoclaving, the medium was passed through 0.2 μm filter (142 mm) under gravity pressure for sterilization using 316 stainless steel sanitary filter holder. Sterilized 50 ml glass test tubes with caps were used for culturing the specimens. Incubators (Powers Scientific SD 33SE) were used at the 4 designated temperatures. Medium of different salinities were obtained by diluting with double distilled water or adding salt according to artificial seawater recipe when mixing natural and artificial seawater before enrichment. ATAGO hand refractometer was used for salinity measurement. Ostrum Lumilux cool white fluorescent tubes were used to provide 14h: 10h (L:D) light. Irradiance was measured by Licor model LI 185 meter with 2B collector.

Experiments on competition among *A. tamarense*, *H. carterae* and *S. costatum* were conducted with a 2-factor design at combinations of 2 temperature levels (19°C, 25°C) and 5 salinity levels (10, 18, 25, 30, and 35). Irradiance was fixed at $1.6 \times 10^{16}$ quanta/(cm²·s). Each treatment was done in four replicates. Inoculation of the cultures with each species was initiated at equal density (500 cells/ml) in 45 ml fresh medium. One ml sample was taken at day 2, 3, 4, 5, 6, 7, 10, 13, 15, and 22, and fixed with Logol’s solution for counting under inverted microscope using Utermoehl’s technique.

RESULTS

Growth of each algal species in the mixed cultures

Fig. 1 shows the growth of *A. tamarense*, *H. carterae* and *S. costatum* in the mixed culture under combinations of two different temperatures (19°C, 25°C) and five salinities (10, 18, 25,
Fig. 1 Growth of three HAB species *A. tamarense*, *H. carterae* and *S. costatum* in mixed cultures under salinities 10(A), 18(B), 25(C), 30(D), 35(E) at 19°C and salinities 10(F), 18(G), 25(H), 30(I), 35(J) at 25°C, respectively.

*S. costatum* grew faster and attained higher final cell densities than the total cell densities of *A. tamarense* and *H. carterae* at salinity of 18–35 at temperatures of 19°C and 25°C.
The cell density of *H. carterae* increased quickly within the cultures at day 2 of the experiment and then dropped quickly after day 5; no cells were found after day 7 at salinities 18 - 35 at 25°C. Up and down variation of *H. carterae* cell numbers also occurred at 19°C except at salinity 10, although more slowly, compared to those at 25°C. However, under salinity of 10°C and 25°C, the cell density of *H. carterae* remained constant after increasing to 10000 cells/ml. *A. tamarense* cell density decreased with time at low salinities of 10 - 25, while at salinity of 30 and 35 at both 19°C and 25°C, the increase of *Alexandrium* cells number was relatively low but could remain at low density during the experiments (Fig. 1).

From Fig. 1, it is clear that the phytoplankton density change showed similar trend among treatment at salinity 18 - 35 at each temperature, but differed from that at salinity 10 at both temperatures.

**Composition changes in the phytoplankton community during the experiment**

<table>
<thead>
<tr>
<th>Salinity</th>
<th>19°C</th>
<th>25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. tamarense</td>
<td>H. carterae</td>
<td>S. costatum</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>0.1</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1 shows the composition of the phytoplankton community composed of *A. tamarense, H. carterae* and *S. costatum* in mixed cultures under salinity 10, 18, 25, 30, and 35 at 19°C and 25°C, respectively at the end of the 22 days experiment. At salinity of 18 - 35 and temperatures of 19°C and 25°C, *S. costatum* was the dominant species and comprised more than 98% of total cell density. At salinity 10 and 19°C, the cell number of *H. carterae* reached to about 25% of total algal cell number at the end of the experiment. At salinity 10 and 25°C, *H. carterae* outgrew the two other species and became the single dominant species within the cultures. *A. tamarense* failed in competing with the other two species in all the treatments, although it remained in the mixed culture at low density.

**DISCUSSION**

The study on the combined effects of temperature, irradiance and salinity on the growth of these three HAB species (Qian and Yan, 2000) showed that temperature (12, 19, 25, 32°C), salinity (10, 18, 25, 30, and 35) and irradiance (0.02, 0.08, 0.3, 1.6 x 10^16 quanta/(cm^2·s)) significantly influenced the growth of all these three species. The optimal growth conditions of *A. tamarense* was 19°C, salinity 30 and 1.6 x 10^16 quanta/(cm^2·s); that of *H. carterae* was 25°C, salinity 10 - 35 and 1.6 x 10^16 quanta/(cm^2·s); and that of *S. costatum* was 25°C, salinity 18 - 35 and 1.6 x 10^16 quanta/(cm^2·s). All the three species showed maximum growth rates at irradiance of 1.6 x 10^16 quanta/(cm^2·s) (the highest irradiance level provided in the experiment). *S. costatum*
had highest growth rate among the three species. *S. costatum* and *H. carterae* were more likely to bloom under high temperature and both species were able to distribute widely in ocean and estuary due to their adaptation to relatively wide salinities. *A. tamarense* grew at a lower rate than the other two species and was more likely to bloom in early spring or late autumn in higher salinity ocean area.

Basically, the results of the competition experiment coincided with the above results. *S. costatum*, which had the highest growth rate, competed successfully over the other two species in all treatments except at salinity 10 at 25°C. *S. costatum* was found to be a wide temperature and salinity adaptation species (Zou et al., 1983). Our experiment also showed that it had high competitive ability. All these findings could probably explain why it is one of the most common HAB species in the world. *A. tamarense* could not compete successfully over two species, especially at low salinities. However, it was able to remain at low density in the presence of the other two high density algae. Together with its vertical migration ability, it can potentially form HAB. Since shellfish could accumulate PSP during long-term exposure, *A. tamarense* could still pose potential threat to human health even at low density. Even worse, as *A. tamarense* is usually not dense enough to discolor seawater as other HAB species (such as *H. carterae* and *S. costatum*) do, it is likely to be ignored in monitoring. Due to its toxic threat to humans, further investigation on the potential threat of this species to coastal waters is recommended.

According to Honjo (1993), the range of water temperature during *H. carterae* bloom in Japan was 15°C - 30°C, where most HABs occurred at 20°C - 25°C. Our results further support these findings. Our observation of rapid drop in *H. carterae* accorded with the phenomena of sudden disintegration of red tide of this species. This observation seemingly reflects the physiological changes of cyst formation (Nagasaki et al., 1996), the presence of bacteria (Yoshinaga et al., 1998) or virus-like particles (Nagasaki et al., 1994) or inhibition by other species. *H. carterae* could adapt to salinity as low as 10 which gives it advantage in competing successfully over the other two species, so it became dominant in the mixed culture at low salinity and 25°C in competition experiments. In fact, it is widely distributed in estuaries where it caused heavy fish mortality in New Zealand, Japan and Canada (Chang et al., 1990; Honjo, 1993; Taylor and Haigh, 1993). Its rapid growth, high adaptability, and toxicity to fish make this species worth further attention.

Similar competition experiments on three contrasting species: the diatom *Thalassiosira pseudonana*, harmful flagellate *Heterosigma carterae* and the toxic dinoflagellate *Alexandrium minutum* were carried out under simulated spring conditions of 150 μmol/(m²·s) irradiance and 12°C temperature; and summer conditions of 366 μmol/(m²·s) irradiance and 18°C temperature (Davidson et al., 1999). Their results showed *Thalassiosira and Heterosigma* to be well-matched competitors under spring conditions, and that *Thalassiosira* dominated in both spring and summer conditions in terms of cell number. The cell number of *Alexandrium* remained low throughout their experiments. So similar to our results, their competition experiments also showed the rapidly dividing diatom species could compete successfully over the other two and that the change of environmental conditions led to the change of the phytoplankton community structure.

A study on the effect of Fe and Mn on the growth of *A. tamarense* under different nutrient levels in monoculture and mixed culture (Huang et al., 2000) showed that under all mixed-culture conditions, the cell number of *S. costatum* and Coscinodiscus sp. was much higher than the cell number of *A. tamarense*. The cell density of *A. tamarense* declined after a while in the experiment. The diatom predominance in competition under set laboratory conditions was in agreement with our results. In all our experiments we applied full enrichment medium (with Si, and N:P = 15:1) and focused on physical factors. However, there could be other influencing factors such as Si, and N:P ratio for the outcome of competition. Furthermore, except for competition for nutrients, there
were possible interactions between and among species in mixed cultures, caused by metabolic excre-
tions from algae (which may inhibit or stimulate the growth of other species). As the growth behav-
ior of each species was also nutrient mediated and influenced by other organisms, further studies are
needed to explore more the function of species competition in the HAB formation mechanism.

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