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# First report on *Gonyaulax polygramma* (Gonyaulacales, Dinophyceae) blooms in the Yeosu waters of the South Sea of Korea

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The aim of this study is to determine the outbreaks of nontoxic *Gonyaulax polygramma* Stein in Yeosu waters in place of harmful *Cochlodinium polykrikoides* Margalef, which has occurred annually in the same region since 1995. The observation of cellular arrangement and structure by electron microscopy showed that *G. polygramma* isolated from Yeosu waters had a few spines connecting with membranes and prominent longitudinal ridges on the cell surface, with a cingular displacement 1.5 times their cell width. Furthermore, the location of the nucleus was posterior of large oval formation according to electron microscopy. On 6 August, 2004, the first bloom of *G. polygramma* occurred, the date of its disappearance was with a maximum cell density of 8,000 cells $ml^{-1}$ on 21 August, 2004. During the period of this study, the horizontal distribution of sea water temperature and salinity showed a strong coastal front, whereas the front of DIN (Dissolved Inorganic Nitrogen) was significantly different between the occurrence and disappearance of *G. polygramma* blooms. These results suggested that the process of the breakdown of stratification by wind and a low level of inorganic nitrogen play important roles in the rapid growth of *G. polygramma*, which is associated with a greater robustness in growth against DIN than that of *C. polykrikoides* in nature.

Key Words : *Cochlodinium polykrikoides*, DIN, Front, *Gonyaulax polygramma*, Outbreak

## 1. Introduction

The coastal waters of Yeosu, off the southern coast of South Korea and part of the South Sea, cover relatively a large area and offer a wide avenue for exchange with oceanic waters and freshwater runoff from the Sumjin River. In particular, a large volume of freshwater meets the sea in Yeosu waters during the summer, which causes variability in salinity in the horizontal and vertical planes. The very important implication was that this season and region saw the massive blooms of *Cochlodinium polykrikoides* Margalef[1]. Since the 1990s, *Cochlodinium* bloom has occurred annually off the southern coast of Korea. The mass mortality of cage-cultured fish due to the bloom of *C. polykrikoides* has been a serious problem for the Korean aquaculture industry for many years[1]. To provide a better understanding of the outbreaks of *C. polykrioides*, numerous researchers have concluded that the intrusion of offshore water might play a critical role in *Cochlodinium*[2~5] bloom, but the precise factors initiating bloom have yet to be determined.

Interestingly, blooms of *Gonyaulax polygramma* Stein, instead of *C. polykrikoides*, in early August, 2004, first occurred in the coastal waters of Yeosu, whose occurrence was the first in Korea as well. It is known that this species is armored, marine planktonic dinoflagellate, and shows wide geographic distribution from cold temperate to even tropical waters[6]. In addition, this species is regarded as nontoxic fish kills, although fish and shellfish kills due to oxygen depletion in relation to cell mineralization have been reported[7]. So far, blooms of *G. polygramma* have been reported in several countries (http://www.nmnh.si.edu/botany/projects/dinoflage/taxa). The present study aimed to provide clues as to why the first blooms arose in Yeosu, based on an examination of fluctuations in water quality, nutrients, and weather conditions.

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## 2. Materials and Methods

For the scanning electron microscope (SEM) observation, the field samples were fixed with 2.5% glutadehyde and filtered through a 5 μm pore size polycarbonate membrane filter under no pressure. The filter was dehydrated by a graded ethanol series to 100% ethanol. The samples on the filter paper were air dried, transferred to aluminum stub to be coated with deionized gold and then examined under a HITACHI SEM (S-3000N, Japan prod.). For the transmission electron microscope (TEM), the fixed samples were washed with 0.1M phosphate buffer (pH 7.2) and dehydrated according to SEM. The filter was placed overnight into two changes of propylene oxide and in a 1:1 mixture of Epon resin and propylene oxide for 8 h in fresh Epon resion. Sections were cut with a glass knife approaching the particles on the membrane filter parallel to the membrane surface. The ultra-thin secions (60-90 nm) were cut with a diamond knife, stained with uranyl acetate followed by lead citrate and observed at 80 kV with JEOL TEM (JEM 1200 EX-II, Japan prod.). Sea water temperature and salinity were measured using an YSI 650 MDS (YSI incorporated, U.S.A.) in August 3, 6, 11, 21, 2004, which was recorded as the beginning and final occurrence of *G. polygramma*. Concentration of dissolved inorganic nutrients ($NO_3$-N, $NO_2$-N, $NH_4$-N, and $PO_4$-P), which passed through Whatman GF/F glass fiber filters, was determined as the procedure of Strickland and Parsons[8]. Chlorophyll *a* was harvested on Whatman GF/F glass fiber filters and then the filters extracted in 90% acetone and measured on a UV-VIS spectrophotometer (Perkin Elmer, LS50B).

## 3. Results

It is known that a clear longitudinal striation plays an important role in discriminating *G. polygramma* from morphologically similar species. Under SEM, cells showed prominent longitudinal ridges (arrow) on the surface, including one large and two tiny spines (arrowhead, Fig. 1a). Cingular displacement was more than 1.5 times their own width (Fig. 1b). Figure 1c-e show a transverse section of *G. polygramma* and the distribution of the main components of cellular organelles. The nucleus was located in the posterior of the cells and formed a large oval. Chloroplasts lied alongside many of the thlakoid lamellae, which abutted along the entire length of the chloroplast. The chloroplasts were separated from each other. The pyrenoid was found localized among the thylakoids at the periphery of the chloroplasts at the base of the cells. Mitochondria were surrounded by electron-dense membranes and possessed many tubular cristae in the endoplasm. The first outbreak of *G. polygramma* in Yeosu waters was on 6 August, 2004, with 2,000 cells $ml^{-1}$ (Fig. 2). In particular, on 9 and 14 August, a significant dense cell density of more than 4,000 cells $ml^{-1}$ occurred, but the cells disappeared on 21 August. In contrast to *G. polygramma*, *C. polykrikoides* showed a considerably low cell density, which was almost less 2,000 cells $ml^{-1}$ during the period of this study. The horizontal distributions of sea temperature, salinity, dissolved inorganic nitrogen (DIN), and phosphate for surface and bottom waters on 3, 6, 11, and 21 August, 2004, are shown in Figs. 3-6. Before the outbreak of *G. polygramma*, a surface water mass of less than 25℃, was found in the coastal area of this study (Fig. 3). Bottom water also showed a strong water front of 20℃, although the water was clearly stratified. A strong front was found here, with a high salinity of 34 psu. A DIN concentration of 5.0 μmol $l^{-1}$ was found in both surface and bottom waters, whereas a high phosphate concentration was not. While water temperature and salinity at the surface and the bottom during the period of *G. polygramma* blooming were similar to characteristic magnitudes shown on 3 August, 2004, strong horizontal distributions of DIN and phosphate were not found (Figs. 4-5). Although the abundance of *G. polygramma* sharply declined, strong fronts of water temperature, salinity, DIN, and phosphate were shown on 21 August, 2004 (Fig. 6).

## 4. Discussion

During the period of this study, relatively high water temperature and salinity persisted horizontally with a strong front in Yeosu waters before the outbreak and after the disappearance of *G. polygramma* Kim et al[9] have suggested that the optimal growth rate of *C. polykrikoides* were shown at a combination of 25℃ and a salinity of 34 psu. It is quite clear that the two species, *C. polykrikoides* and *G. polygramma*, require similar environmental characteristics for rapid growth. However, nutrients in nature were shown in a strong

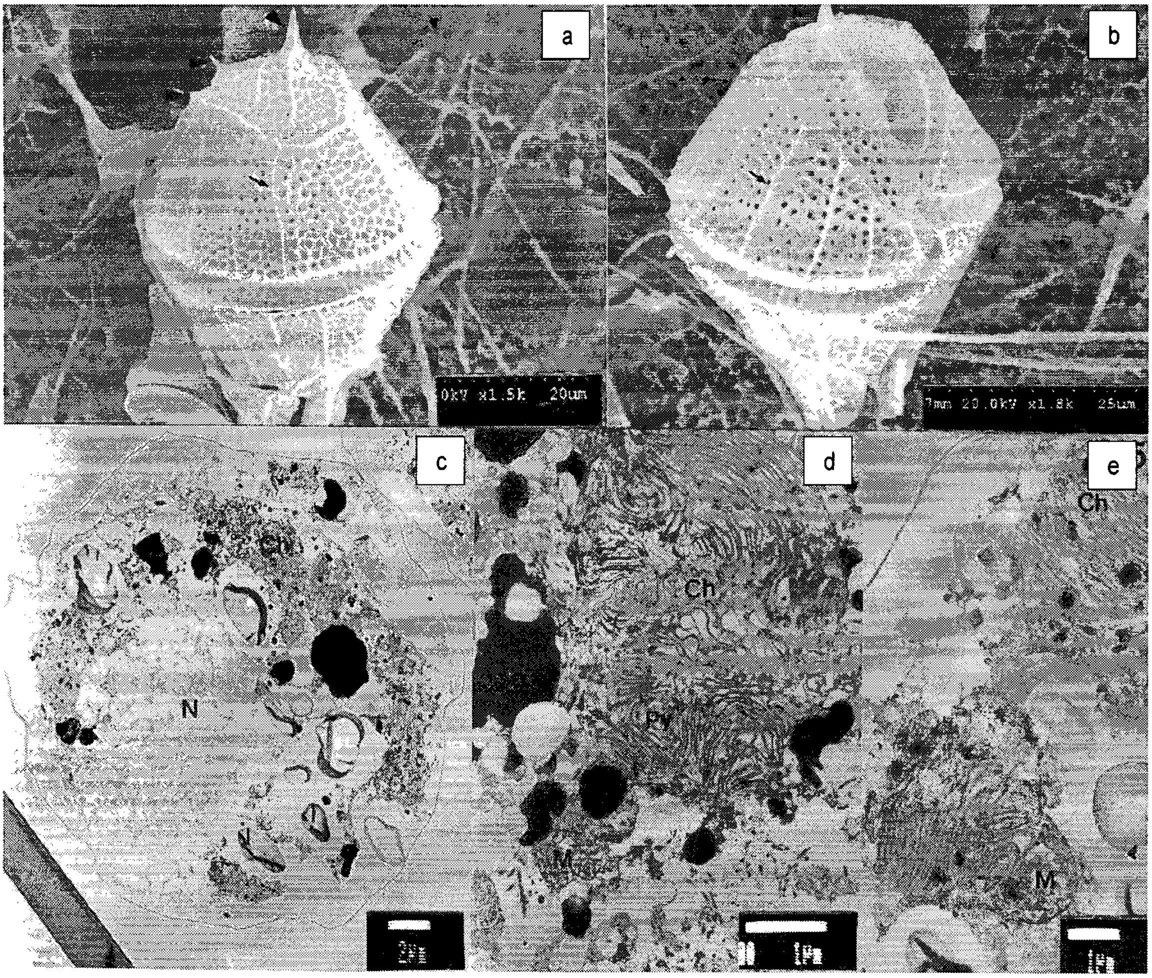

Fig. 1. Electron micrographs of *Gonyaulax polygramma*. A, Dorsal view: three antapical spines (shown by arrowheads) and longitudinal rides (shown by arrows) presented. B, Ventral view: spines (shown by arrowheads) and longitudinal rides (shown by arrows). C, Transverse section of *G. polygramma* cell. N, nucleu; Ch, chloroplast. D, Enlargement of chloroplast. Py, pyrenoid, M, mitochondria. E, Mitochondria with tabular cristae.

front in high concentrations before the occurrence and after the remarkable cell decline of *G. polygramma* (Figs. 3-6). It is thought that high concentrations of nutrients likely play an important role in depressing the cell growth of *G. polygramma* in nature. Likewise, the growth of *C. polykrikoides* can be limited under high concentrations of nutrients[10], but low concentrations of nutrients are associated with higher impact on the growth rate of *G. polygramma* than on that of *C. polykrikoides*. In particular, from our present study DIN is shown to be a more important stimulus or inhibitor of the growth of *G. polygramma* than phosphate. According to Kim[10], half-saturation constants of nitrogen uptake by *C. polykrikoides*

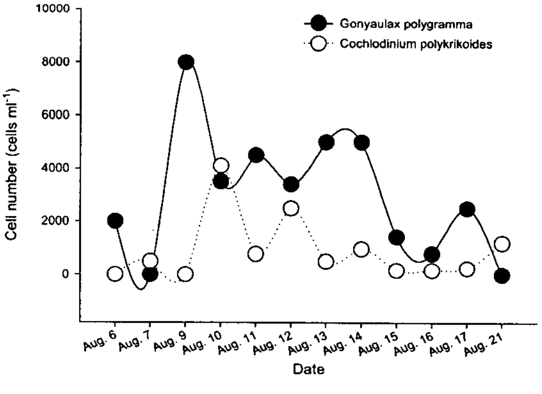

Fig. 2. Daily changes in cell density of *G. polygramma* and *C. polykrikoides* during the period of this study.

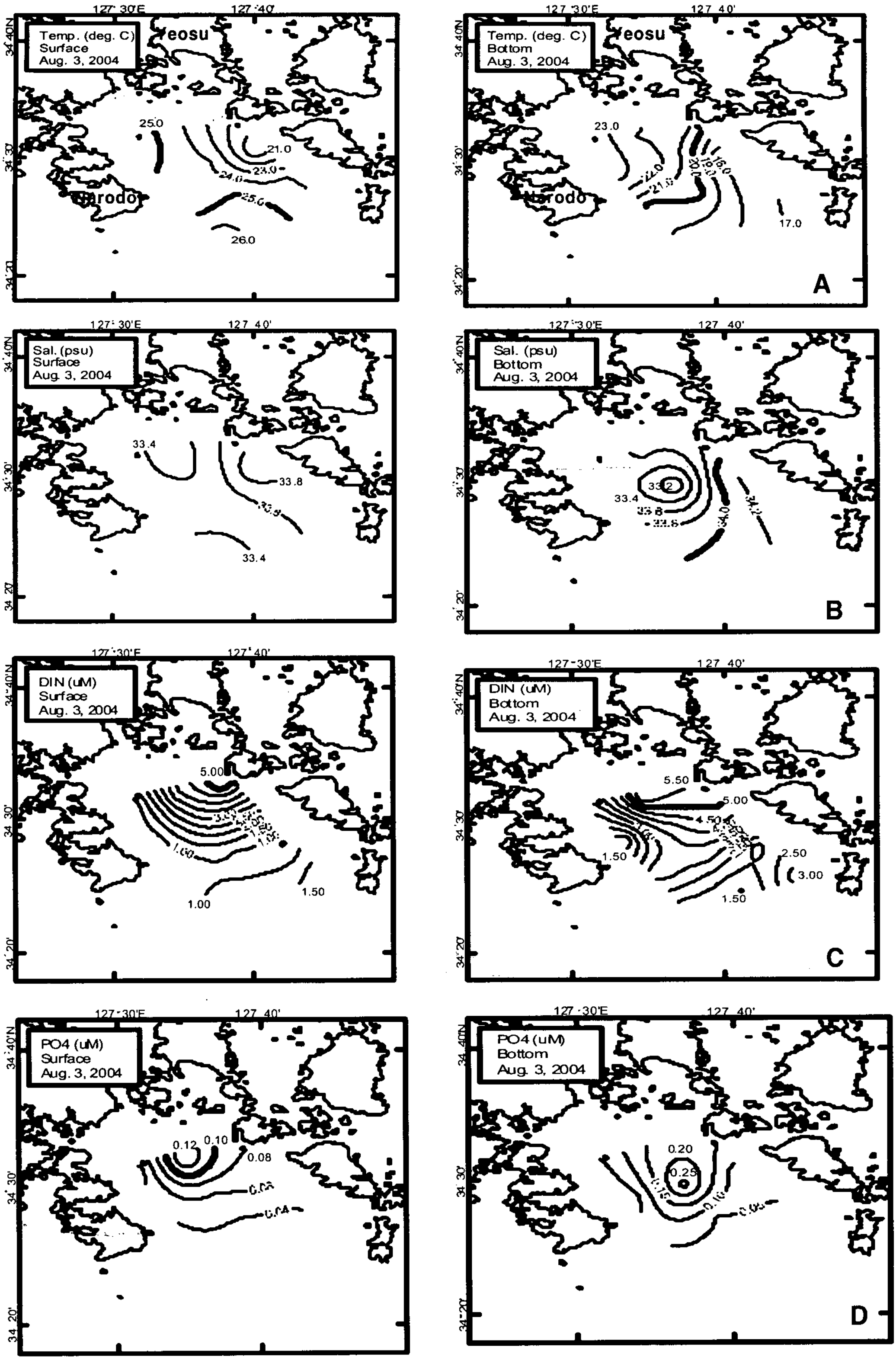

Fig. 3. Horizontal distributions of water temperature (A), salinity (B), DIN (C), and $PO_4$ (D)at surface and bottom on 3 August, 2004.

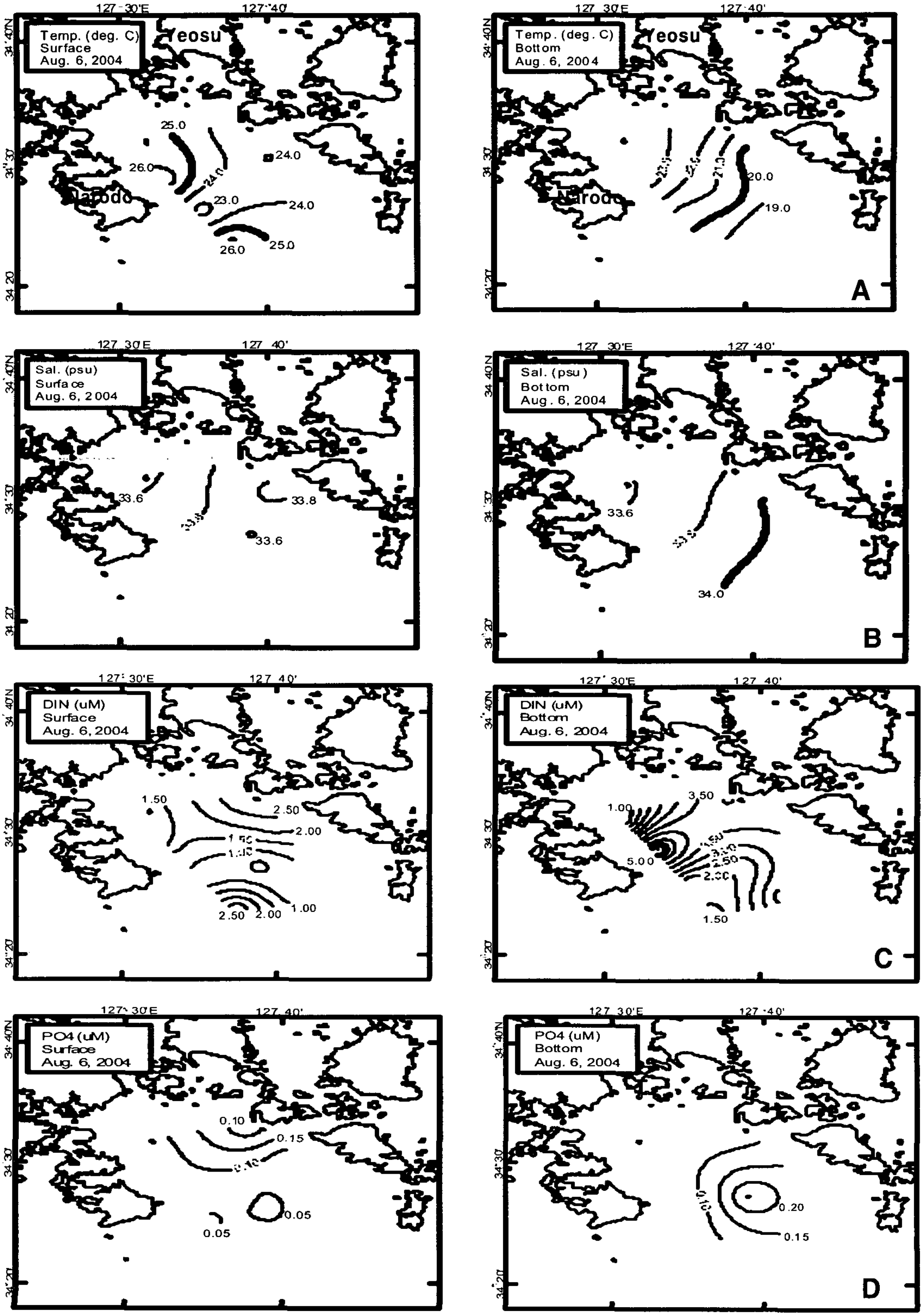

Fig. 4. Horizontal distributions of water temperature (A), salinity (B), DIN (C), and PO₄ (D) at surface and bottom on 6 August, 2004.

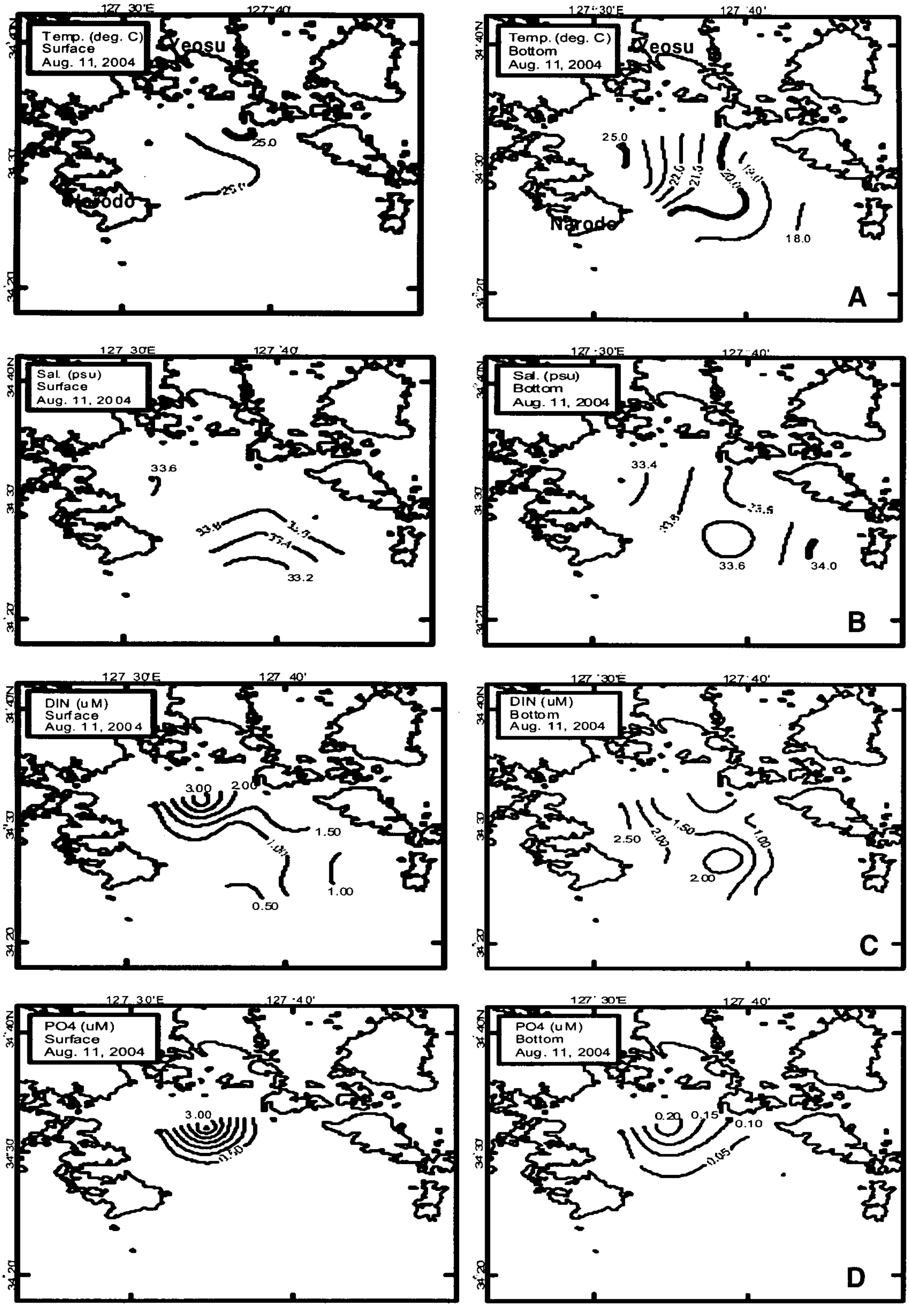

Fig. 5. Horizontal distributions of water temperature (A), salinity (B), DIN (C), and $PO_4$ (D) at surface and bottom on 11 August, 2004.

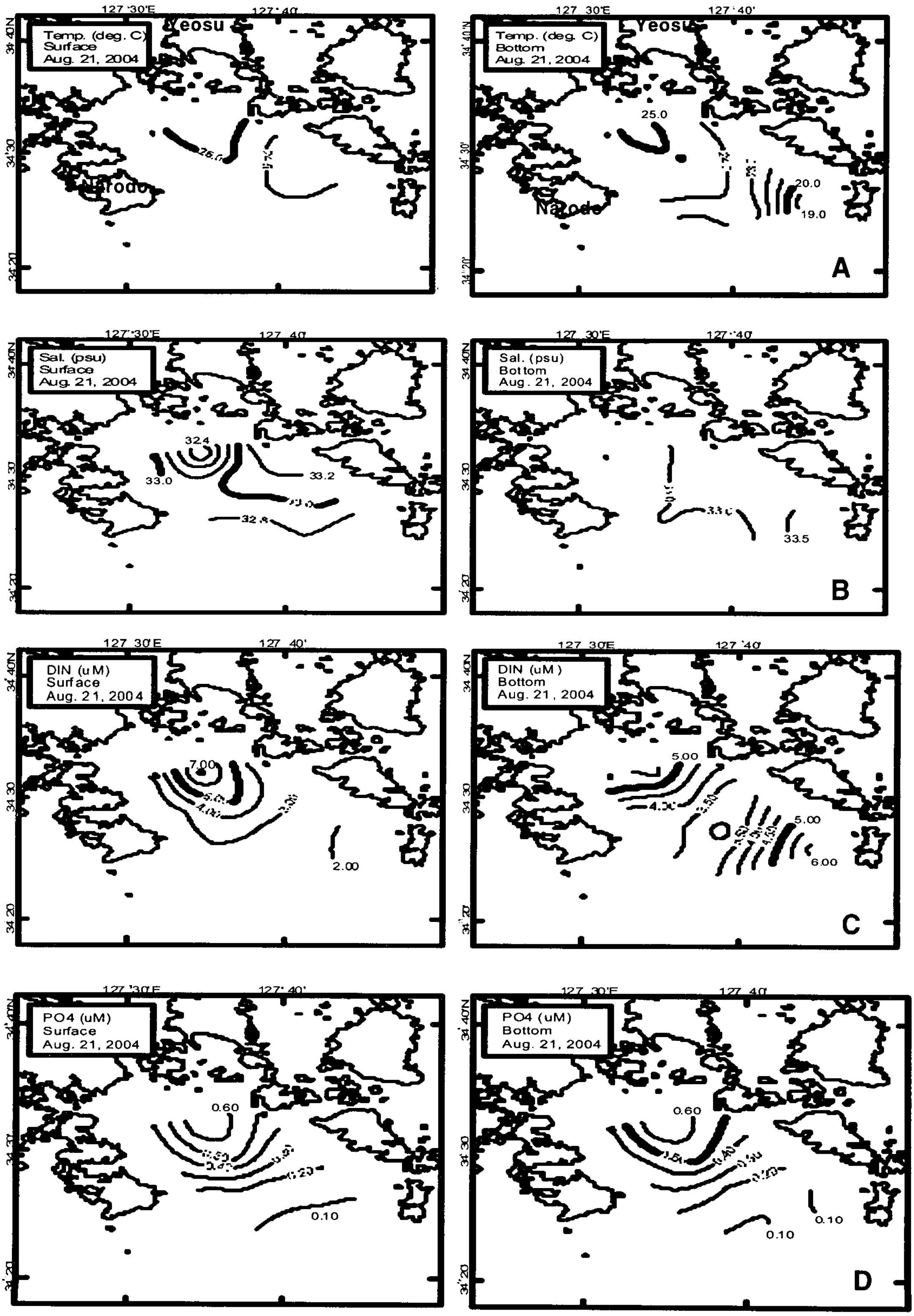

Fig. 6. Horizontal distributions of water temperature (A), salinity (B), DIN (C), and $PO_4$ (D) at surface and bottom on 21 August, 2004.

recorded a somewhat low value against *Alexandrium tamarense* (Lebour) Balech[11], *Gonyaulax polyedra* Stein[12], *Peridinium cinctum* Ehrenberg[13], and *Prorocentrum minimum* (Pavillard) Schiller[14]. In this study, *G. polygramma* showed a higher capability of nitrogen kinetics for sustainable growth than *C. polykrikoides* based on a small concentration of nitrogen in nature, although a little has been studied of the growth rate of *G. polygramma* at ambient concentrations of nitrogen in cultures. Further study needs to be done on evaluating the uptake kinetics and the changes in the assimilation between *G. polygramma* and *C. polykrikoides*.

In summer, nutrient concentrations at the surface of the ocean are usually insufficient to support the production of phytoplankton assemblages[15], which are associated with stratified and nutrient-depleted conditions. On the basis of hydrographic properties, the Yeosu waters are influenced by different minor systems (the Kuroshio current; the Tsushima warm current; and the Korean coastal waters), which can often cause changes in water temperature and contribute to an abundance of nutrients[16]. In this sense, the coastal regions of the South Sea of Korea are the most vulnerable to the influence of offshore water currents among the other waters in Korea. Consequently, the establishment of a strong front of high concentrations of DIN in the Yeosu waters on 3 August, 2004 are be associated with a greater intrusion of the nutrient-enriched offshore current than other nutrient sources including upwelling, freshwater input, and rainfall systems. According to weather conditions (data not shown), there was a shortage of precipitation during the period of this study, indicating that rainfall was not sufficient to supply nutrients to surface waters. However, among weather conditions, wind directly effected the demographic and spatial distribution of high concentrations of DIN after on 3 August, 2004. It is thought that a strong front of high concentrations of DIN on 21 August, 2004 was associated with the influx from enriched offshore water. In summer, freshwater input from rivers reduces surface water temperature and salinity over the study area. Choi[2] has suggested that the influx of low-salinity water induces the promotion of blooms caused by *C. polykrikoides* in the Yeosu region. However, this study showed that freshwater did not play an important role in the progressive growth of *G. polygramma*. Overall, the combined results of the breakdown of the stratification of DIN by wind and a low concentration of organic nitrogen provide support for the outbreaks of *G. polygramma* in the Yeosu waters.

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