



Diurnal vertical migration of *Cochlodinium polykrikoides* during the red tide in Korean coastal sea waters

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Abstract: The diurnal vertical migration of *Cochlodinium polykrikoides* (*C. polykrikoides*), which caused a red tide in the Korean coastal waters of the East Sea/Sea of Japan in September 2003, was examined by determining the time-dependent changes in the density of living cells in relation to the depth of the water column. The ascent of this species into the surface layer (depth of water, 2 m) occurred during 1400-1500. The descent started at 1600 and a high distribution rate (86%) at 15-20 m was observed at 0300. During the ascent, the cells were widely distributed at each depth level from 0600 hr and at 0800-1100, the cells were primarily distributed in the middle layer (0-6 m). The concentration of dissolved inorganic nitrogen was generally $\leq 2.86 \mu\text{mol l}^{-1}$, but at 1400-1500, the concentration in the surface layer reduced to $\leq 0.14 \mu\text{mol l}^{-1}$. Moreover, the concentration gradually increased as the depth increased to ≥ 5 m. These results showed that the nutrient-consumption rate associated with the proliferation of *C. polykrikoides* during a red tide is more influenced by the inorganic-nitrogen resources rather than the inorganic-phosphorus compounds.

Key words: *Cochlodinium polykrikoides*, Diurnal vertical migration, Red tide, Nutrient
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Introduction

The theories on the life strategies of phytoplanktons in freshwater (Reynolds, 1988) and marine environments (Margalef, 1978) have emphasized the scarcity of ecological space in the conditions where all the environmental factors are optimal for growth. Usually, the habitats of these organisms have limited light or nutrient supply. However, the ability to perform diurnal vertical migration affords a competitive advantage to the microorganisms, which can exploit the high light levels near the surface. Although the low concentrations of inorganic nutrients near the surface would normally limit growth, the cells descend to deeper layers during the night, thereby fulfilling their nutrient demands and recharging their intracellular reserves (Eppley *et al.*, 1968; Cullen, 1985; Lieberman and Shilo, 1994).

The pattern of variation in the cellular characteristics during diurnal vertical migration (Jones, 1993; Kamykowski *et al.*, 1995) provides information on the role of cell motility in optimizing biosynthetic patterns, thereby elucidating cell growth and division in response to changing resource distributions in the water column (MacIntyre *et al.*, 1997). Recent studies on *Gymnodinium breve* (Kamykowski *et al.*, 1998), a harmful algal bloom species, revealed that in the nutrient-replete cells that aggregated at the surface during diurnal vertical migration, the cellular concentration of many biochemical constituents, especially lipids, during the light period was lower than the nutrient concentrations in the cells deeper in the water column.

In Korea, harmful blooms of dinoflagellates occur annually over large areas along the southern and eastern coasts in August and September and cause extensive damage to fin-fish farms (Kim *et al.*, 1997b). The causative organism is usually a chain-forming dinoflagellate, *Cochlodinium polykrikoides*. Several marine dinoflagellates are known to exhibit diurnal vertical migrations that are characterized by daytime ascent and nocturnal descent (Eppley *et al.*, 1968; Kozumi *et al.*, 1996). Many studies in various fields have attempted to determine approaches to reduce the damage caused by the *C. polykrikoides* red tide (Kim *et al.*, 1999; Cho *et al.*, 1999) and the present investigation is one such study. Since the fish deaths in the east coast of Korea usually occur at night in the deep waters, the causes of these deaths may be slightly different. Therefore, it is essential to examine the cause of this phenomenon and determine a prevention method that is relevant to the features of the East coast.

To this end, we studied the vertical distribution of red-tide organisms over time and the hydrological properties at the sites where the organisms were present. The results of these analyses facilitated indirect speculation of the optimum conditions and the physiological-activation mechanisms of these organisms.

Materials and Methods

Study area: Fig. 1 is the map of the research area. We performed this investigation in 2003, when the red tide reached up to the eastern coast of Korea. The studies were performed from September 18 to 19; samples were collected from depths of 0 to 20 m in a region

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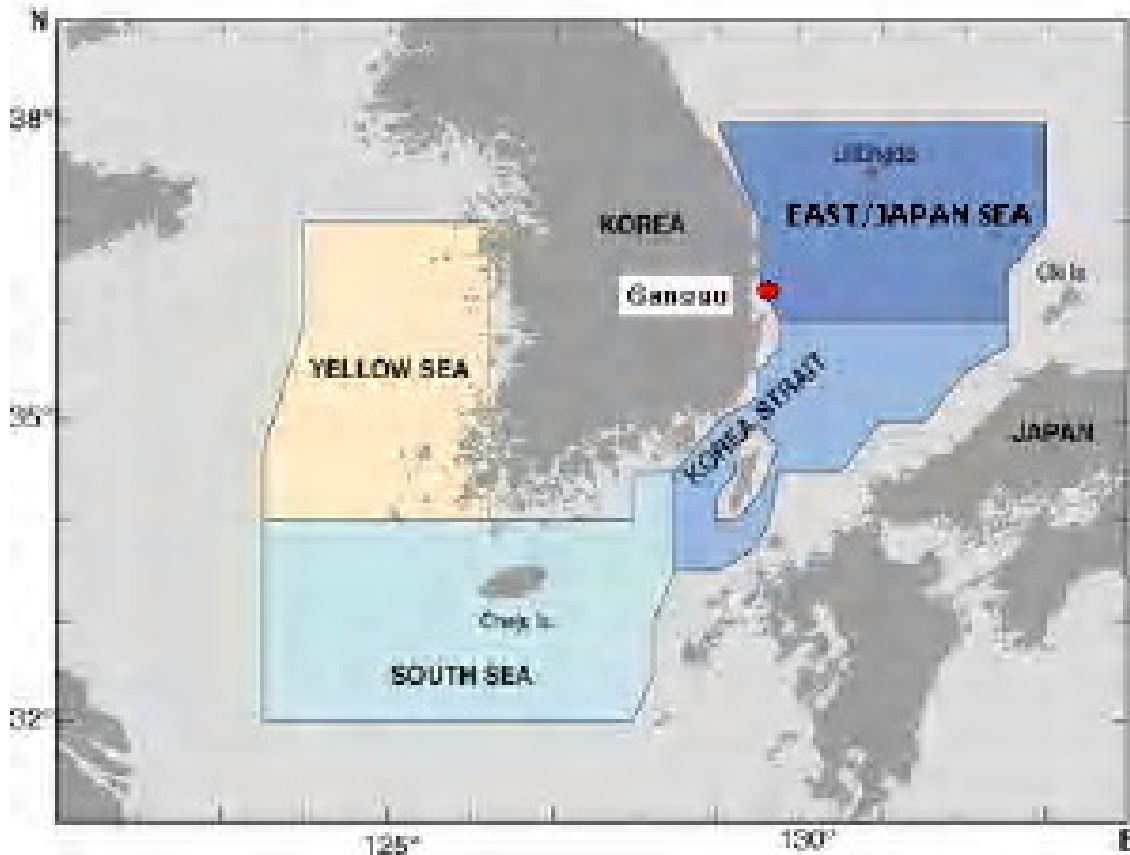


Fig. 1: Location of the sampling station

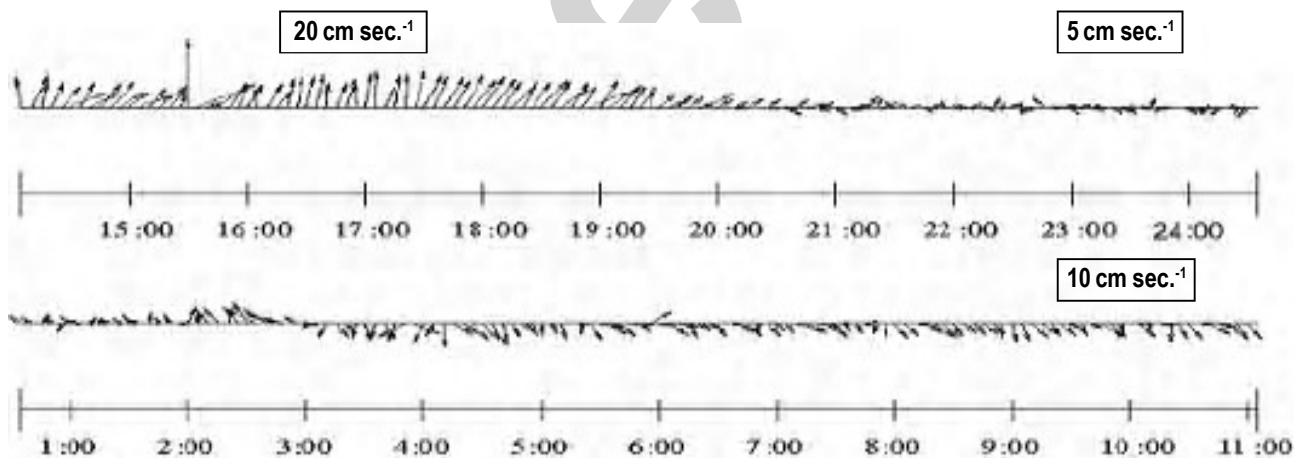


Fig. 2: Vector diagram showing speed of current for a 24 hr period at the surface layer (0-3 m) of Ganggu coast

approximately 0.5 miles from the port of Ganggu ($36^{\circ}22' 747''$ N, $129^{\circ}25' 265''$ E).

Sampling and data analysis: We performed the investigations over a 24 hr sampling period (September 18-19, starting at 1400). The analyses were performed at various locations from the surface to the 20 m layer at 1-5 m vertical intervals using an array of samples collected using a Niskin water sampler (sample collection was performed at 10 depths: 0, 1, 2, 3, 4, 5, 6, 8, 10, 15 and 20 m).

The subsamples for cell counts were transferred to 500 ml polyethylene bottles and the cell counts were performed within 20 min. The cell densities were determined using an inverted microscope (Olympus, AX70; phase-contrast optics, 40 \times magnification) and expressed in terms of cells ml^{-1} . The direction and speed of the current were measured from 1400 to 1100 on September 18-19 by using the current meter (Sontek-MD) on the surface of the water at a depth of 3 m. The intensity of illumination was estimated using an illuminometer (Alex), which was deployed at the same sampling depth.

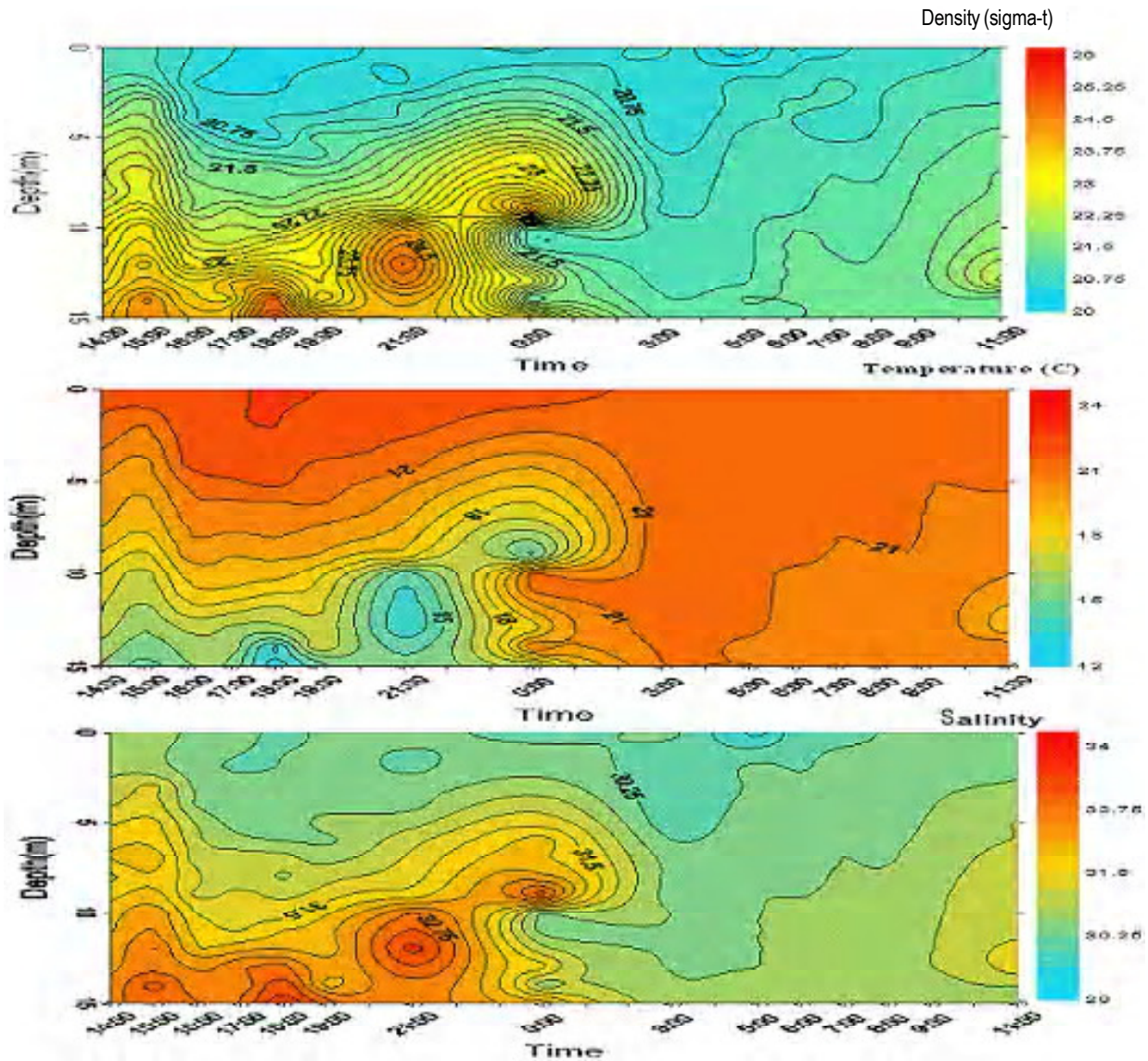


Fig. 3: Depth-time profiles of water salinity, density (sigma-t) and temperature (°C) at different sampling times

Water-quality analyses: The mineral-nutrient content was determined from discrete samples collected from depths of 0, 5, 10, and 15 m at the beginning and end of the observation period. The dissolved inorganic-nutrient content (the sum of $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$, and dissolved inorganic phosphate (DIP) as $\text{PO}_4\text{-P}$ content) was analyzed on board the vessel by using standard colorimetric methods (Grashoff *et al.*, 1983). The chlorophyll α concentration was measured fluorometrically after overnight extraction in 90% acetone at -20°C . The water temperature and salinity of all the samples were simultaneously measured from 1400 to 1100 by using a CTD device (SBE 25; Sea-Bird Co.).

Results and Discussion

The results revealed the changes in the vertical distribution of *C. polykrikoides* that occurred in conjunction with changes in

environmental conditions such as nutrient conditions, light and hydrographical parameters.

Physical conditions: In this study, we tracked the vertical movements of the *C. polykrikoides* cells over time. The northward current was dominant in the initial stage (1400-1900) on September 18 (speed, approximately 20 cm s^{-1}). In the intermediate stage, both northward and eastward currents had low speeds (less than 5 cm s^{-1}). In the latter stages, the southward current had a speed of approximately 10 cm s^{-1} (Fig. 2). During the survey period, the speed of the currents in the examined coastal areas was relatively low (less than 10 cm s^{-1}). Therefore, the mass of *C. polykrikoides* did not show any remarkable movement. Since small-scale turbulence can directly influence cell trajectories by causing cell dispersion and disorientation (White, 1976) and also cause breakage or loss of the longitudinal flagella (Thomas and Gibson,

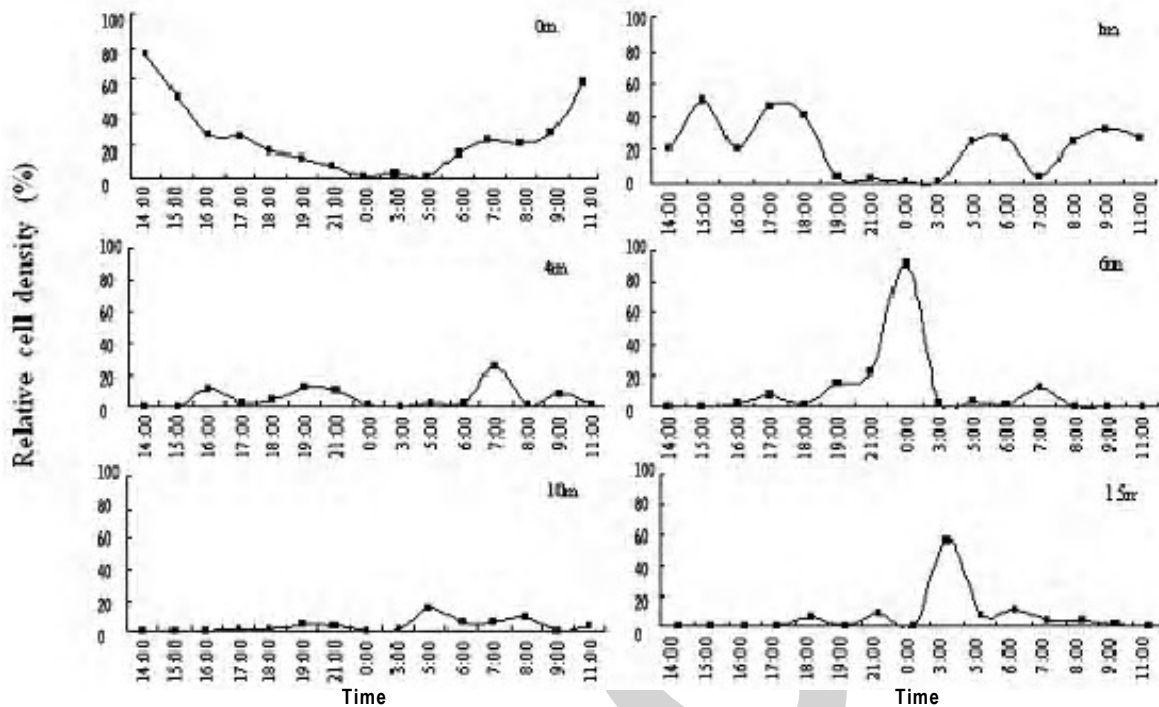


Fig. 4: Vertical distribution of *C. polykrikoides* in Ganggu coast at different sampling times (% relative cell density)

1990), the absence of any remarkable movement facilitated our investigation. The water temperatures showed little variation throughout the study period [range, 12.0-21.6°C (Fig. 3)]. The temperature at the surface was higher than 21°C; the temperature at the middle layer was between 18 and 21°C and the temperature at the bottom layer (deeper than 10 m) was between 12 and 18°C. Between 2300 on 18th September and 1100 on 19th September, the cold water passed into the lower layers (deeper than 10 m).

Although the results obtained above have shown that the water-temperature changes were dependent on the current flow, the diagram of the temperature distribution does not show correlation with the movement of the species. Temperature was likely to influence the specific growth rate, and temperatures lower than the optimal values were likely to reduce the specific growth rate (Carbonel and Valentin, 1999; Lonin and Tuchkovenko, 2001). In a previous study, *C. polykrikoides* was observed to survive after laboratory incubation at low temperature (+10°C) (Kim *et al.*, 1997a). However, in this study, the temperature range (15-21°C) had little effect on the growth rate of *C. polykrikoides*. Therefore, we deduced that while water temperature was capable of affecting the species activation, it did not influence the vertical movement of the species.

The salinity ranged from 30.2 to 32.8 and showed little variation over time. Generally, the salinity was more than 30 and was hardly affected by freshwater input. However, the low salinity values recorded between 1400 and 1800 (less than 31) suggested that the input did have an effect on salinity, although the effect itself was insignificant. Fig. 3 shows the sigma-t plot for seawater density. In general, the water temperature and salinity had similar distributions,

which showed that these 2 factors influenced the water density. These findings also indicated that external freshwater input does not have a significant effect on the movement of the red-tide organisms. However, the internal tidal movements may cause the organisms to flow out offshore; in such cases, the organisms flow back to the surface layer due to the wind forces and are therefore observed in the surface layer.

Vertical distribution of *C. polykrikoides*: Fig. 4 and 5 show the vertical distribution of *C. polykrikoides* density over time. High cell density (more than 500 cells ml⁻¹) was observed at the level of the red-tide alert in Korea. At the 0300, low concentration (≤ 40 cells ml⁻¹) of *C. polykrikoides* was showed partly by its depth, but high distribution rates (86%) were observed at a depth of 15 m. Between 1400 and 1500, the density at the surface was approximately 80% (Fig. 4). The species started to descend from 1600, and between 1900 and 2100, they were evenly distributed over the entire water column. The distribution rate at the lower levels began to increase from 2100, and at 0300, the cells were concentrated at a depth of 15 m. The velocity of descent to the 15-20 m bottom layer was 1.4-1.8 m hr⁻¹, but the time required to move from the bottom to 0 m (surface layer) was 9 hr. During the ascent, the cells were widely distributed over the water column from 0600. The distribution rate at 0 m at 0600-0700 was 15-23%. The cells rarely appeared at 10-15 m at 0800, and they were primarily distributed in the middle layer (0-6 m) at 0800-1100 (Fig. 4 and 5). The combined propulsive effect of this species produces a characteristic swimming speed and direction, which was determined to be 1.4-2.5 m hr⁻¹. Thus, these results established that the members of this species showed diurnal vertical

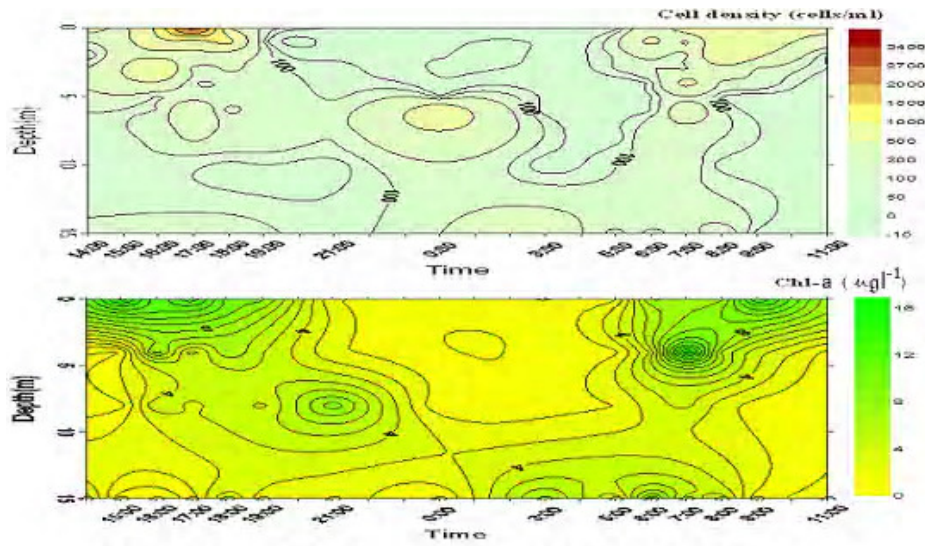


Fig. 5: Temporal changes in the vertical distribution of chlorophyll α concentration and cell density at different sampling times

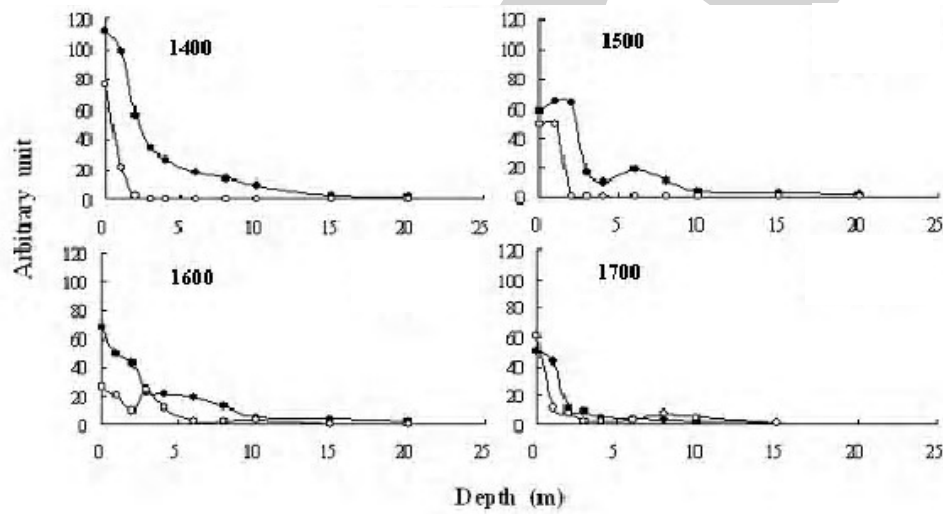


Fig. 6: Vertical distributions of relative cell density (○-%) and light intensity (●-cdsr m^{-2}) at 1400, 1500, 1600 and 1700

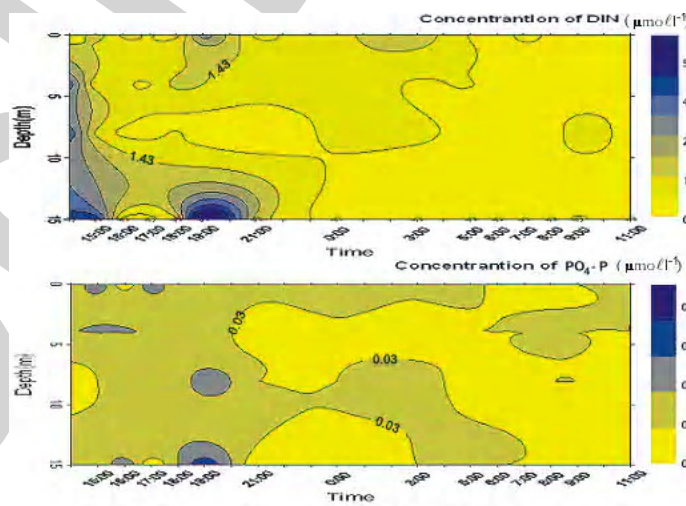


Fig. 7: Temporal changes in the vertical distribution of dissolved inorganic nitrogen (DIN) and $PO_4\text{-P}$ concentrations at different sampling times

migration over a depth of 15-20 m. Swimming speed is traditionally estimated from the integrated population movement (which is determined from cell counts) during diurnal vertical migration. The speed estimates for different species are generally within the same range ($0.2\text{-}2.0\text{ m hr}^{-1}$), although some species travel at faster speeds (greater than 5 m hr^{-1}) (Levandowsky and Kaneta, 1987; Kamykowski, 1995). Raven and Richardson (1984) suggested that dinoflagellates are among the faster phytoflagellate swimmers. At 0300, in deep water, the division chain of *C. polykrikoides* consisted of ≤ 2 cells. However, the distribution rate increased during the ascent to the surface and the division chain increased to ≥ 4 cells. In Fig. 5, we have also shown the vertical distributions of red-tide organisms against the chlorophyll α concentrations. The distributions of the chlorophyll α concentration and the cells were similar. On the surface, the highest chlorophyll α concentration was observed between 1400 and 1800. At night, the concentration was rather higher than the cell distribution at a depth of 10 m, and in the morning, the concentration was higher at a depth of approximately 5 m.

Fig. 6 shows the vertical distribution of *C. polykrikoides* cells as a function of light at 1400, 1500, 1600 and 1700 on 18th September 2003. The survey was performed under cloudless weather and at a depth of 0-2 m, the distribution of illuminance intensity was similar to that of the cell density. The maximum intensity ($98\text{-}111\text{ cdsr m}^{-2}$) on the surface layer (depth, less than 1 m) was observed at 1400. Between 1400 and 1500, the intensity of illuminance on the surface layer suddenly reduced (from 111.4 cdsr m^{-2} to 58.3 cdsr m^{-2}), and the intensity gradually decreased over a depth of 3-20 m. From 1600 onwards, the cells were distributed widely over the water column with light. In this study, the variation during the summer red-tide period was primarily observed in the upper layers (0-6 m). The vertical profiles indicate that the motile *C. polykrikoides* avoided the surface. The radiation levels during the calm and cloudless day may have exceeded the optimal levels for the microorganisms, and the cells may have migrated downwards to avoid photoinhibition (Vincent *et al.*, 1988; Elser, 1985). This active orientation to light and gravity depends on the existence of sense organelles, most of which are poorly characterized in dinoflagellates (Kamykowski, 1995). Generally, the light-sensitive area for phototaxis is located on the ventral side just under the sulcus and is often found near the junction of the sulcus and cingulum and below the base of the longitudinal flagellum.

The vertical movement during the red tide may have been caused by light. However, the cells started to ascend at 0500 before sunrise (on 19th September, sunrise occurred at 0600), and they started to descend at 1600, irrespective of the light conditions (on 18th September, sunset occurred at 1427). Levandowsky and Kaneta (1987) speculated that the crystalline inclusions found in some dinoflagellate species may provide a true gravity-sensing apparatus analogous to the statoliths in metazoa. In the present

study, the observed speed of the *C. polykrikoides* descent ($1.4\text{-}1.8\text{ m hr}^{-1}$) was lower than the speed of ascent ($1.7\text{-}2.2\text{ m hr}^{-1}$). This finding implies that gravitational effects may negatively influence the migration velocity in the downward direction. Therefore, the initiation of upward migration of *C. polykrikoides* in the absence of light can be ascribed to a similar rhythm that is unrelated to gravity.

Vertical distribution of mineral nutrients: Fig. 7 is a spatiotemporal depiction of the mineral nutrient concentrations [the dissolved inorganic nitrogen (DIN) was estimated as the sum of the $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NH}_4\text{-N}$ concentrations]. Between 1400 and 1500, the DIN value was low at the deeper regions, where the cell density was high, while it was high in the regions with low cell density. Between 1800 and 2100, when the cell density in the middle layer was high, the DIN values at the surface and lower layers were relatively high. Between 2300 and 1100 on the next day, the DIN was generally low ($2.86\text{ }\mu\text{mol l}^{-1}$).

These results indicate a relationship between the activation of red-tide organisms and the nitrogen sources, which in turn, implies that the red-tide organisms may show a relatively higher uptake rate of nitrogen sources. Unlike DIN, the dissolved inorganic phosphate $\text{PO}_4\text{-P}$ (DIP) did not show any particular relationship with the cell density. Therefore, the concentration of DIP may barely affect the distribution of the species.

C. polykrikoides showed rapid growth on media with inorganic nitrogen compounds and grew at a slower rate on organic nitrogen compounds. In contrast, the organism effectively utilized a variety of both inorganic and organic phosphorus compounds as P sources (Kim *et al.*, 2007). Thus, the low consumption rate of DIP implies that the cells do not require high DIP concentrations for proliferation.

Recent studies have also reported that under depleted-inorganic-nutrient conditions, the *C. polykrikoides* red-tide outbreaks occur using dissolved organic nutrients (Lee *et al.*, 2007). The nutrients in red-tide waters may originate from submarine ground water, and these nutrients are rapidly utilized by phytoplankton. The waters show low DIN or DIP values, but high DON and DOP values (caused by rapid remineralization of the biota) when the groundwater inputs are reduced during neap tides.

Since mineral nutrients are uniformly distributed in the upper water column, the observed vertical movements may have been performed in the search for optimal optical depth. In this investigation, the highest relative cell density of the species was observed when the concentration of DIN gradually increased in deeper water during daytime. In darkness, the concentration of DIN was generally low. There is very limited information on the ecology and trophic role of planktonic heterotrophic dinoflagellates in marine coastal ecosystems. The inability of flagellates to use nitrate at high rates in darkness has not been clarified.

On the basis of our results for the comparison of the 2 major nutrients, nitrogen was presumed to show more effect on the proliferation of the species red tide.

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