

# Persistent whole-bay red tide of *Noctiluca scintillans* in Manila Bay, Philippines

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**Abstract**—*Noctiluca scintillans*, which contains a photosynthetic endosymbiont, *Pedinomonas noctilucae*, formed perennial red tides in Manila Bay, Philippines, occasionally covering almost whole area since 2001. A whole-bay scale red tide of the green *Noctiluca* in Manila Bay is described as the first step to elucidate the formation mechanism of the large scale blooming of the organism. A field survey was conducted in March 2004, when greenish discoloration due to *N. scintillans* was observed in the whole bay. The bloom persisted at least for two months from late February to late April 2004 as revealed by SeaWiFS images and sea-truth observations. Chlorophyll *a* varied from 0.55 to 522  $\mu\text{g L}^{-1}$  in the upper 10-m water column, 28 to 94% of which was ascribed to *N. scintillans* with a mean of  $75 \pm 17\%$  ( $n=60$ ) as revealed by HPLC analysis of bio-marker pigments with CHEMTAX interpretation. *In-situ* specific growth rate was too low for the continuous dominance of *N. scintillans* in the bay, where potentially fast growing diatoms occurred as secondary dominant species. While the endosymbiosis of *P. noctilucae* provides competitive advantages for *N. scintillans* under conditions with low availability of external food supply or insufficient light intensity for photosynthesis of the symbiont, no suitable explanation was obtained for the persistent dominance. Trophic status of the green *Noctiluca* appears to be crucial.

**Key words:** *Noctiluca scintillans*, *Pedinomonas noctilucae*, red tide, symbiosis, Manila Bay, growth, feeding

## Introduction

*Noctiluca scintillans* (Macartney) Ehrenberg, a heterotrophic dinoflagellate is one of the common red tide organisms. The organism contains the photosynthetic green flagellate *Pedinomonas noctilucae* as an endosymbiont in the coastal waters of Southeast and South Asia, and adjacent waters (Elbrächter and Qi 1998), and thus called green *Noctiluca* locally due to its color. The symbiont is found only within the host cell and not in seawater, because the optimum pH of the symbiont is 4.5 to 5.0 and it does not survive in the seawater (Okaichi et al. 1991). Dense blooms of the green *Noctiluca* frequently occur in coastal waters of the Southeast Asia including Jakarta Bay (Adnan 1984 and the references), Ambon Bay (Sidabutar et al. 2001) and the Gulf of Thailand (Suvapepun 1989, Menasveta 2000, Cheevaporn and Menasveta 2003). The blooms occasionally cause mass mortality of fish (Adnan 1989) and reduction of harvests of shrimp aquaculture (M. Ahmed, pers. comm; Putth et al. this volume). Furthermore, as inferred from blooming of *Noctiluca* in temperate waters despite lack in direct evidence in the green *Noctiluca* as published literature, dense blooms of

*Noctiluca* may cause formation of anoxia, impacts on fisheries (Caddy and Bakun 1995) and toxicity by ammonia (Okaichi and Nishio 1976). Therefore, the prevention of the dense blooms is of public concern in the Southeast Asia.

Various physical and biological processes are evolved in the dense accumulation of biomass, viz., active growth over mortality, drifting by wind stress or current, and local aggregation by motility or buoyancy. *N. scintillans* without the symbiont, which is called the red *Noctiluca* hereafter, is known to regulate its specific gravity through changes in intracellular ammonia content (Elbrächter and Qi 1998). Cells are negatively buoyant and dispersed throughout the water column in early bloom conditions, and become positively buoyant with the increased amount of ammonia along with the development of blooms (Elbrächter and Qi 1998). The positive buoyancy explains a localized build-up of biomass. For example, *N. scintillans* accumulates in frontal areas (Schaumann et al. 1988). Streak-shape red tides are very common in this species. These are likely a consequence of a combined effect of the positive buoyancy and physical processes such as presence of convergence, or gentle wind-driven drifting. The convergence mechanically concentrates *N. scintillans*, reduces diffusivity and favors the maintenance

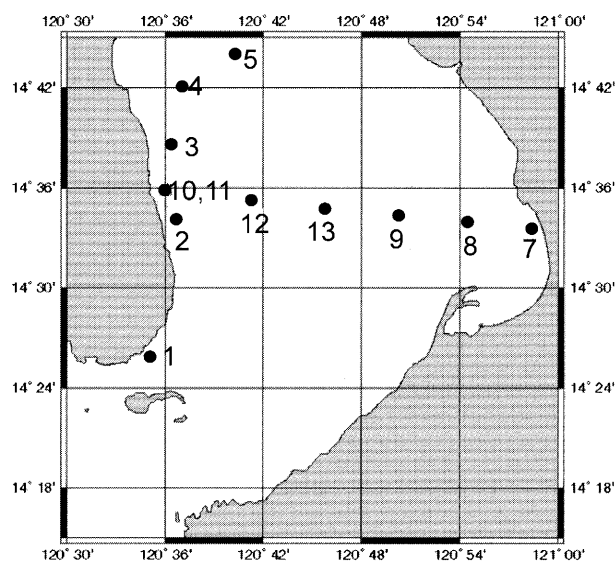
of a dense population (Le Fèvre and Grall 1970).

Our understanding on the formation processes of the *N. scintillans* bloom is limited to that without the symbiont, and does not warrant applying the knowledge to the green *Noctiluca*. Furthermore, recently large-scale blooming of the green *Noctiluca* tends to occur in some Southeast Asian waters. In Manila Bay, whole-bay red tides of the green *Noctiluca* occur since 2001, which prevails in the bay almost throughout the year, particularly in the western part (Borja and Furio, unpublished). Such a large scale blooming is hardly explained simply by the combination of the buoyancy and physical processes. In 1980's and 1990's, the bay was known for recurrence of blooms of PSP causing dinoflagellates *Pyrodinium bahamense* var. *compressum* and *Gymnodinium catenatum* (Furio and Gonzales 2002). However, these species have not formed blooming since 2000, and the blooming species has changed to the green *Noctiluca* (Borja and Furio, unpublished; Hansen et al. 2004).

A large bloom of the green *Noctiluca* developed along the coast of the bay late February as found by SeaWiFS chlorophyll images (W. Takahashi, per. comm.) and sea-truth observations by BFAR, and covered the whole bay by late March. The bloom persisted at least until the end of April. The high chlorophyll *a* was also observed afterward in SeaWiFS or MODIS/aqua chlorophyll images, but no sea-truth was made to confirm the blooming species. As the first step to elucidate the formation mechanism of the large scale blooming of the organism, a field observation was made to describe the distribution of the green *Noctiluca* in relation with environmental conditions on March 19 to 23, 2004 (Fig. 1). An additional observation was made in March 2002 in the vicinity of Stns. 10 and 11 off Limay, Bataan. This report summarizes the results obtained in the field surveys, and full description of subjects dealt here will be published.

## Hydrography and Nutrients of Manila Bay

Manila Bay is a semi-enclosed body of water with a mean depth of 17 m. Pampanga River provides a major freshwater influx of the bay (49% of the total) flowing into the northwestern part (Jacinto et al. 1998 <<http://data.ecology.su.se/MNODE/Asia/Philippines/manilabay/Manilabud.htm>>). The climate of the bay is characterized by two pronounced seasons; dry from November to May and wet from June to September. In general, August is the rainiest, and February is the driest. There are three major wind regimes in the bay; northeast monsoon from October to January, southeasterly from February to May and the southwesterly trade winds from June to September. The circulation pattern of the surface water is influenced by the interplay of the surface wind stress, tidal forces and freshwater discharges (de las Alas and



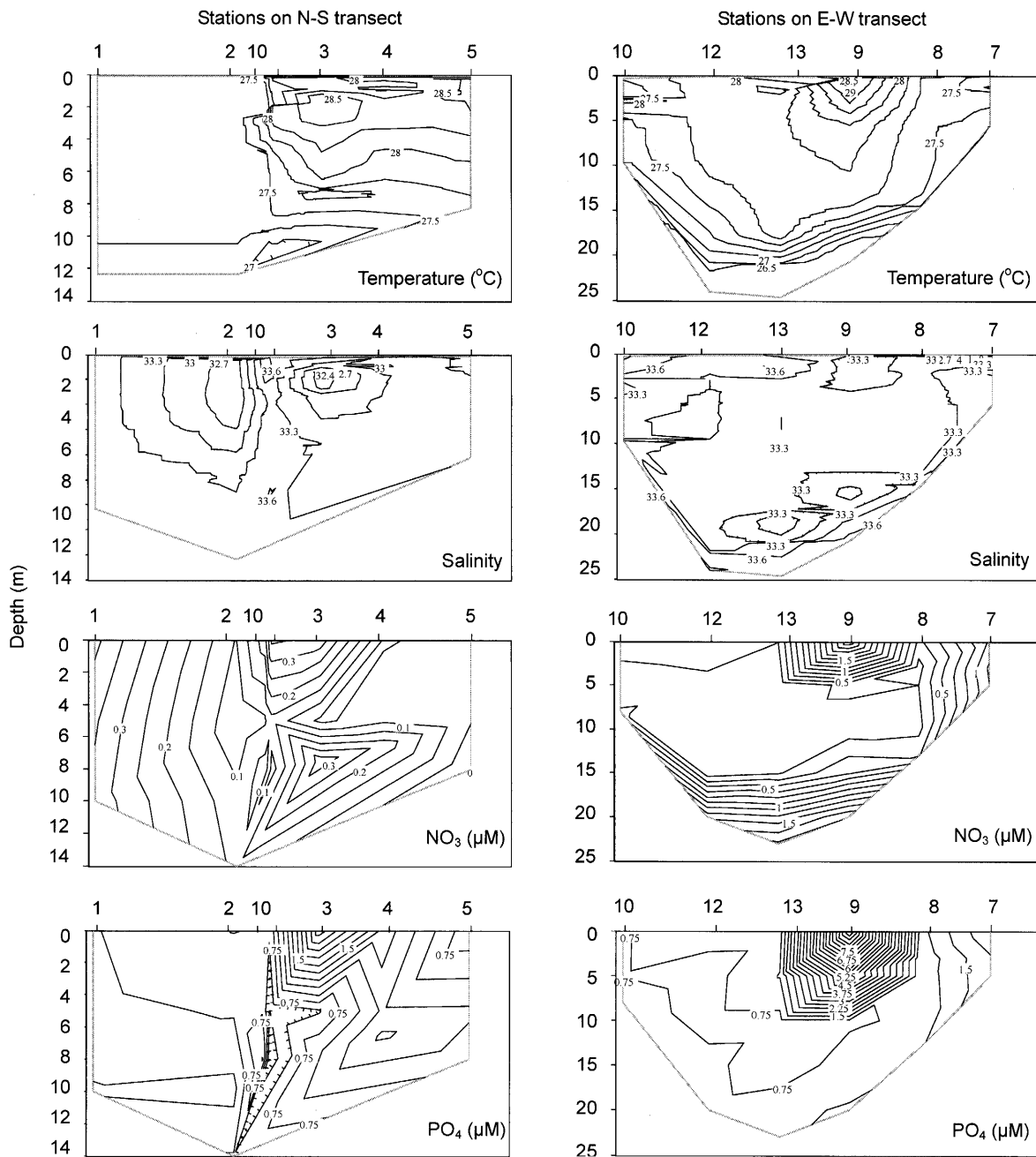
**Fig. 1.** Station locations. Two transactions were occupied in March 2004: north-south (from Stns. 1 to 5) and east-west (from Stn. 7 to 13). Growth rate of the green *Noctiluca* was obtained at a fixed station near Stn. 10, where a 30 h time-series observation was made.

Sodusta 1985). During the southeasterly wind season, a single anticyclonic gyre dominates the bay.

During the 2004 observation, thermal stratification was weak with a thermocline located near the bottom on the east-west transect (Fig. 2). A surface temperature rise was observed at Stn. 9, which was coincided with a distinctively high chlorophyll patchiness exceeding  $520 \mu\text{g/L}$  (Fig. 3), suggesting the warming due to thermal loss of absorbed light energy by chlorophyll *a* (Lewis et al. 1983). Surface salinity as determined by a CTD varied above 31.37, showing a typical condition in the dry season with a minor influence of freshwater inflow (Fig. 2). The lowest salinity was observed at Stn. 7, showing an influence of a discharge from Metropolitan Manila area. This was in good agreement with both nitrate and phosphate distribution where a horizontal was observed on the east-west transect (Fig. 2). During our observation, influx of the nutrients was likely transported northward by the anticyclonic current, which tended to retain the nutrient within the bay (de las Alas and Sodusta 1985). Both dissolved inorganic nitrogen (nitrate+nitrite+ammonia) and phosphate were not exhausted, and phosphate was relatively rich in terms of the Redfield ratio (Fig. 3).

## Distribution of the Green *Noctiluca*

Biomarker pigment distributions (chlorophylls and carotenoids) were used to infer spatial variations in phytoplankton biomass and composition. Phytoplankton collected on GF/F filters were frozen and brought back to laboratory in



**Fig. 2.** Vertical profiles of temperature (top), salinity (second row), nitrate (third row) and phosphate (bottom) on the north-south (left) and east-west (right) transect. Note that vertical scaling differs between the two transects. Temperature and salinity were measured using a YSI CTD. Nitrate and phosphate were determined using samples obtained by a van Dorn sampler.

liquid nitrogen. Biomarker pigments were analyzed after Zapata et al. (2000). Chlorophyll *a* was generally high varying from 0.55 to 522  $\mu\text{g/L}$  (Fig. 4). The highest concentration was found in the surface of Stn. 9. While low concentrations were observed at Stns. 1 and 2, chlorophyll *a* fluctuated above 4  $\mu\text{g/L}$  in the upper 10-m water column at stations on the east-west transect and north of Stn. 10 on the north-south transect. Cellular abundance of the green *Noctiluca* showed a similar distribution pattern to that of chlorophyll *a* with the highest abundance in the surface of Stn. 9 and low standing stock at Stns. 1 and 2. The green *Noctiluca* tended to distrib-

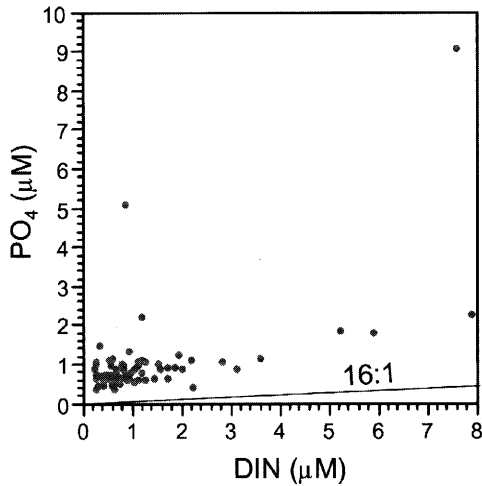
ute near the surface on the east-west transect. In contrast, at stations on the north-south transect, maximum abundance was found in the subsurface layers (Fig. 4). The similarity in the distribution between chlorophyll *a* and numeric abundance of the green *Noctiluca* indicates the green *Noctiluca* contributed to a major portion of chlorophyll *a*.

This was examined by evaluating chlorophyll *a* amount of *P. noctilucae*. In the biomarker composition, divinyl chlorophyll *a* and prasinoxanthin were of trace amount. Then, we used following diagnostic pigment; peridinin, 19'-butanoyloxyfucoxanthin, fucoxanthin, violaxanthin, 19'-

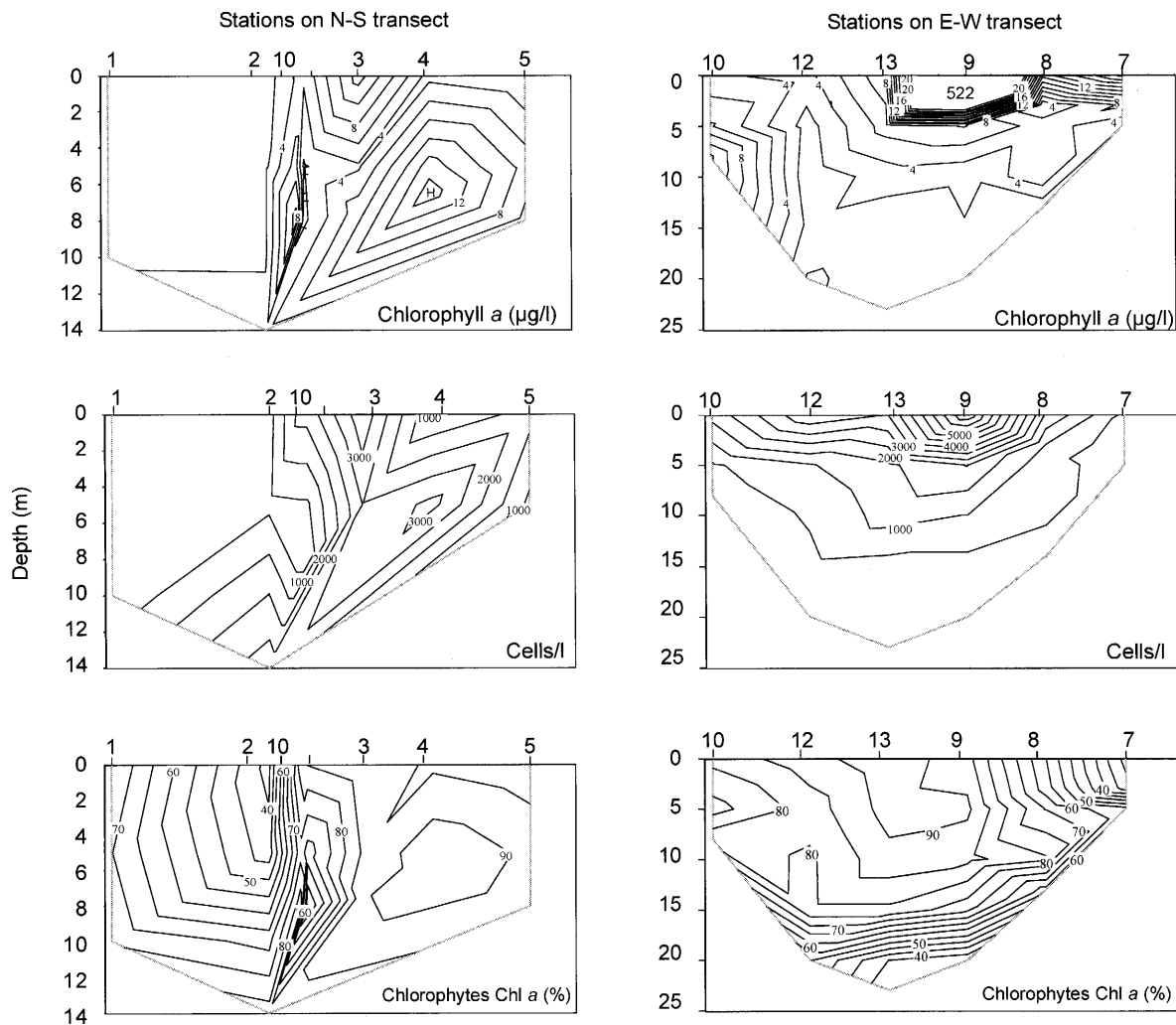
hexanoyloxyfucoxanthin, alloxanthin, zeaxanthin, lutein, chlorophyll *b* chlorophyll *a* to estimate chlorophyll *a* amount of dinoflagellates, chrysophytes, diatoms, chlorophytes, hap-

tophytes, cryptophytes and cyanobacteria by CHEMTAX (Mackey et al. 1996). According to microscopic observation, no significant amount of chlorophytes was observed. Since major pigments of *P. noctilucae* are neoxanthin, antheraxanthin, violaxanthin, zeaxanthin, lutein, chlorophyll *b*, chlorophyll *a* and  $\beta$ -carotene (Furuya and Lirdwitayaprasit 2000), chlorophyll *a* of *P. noctilucae* was included in that assigned to chlorophytes. Therefore, we ascribed chlorophyll *a* allocated to chlorophytes to *P. noctilucae* (Fig. 4). The green *Noctiluca* was found to contribute much to total chl *a* varying to 94%.

However, although with low contributions, there were many phytoplankton species. Microscopy revealed that there were considerable number of secondary dominant species which included *Chaetoceros* spp., *Pleurosigma* spp., *Rhizosolenia* spp., *Thalassiosira* spp., *Haslea gigantea*, *Ceratium fusus*, *Alexandrium tamiyavanichii*, *Gymnodinium catenatum* and *Goniodoma polyedricum*. Since nutrient supply appeared to be enough (Figs. 2 and 3), these species likely grew *in situ*. In particular, there were potentially fast



**Fig. 3.** Relationship between dissolved inorganic nitrogen and phosphate. The 16 : 1 line denotes the Redfield ratio.



**Fig. 4.** Vertical profiles of chlorophyll *a* (top), cellular abundance of the green *Noctiluca* (middle) and chlorophyll *a* of chlorophyte-type phytoplankton (bottom) on the north-south (left) and east-west (right) transect. Note that vertical scaling differs between the two transects.

growing diatoms such as, *Bacteriastrium delicatulum*, *Chaetoceros pseudocurvisetum*, *Chaetoceros coarctatus* and other *Chaetoceros* species. Then, why the green *Noctiluca* was so successful in Manila Bay for more than two months? For this growth rate of the green *Noctiluca* was evaluated.

## Growth Activity

*In situ* growth of the natural population was analyzed by the cell cycle analysis (Furuya et al. in press). The method is based on temporal changes in nuclear DNA content and is free from possible influence of by feeding of grazer, advective transport of populations and so-called bottle effects associated with bottle incubations. Hourly sampling was conducted at a fixed station near Stn. 10 by vertical hauls of a 64  $\mu\text{m}$  mesh plankton net. Nuclear DNA content of the green *Noctiluca* was determined by microfluorometry of 4',6-diamidino-2-phenylindole (DAPI) staining to estimate fractions of G1, S, and G2+M phase in order to calculate specific growth rate  $\mu$  (Carpenter and Chang 1988).

Phased cell division was observed with maxima of both S and G2+M phase during early morning. G2+M phase showed three maxima between three to seven hours with several smaller peaks during the daytime. Thus, cell division was most frequent early in the morning, but occurred during the daytime as well. This weak synchronism contrasts clearly with the phased cell division of the red *Noctiluca* in the German Bight (Uhlrig and Sahling 1995), where a distinct maximum of dividing cells was observed during night. Timing of cell division is earlier than in our observation.

Specific growth rate of the natural population was estimated to be  $0.16 \text{ d}^{-1}$  (Furuya et al. in press). Those of natural population of the red *Noctiluca*, that is, *Noctiluca* without the symbiont varied between  $0.01$  and  $0.12 \text{ d}^{-1}$  in the German Bight (Uhlrig and Sahling 1995), and from  $-0.02$  to  $0.28 \text{ d}^{-1}$  in Seto Inland Sea (Nakamura 1998). Owing to scarcity of available information, difference in the methods used and oceanographic conditions limit direct comparison of our result with others'. Furthermore, nutrition distinctly differs between two types of *Noctiluca* (Hansen et al. 2004, Furuya et al. in press). However, our result is within a range of values reported for the red *Noctiluca*. Potentially maximum growth rate of phytoplankton at  $28^\circ\text{C}$  is around  $3.5 \text{ d}^{-1}$  (Eppley 1972). Thus, the *in situ* rate of the green *Noctiluca* was far below the maximum rate. Under optimum laboratory conditions, clone cultures of the green *Noctiluca* showed higher specific growth rate ( $0.33 \pm 0.100 \text{ d}^{-1}$ ,  $n=6$ ; Furuya et al., in press) than that *in situ*. Although the difference in the growth rate between *in situ* and cultures suggests that *in situ* growth was under limitation, even the rates under optimum conditions are still much below the potential maximum. Therefore, fast growing diatoms which co-existed in Manila Bay could

exceed the growth of the green *Noctiluca* with ample supply of nutrients (Fig. 2). However, it did not occur. Then, why was the green *Noctiluca* so successful?

## Summary and Conclusion

Furuya et al. (in press) showed from laboratory experiments using cultures that the symbiosis secures survival of the green *Noctiluca* under food limitation but does not stimulate fast growth even with sufficient supply of light and nutrient salts. Obviously, the green *Noctiluca* is a slow grower compared with coexisting diatoms and dinoflagellates which were secondary dominant in Manila Bay. The question is what is the competitive advantage over other plankton species. A clue was obtained in the nutrient composition. Manila Bay was characterized by the relative abundance of phosphate (Fig. 3). Although nutrient salts were not depleted during the observation, the relative abundance seemed to give an advantage to the green *Noctiluca*. That is, since *Noctiluca* tends to accumulate ammonia within its cell, this eliminates possible nitrogen deficiency for the symbiont. Thus, in case of shortage of nitrogen supply, growth of phytoplankton will be limited, but not in *P. noctilucae*.

Another possible advantage was indicated in March 2002 off Limay. There was a dense large scale blooming, in which many green *Noctiluca* contained one to several food vacuoles with *Gymnodinium catenatum* and *Rhizosolenia* spp., both were dominant. *Noctiluca* in temperate waters is known for its active grazing (Nakamura 1998). The active grazing can reduce abundance of co-occurring phytoplankton, potential competitors for nutrient salts. Thus, even with the low growth rate, once it becomes dominant, the grazing may control growth of the competitor. However, our observation was qualitative, and it is not clear how much impact was set to prey phytoplankton. This should be addressed to clarify the competitive advantage of the green *Noctiluca*. Moreover, little is known whether the green *Noctiluca* is a prey of any animal. If it stands at a dead end of trophic chain, biomass accumulation is possible for the slow grower. Hence, evaluation of trophic status of the green *Noctiluca* in planktonic ecosystem is an important question.

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