

## Ammonium and phosphate pools of *Noctiluca scintillans* and their supplies to the water column in Harima Nada, the Seto Inland Sea, Japan

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**Abstract :** The contribution of ammonium and phosphate contained in *Noctiluca scintillans* to the water-column nutrient pools were investigated in Harima Nada, the Seto Inland Sea, Japan. Ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) and phosphate ( $\text{PO}_4^{3-}\text{-P}$ ) concentrations varied widely from undetectable to  $36.5 \mu\text{M}$  and to  $1.6 \mu\text{M}$ , respectively. Percentages of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  contributed by *Noctiluca* were estimated based on population densities and the cellular nutrient contents. The  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  contained in *Noctiluca* contributed to the water column  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  pools ranging from 0 to 119% and to 80%, respectively. The estimated high percentages of both macro-nutrients were usually found in the middle of spring (April to June) where *Noctiluca* red tides were observable, indicating that during this period the nutrient regeneration by *Noctiluca* should not be ignored. The temporary enhancement of nutrients at the sea surface by *Noctiluca* during Spring season when low ambient nutrient concentration is characterized, effectively supports the growth of autotrophs and results in successive phytoplankton blooms.

**Key words :** *Noctiluca scintillans*, nutrient pools, nutrient regeneration, the Seto Inland Sea

### 1. Introduction

*Noctiluca scintillans* is one of the important red tide heterotrophic dinoflagellate which is abundant in tropical and subtropical coastal waters (e. g. HUANG and QI, 1997 ; OKAICHI and NISHIO, 1976 ; TADA *et al.*, 1997, UHLIG and SAHLING 1990). The extraordinary mass development of this organism dyes sea surface with large red streaks when optimum physico-chemical and biological factors are suitable for cell division and accumulation. This phenomena, so-called *Noctiluca* red tide, has been studied in ecological aspects in global to regional level (e. g. ADNAN, 1989 ; OKAICHI and NISHIO, 1976 ; OMORI and HAMNER, 1982 ; PORUMB, 1992 ; SCHAUMANN *et al.*, 1988 ; UHLIG and SAHLING, 1990 ; HUANG and QI, 1997). In the Seto Inland Sea, Japan, seasonal variation in population density and *Noctiluca* biomass have been studied (TADA *et al.*, 1997) together with biophilic

cellular carbon and nitrogen contents (TADA *et al.*, 2000). *Noctiluca* has been ecologically considered to be a nutrient regenerator in the coastal seas due to its high cellular contents of ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) (OKAICHI and NISHIO, 1976 ; SCHAUMANN *et al.*, 1988 ; MONTANI *et al.*, 1998) and phosphate ( $\text{PO}_4^{3-}\text{-P}$ ) (SCHAUMANN *et al.*, 1988 ; MONTANI *et al.*, 1998). The high concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  which contaminate the sea surface after cell lysis, are subsequently utilized by autotrophs. *Noctiluca* ingests particulate organic matter (POM) such as phytoplankton, detritus (FUKUYO *et al.*, 1990) and bacterial size particles (KIRCHNER *et al.*, 1996), and transforms those organic materials into inorganic nutrients inside the cell by metabolic activity. Although protozoan nutrient regeneration has been considered to be less important than that of the microheterotrophs (HARRISON, 1980, 1992 ; CARON and GOLDMAN, 1990), vertical migrating of the species may transport a significant fraction of their regenerated nitrogen ( $\text{NH}_4^+\text{-N}$ ) to

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the sea surface where it is easily accessible by primary producers (LONGHURST and HARRISON, 1988 ; LONGHURST *et al.*, 1989 ; HARRISON, 1992). In this study, the authors conducted a long-term study of *Noctiluca* abundance in Harima Nada over the period beginning May 1995 and lasting until December 1998. The cellular  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  contents had been measured and data were used to estimate the nutrient pools of *Noctiluca* in the study area. The study further examines the role of *Noctiluca* as the nutrient regenerator in the Seto Inland Sea.

## 2. Materials and methods

### Collection of *Noctiluca* and water samples

Seawater samples were collected almost monthly from May 1995 to December 1998 at the depths of 0, 5, 10, 20, and in deeper water 2m from the bottom at Stn. NH ( $34^\circ 28' \text{N}$ ,  $134^\circ 24' \text{E}$ ) located in Harima Nada, the Eastern

part of the Seto Inland Sea, Japan (Fig.1). A 10- $\ell$  Vandorn type water bottle was used to collect water samples except the sea surface sample that was collected using a 15- $\ell$  plastic bucket. Water samples were poured into twice-rinsed 5  $\ell$ -capacity polyethylene bags and stored in the dark at surface ambient water temperature during transportation to the land laboratory. Within a few hours after collection, samples were filtered using a Whatman GF/F filter. Duplicate filtrate samples were immediately frozen in a freezer ( $-20^\circ\text{C}$ ) for subsequent analysis of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$ . *Noctiluca* abundance was estimated from 1  $\ell$  water filtration through 300  $\mu\text{m}$ -open mesh screen. Cells retained on the screen were fixed with formaldehyde solution (1% final concentration) and counted under a stereomicroscope at  $10\times$  magnification. In case of high *Noctiluca* density in the samples, serial dilutions to appropriate cell number were performed before counting.

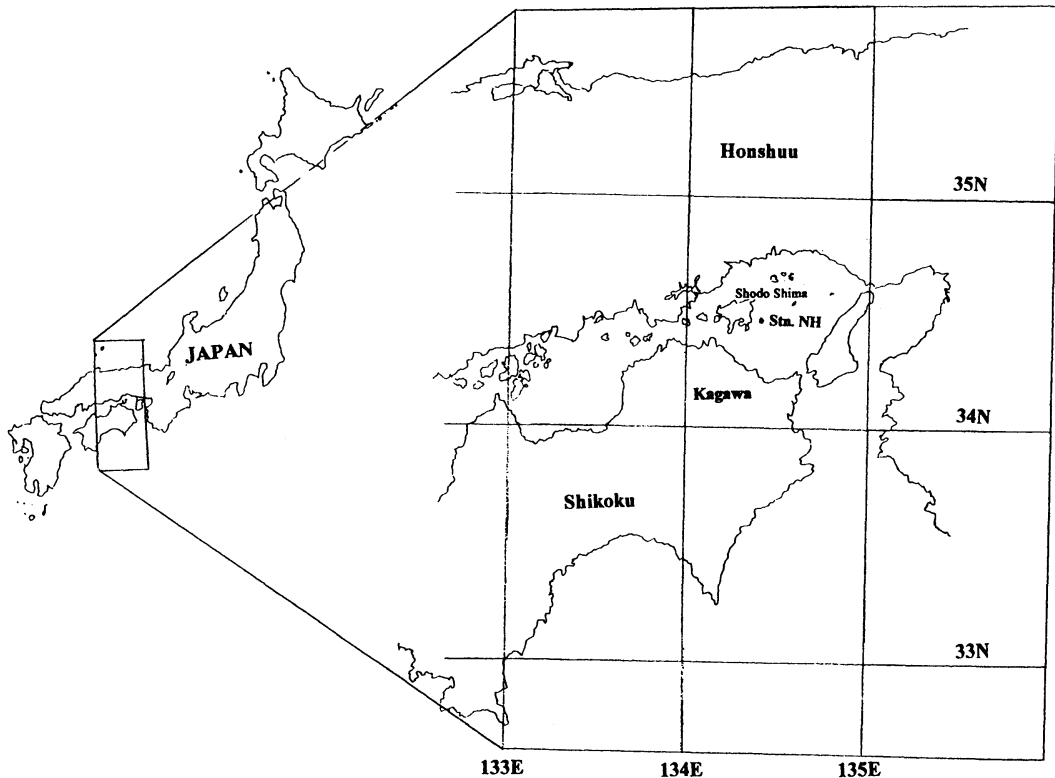


Fig. 1. The location of sampling station, Stn. NH, in Harima Nada, eastern part of the Seto Inland Sea, Japan.

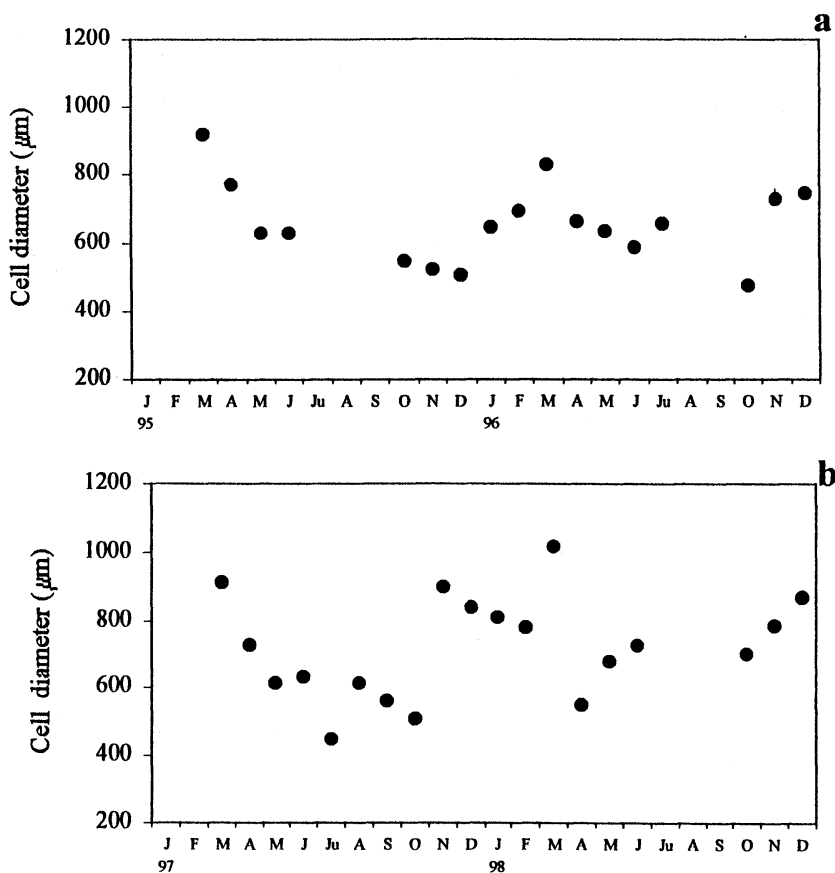


Fig. 2. Monthly changes in *Noctiluca* mean cell diameter during a) 1995–1996. b) 1997–1998.

*Noctiluca* samples used for determination of cellular nutrient contents were collected by carrying out a vertical tow of a zooplankton net (300 µm opening mesh) at a depth of 20m in April, May, June, October, November and December 1996.

#### ***Noctiluca* cellular content and cell size determination**

The details of the method for determining *Noctiluca* cellular nutrient contents are given elsewhere (MONTANI *et al.*, 1998). Buoyant *Noctiluca* cell collected by a zooplankton net were siphoned off the top of net-plankton samples. Cells were washed to eliminate other plankton species with 0.2 µm filtered seawater for 3–5 times by repeating the siphon process. *Noctiluca* cell were carefully placed onto a 0.45 µm Milipore filter (cellulose nitrate membrane).

*Noctiluca* cells together with the filter were homogenized and sonicated in an ice-cold water bath. Double-distilled water was used to dissolve the inorganic nutrients from the samples. The liquid phase was then filtered using a 0.45 µm disposable disk membrane filter for further analysis of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  using the Technicon Auto-Analyzer II (GRASSHOFF *et al.*, 1983). All samples were prepared in replicates ( $n=2\sim6$ ).

*Noctiluca* cell diameter was measured for different collection period of the year by observing 20 live cells from the plankton net sample under a light microscope with a micrometer at 100 × magnification. No fixing solution was used to fix the cells but we reduced the sea water volume on the Sedgwick-Rafter counting chamber so that the movement of *Noctiluca* was restricted. *Noctiluca* cell volumes were

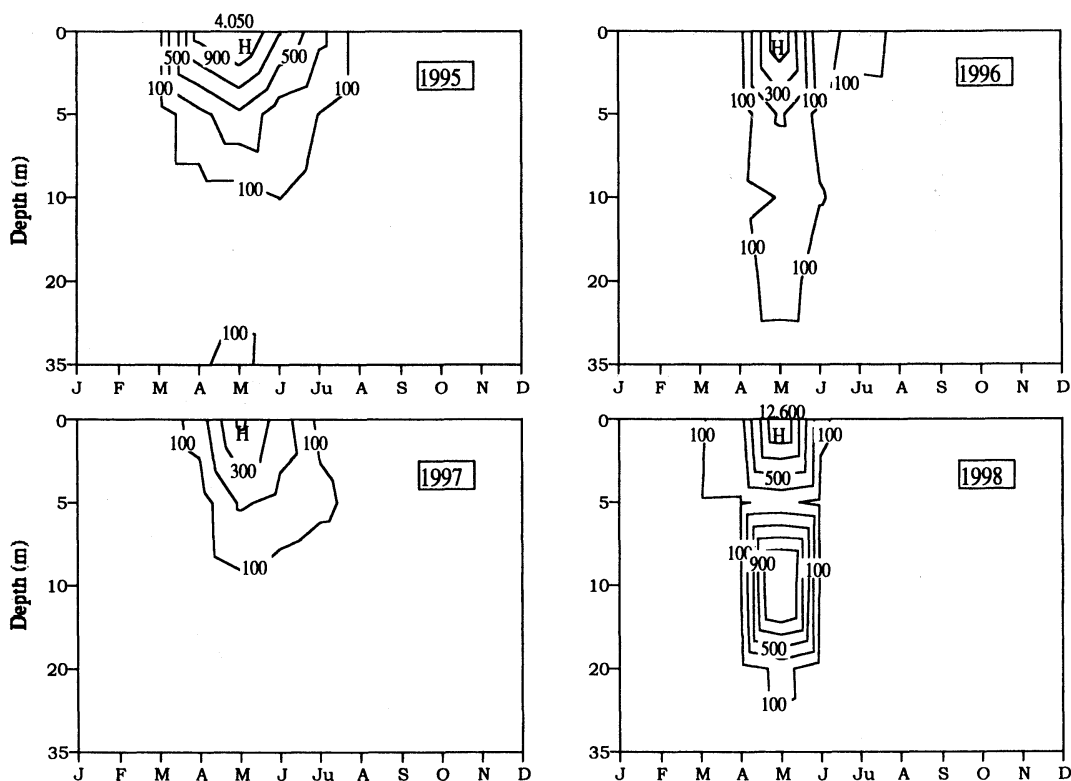


Fig. 3. Vertical profiles and monthly changes in *Noctiluca* population density at Stn. NH during 1995-1998.

calculated assuming spherical shape.

#### Estimation of ammonium and phosphate pools of *Noctiluca*.

The ammonium and phosphate contents of *Noctiluca* was measured in the 1996 samples and used to estimate the *Noctiluca* inorganic nutrient pools based on its cell volume. We also observed seasonal changes in *Noctiluca* cell diameter during the study period. The largest cells were usually observed in March with diameters decreasing from March to July of October and then increasing toward December (Fig. 2 a, b). For the remaining months that we were not able to measure cellular nutrient contents due either to low cell densities in the water column (in August and September) or due to the difficulty of separating *Noctiluca* from other contaminated zooplankton in the net-samples (in July). We used linear correlation equations between *Noctiluca* cell volume and cellular  $\text{NH}_4^+$

N and  $\text{PO}_4^{3-}\text{-P}$  to calculate nutrients content of the cell.

### 3. Results

#### The abundance of *Noctiluca scintillans* in the Seto Inland Sea

The high population density of this organism was clearly noticed once or twice every year during the study period (note that our monitoring was carried out at almost monthly intervals). Population densities ranged from 0 to 12,600 cell  $\text{l}^{-1}$ . The peak cell densities at the sea surface occurred in April to May 1995, and March to May 1998 (Fig. 3). In April 1995 a density of 155 cell  $\text{l}^{-1}$  was unexpectedly found in the bottom layer. There were small cell density peaks reaching to 500 cell  $\text{l}^{-1}$  in May 1996 and May 1997 (Fig. 3). No *Noctiluca* density greater than 1,000 cell  $\text{l}^{-1}$  was found in the 1997 samples. However, a moderately high cell density was counted at that time (426 cell  $\text{l}^{-1}$ ).

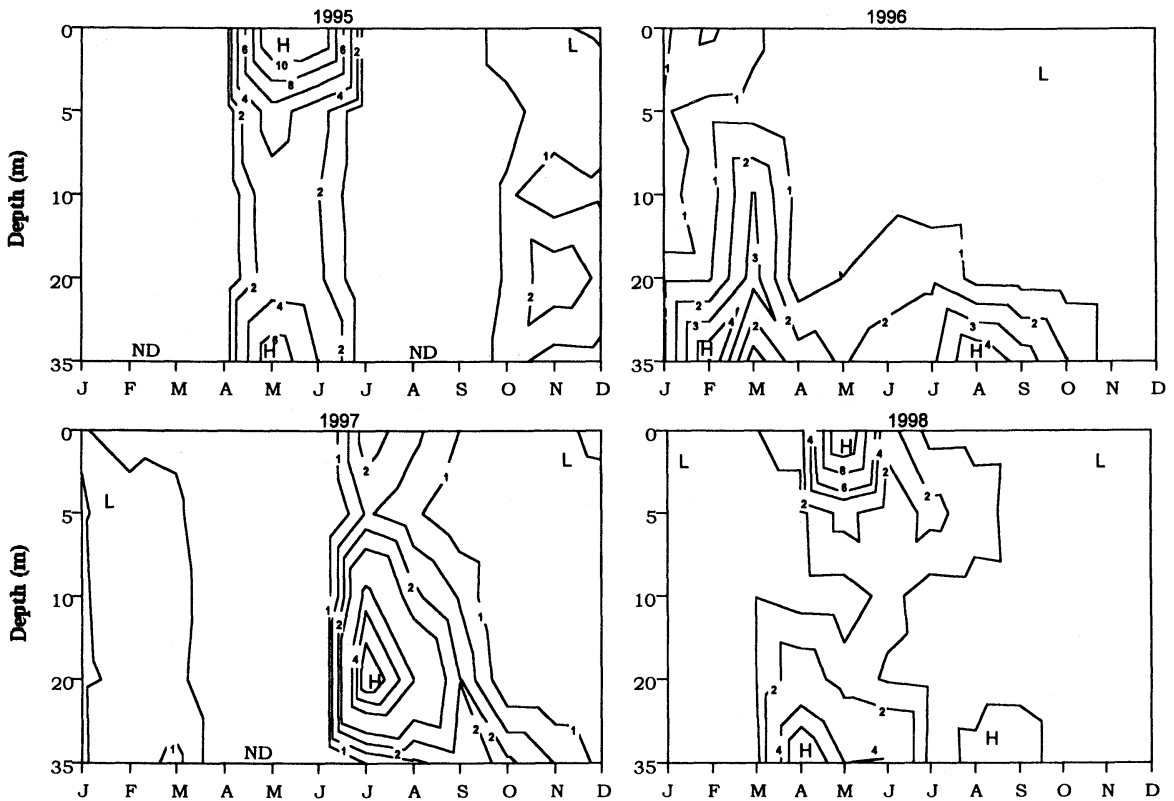


Fig. 4. Vertical profiles and monthly changes in  $\text{NH}_4^+\text{-N}$  concentrations ( $\mu\text{M}$ ) at Stn. NH during 1995-1998.

### The seasonal variation in $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ in the study area

The  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  concentrations of seawater samples varied widely from undetectable to  $36.5\mu\text{M}$  and to  $1.6\mu\text{M}$  for  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$ , respectively. Ammonium nitrogen concentration usually increased in middle of spring. The highest concentration of  $36.5\mu\text{M}$  was found concurrently with  $718\text{ cell l}^{-1}$  *Noctiluca* density in June 1995 (Fig. 4). Low ammonium level spring and summer were found with the exception of samples taken in 1996. Low  $\text{NH}_4^+\text{-N}$  concentrations, however, were measured in autumn and winter waters for most of the samples throughout the monitoring period.

Seasonal changes in  $\text{PO}_4^{3-}\text{-P}$  concentrations experienced the same seasonal trends as the concentrations of  $\text{NH}_4^+\text{-N}$  concentration (Fig. 5). Phosphate and  $\text{NH}_4^+\text{-N}$  concentration increased concurrently with the increase in

*Noctiluca* cell density in May 1995 and May 1998. Low  $\text{PO}_4^{3-}\text{-P}$  concentrations were found in early spring and summer as a result of phytoplankton uptake. We observed that nutrient flux from water-sediment interface occasionally induced high concentration of both nutrients found in a bottom layer.

### The estimation of ammonium and phosphate provided by *Noctiluca*

The intracellular  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  contents of *Noctiluca* were calculated based on the correlation between cell volume and cellular inorganic nutrient contents as :

$$\begin{aligned} \text{Noctiluca cellular } \text{NH}_4^+\text{-N (pmol/cell)} \\ = 2 \times 10^{-5} v + 88.89 \end{aligned} \quad (1)$$

$$\begin{aligned} \text{Noctiluca cellular } \text{PO}_4^{3-}\text{-P (pmol/cell)} \\ = 3 \times 10^6 v - 129.13 \end{aligned} \quad (2)$$

where  $v$  is the *Noctiluca* cell volume ( $\mu\text{m}^3$ ).

The *Noctiluca* ammonium nitrogen and  $\text{PO}_4^{3-}\text{-P}$

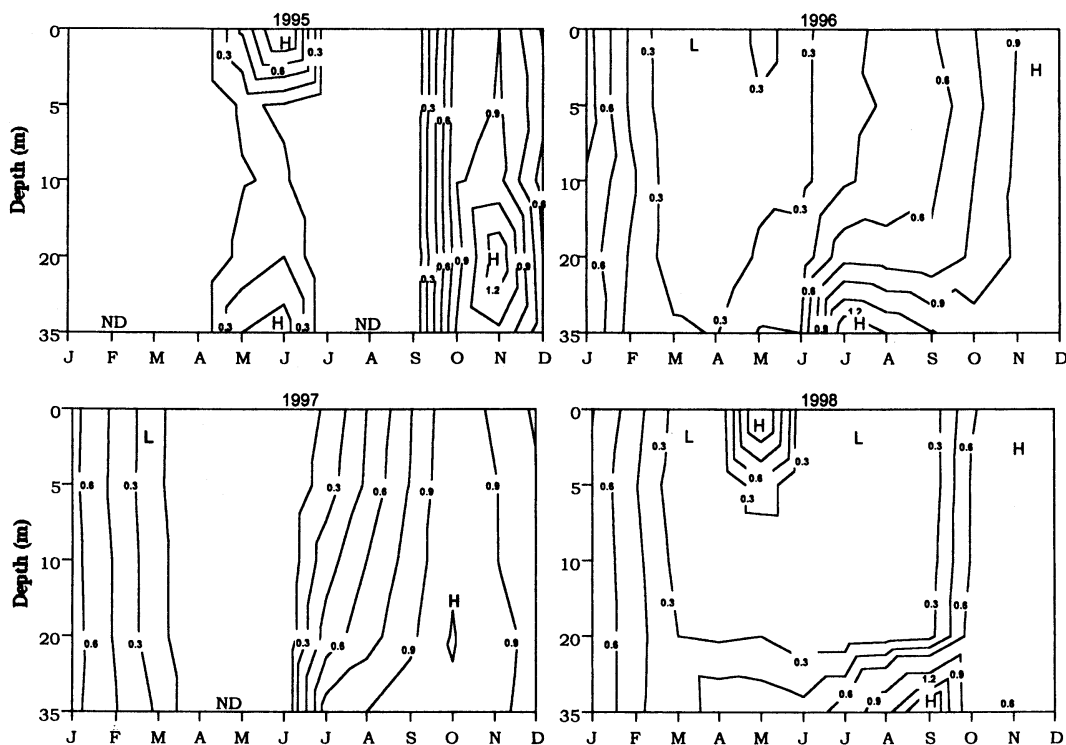


Fig. 5. Vertical profiles and monthly changes in  $\text{PO}_4^{3-}\text{-P}$  concentrations ( $\mu\text{M}$ ) at Stn. NH during 1995-1998.

P contents of has been reported in a previous study (MONTANI *et al.*, 1998). The estimated seasonal changes in *Noctiluca* cellular nutrient content is shown in Fig. 6. *Noctiluca* cell diameter decreased from the largest cells found in the March sample to the smallest cells found in April, July and October samples. Cell diameters then increased toward December (unpublished data). The same trend in changes in cellular nutrient content was obtained. The highest *Noctiluca* cellular  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  contents were found in the March 1998 samples (11.2 and 1.5  $\text{nmol cell}^{-1}$  for  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$ , respectively) when the cell diameter was 1019.2  $\mu\text{m}$ . The lowest cellular nutrient content was found in the July 1997 samples (1.0 and 0.01  $\text{nmol cell}^{-1}$  for  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$ , respectively) when the cell diameter averaged 448  $\mu\text{m}$  (Fig. 6).

Nutrient pools of *Noctiluca* were estimated by the multiplication of intra-cellular nutrient contents and cell standing stocks in 0-33 m

depth water-column. High *Noctiluca*  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  pools comparing to the ambient nutrient pools were found in May and June 1995, April and May 1996, 1998 and March 1997. These led the percentages of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  contained in *Noctiluca* to the ambient nutrient standing stocks ranged from 6.5-119% and from 5.1-80.0%, respectively. Low percentages of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  pools to the ambient nutrient concentrations were found in other periods of the year (Fig. 7).

#### 4. Discussion

##### Seasonal variation in *Noctiluca* abundance

*Noctiluca* abundance has been studied globally (e. g. western coast of Brittany-FÈVRE and GRALL, 1970; German Bight-UHLIG and SAHLING, 1982, 1990; SCHAUMANN *et al.*, 1988; Romanian Black Sea-PORUMB, 1992; Dapeng Bay, the South China Sea-HUANG and QI, 1997; Seto Inland Sea-TADA *et al.*, 1997). HUANG and QI (1997) reported that during the peak period of

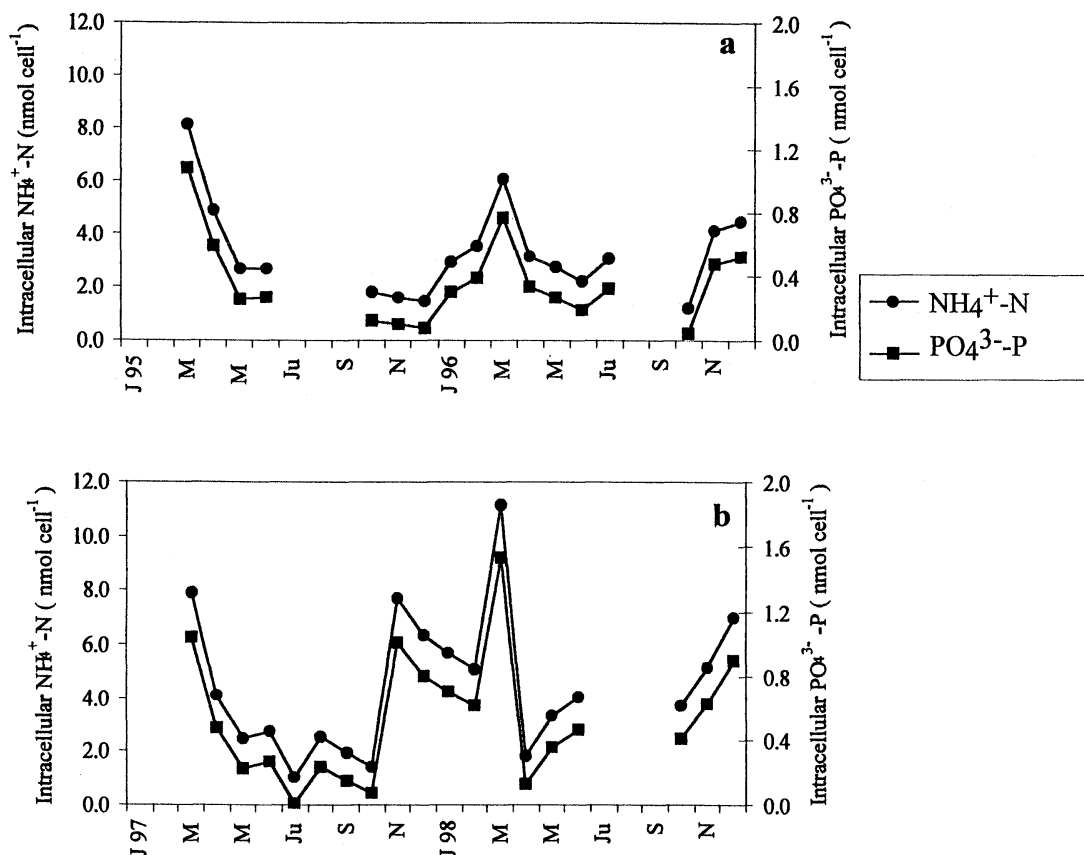


Fig. 6. Monthly changes in *Noctiluca* cellular  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$ -P contents ( $\text{nmol cell}^{-1}$ ) calculated based on the difference in cell volume during a) 1995-1996, b) 1997-1998

abundance between March and May in Dapeng Bay, South China Sea, the average density of *Noctiluca* had a significantly positive relationship with average water temperature. Our study documents the same phenomenon. Other physical factors effecting on the appearance of *Noctiluca* population are wind and tidal current (UHLIG and SAHLING, 1990). UHLIG and SAHLING (1990) also stated that *Noctiluca* growth is influenced by light intensity. Cell growth in nature could be influenced by increasing day length from December toward June (in the German Bight). In spring, water temperature would be the dominating growth parameter. Moreover, hydrographical features, such as fronts, have been postulated as an indicator in outbreaks of the red tide (FÈVRE and GRALL, 1970). The calm weather and weak stratification of the water column with a warm surface

layer obviously aids in generating a *Noctiluca* patch (SCHAUMANN *et al.*, 1988). However, PORUMB (1992) reported the most abundance of *Noctiluca* (over 50% of its density) in Romanian Black Sea water was observed between 50 and 75m depth in summer. He suggested that the stratification of water masses and strong heating of the superficial water in August may cause a numerical decrease in the *Noctiluca* population in the surface water. High temperature in the surface layer (over 21°C was stated by PORUMB, 1992, in Romanian Black Sea waters) modifies the behavior of *Noctiluca* which, by modifying their buoyancy, succeeds in avoiding layers that are over heated.

Apart from the factors mentioned above, the primary factor in *Noctiluca* multiplication is the presence of food (PORUMB, 1992). *Noctiluca* is well-known for its grazing behavior on a

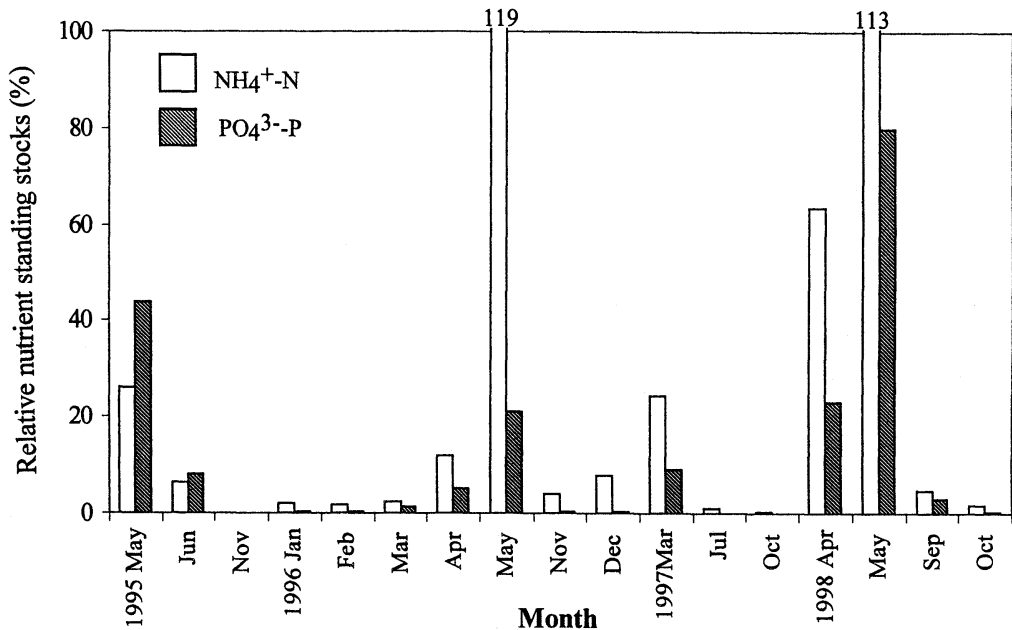


Fig. 7. Percentages of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  pools contained in *Noctiluca* cells to the ambient nutrient concentrations in the water column during 1995–1998

wide variety of foods, including phytoplankton (mainly diatoms and flagellates), zooplankton, fish eggs, detritus (HATTORI, 1962; FUKUYO *et al.*, 1990), and copepods eggs (KIMOR, 1979; DANN, 1987). HUANG and QI (1997) suggested that during the peak period of the *Noctiluca* abundance, the average density of *Noctiluca* at stations in Dapeng Bay showed a significantly negative relationship with average Chlorophyll *a* concentration. This indicated that *Noctiluca* exerts some predation pressure on the phytoplankton. Cyanophytes and bacteria may also serve as a food source for *Noctiluca*. SWEENEY (1970) observed cyanophyte *Trichodesmium* sp. within *Noctiluca* food vacuole. KIRCHNER *et al.* (1996) reported that bacteria and bacterial sized fluorescent latex microspheres can be removed from the water by entrapment in the mucoid web of *Noctiluca*. They also suggested that *Noctiluca* ingests bacteria in the winter when other food source are scarce. In the Seto Inland Sea, we found that a decrease in phytoplankton biomass occurred concurrently with an increase in *Noctiluca* population (TADA *et al.*, 1997).

#### Ammonium and phosphate provided by *Noctiluca*

The regeneration and supply of limiting nutrients in coastal surface waters are ecologically important especially in temperate regions where there are the high seasonal fluctuations in nutrient concentrations and other physical factors, such as water temperature. Organisms which contribute to nutrient regeneration have been seriously investigated for decades (HARRISON, 1980, 1992). It was suggested that microheterotrophs account for considerably more of the energy transfer and nutrient cycling within the plankton community than the grazing concept [phytoplankton-zooplankton-fish] held (HARRISON, 1992). The regeneration by micrograzers excretion, for example of heterotrophic bacterivorous and herbivorous, however, has also been studied extensively. CARON and GOLDMAN (1990) suggested that protozoans may be important nutrient remineralizers. This observation was based on the abundance of protozoa in coastal and oceanic waters, the fact that they are major consumers of bacteria and autotrophic picoplankton, their high biomass-specific metabolism, and field observations



that often associated high nutrient regeneration rates with the particle size class in which they fall (CARON and GOLDMAN, 1990; HARRISON, 1992).

Nutrient regeneration contributed by *Noctiluca* has been considered by OKAICHI and NISHIO (1976). Okaichi and Nishio pointed out that the harmfulness of this dinoflagellate is due to the high  $\text{NH}_4^+$ -N content in the cell. In addition ammonium possibly contaminates the surrounding water when cell lysis occurs. This topic has been subsequently discussed by a few additional authors. SCHAUMANN *et al.* (1988) has concluded based on the reports of German scientists that the dense accumulation of *Noctiluca* cell in the red tide patch heavily influenced the chemical and biological balance of the pelagic ecosystem. Aging *Noctiluca* cells ascend to the water surface which is in part achieved by active intracellular ion exchange and  $\text{NH}_4^+$ -N accumulation which reduces the specific weight of the cell. Part of this  $\text{NH}_4^+$ -N is then subsequently secreted together with  $\text{PO}_4^{3-}$ -P near to at the surface of the water column. This observation has been evidently supported by our findings that patches of *Noctiluca* red tide provided 16- and 25-fold greater concentrations of  $\text{NH}_4^+$ -N and  $\text{PO}_4^{3-}$ -P (maximum 86 and 4.6  $\mu\text{M}$ , respectively). Concentrations were greater in the uppermost layer (0–10 cm depth) of the water column compared with concentrations in the ambient sea water (MONTANI *et al.*, 1998). We also reported the intracellular nutrient contents of *Noctiluca* which are used to estimate  $\text{NH}_4^+$ -N and  $\text{PO}_4^{3-}$ -P pools in this study.

The increase in *Noctiluca* population density enhances  $\text{NH}_4^+$ -N and  $\text{PO}_4^{3-}$ -P concentrations on the sea surface due to its mass nutrient accumulation in the cell which is secreted as waste or released to the water after cell lysis (SCHAUMANN *et al.*, 1988, MONTANI *et al.* 1998). MONTANI *et al.* (1998) reported that the  $\text{NH}_4^+$ -N concentrations at the center of *Noctiluca* red tide between 0–10cm depth were markedly higher than those of the next 10cm depth layer (10–20cm). In addition to direct nutrient regeneration, a large amount of organic detritus and dissolved organic matter (DOM) produced within the red tide may stimulate microbial activities (SCHAUMANN *et al.*, 1988) which is

believed to provide an important fraction of regenerated nutrient. In this study, we can point out that  $\text{NH}_4^+$ -N and  $\text{PO}_4^{3-}$ -P pools contained in *Noctiluca* in Harima Nada, the Seto Inland Sea, accounted for 0–119% and 0–80%, of the ambient nutrient pools respectively during spring. These high percentages are important ecologically in relation to the biogeochemical cycles of the biophilic elements, mainly nitrogen and phosphorus, in marine environment.

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