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Implications for Top-Down Control of Phytoplankton Species Succession Within a Large Coastal Mesocosm

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Abstract

Mesocosm studies allow accurate ecosystem analysis since mass is conserved in the closed system. Here, a mesocosm (5 m in diameter and 18 m in depth) established in the Seto Inland Sea offers the additional advantage of incorporating a vertical mixing system to control the physical environment so that it resembles the characteristics of surrounding water (stratification at 5 m; 0-5 m mixing layer). We performed three independent mesocosm experiments in 1991, 1992 and 1994 under the same physical condition but with different chemical, biological and ecological conditions: higher nutrient availability in 1992 and presence of *Gymnodinium mikimotoi* red tide in 1994. Phytoplankton species succession was monitored in addition to several other chemical, biological and ecological parameters. Tracer (carbon stable isotope) experiments were performed in parallel to determine zooplankton grazing activity. The short-term phytoplankton succession, in some cases, was likely regulated by zooplankton grazing (top-down control) in addition to nutrient availability (bottom-up control). This type of information is invaluable for future environmental management of coastal and estuarine waters.

KEYWORDS: *mesocosm, nutrient availability, PLT, top-down control, zooplankton*

1. Introduction

Characterization of phytoplankton bloom and/or succession in eutrophic waters is essential for effective water environment management. Thus, ecological data, in addition to chemical, biological and physical data is required. We previously analyzed the food web structure of coastal waters using stable carbon isotope (Koshikawa *et al.*, 1996, 1999). Although those analyses clarified the photosynthetic and bacterial carbon cycles within coastal food webs, they did not provide any insight or guidance for water environment management.

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Ecosystem changes are affected by various factors such as physical, chemical, biological and ecological factors (Harada *et al.* 1996). However, for effective water environment management, it is important to understand whether control of the planktonic ecosystem is bottom-up (nutrient availability determines the structure of the ecosystem) or top-down (predators determine the structure of the ecosystem). In reality, however, it is difficult to distinguish top-down from bottom-up control in nature. Nonetheless, we should continue to seek information regarding top-down and bottom-up control. Based on above-mentioned ideas, we underline the importance of the analyzes of grazing and nutrient availability. The analyzes may implicate some ideas for future biomanipulation.

Mesocosms provide an excellent means for conducting ecosystem analysis because an actual water column at a given site is enclosed, and thus the chemical, biological and ecological conditions within the mesocosm resemble those in nature. Moreover, mesocosms are closed systems with respect to mass, which enables repetitive sampling from the same body of water in order to analyze the causality of observed ecosystem changes. Furthermore, mesocosms also permit the manipulation of variables such as the nutrient conditions in order to better understand how ecosystems operate under different conditions. This is the strength of mesocosm experiments compared to field analyses.

The mesocosm used in this study was developed in the Seto Inland Sea, Japan, and it incorporates a vertical mixing system (Figures 1 and 2) (Watanabe *et al.*, 1995); turbulence is sufficient to keep non-motile phytoplankton such as diatoms in suspension and the physical conditions (mixing regime) in the water column can be manipulated, both aspects which were major problems in previous mesocosm designs (Sanford *et al.*, 1997).

Phytoplankton succession within the mesocosm was analyzed using data obtained in 1991, taking into account features of phytoplankton species e.g. silica requirement, motility, size, however, we could not distinguish between top-down and bottom-up control (Harada *et al.* 1996). Data indicating the varying species interactions at different nutrient levels are insufficient at present. Also, information concerning grazing activities are necessary. Thus, we analyzed top-down and bottom-up control using PLT (a parameter for zooplankton grazing activity: defined in Koshikawa *et al.* 1996,1999) in this paper.

We defined top-down and bottom-up control as follows. Top-down control is considered to control the system when phytoplankton abundance is lowered by high PLT* (: see Material and Method) while nutrient levels remain stable. Bottom-up control is considered to be active when phytoplankton abundance is lowered when nutrients are decreased and PLT* is also low (see also Discussion).

Here, we analyzed phytoplankton succession for different chemical, biological and ecological conditions occurring during summer based on the results of experiments conducted in 1991, 1992 and 1994. Based on the results of stable carbon isotope experiments (Koshikawa *et al.*, 1996, 1999), which were conducted in parallel with the three mesocosm experiments, we discuss factors affecting the succession of short-term phytoplankton blooms in the experimental area during summer.

2. Materials and Methods

2.1 General Description of Mesocosm

The mesocosm from which the data used in this study was collected was developed in a cove off the Ieshima

Islands in the Seto Inland Sea, Japan as described in Watanabe *et al.* (1995). Briefly, the enclosure was 5 m in diameter and 18 m deep and constructed from translucent, non-toxic ethylenevinylacetate reinforced with

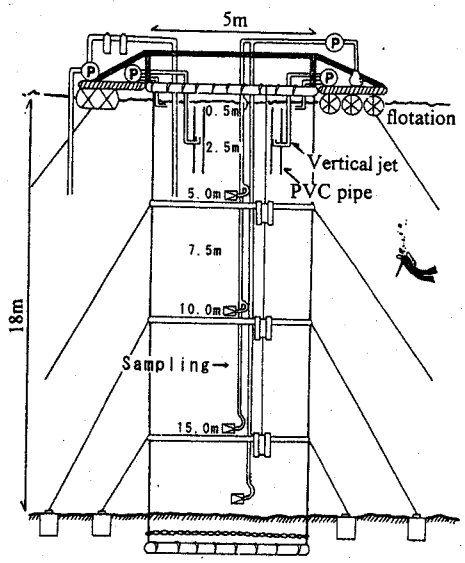

Figure 1 Schematic side profile view of the mesocosm. P indicates pump (after Harada et al. (1996))

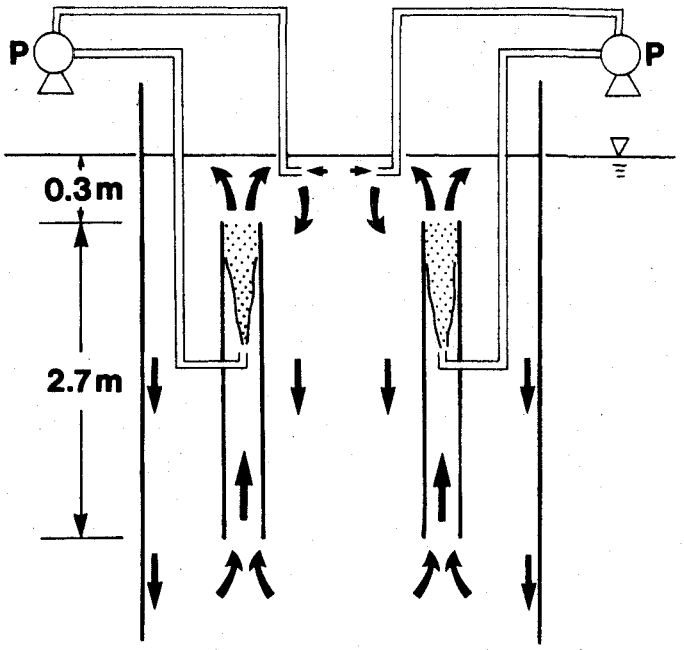

Figure 2 Schematic view of the internal circulation system (after Watanabe et al.(1995))

polyester grids (flexible thus free from tidal effects). The top rim of the enclosure was fixed to a flotation module and the weighted bottom rim was positioned in the sediment by scuba divers. The surface layer (0-5 m) was vertically mixed using a circulation system comprised of vertical jets connected to two PVC pipes (L = 2.7 m, φ = 21 cm) installed within the surface layer and pipes suspended at various depths in the water column, which produced very slow vertical circulation (Watanabe *et al.*, 1995). The other manipulation was nutrient enrichment (Watts and Bigg, 2001). Each year, the depth and level of enrichment was determined and nutrients were mixed into the water column by adjusting the vertical depth of the horizontal jet nose.

2.2 Experimental Design

We attempted to establish different chemical, biological and ecological conditions in each of the three mesocosm experiments by means of nutrient enrichment and selection of the starting date. The factors for nutrient enrichment are quantity, timing and enrichment depth of nutrients. Starting date affects initial conditions of the experiments.

In 1991, the mesocosm was deployed from July 24 to August 12. Before enclosing the water column, a bloom of centric diatoms (mainly *Chaetoceros curvicetus, C. distance, C. pseudocurvicetus* and *Skeletonema costatum*) had occurred at the site (Figure 3(b)). Nutrients were added on July 24 (500 g $NaNO_3$, 60 g $NaH_2PO_4 \bullet 2\ H_2O$, 50 g $Na_2SiO_3 \bullet 9\ H_2$ each corresponds to 16, 1.1 and 1.1 μM) to the whole mesocosm. The same nutrients were added to only (250 g $NaNO_3$, 30 g $NaH_2PO_4 \bullet 2\ H_2O$, 50 g $Na_2SiO_3 \bullet 9\ H_2O$ each corresponds to17, 1.2 and 3.3μM) to the lower portion (below 6 m) of the mesocosm on July 30, August 4 and August 9.

In 1992, the mesocosm was deployed from July 31 to August 11. Before enclosing the water column, a small bloom of centric diatoms (mainly *Chaetoceros distance, C. lauderi, C. lorenzianus, Dactyliosolen mediterraneus* and *Skeletonema costatum*) had occurred at the site (see also Figure 4(b)). Nutrients at levels several times higher than those of 1991 were added only once on July 31 (1500 g $NaNO_3$, 180 g $NaH_2PO_4 \bullet 2H_2O$, 450 g $Na_2SiO_3 \bullet 9\ H_2O$ each corresponds to 86. 3.3 and 17 μM) to the 0- 10-m water column.

In 1994, the mesocosm was deployed from July 31 to August 10. Before enclosing the water column, a red tide of *Gymnodinium mikimotoi* had occurred at the site (Figure 5(b)). Nutrients at levels similar to that used in 1991 were added only once on July 31 (334 g $NaNO_3$, 31 g $NaH_2PO_4 \bullet 2\ H_2O$, 76 g $Na_2SiO_3 \bullet 9\ H_2O$ each corresponds to 11, 0.6 and 1.7 μM) to the entire water column.

2.3 Measurements

Vertical profiles (depths: 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 m) of pH, DO and seawater temperature were measured at 9:00 am almost daily with a Surveyor II (Hydrolab Co.: Watanabe et al. 1995).

Seawater samples were collected at fixed depths (0.5, 2.5, 5.0, 7.5, 10.0 and 15.0 m) at 9:00 am almost every day. Part of each sample was filtered through a Whatman GF/F glass fiber filter, and filtrates were stored at -20°C until analysis of NO_2+NO_3-N, NH_4-N, PO_4-P and $Si(OH)_4$-Si (by Technicon AutoAnalyzer) in a laboratory at the National Institute for Environmental Studies. Every other day, another portion of each seawater sample from each depth was used to identify and enumerate phytoplankton and zooplankton species (Watanabe

et al., 1995).

Nutrients, phytoplankton and zooplankton species, and PLT* (see below) as a grazing index (see below) within the 0-5 m portion of the water column (averages at 0, 2.5 and 5 m) were monitored for 11 days. The variations in nutrients at 0 and 2.5 m were similar with those in 0-5 m average.

2.4 Tracer Experiment Using Stable Carbon Isotope

Tracer experiments were conducted as previously (Koshikawa *et al.*, 1996); seawater was collected at 0.5 m from the mesocosm at 9:00 am using a 10-l Van Dorn sampler, and then transferred to acid cleaned 4.5-l transparent polycarbonate bottles into which [^{13}C] $NaHCO_3$ (ISOTEC Inc. 91.5 mg per bottle) was introduced, since the use of radioactive isotopes in the field is strictly prohibited in Japan. The bottles were suspended *in situ* for 4 h, until 13:00, at the depth of 0.5 m depth at the collection site. Incubated samples were fractionated into different size fractions by sequential filtration using plankton nets (200, 100, 20 μm mesh size) and filtered on precombusted glass fiber filters (Whatman GF/F; 450°C for 4 h) (Koshikawa *et al.*, 1996). Particles collected in each of the plankton nets were washed with GF/F-filtered seawater to remove particles smaller than plankton net mesh size. After washing, the particles were collected in GF/F-filtered seawater and filtered on precombusted GF/C filters(450°C for 4 h). All filters were stored at -20°C until analysis of POC (particulate organic carbon) and ^{13}C abundance (atom%) determined using an Elemental Analyzer (Fisons EA1108) and an Isotope Ratio Mass Spectrometer (Finnigan Mat 252/B). The amount of net tracer transformation from dissolved to particulate carbon for each size was fraction calculated as the amount of excess ^{13}C ($^{13}C_{ex}$) against natural abundance, as follows (equation 1)

$$^{13}C_{ex}\ (\mu g\ ^{13}C\ l^{-1}) = (a_s - a_n) \times POC \qquad (1)$$

where a_s and a_n are the ^{13}C atom% in the incubated sample of a given size-fraction, and in a natural sample, respectively, and POC is the particulate organic carbon [μg l^{-1} C] in a given fraction. Grazing activity, which we defined as the percentage label transfer (PLT), was calculated using the sum of $^{13}C_{ex}$ accumulated in the >200 μm and 100-200 μm fractions ($^{13}C_{ex,>100\mu m}$) as a percentage of all the ^{13}C excess ($^{13}C_{ex,all}$) as follows (equation 2)

$$PLT\ (\%) = (^{13}C_{ex,>100\mu m} / ^{13}C_{ex,all}) \times 100 \qquad (2)$$

PLT used in this study represents the proportion of particulate carbon produced by phytoplankton during the 4-h of incubation that could be transferred and retained in metazooplankton by their feeding activities. PLT is a time-dependent value and differs from the energetic efficiency parameter proposed by Lindeman (1942). As the aim of this study was to qualitatively ascertain temporal changes in grazing activities based on the carbon transfer from phytoplankton to metazooplankton, we considered PLT to be sufficient for this purpose. Koshikawa et al. (1996, 1999) defined PLT in the photosynthetic pathway and that of bacterial pathway. Here we use only the former PLT.

The absolute values of PLT were differed in 1991, 1992 and 1994 experiments due to the differing abundances of phytoplankton and zooplankton. While absolute PLT values are not necessary for our analysis,

relative variation of PLT should be determined for each year. Thus, PLT in each year was modified as follows;

$$PLT^* = PLT(i) / PLT(average) \quad (3)$$

where PLT(i) is PLT of day i and PLT(average) is average PLT in each year. Thus, we were able to determine relative variation of PLT for each day during the experiments run in different years.

3. RESULTS

Each year, mixing regimes, and thus variations in seawater temperature, DO, etc. were similar to those reported by Watanabe et al. (1995) as stratification developed at 5 m with surface mixing layer.

3.1 1991 Experiment

Nutrient levels decreased quickly during the initial 6 days (Figure 3(a)). Centric diatoms were abundant initially, but decreased quickly (extraordinarily large cells such as *Coscinodiscus* may not have been sufficiently suspended by the circulation system, whereas the major species remained suspended) over the 6-day course of the experiment (Figure 3 (b)) with timing that was nearly coincident to that of the silicate decrease (Figure 3(a) and (b)). After the centric diatom bloom, pennate diatoms showed a small peak in abundance (Figure 3(b)). Dinoflagellates (mainly *Prorocentrum triestinum*) were dominant during the latter half of the experimental period, during which period all nutrients were in short supply (Figure 3(a) and (b)). Zooplankton and PLT* showed similar variations (Figure 3(c) and (d)). At the beginning of the experiment, copepods were dominant, whereas doliolids were dominant at the end of the experiment. At the latter sampling periods, higher PLT*s were recorded (Figure 3(d)), while PLT* was low during the middle period of the experimental period when zooplankton were of very low abundance (Figure 3 (c) and (d)).

3.2 1992 Experiment—different nutrient levels

All nutrients were abundant during the early period of the experiment (Figure 4(a)), with silicate decreasing by the middle of the experiment (Figure 4(a)). Centric diatoms decreased before silicate was exhausted (Figure 4(b)). Copepods were dominant within the zooplankton community (Figure 4(c)). PLT* was high only at the beginning of the experimental period, suggesting that zooplankton were active only during the initial few days (Figure 4(d)). Doliolids were not abundant at the end of the experimental period (0.6 inds l^{-1}), but were expected to have increased, as in 1991 and 1994, if the experimental period had been longer.

3.3 1994 Experiment—different initial plankton species compositions

All nutrients were exhausted by the middle of the experimental period (Figure 5(a): The axes of (a) are different from those used in the other figures). Dinoflagellates (*Gymnodinium mikimotoi*) were predominant at

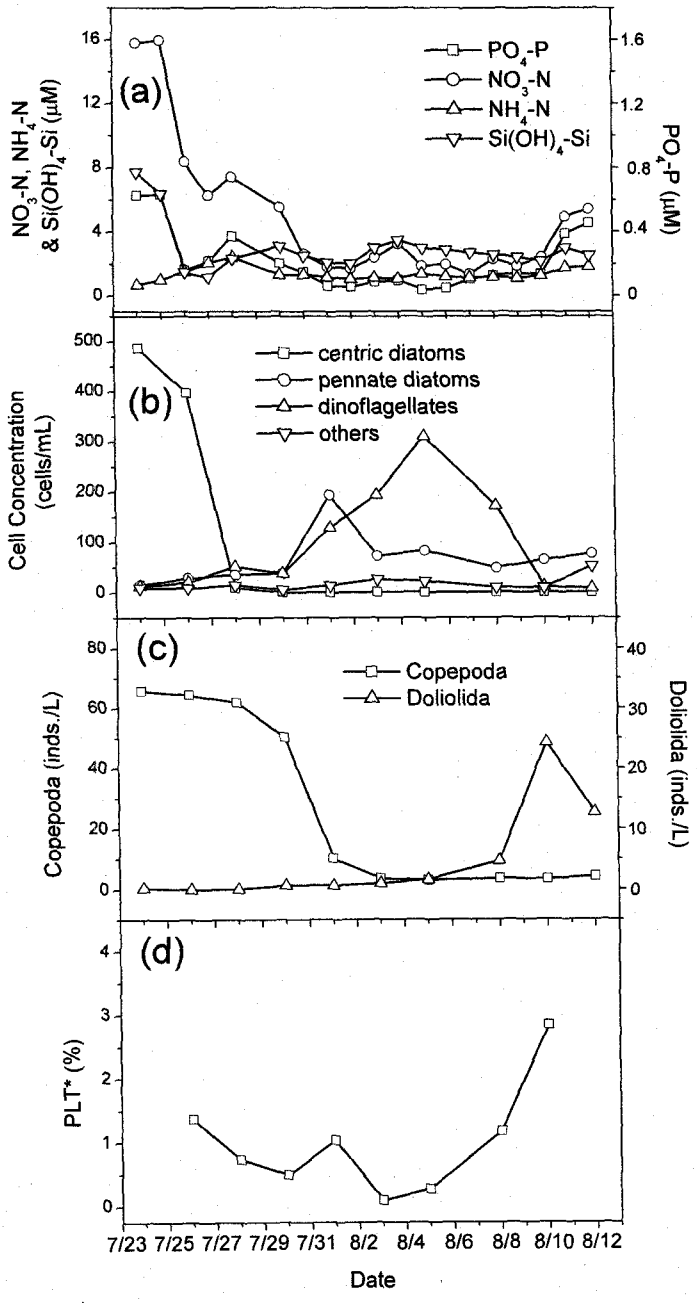

Figure 3 Time-series data in the 1991 experiment (0-5 m) for (a) nutrient concentrations; (b) phytoplankton abundance and distribution; (c) zooplankton abundance and distribution; (d) PLT * (after Koshikawa *et al.* 1996)

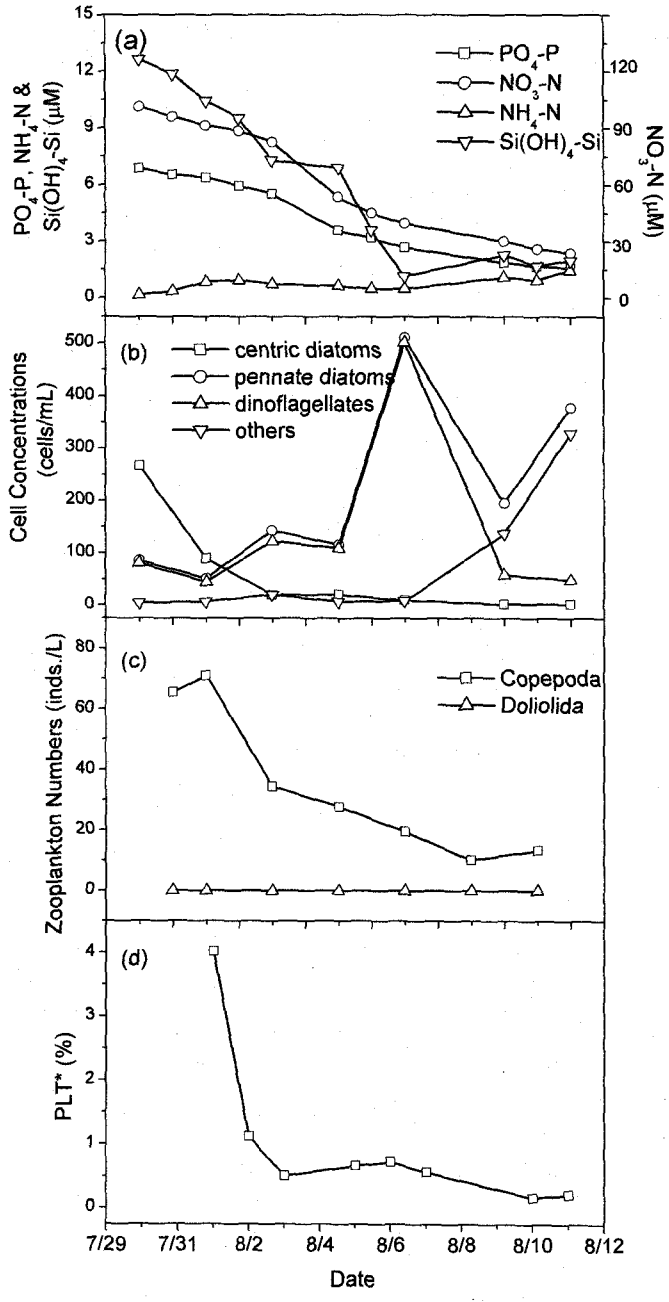

Figure 4 Time-series data in the 1992 experiment (0-5 m) for (a) nutrient concentrations; (b) phytoplankton abundance and distribution; (c) zooplankton abundance and distribution; (d) PLT*

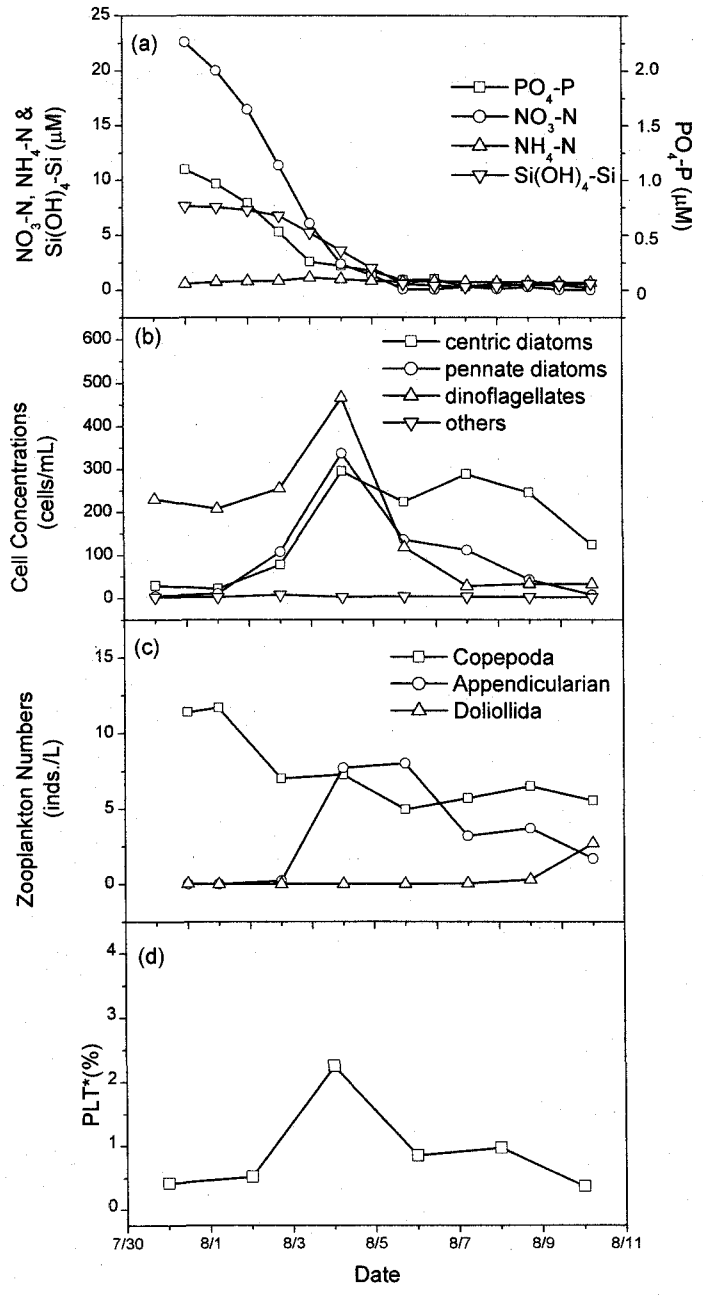

Figure 5 Time-series data in the 1994 experiment (0-5 m) for (a) nutrient concentrations; (b) phytoplankton abundance and distribution; (c) zooplankton abundance and distribution; (d) PLT * (after Koshikawa *et al.* 1999)

The beginning of the experimental period and increased following nutrient enrichment (Figure 5(a) and (b)). Centric and pennate diatoms also increased following nutrient enrichment (Figure 5(b)). Dinoflagellates and pennate diatoms decreased to nearly zero cells ml^{-1} by the end of the experiment (Figure 5(b)). However, there was no decrease in centric diatom abundance despite the lack of nutrients, until Aug. 8 (Figure 5(a) and (b)). Copepods were comparatively abundant throughout the experimental period (Figure 5(c)). Appendicularian and doliolids increased during the middle and end of the experimental period, respectively. High PLT* was observed during the middle of the experimental period when appendicularian became dominant (Figure 5(c) and (d)). PLT*s remained constant, although below peak levels, during the latter half of the experimental period (Figure 5(c) and (d)).

4. DISCUSSION

In order to analyze phytoplankton succession, an experiment with high nutrient enrichment was conducted in 1992, and experiment during a red tide bloom was done in 1994. These experiments provided insights into various phytoplankton-zooplankton interactions under different nutrient conditions.

We previously proposed that both bottom-up (nutrient availability) and top-down (grazing activity) control were exerted on phytoplankton succession in 1991 (Harada *et al.*, 1996). In particular, we suggested that the rapid decrease in centric diatoms at the beginning of the experimental period in 1991 could be explained both by the effects of low nutrient availability and high grazing pressure (Figure 3). However, although the results of the 1992 and 1994, the experiments suggest the greater role of grazing (top-down control). In particular, at the beginning of the 1992 experiments, centric diatoms decreased quickly even when nutrients were abundant (Figure 4 (a) and (b)). Furthermore, at the middle of the 1994 experiments, centric diatoms did not decrease until Aug. 8, even when all the nutrients were exhausted (possibly nutrients were not depleted inside the cells of the phytoplankton that emerged during this period.) (Fig.5 (a) and (b)). These results can be explained by the patterns of PLT* in 1992 and 1994: 1) in 1992, PLT* was high at the beginning of the 1992 experiments, implying that strong effects of grazing resulted in phytoplankton decrease and 2) in 1994, PLT* was low at the end of the 1994 experiments, implying that weak effects of grazing resulted in maintenance of phytoplankton.

Moreover, the decrease in dinoflagellates and pennate diatoms at the end of both 1991 and 1994 could be explained by the grazing pressure of doliolids. In contrast, in 1992, doliolids were not evident at the end of the experimental period (0.6 inds l^{-1}), resulting in the increase in pennate diatoms and dinoflagellates. The present results underline the significant effect of zooplankton grazing, together with nutrient availability, on phytoplankton species succession.

We did not carry out detailed analysis of the ecological relationships among zooplankton, planktivorous and pistivourous fish within the mesocosm. However, the presence of fish inside the mesocosm was confirmed each year. Thus, we believe our analyses on the relationship between phytoplankton and zooplankton are applicable to natural conditions. However, a precise characterization of the relationships of phytoplankton and zooplankton with planktivorous and piscivorous fish needs to be performed, preferably in larger scale and/or at near natural environments (more so than our mesocosm) before considering any actual biomanipulations.

Our definition of top-down and bottom-up control might be inaccurate when both phytoplankton and zooplankton composition is affected simultaneously by other factors, such as temperature.

It is important to note that the present results cannot rule out the potential importance of nutrient availability due to the short-term nature of the experiment period. Nutrient availability appears to more strongly impact the dominant species, as shown in the 1994 experiment. More research, including laboratory studies, is necessary.

5. CONCLUSIONS

Mesocosm experiments conducted on either a highly nutrient enriched mesocosm (1992) or an mesocosm initially in a state of red tide bloom (1994), together with carbon isotope experiments and resulting PLT* values, indicated that the short-term dominant phytoplankton species succession (2-3 weeks) was likely to be affected mainly by grazing activity (top-down control) together with the control of nutrient availability (bottom-up control).

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