

To sink or to be lysed? Contrasting fate of two large phytoplankton species in Lake Biwa

Maiko Kagami,¹ Tek Bahadur Gurung,² Takehito Yoshida,³ and Jotaro Urabe⁴
Center for Ecological Research, Kyoto University, Kamitanakami 509-3, Shiga, Otsu, Japan

Abstract

We estimated the contributions of sedimentation and cell lysis to the loss of two large phytoplankton species (*Fragilaria crotonensis* and *Staurastrum dorsidentiferum*) in Lake Biwa. Major loss process differed between species, and large phytoplankton did not necessarily function as a nutrient sink. The spring bloom of *F. crotonensis* was terminated by nutrient depletion and a subsequent increase in sedimentation rate. Although this species could be occasionally grazed by zooplankton, sedimentation eliminated nutrients from the surface. In contrast, the summer bloom of *Staurastrum dorsidentiferum* crashed mainly as a result of cell lysis caused by fungal infection within the surface mixing layer, which accounted for more than 75% of the mortality rate of this species. Cell lysis of *S. dorsidentiferum* may lead to nutrients within the surface mixing layer, supporting the production of bacteria and zooplankton. The different loss process of these two species implies that the function of phytoplankton in material flows cannot be assessed by cell size alone. Fungal parasitism can result in a different fate, which may play an important role in material cycling in lakes.

Phytoplankton production is lost from the pelagic zone through various processes, such as sedimentation, zooplankton grazing, and cell lysis due to pathogenic infection and physiological death (Reynolds 1984). Because cell or colony size of phytoplankton is one of the important factors determining sedimentation rates and vulnerability to zooplankton grazing (Smayda 1970; Lampert 1974), loss processes have been thought to differ between large and small phytoplankton species (Malone 1980; Legendre and Le Fèvre 1991; Kjørboe 1993).

Different loss processes of phytoplankton cause different material flows in aquatic ecosystems (Legendre and Le Fèvre 1991). Because small phytoplankton species (<20 μm) are in general highly vulnerable to zooplankton grazing, especially filter-feeding cladoceran zooplankton such as *Daphnia* (Lampert 1974; Sterner 1989; Kagami et al. 2002), their production is likely to enter the grazing chain and support the production of higher trophic levels. Large phytoplankton species (>20 μm) are, in contrast, less vulnerable to zooplankton grazing, although some large

phytoplankton species are grazed by calanoid copepods (Sommer et al. 2001; Kagami et al. 2002). According to the Stokes law, large phytoplankton sink faster than small phytoplankton (Smayda 1970; Kjørboe 1993). It is therefore believed that large inedible phytoplankton species are lost from the pelagic zone mainly through sedimentation, resulting in exporting the material to the profundal zone and supporting the production of benthic organisms (Malone 1980; Legendre and Le Fèvre 1991; Fitzgerald and Gardner 1993). Thus, size structure of phytoplankton, which affects the fate of phytoplankton production, can be crucial in channeling and determining the material flows in aquatic ecosystems. Cell lysis of phytoplankton may also affect material flows within the microbial food web if cell contents are released as dissolved organic matter and nutrients (van Boekel et al. 1992; Brussaard et al. 1996). It is still unknown, however, whether cell lysis occurs to a similar extent between different phytoplankton species with similar size.

In Lake Biwa, the largest lake in Japan with a surface area of 674 km² and a maximum depth of 104 m, phytoplankton species forming large colonies or having a large cell size (>20 μm), such as *Fragilaria crotonensis* (diatom) and *Staurastrum dorsidentiferum* (green algae), occur abundantly and contribute more than 50% to the total primary production during the stagnant period from spring to fall (Nakanishi 1976; Tezuka 1984; Kagami et al. 2002). Because of its large cell size (40–60 μm), *S. dorsidentiferum* is hardly grazed by zooplankton (Okamoto 1984; Kawabata 1987; Kagami et al. 2002). Because *F. crotonensis* develops large belt-like colonies (40–170 μm), it is also less vulnerable to *Daphnia* grazing (Kawabata 1987). Thus, a large part of primary production in Lake Biwa may be transported to the profound zone without being consumed by zooplankton. Nonetheless, estimations of the material fluxes indicated that the grazing rate of the zooplankton community in the pelagic zone corresponded closely with the primary production rate, and that the sedimentation rate of organic matter is only 10% of primary production during the stagnant period (Hama et

¹To whom correspondence should be addressed. Present address: Netherlands Institute of Ecology (NIOO-KNAW), Center for Limnology, Rijksstraatweg 6, 3631AC, Nieuwersluis, The Netherlands (m.kagami@nioo.knaw.nl).

²Present address: Fisheries Research Division, Nepal Agricultural Research Council, Katmandu, Nepal.

³Present address: Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York, 14853.

⁴Present address: School of Life Sciences, Tohoku University, Aoba, Sendai, 980-8578, Japan.

Acknowledgments

We thank M. Nakanishi and members of CER, Kyoto University, for their invaluable discussion. We are grateful to T. Ueda and T. Koitabashi for assistance in the field. We thank E. van Donk and A. Verschoor for comments.

This study was supported by a grant-in-aid for scientific research (10308025) and for Creative Basic Research (09NP1501) from the Ministry of Education, Science, Sports, and Culture of Japan, and a research fellowship from the Japan Society for the Promotion of Science for Young Scientists.

al. 1990; Nakanishi et al. 1992; Yoshimizu et al. 2001). Furthermore, the elimination rate of algal biomass (i.e., primary production rate—sedimentation rate) within the surface mixing layer correlated positively with the zooplankton biomass (Yoshimizu et al. 2001). Those evidences suggest that a large part of primary production is channeled into zooplankton, even if large phytoplankton species were not efficiently grazed (Okamoto 1984; Urabe et al. 1996). We therefore hypothesized that the large phytoplankton species in this lake do not necessarily sink to the bottom but disappear within the surface mixing layer as a result of cell lysis, which in turn may indirectly support zooplankton production via the microbial food web.

To test the hypothesis, we first examined the population dynamics of the two dominant large phytoplankton species, *F. crotonensis* and *S. dorsidentiferum*, over the course of 14 months in Lake Biwa. At the same time, gross growth rates of these species were estimated from laboratory experiments under ambient conditions and compared with net population growth rates to calculate the total loss rate in the surface mixing layer (>30 m). In order to assess the relative contributions of different loss factors to the total loss rates, sedimentation, cell lysis, and zooplankton grazing were also quantified.

Methods

Sampling, enumeration of phytoplankton, and chemical analysis—Phytoplankton sampling was done weekly from April 1997 to July 1998 at a pelagic site in the north basin of Lake Biwa. At each sampling date, lake water was collected from 8 depths (0, 2.5, 5, 10, 15, 20, 30, 45 m) with a 10-liter modified van Dorn sampler. Five hundred mL of lake water was fixed with 0.4% Lugol's solution (final concentration) and stored in the dark for enumeration of phytoplankton cells. The remaining water was used to analyze nutrient concentrations. For laboratory experiments, additional lake water was collected at 2.5 m and 10 m depth and brought to a nearshore laboratory while keeping them in a cool, dark place. Water temperature and light intensity were measured by a conductivity-temperature-depth (CTD) profiler (Sea Bird Electronics, USA) equipped with an underwater quantum meter (QSP-200L: Biospherical Instruments). The surface mixing layer was defined as a layer above the depth where water temperature changed 1.2°C per 1 m, which corresponded to the epilimnion during the stagnant period. The compensation depth was defined as the depth where 1% of photosynthetically active radiation at the surface remained.

Sedimentation rate of phytoplankton was measured monthly by use of sediment traps composed of four cylinders of 12 cm internal diameter and 70 cm height (Yoshimizu et al. 2001). These traps were moored at 30 m depth, which was always below the thermocline. To avoid the invasion of large amphipods (*Jesogammarus annandalei*), we installed a 1 mm mesh net on the top of each cylinder. After the traps were settled for 24 h, sedimented particles were collected and fixed with 0.4% Lugol's solution (final concentration) for the enumeration of phytoplankton.

Phytoplankton cells in the 500 mL samples were concentrated to 20 mL by sedimentation for at least 2 d, and counted by Sedgwick-Rafter cell at a 200 × magnification. The living and dead cells of *F. crotonensis* and *S. dorsidentiferum* were enumerated separately. We defined that cells with at least one optically distinguishable chloroplast were living (photosynthetically active) (Knoechel and Kalff 1975), and those without intact chloroplasts (empty cells) were dead. Note that this definition might underestimate the cell lysis rate because cells may die before the entire chloroplast disappears. However, we applied this definition in order not to overestimate the effects of cell lysis on population dynamics of phytoplankton species. We also estimated the fungal infection rate on phytoplankton cells under a phase contrast microscope at a 400 × magnification (Kagami and Urabe 2002). We only examined the number of the empty cells that were infected with zoospores or sporangia of fungi, because fungi behind/beneath cells with protoplasm were not visible.

For the measurements of dissolved inorganic nitrogen (DIN: ammonium + nitrite + nitrate) and soluble reactive phosphorus concentrations (SRP), lake water was passed through a precombusted GF/F filter. Ammonium, nitrite, and nitrate nitrogen were determined according to the methods of Sagi (1966), Bendschneider and Robinson (1952), and Mitamura (1997). SRP in the filtrate was analyzed by the ascorbic acid molybdenum blue method as described in Murphy and Riley (1962). For the determination of soluble reactive silicate concentrations (SRSi), lake water was filtered through a 0.2 μm Nuclepore filter, and further analyzed by the ascorbic acid molybdenum yellow method as described in Wetzel and Likens (2000).

Estimation of phytoplankton growth rate under different nutrient conditions—Short-term laboratory experiments were performed on each sampling date at a nearshore laboratory; ambient lake water was used to determine cell-specific growth rate of *S. dorsidentiferum* and *F. crotonensis*. Lake water containing phytoplankton assemblages from 2.5 m and 10 m depth were filtered through 200 μm mesh to remove large zooplankton. Then, the filtrate of each depth was diluted 30 times with 0.2 μm filtrate of the same lake water in order to reduce the possibility of changing nutrient levels during the incubation and grazing loss by small zooplankton (<200 μm). The 0.2 μm filtrate was prepared by gentle filtration through 0.2 μm Nuclepore filters. The diluted water from each depth was divided over two tissue plates with 96 holes (1.5 mL per hole; Iwaki). Nitrogen ((NH₄)₂SO₄ 15 μmol L⁻¹), phosphorus (KH₂PO₄ 1.5 μmol L⁻¹) and silica (Na₂SiO₃ 20 μmol L⁻¹) were added to one of the two plates (enriched treatments). The concentrations of these nutrients were chosen on the basis of the previous study (Kagami and Urabe 2001). The other plate received no nutrients and served as a control treatment. These plates were placed for 2 d in an incubator where temperature was set at ambient temperature of 2.5 m or 10 m. Light intensity was set at the averaged intensities at 2.5 m depth (480 μmol quanta m⁻² s⁻¹) and 10 m depth (50 μmol quanta m⁻² s⁻¹) during the study period, but length of the light period was adjusted to that of the actual

sampling date (sunrise to sunset). All holes were scanned on an inverted microscope at 100 × magnification and the initial and final cell numbers of the target phytoplankton species were counted. During this procedure, we carefully checked live and dead cells judged by the existence of intact chloroplasts. For the calculation of cell-specific growth rates, we excluded data of the holes where algal cells died during 2-d incubation, because inclusions of these data cause the underestimation of the growth rates due to cell lysis.

Data analysis—Net growth rate of each phytoplankton population (r : d^{-1}) between two samplings (x days' interval) was estimated as follows,

$$r = \frac{\ln(N_t) - \ln(N_{t+x})}{x} \quad (1)$$

where N_t and N_{t+x} are the integrated numbers (cells m^{-2}) of living cells in the layer from 0 m to 30 m depth at times t and next sampling date ($t+x$). Because sediment traps were suspended at 30 m depth, we focused on the population densities above this depth.

Gross growth rates of the population (μ) were calculated from cell-specific growth rates estimated in control treatment of the short-term laboratory experiments and actual vertical distributions of phytoplankton cells. The cell-specific growth rates at 2.5 m ($\mu_{2.5\text{ m}}^*$) and 10 m depth ($\mu_{10\text{ m}}^*$) in the experiments were estimated from the changes in the cell numbers during the 2-d incubation by assuming exponential growth. By using $\mu_{2.5\text{ m}}^*$ and $\mu_{10\text{ m}}^*$, the cell numbers at day $t+1$ (N_{t+1}) were calculated as follows;

$$N_{t+1} = N_{t,0-5\text{ m}} \times e^{\mu_{2.5\text{ m}}^*} + N_{t,5-15\text{ m}} \times e^{\mu_{10\text{ m}}^*} + N_{t,15-30\text{ m}} \quad (2)$$

where $N_{t,0-5\text{ m}}$, $N_{t,5-15\text{ m}}$, $N_{t,15-30\text{ m}}$ are integrated cell numbers (cells m^{-2}) at day t at each layer. Because the average 1% compensation depth during the study period was 15.5 m, cell growth rates below 15 m depth were assumed to zero. The gross growth rate (μ) was then calculated as,

$$\mu = \ln(N_{t+1}) - \ln(N_t) \quad (3)$$

Loss rate (l) was calculated from the difference between the gross growth rate (μ) and net population growth rate (r) as $l = \mu - r$. Finite loss rate (\bar{L} : cells $\text{m}^{-2} \text{d}^{-1}$) was calculated according to Caswell (1972) as follows,

$$\bar{L} = \frac{\mu - r}{r} (e^r - 1) \times N_0 \quad (4)$$

The finite loss rate includes sedimentation rate, grazing rate and cell lysis (Sommer 1984a). Sedimentation rate (\bar{N}_s , cells $\text{m}^{-2} \text{d}^{-1}$) was estimated from the number of living cells that were collected in the sediment traps during 24 h suspending. Sinking velocity ($\text{m} \text{d}^{-1}$) was calculated by dividing the cell numbers collected in the traps (cells $\text{m}^{-2} \text{d}^{-1}$) by those in the water column above the trap (cells m^{-3}) (Hargrave and Burns 1979). Cell lysis was

defined as the complete loss of the chloroplasts. Mortality rate due to cell lysis (\bar{N}_c , cells $\text{m}^{-2} \text{d}^{-1}$) was estimated according to Sommer (1984a),

$$\bar{N}_c = \bar{N}_{ds} + N_{t+1,d} - N_{t,d} \quad (5)$$

where \bar{N}_{ds} (cells $\text{m}^{-2} \text{d}^{-1}$) is the number of dead cells collected in the sediment traps, $N_{t,d}$ and $N_{t+1,d}$ (cells m^{-2}) are the numbers of dead cells in the layer (0 m to 30 m depth) layers at the start and end of the exposure time of the sediment trap, respectively. Because sediment traps were suspended only for 1 d while the population density in the surface layer was measured at interval (x) of 5 to 8 d, $N_{t+1,d}$ was estimated indirectly by using the number of dead cells at the next sampling date ($N_{t+x,d}$), as

$$N_{t+1,d} = N_{t,d} \times e^{\frac{\ln(N_{t+x,d}) - \ln(N_{t,d})}{x}} \quad (6)$$

The loss rate that was not accounted for by either sedimentation or cell lysis was treated as unexplained loss (\bar{N}_u , cells $\text{m}^{-2} \text{d}^{-1}$).

$$\bar{N}_u = \bar{L} - (\bar{N}_s + \bar{N}_c) \quad (7)$$

Note that \bar{N}_u includes grazing loss rates.

Results

Environmental conditions—Surface water temperature increased and thermal stratification developed from May (Fig. 1A,B). After September, surface water temperature declined and the thermocline moved to deeper depths. In January, water temperature decreased to 9°C and the lake water became vertically isothermal until April. The compensation depth ranged from 10 to 21 m with a mean of 15.5 m. It was deeper than the mixing layer from May to September but placed within that layer in the other months.

Dissolved inorganic nitrogen (DIN) concentration in the surface layer showed clear seasonal changes, decreasing from spring to summer and increasing from autumn to winter (Fig. 1C). Similar to DIN, soluble reactive phosphorus (SRP) concentration in the layer tended to increase from autumn to winter. During summer, SRP was low and close to the detection limit of the present analytical method (0.04 $\mu\text{mol L}^{-1}$). Soluble reactive silicate (SRSi) concentration was low in spring, increased from July onwards and reached to a maximum in September, and remained high until next spring.

Population dynamics of Staurastrum dorsidentiferum—*S. dorsidentiferum* was abundant in summer and fall in 1997 (Fig. 2). When the density of living cells decreased from October to November, empty dead cells of this species appeared abundantly in the surface layer. In that period, the vertical distribution of living cells moved to deeper layers in the course of the expansion of the surface mixing layer (Fig. 2B) while the empty cells tended to accumulate within the surface layer (Fig. 3, Kagami and Urabe 2002). The cell number increased again from April 1998.

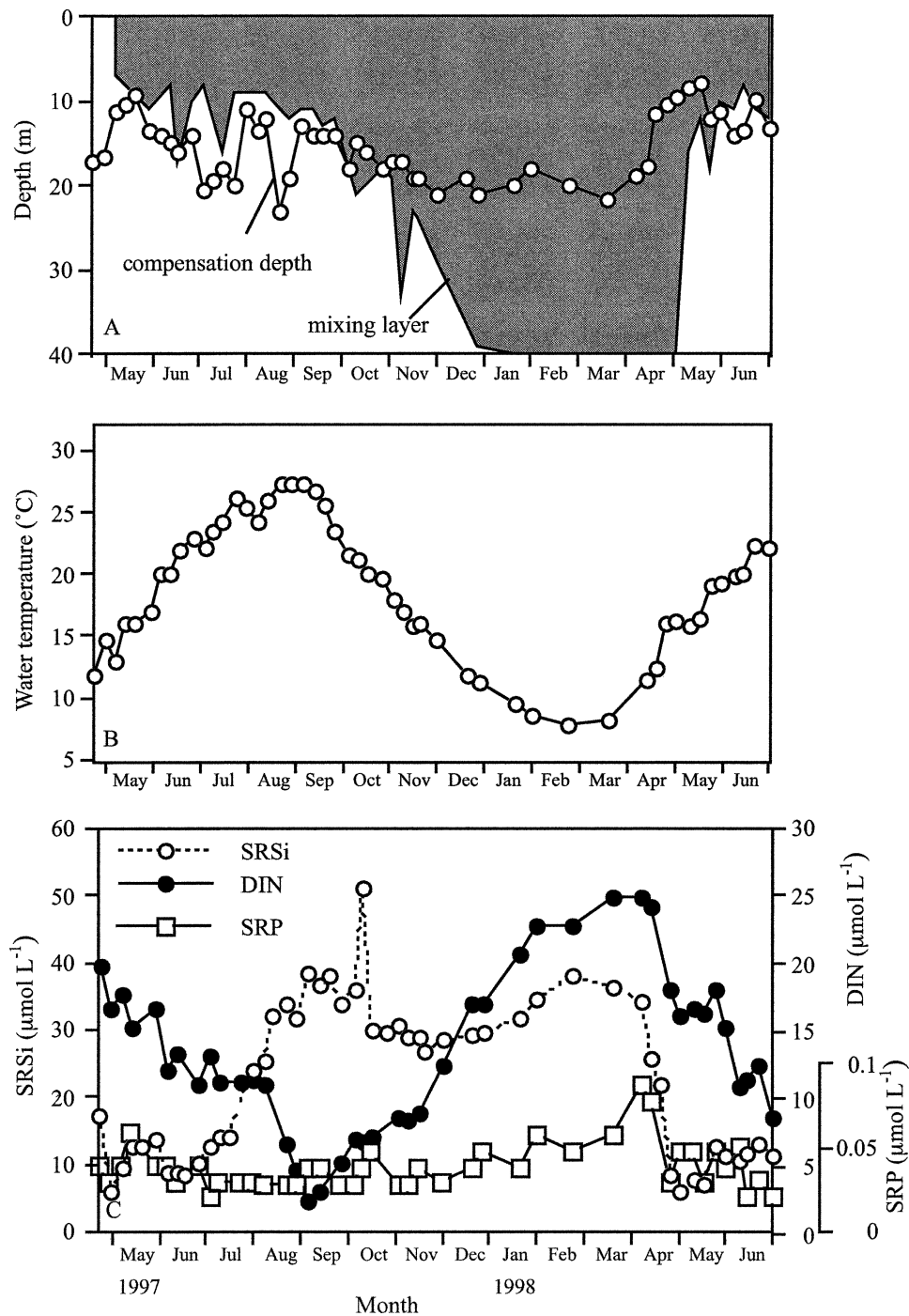


Fig. 1. (A) Temporal changes in mixing layer and compensation depth, (B) water temperature at 0 m depth, and (C) concentrations of soluble reactive silicate (SRSi), soluble reactive phosphorus (SRP), and dissolved inorganic nitrogen (DIN) from April 1997 to June 1998.

Cell-specific growth rate of *S. dorsidentiferum* in the control treatment at 2.5 m depth ($\mu^*_{2.5 m}$) was around 0.2 d^{-1} and sometimes exceeded 0.4 d^{-1} from spring to fall, but was less than 0.1 d^{-1} in winter (Fig. 4A). Compared with the control, the cell-specific growth rate in the enriched treatment was at most dates higher except during winter, suggesting that the gross growth rate of this

species was nutrient limited from spring to fall. Cell-specific growth rate at 10 m depth ($\mu^*_{10 m}$) was lower than that at 2.5 m depth ($\mu^*_{2.5 m}$) and almost similar between the treatments regardless of seasons (Fig. 4B).

From September to November, when the gross growth rate (μ) of *S. dorsidentiferum* population decreased slightly, net growth rate of the population (r) decreased temporally

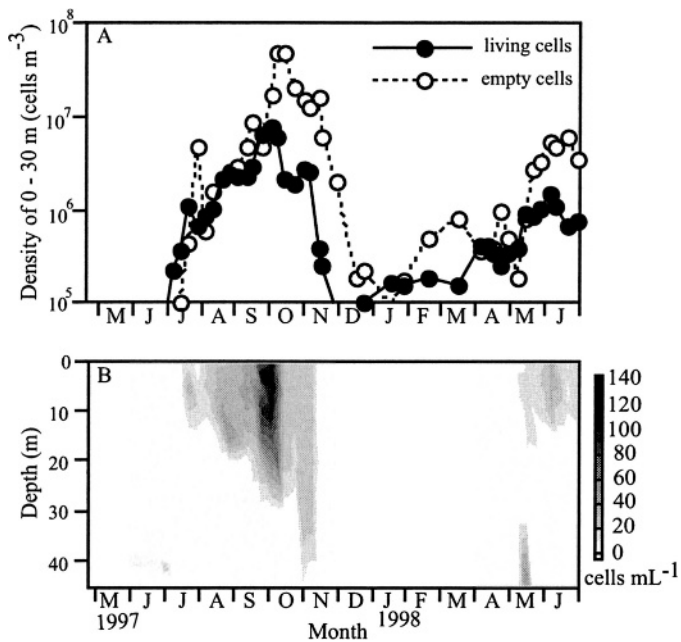


Fig. 2. (A) Temporal changes in living and empty cells, and (B) vertical profiles of cell density (cells mL⁻¹) of *S. dorsidentiferum* from April 1997 to June 1998.

much more and sometimes showed negative values (Fig. 4C,D). As a result, high finite loss rate (\bar{L} : cells m⁻² d⁻¹) was found during this period (Fig. 4D).

The majority of the cells collected in the sediment trap were empty cells (Table 1), especially from September to November when the population density decreased (Fig. 2A). Sinking velocities of the empty cells ranged from 1 to 10 m d⁻¹ (Table 1), and were always much higher than those of the living cells (paired *t*-test, *t* = 3.478, *p* = 0.01). Mortality rates due to cell lysis of *S. dorsidentiferum* were higher than the sedimentation rate

at most dates (Table 2), except for October 1997 and June 1998 when the estimates of the mortality rate due to cell lysis were negative. *S. dorsidentiferum* cells were infected by the parasitic fungus, *Rhizophydium couchii* (Fig. 5), and the percentages of infected empty cells reached more than 80% when the population density decreased (Fig. 6). The percentage of infected cells showed similar temporal pattern as that of the finite loss rate especially in October when the finite loss rate was high (Fig. 6), suggesting that the major loss factor in this season was fungal parasitism.

Population dynamics of Fragilaria crotonensis—*F. crotonensis* occurred abundantly during spring 1997 and 1998 in the layers shallower than 10 m deep (Fig. 7). It also appeared during late fall but the density was very limited. Living and empty cells showed similar seasonal change patterns, and the number of their empty cells was always lower than that of the living cells. In contrast to *S. dorsidentiferum*, there was no trend for the empty cells of *F. crotonensis* to accumulate in the surface layer.

Cell-specific growth rates of *F. crotonensis* in the control treatment at 2.5 m ($\mu^*_{2.5 m}$) were around 0.4 d⁻¹ in March, and decreased to 0.1 d⁻¹ by the end of April (Fig. 8A). Growth $\mu^*_{2.5 m}$ in the enriched treatment was relatively constant, and as high as 0.4 d⁻¹. Cell-specific growth rates at 10 m ($\mu^*_{10 m}$) were lower than those at 2.5 m in both control and enriched treatments. Similar to 2.5 m depth, the effect of nutrient enrichment was remarkable in April at 10 m depth, suggesting that the growth of this species was nutrient limited not only at 2.5 m depth but also at 10 m depth. Gross growth rate (μ) of *F. crotonensis* population decreased in mid-April, followed by decrease of the net growth rate of the population (*r*) and the increase of loss rate (*l*) (Fig. 8C,D). As a result, finite loss rate (\bar{L} : cells m⁻² d⁻¹) was high in late April (Fig. 8D). *F. crotonensis* collected in the sediment trap were mainly living cells (Table 1). Sinking velocities of the living and empty cells ranged from 0 to 5 m d⁻¹, and did not differ

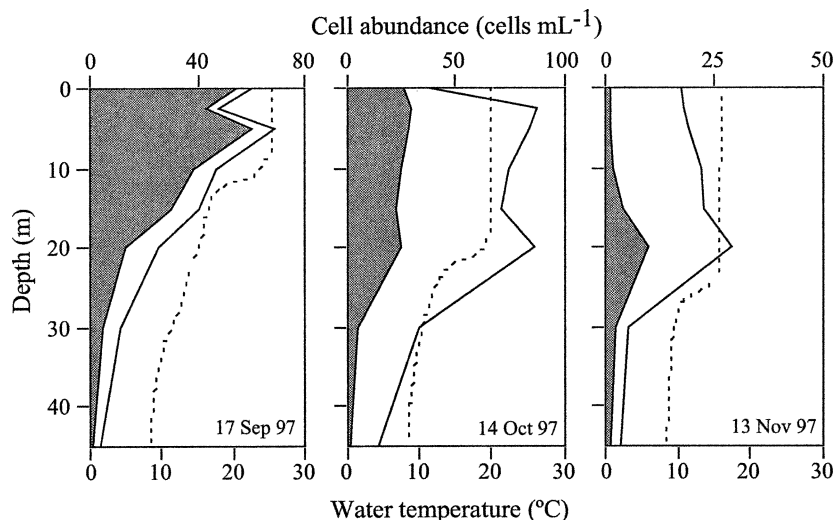


Fig. 3. Vertical profiles of water temperature (dotted line) and number of living cells (shaded area) and empty cells (open area) of *S. dorsidentiferum*.

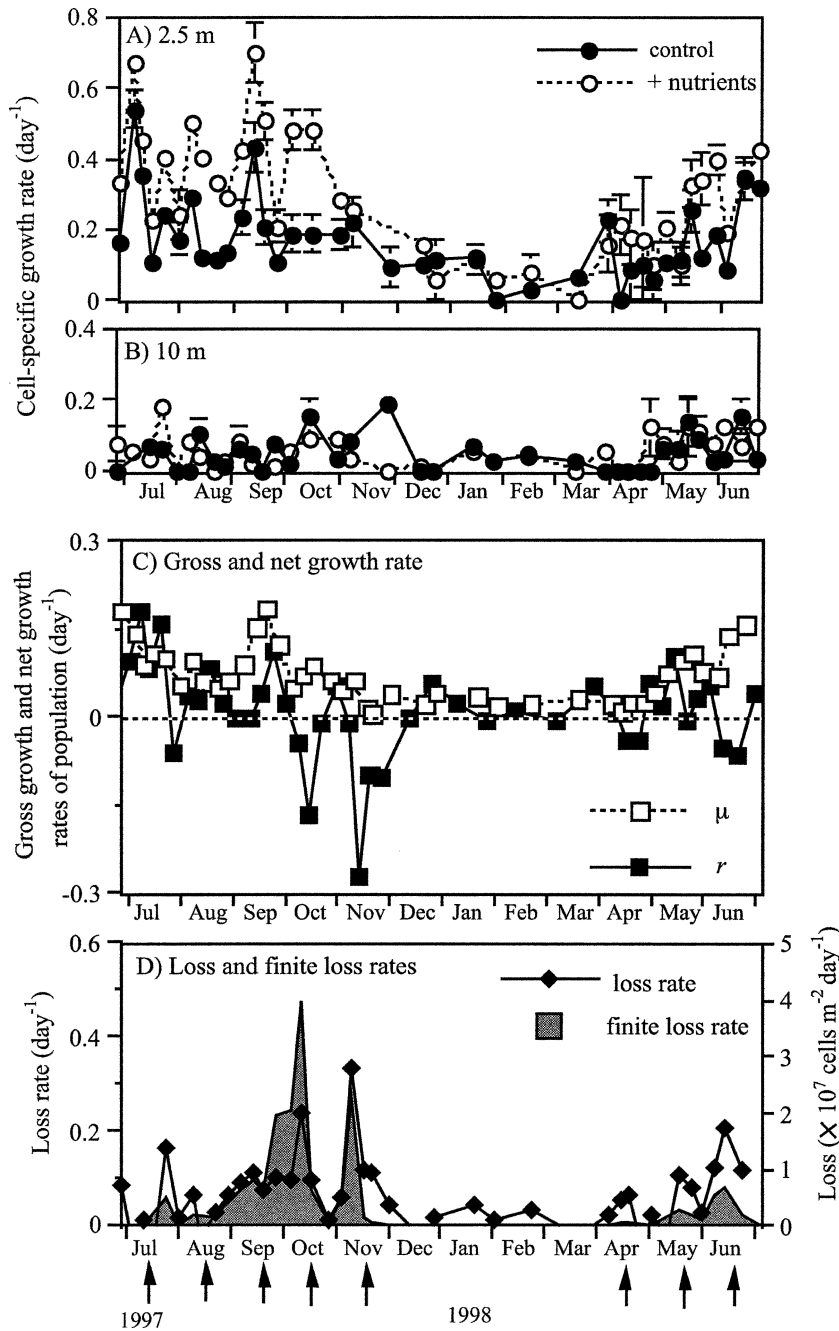


Fig. 4. Population dynamics of *S. dorsidentiferum* in the layer from 0- to 30-m depth from July 1997 to June 1998. (A) Cell-specific growth rate ($\mu^*_{2.5 m}$) with and without nutrients under 2.5-m depth condition. Vertical lines are standard errors. (B) Same as (A) but under 10-m condition ($\mu^*_{10 m}$). (C) Gross growth rate (μ) and net growth rate (r) of the population, and (D) loss rate (l) and finite loss rate (\bar{L}). Arrows in the graph indicate the date when sedimentation and mortality rate due to cell lysis were estimated.

significantly between living and empty cells (paired *t*-test: $t = 0.148$, $p = 0.896$), which was a contrast to *S. dorsidentiferum*. Sedimentation loss rates contributed to 70% of the total loss rates in *F. crotonensis* population in mid-May (Table 2). In mid-April and June, however, neither sedimentation nor cell lysis rates had large contributions to the total loss rates. Throughout the study

period, fungal parasites were not found in *F. crotonensis* population.

Discussion

S. dorsidentiferum—In *S. dorsidentiferum* population, the gross growth rate seemed to be limited by nutrients at

Table 1. Number of living and empty cells collected in the sediment trap. Sinking velocity was calculated by dividing the sedimentary flux by the concentration of settling particles above the trap according to Hargrave and Burns (1979).

	Density of sedimented cells ($\times 10^5$ cells $m^{-2} d^{-1}$)		Sinking velocity ($m d^{-1}$)	
	Living cells	Empty cells	Living cells	Empty cells
<i>Staurastrum dorsidentiferum</i>				
15 Jul 97	5	6	0.13	6.00
12 Aug 97	14	36	0.14	2.32
17 Sep 97	43	717	0.15	8.00
14 Oct 97	125	1,175	0.57	2.41
17 Nov 97	14	635	0.56	10.48
15 Apr 98	22	14	0.64	3.25
20 May 98	17	67	0.19	2.39
19 Jun 98	10	41	0.18	0.68
<i>Fragilaria crotonensis</i>				
15 Apr 98	95	0	0.69	0.00
20 May 98	125	32	4.66	5.36
19 Jun 98	3.3	2	0.14	0.31

2.5 m depth from spring to fall, because the cell-specific growth rate at 2.5 m depth was higher in the enriched treatment than that in the control treatment (Fig. 4A). On the other hand, cell-specific growth rate at 10 m depth was not stimulated by nutrient enrichment regardless of season (Fig. 4B), suggesting that at 10 m depth the growth of this species is limited by factors other than nutrients such as light and water temperature. Because water temperature did not largely differ between 2.5 m and 10 m depths during the stagnant period, the specific growth rate at 10 m depth was likely to be limited by the low light intensity.

From September to November, the gross growth rate decreased gradually and low net growth rates (<0) were frequently found (Fig. 4C), suggesting that relatively low gross growth rate and high loss rate jointly decreased the population density in this period. In October, the mixing depth moved to below the 1% compensation depth (Fig. 1A). This expansion of the surface mixing layer decreased mean light intensity in the surface mixing layer (Kagami and Urabe 2002). This decrease in the mean light intensity together with the decline in water temperature

could have limited the gross growth rate of *S. dorsidentiferum* in October. Kagami and Urabe (2002) showed that under low light intensity *S. dorsidentiferum* was easily infected by the chytrid parasite *Rhizophyidium couchii*. *S. dorsidentiferum* cells became empty shortly after being infected by this fungus (Kagami and Urabe 2002). It should be noted that a large number of empty cells was observed in the surface mixing layer from October to November (Fig. 4) when the loss rate of *S. dorsidentiferum* was high (Fig. 3C,D). In addition, the mortality rate due to cell lysis often took higher values than the sedimentation rate (Table 2). Furthermore, a large number of the cells (80% in maximum) were infected by the chytrid parasite *Rhizophyidium couchii*, and the finite loss rates were well correlated with the infection percentages of empty cells (Fig. 6). These results indicate that most of *S. dorsidentiferum* cells were lysed by fungal infection in the surface mixing layer before sinking to the bottom. Evidently the decrease in mean light intensity due to expansion of the surface mixing layer in the autumn ultimately decreased the population density of *S. dorsidentiferum* not only by

Table 2. Finite loss rate of sedimentation, cell lysis, and unexplained losses of *Staurastrum dorsidentiferum* and *Fragilaria crotonensis* populations in Lake Biwa. Values before corrected are shown in parentheses.

	Sedimentation	Cell lysis	Unexplained	Total loss
<i>Staurastrum dorsidentiferum</i> ($\times 10^5$ cells $m^{-2} d^{-1}$)				
15 Jul 97	4.9	13.2	-82.2	-64.1
12 Aug 97	14.4	52.9	-130.8	-63.5
17 Sep 97	42.5	488.6	122.5	653.6
14 Oct 97	124.6	0 (-5.3)	508.1	632.7
17 Nov 97	14.0	460.9	-395.9	79.0
15 Apr 98	21.8	30.2	11.7	63.7
20 May 98	16.5	91.1	98.9	206.6
19 Jun 98	10.2	0 (-65.4)	191.7	201.9
<i>Fragilaria crotonensis</i> ($\times 10^7$ cells $m^{-2} d^{-1}$)				
15 Apr 98	95	35	237	367
20 May 98	125	5.3	52.7	183
19 Jun 98	3	9	248	261

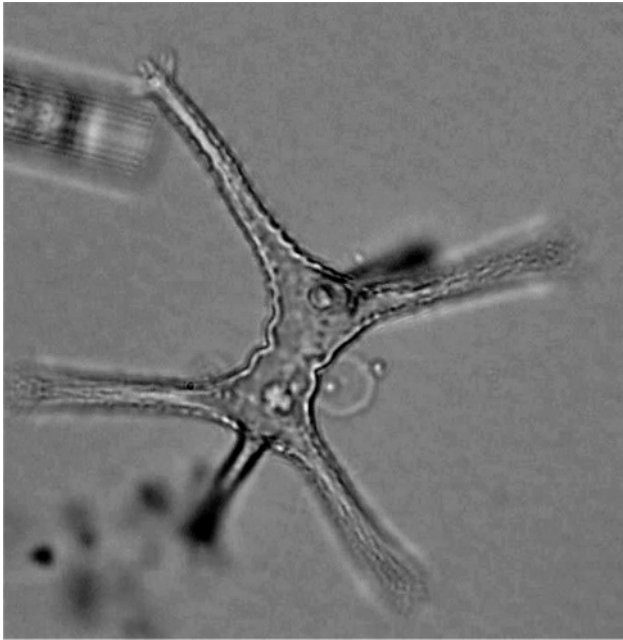


Fig. 5. Microscopic photograph of the parasitic fungi, *Rhizophydium couchii*, infecting the host *S. dorsidentiferum*.

decreasing the gross growth rate but also by increasing the mortality rate due to the fungal infection. Interestingly, empty cells of *S. dorsidentiferum* sank faster than living cells (Table 1). Sinking velocity of *S. dorsidentiferum* might be related to cell activity or cell contents such as lipids that would be consumed and thus lost by fungal infection.

In the present study, however, the total loss rate of *S. dorsidentiferum* population could not be explained by the fungal infection alone (Table 2). In July and August, the total loss rate (\bar{L}) was negative. These negative values were caused by slightly higher values in the net growth rate of population (r) compared with the gross growth rate of population (μ) (Fig. 4C). Because the population grew in this period, we might underestimate the gross growth rate of population (μ). The possibility suggests that total and thus unexplained loss rates may be higher than those shown in Table 2. The unexplained loss rate may be directly related with grazing of a copepod *Eodiaptomus japonicus* that dominated within the crustacean plankton in summer and autumn as well as the amphipod *Jesogammarus*

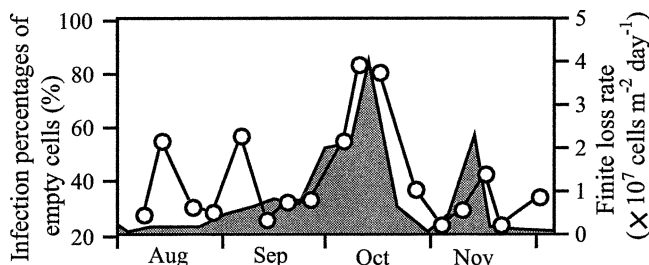


Fig. 6. Finite loss rate (\bar{L}) of *S. dorsidentiferum* (shaded area) and infection percentage of empty cells of this species (open circle) from August to November 1997.

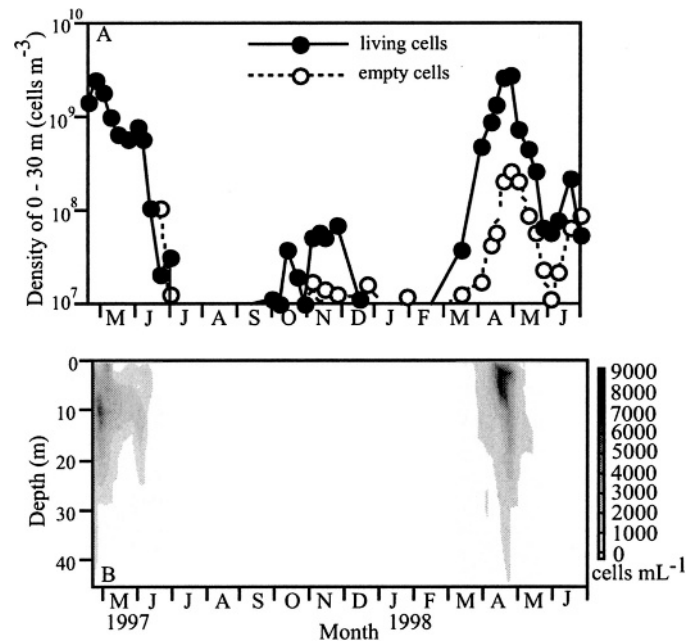


Fig. 7. (A) Temporal changes in living and empty cells, and (B) vertical profiles of cell density of *Fragilaria crotonensis* from April 1997 to June 1998. The density of empty cells was not measured from April to May 1997.

annandalei, because *S. dorsidentiferum* cells were found in their guts (Kawabata 1987; Ishikawa unpubl. data). However, grazing pressure by *E. japonicus* on *S. dorsidentiferum* was previously shown to be low (Kagami et al. 2002). In addition, *J. annandalei* was not so abundant in fall when *S. dorsidentiferum* dominated (Ishikawa and Urabe 2002). Therefore, the unexplained loss rate could not be well explained by grazing. It should be noted that unexplained loss rates calculated here accompany the artifacts in estimating cell lysis and sedimentation loss rates. In October 1997 and June 1998, we corrected mortality rates due to cell lysis to zero because they took negative values (Table 2). It is likely that negative values of the mortality rate due to cell lysis were caused by differences in time intervals between the sediment trap sampling and the lake water sampling (Eq. 6). In the present study, sediment traps were moored for 24 h once a month, while the lake water sampling was done at 7-d intervals. If a large number of empty cells sank when the sediment trap was not moored, the mortality rate due to cell lysis would be underestimated. Such a possibility implies that the mortality due to cell lysis could have been much higher than that estimated here, especially in October.

F. crotonensis—*F. crotonensis* occurred abundantly in spring, but the decrease in gross growth rates and subsequent increase in the loss rate depleted this population in May. From April to May, cell-specific growth rates of this species were lower in the control than the enriched treatments (Fig. 8). During this period, nutrient (DIN, SRP, SRSi) concentrations decreased. These results in-

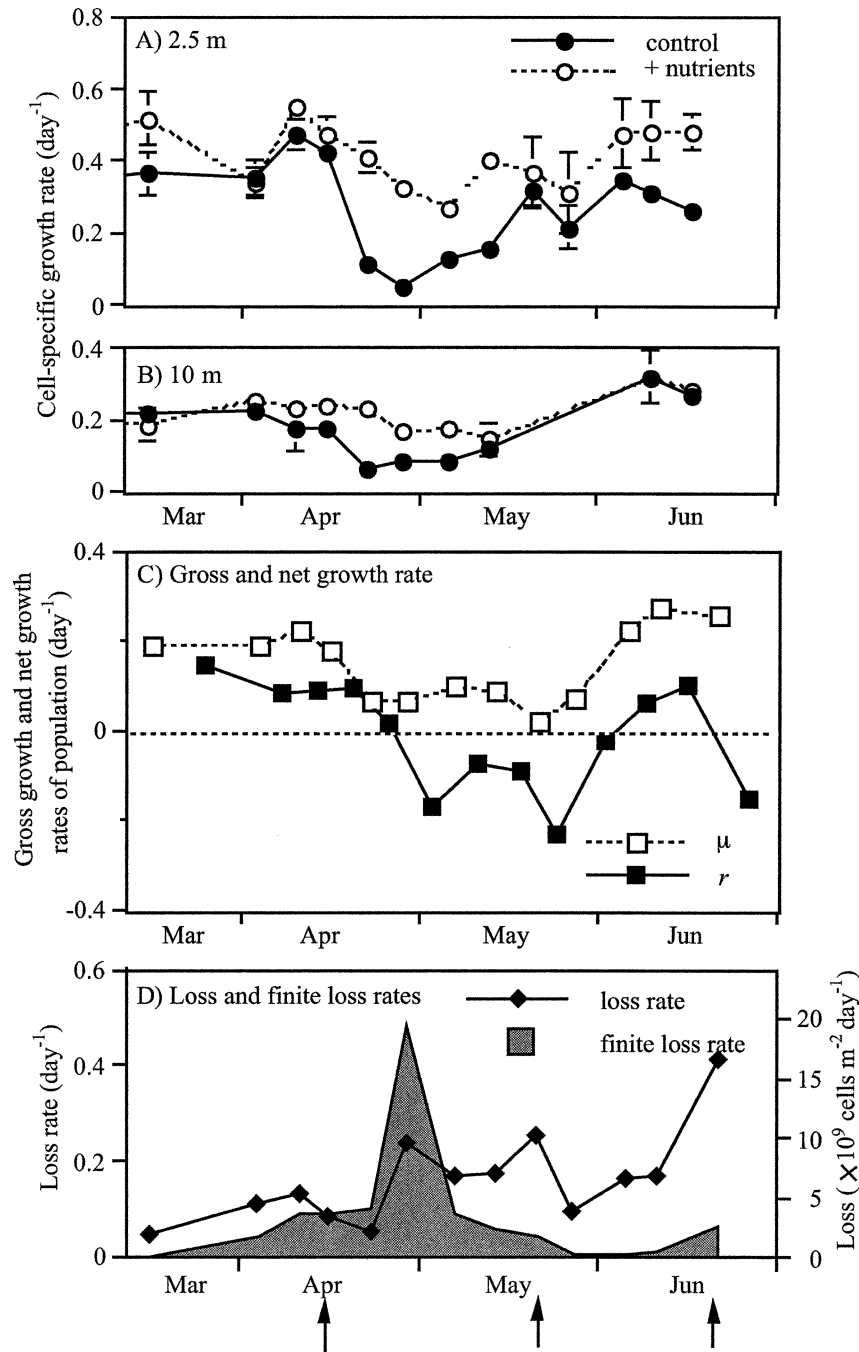


Fig. 8. Population dynamics of *Fragilaria crotonensis* in the layer from 0- to 30-m depth from March to June 1998. (A) Cell-specific growth rate ($\mu_{2.5 m}$) with and without nutrients under 2.5-m depth condition. Vertical lines are standard errors. (B) Same as (A) but under 10-m condition ($\mu_{10 m}$). (C) Gross growth rate (μ) and net growth rate (r) of the population, and (D) loss rate (l) and finite loss rate (L). Arrows in the graph indicate the date when sedimentation and mortality rate due to cell lysis were estimated.

dicate that the gross growth rate of *F. crotonensis* population was highly limited by nutrients from April to May. In May, sinking velocity of this species was high, suggesting that the increase in the loss rate in May was derived from the increased sinking rate. It is known that sinking velocity of phytoplankton species relates to the

onset of thermal stratification (Lund 1971; Knoechel and Kalff 1975; Reynolds et al. 1982), and/or to physiological or nutritional status of algal cells (Eppley et al. 1967; Reynolds and Wiseman 1982; Sommer 1984b). In the present study, sinking velocity of *F. crotonensis* was much lower in April and June when thermal stratification

developed, but was higher in May when its growth rate was limited by nutrients. Thus, it is likely that the increased sinking velocity in May was related to the depletion of nutrient supplies. Considering the similar sinking velocity between living and dead cells of this species, however, the increased sinking velocity in May cannot be attributable to changes in physiological or nutritional status within the cells. In May 1998, the lake was rapidly stratified (Fig. 1A). These rapid changes in water stratification regimes might accelerate the sinking velocities of both dead and living *F. crotonensis*.

In April and June, in contrast to May, neither sedimentation nor cell lysis contributed significantly to the loss rate of *F. crotonensis* (Table 2). A possible factor for those unexplained losses is zooplankton grazing. In Lake Biwa, *J. annandalei* was abundant from April to June (Ishikawa and Urabe 2002). During this period, *J. annandalei* migrated from the bottom up to 20 m depth just below the thermocline at night to feed and many *F. crotonensis* cells were found in their gut (Ishikawa unpubl. data). Thus, it is likely that *J. annandalei* population grazed *F. crotonensis* cells above 30 m depth, before these cells sank and were caught in the sediment traps. In addition, *E. japonicus*, abundantly occurred in April and June when the unexplained loss rate increased (Yoshida et al. 2001). *E. japonicus* is known to graze *F. crotonensis* efficiently (Okamoto 1984; Kawabata 1987; Kagami et al. 2002). These evidences suggest that the unexplained loss of *F. crotonensis* cells in April and June could be mainly due to zooplankton grazing.

Roles of large phytoplankton species in material flows—The present study showed that major loss processes differed between two large phytoplankton species. Specifically, loss of *F. crotonensis* was explained by sedimentation and grazing by *E. japonicus* and *J. annandalei*, while loss of *S. dorsidentiferum* was mainly caused by cell lysis (Table 2). Because both species comprised maximally 90% of the total phytoplankton biomass during their periods of dominance (Yoshida et al. 2001; Kagami 2002), the differences in their loss processes would bring up the different consequences to plankton community structure and material cycling in this lake. Sedimentation of *F. crotonensis* transports organic matter and nutrients to the lake bottom. Thus, as a classical view of pelagic-benthic coupling (Fitzgerald and Gardner 1993), this species functions to transport organic matter produced in the surface layer to benthic animals, such as *J. annandalei*, in the profound zone.

S. dorsidentiferum lost their cytoplasm by fungal parasitism, suggesting that most nutrients and organic matter within their cells were released and regenerated within the surface mixing layer. Cell lysis rates accounted for more than 75% of the decline of the *S. dorsidentiferum* population. High cell lysis rates have also been found in the marine systems (Brussaard et al. 1996; Agustí et al. 1998; Agustí and Duarte 2000). Brussaard et al. (1996) showed that cell lysis accounted for 75% of the decline of a *Phaeocystis* bloom, which is comparable to our estimates for Lake Biwa. In the Mediterranean sea, Agustí and Duarte (2000) estimated that cell lysis represented about

25% of the gross primary production. When the finite loss rate of cell lysis of *S. dorsidentiferum* in Lake Biwa is converted to carbon units by means of cell-specific carbon contents (3,345 pg cell⁻¹, Verity et al. 1992), the loss flux corresponds to 163 mg C m⁻² d⁻¹. This number accounts for 25% of primary production in September (660 mg C m⁻² d⁻¹; Yoshimizu et al. 2001). The value of primary production in Lake Biwa was likely an underestimation of a gross primary production because it was measured by C¹³ method. In addition, the organic carbon in cell walls is likely lost by sedimentation without being consumed within the surface mixing layer. Thus, the contribution of cell lysis of *S. dorsidentiferum* to the loss of the gross primary production would be smaller than 25%. Given that our estimation of cell lysis rate was based only on single phytoplankton species, however, cell lysis rate in whole phytoplankton community in Lake Biwa is probably higher than estimated here. Therefore, the present estimate, together with the result in the Mediterranean sea (Agustí and Duarte 2000), does suggest that cell lysis is an important loss process of primary production in summer not only in marine ecosystems but also lakes.

High mortality of *S. dorsidentiferum* due to cell lysis caused by fungal parasitism may explain the contradictory observations reported in Lake Biwa, where zooplankton eliminated a large part of primary production (Yoshimizu et al. 2001) in spite of the fact that large less edible phytoplankton species dominated. The parasitic fungus can be grazed by zooplankton when it is in the zoospore stage (Kagami et al. 2004). In addition, through DOM release, cell lysis of phytoplankton stimulates growth of microorganisms (van Boekel et al. 1992; Brussaard et al. 1996), which in turn can be grazed by zooplankton (Yoshida et al. 2001). Thus, although zooplankton cannot always utilize the production of large phytoplankton directly, they may use a large part indirectly by grazing on fungal zoospores and heterotrophic organisms in the microbial food web (Azam et al. 1983). Because of such indirect processes, the sedimentation rate was low in Lake Biwa (Urabe et al. 2005) when large inedible phytoplankton species dominated during summer.

Large phytoplankton species that are less vulnerable to zooplankton grazing may be lost from the surface layers by sinking. However, the present study showed that cell lysis due to fungal infection was a major loss factor to *S. dorsidentiferum* but not to *F. crotonensis* in Lake Biwa, suggesting that fate of the production of large phytoplankton differs among species. Because cell lysis within the surface mixing layer will cause retention of nutrients, it may serve to sustain the pelagic food webs in the upper mixed layer. Recent studies have shown that viral infection is one of the important loss factors of eukaryotic marine phytoplankton (Fuhrman 1999). Other than fungi, viruses may also contribute to cell lysis of algal species (Kirchman 1999). In addition to cell and colony size that relates functionally to sinking velocity and vulnerability to zooplankton grazing, other species-specific features, such as vulnerability to fungal and viral infections, should be taken into account when the function of phytoplankton community structure in the material flow is considered.

References

- AGUSTI, S., AND C. M. DUARTE. 2000. Strong seasonality in phytoplankton cell lysis in the NW Mediterranean littoral. *Limnol. Oceanogr.* **45**: 940–947.
- , M. P. SATTÀ, M. P. MURA, AND E. BENAVENT. 1998. Dissolved esterase activity as a tracer of phytoplankton lysis: Evidence of high phytoplankton lysis rates in the northwestern Mediterranean. *Limnol. Oceanogr.* **43**: 1836–1849.
- AZAM, F., T. FENCHEL, J. G. FIELD, J. S. GRAY, L. A. MEYER-REIL, AND F. THINGSTAD. 1983. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* **10**: 257–263.
- BENDSCHNEIDER, K., AND R. J. ROBINSON. 1952. A new spectrophotometric method for the determination of nitrite in sea water. *J. Mar. Res.* **11**: 87–96.
- BRUSSAARD, C. P. D., G. J. GAST, F. C. VAN DUYL, AND R. RIEGMAN. 1996. Impact of phytoplankton bloom magnitude on a pelagic microbial food web. *Mar. Ecol. Prog. Ser.* **144**: 211–221.
- CASWELL, H. 1972. On instantaneous and finite birth rates. *Limnol. Oceanogr.* **17**: 787–791.
- EPPLEY, R. W., R. W. HOLMES, AND J. D. H. STRICKLAND. 1967. Sinking rate of marine phytoplankton measured with a fluorometer. *J. Exp. Mar. Biol. Ecol.* **1**: 191–208.
- FITZGERALD, S. A., AND W. S. GARDNER. 1993. An algal carbon budget for pelagic-benthic coupling in Lake Michigan. *Limnol. Oceanogr.* **38**: 547–560.
- FUHRMAN, J. A. 1999. Marine viruses and their biogeochemical and ecological effects. *Nature* **399**: 541–548.
- HAMA, T., K. MATSUNAGA, N. HANDA, AND M. TAKAHASHI. 1990. Nitrogen budget in the euphotic zone of Lake Biwa from spring to summer, 1986. *J. Plankton Res.* **12**: 125–131.
- HARGRAVE, B. T., AND N. M. BURNS. 1979. Assessment of sediment trap collection efficiency. *Limnol. Oceanogr.* **24**: 1124–1136.
- ISHIKAWA, T., AND J. URABE. 2002. Population dynamics and production of *Jesogammarus annandalei*, an epidemic amphipod, in Lake Biwa, Japan. *Freshwater Biol.* **47**: 1935–1943.
- KAGAMI, M. 2002. Population dynamics and functions of large phytoplankton in Lake Biwa. Ph.D. thesis, Kyoto Univ.
- , AND J. URABE. 2001. Phytoplankton growth rate as a function of cell size: An experimental test in Lake Biwa. *Limnology* **2**: 111–117.
- , AND ———. 2002. Mortality of the planktonic desmid, *Staurastrum dorsidentiferum*, due to interplay of fungal parasitism and low light conditions. *Verh. Int. Ver. Theor. Angew. Limnol.* **28**: 1001–1005.
- , T. YOSHIDA, T. B. GURUNG, AND J. URABE. 2002. Direct and indirect effects of zooplankton on algal composition in situ grazing experiments. *Oecologia* **133**: 356–363.
- , E. VAN DONK, A. DE BRUIN, M. RIJCKERBOER, AND B. W. IBELINGS. 2004. *Daphnia* can protect diatoms from fungal parasitism. *Limnol. Oceanogr.* **49**: 480–485.
- KAWABATA, K. 1987. Ecology of large phytoplankton in Lake Biwa: Population dynamics and food relations with zooplankton. *Bull. Plankton Soc. Jpn.* **34**: 165–172.
- KJØRBOE, T. 1993. Turbulence, phytoplankton cell size, and the structure of pelagic food webs. *Adv. Mar. Biol.* **29**: 1–72.
- KIRCHMAN, D. L. 1999. Phytoplankton death in the sea. *Nature* **398**: 293–294.
- KNOEHEL, R., AND J. KALFF. 1975. Algal sedimentation: The case of diatom blue-green succession. *Verh. Int. Verein. Limnol.* **19**: 745–754.
- LAMPERT, W. 1974. A method for determination of food selection by zooplankton. *Limnol. Oceanogr.* **18**: 995–998.
- LEGENDRE, L., AND J. LE FÈVRE. 1991. From individual plankton cells to pelagic ecosystems and to global biogeochemical cycles, p. 261–300. *In* S. Demers [ed.], *Particle analysis in oceanography*. Springer-Verlag.
- LUND, J. W. D. 1971. An artificial alteration of the seasonal cycle of the plankton diatom *Melosira italica* subsp. *Subarctica* in an English lake. *J. Ecol.* **59**: 521–533.
- MALONE, T. C. 1980. Algal size, p. 433–463. *In* I. Morris [ed.], *The physiological ecology of phytoplankton*. Univ. of California Press.
- MITAMURA, O. 1997. As improved method for the determination of nitrate in freshwaters based on hydrazinium reduction. *Mem. Osaka Kyoiku Univ. Ser. III* **45**: 297–303.
- MURPHY, J., AND J. P. RILEY. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta.* **27**: 31–36.
- NAKANISHI, M. 1976. Seasonal variations of chlorophyll *a* amounts, photosynthesis and production rates of Macro- and Microphytoplankton in Shiozu Bay, Lake Biwa. *Physiol. Ecol. Jpn.* **17**: 535–549.
- , Y. TEZUKA, T. NARITA, O. MITAMURA, K. KAWABATA, AND S. NAKANO. 1992. Phytoplankton primary production and its fate in a pelagic area of Lake Biwa. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* **35**: 47–67.
- OKAMOTO, K. 1984. Size-selective feeding of *Daphnia longispina hyalina* and *Eodiaptomus japonicus* on a natural phytoplankton assemblage with the fractionizing method. *Mem. Fac. Sci. Kyoto Univ. (Ser. Biol.)* **9**: 23–40.
- REYNOLDS, C. S. 1984. *The ecology of freshwater phytoplankton*. Cambridge Univ. Press.
- , J. M. THOMPSON, A. J. D. FERGUSON, AND S. W. WISEMAN. 1982. Loss processes in the population dynamics of phytoplankton maintained in closed systems. *J. Plankton Res.* **4**: 561–600.
- , AND S. W. WISEMAN. 1982. Sinking losses of phytoplankton in closed limnetic systems. *J. Plankton Res.* **4**: 489–522.
- SAGI, T. 1966. Determination of ammonia in sea water by the indophenol method and its application to the coastal and offshore waters. *Oceanogr. Mag.* **18**: 43–51.
- SMAYDA, T. J. 1970. The suspension and sinking of phytoplankton in the sea. *Oceanogr. Mar. Biol. Ann. Rev.* **8**: 353–414.
- SOMMER, U. 1984a. Population dynamics of three planktonic diatoms in lake Constance. *Holarctic Ecol.* **7**: 257–261.
- . 1984b. Sedimentation of principal phytoplankton species in Lake Constance. *J. Plankton Res.* **6**: 1–14.
- , F. SOMMER, B. SANTER, C. JAMIESON, M. BOERSMA, C. BECKER, AND T. HANSEN. 2001. Complementary impact of copepods and cladocerans on phytoplankton. *Ecol. Lett.* **4**: 545–550.
- STERNER, R. W. 1989. The role of grazes in phytoplankton succession, p. 107–170. *In* U. Sommer [ed.], *Plankton ecology: Succession in plankton communities*. Springer.
- TEZUKA, Y. 1984. Seasonal variations of dominant phytoplankton, chlorophyll *a* and nutrient levels in the pelagic regions of Lake Biwa. *Jpn. J. Limnol.* **45**: 26–37.
- URABE, J., K. KAWABATA, M. NAKANISHI, AND K. SHIMIZU. 1996. Grazing and food size selection of zooplankton community in Lake Biwa during BITEX '93. *Jpn. J. Limnol.* **57**: 27–37.
- , T. YOSHIDA, T. B. GURUNG, T. SEKINO, N. TSUGEKI, K. NOZAKI, M. MARUO, E. NAKAYAMA, AND M. NAKANISHI. 2005. Production-to-respiration ratio and its implication in Lake Biwa, Japan. *Ecol. Res.* **20**: 367–375.
- VAN BOEKEL, W. H. M., F. C. HANSEN, R. RIEGMAN, AND R. P. M. BAK. 1992. Lysis-induced decline of *Phaeocystis* spring bloom and coupling with the microbial foodweb. *Mar. Ecol. Prog. Ser.* **81**: 269–276.

- VERITY, P. G., C. Y. ROBERTSON, C. R. TRONZO, M. G. ANDREWS, J. R. NELSON, AND M. E. SIERACKI. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnol. Oceanogr.* **37**: 1434–1446.
- WETZEL, R. G., AND G. E. LIKENS. 2000. *Limnological analyses*. Springer.
- YOSHIDA, T., T. B. GURUNG, M. KAGAMI, AND J. URABE. 2001. Contrasting effects of a cladoceran (*Daphnia galeata*) and a calanoid copepod (*Eodiaptomus japonicus*) on algal and microbial plankton in a Japanese lake, Lake Biwa. *Oecologia* **129**: 602–610.
- , M. KAGAMI, T. B. GURUNG, AND J. URABE. 2001. Seasonal succession of zooplankton in the north basin of Lake Biwa. *Aquat. Ecol.* **35**: 19–29.
- YOSHIMIZU, C., T. YOSHIDA, M. NAKANISHI, AND J. URABE. 2001. Effects of zooplankton on the sinking flux of organic carbon in Lake Biwa. *Limnology* **2**: 37–43.

Received: 23 September 2005

Accepted: 11 May 2006

Amended: 1 June 2006