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On the ecology of a *Cryptomonas phaseolus* population forming a metalimnetic bloom in Lake Cisó, Spain: Annual distribution and loss factors[1]

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Abstract

Cryptomonas phaseolus was present throughout the water column during holomixis in Lake Cisó (Spain), despite the presence of sulfide. During stratification, *C. phaseolus* formed a layer with abundances up to 10^5 cells ml^{-1} at the metalimnion, where oxygen and sulfide were present simultaneously at low concentrations. Light at this depth was between 1 and 20% of surface incident radiation. Loss factors were studied during 2 years. Outwash was negligible most of the year, but could reach 30% of total losses during certain short periods in winter. *Cryptomonas phaseolus* was found to sink with speeds up to 55 cm d^{-1}, which is in accord with velocities calculated with Stokes' law. There was a single sedimentation peak in spring accounting for 40–60% of total losses. Decomposition was the most important loss factor for most of the year. *Cryptomonas phaseolus* cells were always actively growing, but losses balanced production for most of the study period. Average cell volume increased during mixing and decreased during stratification. Doubling times were minimal (7 d) in March at the onset of stratification and slowly increased through spring and summer, reaching a maximum just before mixing (around 50 d) and decreasing again through winter until the next March minimum.

The presence of an algal population in a natural system is the result of a dynamic balance between production and loss factors. Most algal studies have looked at the populations from the production side of the equation (*see* Kalff and Knoechel 1978), and many workers have recognized the need for more studies concerning algal loss factors (Jewson et al. 1981; Reynolds 1984; Forsberg 1985). Thus, our first point of interest was to assess losses for an algal population.

Metalimnetic blooms of various phytoplankton species have been found in many lakes (Ichimura et al. 1968; Fee 1976; Konopka 1982; Pick et al. 1984). However, there is still little knowledge about their physiology and ecology. Specifically, loss factors affecting the metalimnetic blooms have been almost always ignored. Knowledge of such factors, however, is necessary to discern between different hypotheses about the physiological state of metalimnetic algae. Kiefer et al. (1972), among others, proposed that accumulation due to passive sinking and, therefore, inactive cells, formed such blooms in the sea. Fee (1976) and others thought the populations were actively growing and forming the bloom in situ. Pick (1984; Pick et al. 1984) suggested that loss factors balanced production and, therefore, that metalimnetic blooms would be at a sort of dynamic equilibrium, but no data supporting such views were gathered. We tried to determine which of these hypotheses was correct for the metalimnetic population studied.

Finally, the Cryptophyceae constitute a ubiquitous but little known group of algae. Laboratory studies have been performed on several aspects of their physiology (Morgan and Kalff 1979; Cloern 1977; Lichtlé 1979). Some autecological studies have been carried out recently (Morgan and Kalff 1975; Sommer 1982; Taylor and Wetzel 1984; Braunwarth and Sommer 1985). One frequently cited aspect is their apparent resistance to sedimentation (Burns and Rosa 1980; Livingstone and Reynolds 1981; Sommer 1984; Reynolds 1984). Moreover, metalimnetic blooms of such organisms have been noted (Takahashi and Ichimura 1968; Ichimura et al. 1968; Bohr 1976), but little is known about their ecology.

We studied, during 2 years, the development of a population of *Cryptomonas*

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phaseolus in Lake Cisó, a small monomictic lake (surface area 487 m^2, maximal depth 9 m) which turns completely anaerobic during winter, with sulfide up to the surface, and stratifies during summer.

Cryptomonas phaseolus is distributed throughout the water column during mixing, when it is the only eucaryotic phototroph in the lake. Phototrophic bacteria are the main producers during this time of year (Guerrero et al. 1985*a*; van Gemerden et al. 1985). During stratification *C. phaseolus* forms a layer in the metalimnion, where it constitutes about 95% of the biomass and stains the water brown. Therefore, autecological studies can be performed on natural samples, which are in effect highly enriched cultures of the organism. Phototrophic bacteria can be found below this layer, in the hypolimnion. At this time, *C. phaseolus* is responsible for most primary production in the lake.

Here we report on population losses due to outwash through the lake outlet, to decomposition, and to sedimentation. Losses are compared to actual biomass changes and a balance of carbon flux through the population built. The study is relevant to loss factors of phytoplankton, physiological state of metalimnetic populations, and ecology of the Cryptophyceae.

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Methods

Limnological methods—A floating platform described by Guerrero et al. (1985*a*) was fixed above the deepest part of Lake Cisó. Briefly, the platform was formed by a central square with a hole to lower sampling equipment and four arms with floats and winches. The whole platform was tied to anchors or to shore. Temperature, light, and turbidity profiles were taken at this location. Temperature was measured with a temperature probe (YSI-33 S-TS; Yellow Springs Instr.), light penetration was determined with a submersible quantum meter (Crump), and turbidity with a turbidity meter (Philips Shenk TM-F0 100). Then, a sampling cone (Guerrero et al. 1985*a*) connected to a pump through PVC tubing was carefully lowered, 5 cm at a time, from slightly above to slightly below the turbidity discontinuity. Every 5 cm, water was pumped and 20 ml was filtered through Whatman GF/C filters in Swinnex filter holders. The filters were colored according to the dominant organisms at each depth. Above the high turbidity layer, filters were whitish. Then, one or two filters were dark brown, indicating the presence of *Cryptomonas,* the following was brownish pink, and the rest deep purple due to the presence of Chromatiaceae (purple sulfur bacteria). In this way, the depth of the maximal concentration of *C. phaseolus* could be detected exactly and samples taken accordingly.

The cone was lowered to the desired depths and water pumped to the surface, where it was immediately distributed into sampling bottles. Ten milliliters were fixed with 1 ml of Formalin (4% final concn) in glass, screwcapped tubes for direct counts. Replicate 10-ml samples were fixed with 10 ml of double-strength sulfide antioxidant buffer (Guerrero et al. 1985*a*) in screwcapped tubes, and sulfide was measured in the laboratory with a silver sulfide electrode (Corning 476065) and a reference electrode (Philips RH 44/2-SD/1), coupled to a custom-made millivoltmeter.

Oxygen was measured in duplicate samples by the modified Winkler titration of Ingvorsen and Jørgensen (1979), which involved filling BOD bottles, precipitating sulfide with 1 M zinc acetate and 1 N NaOH, pipetting the supernatant into screwcapped tubes and then performing the usual Winkler assay. This modification was mandatory due to the presence of sulfide, which interferes with the regular Winkler method.

Abundance and biomass—Cell numbers were determined by acridine orange direct counts (Zimmermann and Meyer-Reil 1974). Filters were observed at 400× in an epifluorescence microscope (Olympus BH). The technique was checked with the standard Lugol's solution fixation, followed by sedimentation and counting with an inverted microscope; no significant differences could be found between the two counting methods.

Cell volumes were determined by mea-

suring length (l) and width (w) of at least 40 cells under epifluorescence and using the formula: volume = $(\pi/12)w^2(l + w/2)$, which assumes *Cryptomonas* to be a hemisphere plus a cone.

Outwash—The lake receives most of its water through seepage (although some surface runoff also occurs). There is one surface outlet in fall and winter, which dries up when the water level drops in spring. During the following period, water losses are exclusively due to evaporation. A weir was built in the outlet, and water outflow (W_O) was measured every sampling date. Cell concentration in the water leaving the lake (C_O) was also measured. In this way, losses due to outwash (O) could be calculated (W_O times C_O).

Decomposition—Decomposition experiments were run both in the laboratory and in the field. In the first case, a sample from the *Cryptomonas* layer was incubated at 30°C. Periodically, subsamples were fixed and cells counted by epifluorescence. Some samples were incubated in the light and some in the dark. Controls killed with Formalin were also incubated and counted. In the field experiments, glass tubes were filled with water from the layer, tightly capped, and deployed in the sediment traps at 3 and 7 m (*see sedimentation method*). On every sampling date during summer 1985, two replicates were taken from each depth, fixed with Formalin, and counted by epifluorescence.

In each case, the exponential coefficient of decomposition (k_d) was calculated by regression:

$$\ln C_t = -k_d t + \ln C_0 \qquad (1)$$

where C_t and C_0 are the cell concentrations at the end and at the beginning of the experiment, and t is the time between samples.

With the coefficients obtained at different temperatures, a Q_{10} could be calculated for decomposition:

$$Q_{10} = (k_{d1}/k_{d2})^{10/(T1-T2)} \qquad (2)$$

where k_{d1} and k_{d2} are the coefficients of decomposition at T_1 and T_2 degrees Celsius of temperature. From all the decomposition experiments, an empirical relationship between k_d and temperature (T) was derived:

$$-k_d = -7.2 \times 10^{-3}\, T - 0.05. \qquad (3)$$

Sedimentation—Sediment traps were deployed at 3 and 7 m in the lake. (Detailed description and performance of traps will be published separately.) Briefly, the traps were made of glass tubes suspended in groups of eight from winches mounted on the surface platform. There were traps covered by awnings to measure accumulation of cells in the tubes due to resuspension and uncovered traps to measure accumulation due to both resuspension and vertical sedimentation. In this way, sedimentation flux (S) could be calculated:

$$S = \frac{(N_t - C_0)V}{At} - \frac{(NC_t - C_0)VC}{ACt} \qquad (4)$$

where S is the sedimentation flux in cells $m^{-2}\ d^{-1}$, N_t and NC_t are the cell concentrations inside the uncovered and covered traps on day t, V and VC are the volumes of the traps, and A and AC the surface areas. Finally, C_0 is the cell concentration at the depth of the trap on day 0 (i.e. the previous sampling date).

Knowing the average cell concentration in the lake just above the trap mouth $[(C_0 + C_t)/2]$, we could calculate sinking speeds (v_s) according to

$$v_s = S/[(C_0 + C_t)/2]. \qquad (5)$$

Since the traps had no fixative, decomposition could take place during deployment. Therefore, a correction was applied. The real sedimentation flux (SD') was calculated as:

$$SD' = \frac{S'V}{At} - \frac{SC'VC}{ACt} \qquad (6)$$

where S' and SC' were calculated as follows.

The change of cell number in the traps with time can be expressed as:

$$\frac{dN_t}{dt} = S' + C_0(-k_d)\exp(-k_d t) + \int_i^t S'\, dt_i(-k_d)\exp[-k_d(t - t_i)] \qquad (7)$$

where the left-hand term is the variation of cell numbers within the traps with time (be-

tween two consecutive sample dates). The first term on the right-hand side is sedimentation flux. The second term is the change due to decomposition of the cells initially in the trap according to Eq. 1, and the third term is the sum of changes due to decomposition experienced by each group of cells (S' dt) sedimenting into the trap at each instant t_i and remaining in the trap during $t - t_i$ days. Solving Eq. 7 gives

$$N_t = C_0\exp(-k_d t) + \frac{S'}{k_d}[1 - \exp(-k_d t)], \quad (8)$$

and from this

$$S' = \frac{[N_t - C_0\exp(-k_d t)]k_d}{[1 - \exp(-k_d t)]} \quad (9)$$

where k_d can be calculated from Eq. 3. SC' could be calculated analogously.

Thus, true sedimentary flux (SD') can be calculated if we know the initial and final concentration of cells within the traps and the average temperature. Calculations for uncovered and covered traps were done separately, and then the two fluxes were subtracted as in Eq. 6. Again, true sinking speeds could be calculated by dividing these fluxes by the average concentration of cells just above the traps.

Integration methods, production, and doubling times—Values of outwash, decomposition, and sedimentation were integrated for a water column with 1 m^2 of surface area for every period between sampling dates. Actual changes in biomass were calculated by subtracting integrated cell numbers every two consecutive dates. Thus, all these processes were in cells m^{-2} period^{-1} and could be compared directly. It is understood that gross production = net production + respiration and excretion, and that net production = biomass changes + losses. Since we measured neither $^{14}CO_2$ incorporation nor respiration nor excretion, nothing can be said about gross production. But since we measured biomass changes and losses, adding them up gives an estimate of net production. Since not all possible loss factors were studied (i.e. predation), net production will be underestimated. Thus, we will refer to such calculated values as minimal net production. Dividing production by average integrated biomass for the period, we could calculate (minimal) growth rate (μ); from this, we obtained (maximal) doubling time (D_t):

$$D_t = \ln 2/\mu. \quad (10)$$

Growth rates and doubling times were also calculated in a different way. Increases of cells for a given volume of water, due to actual biomass changes were added to decreases due to all loss factors and to initial biomass (C_0). This procedure gave the "theoretical" concentration of cells at the end of a given time period (C_t). If we assume exponential growth between the two sampling dates,

$$\mu = \frac{\ln(C_t/C_0)}{t} \quad (11)$$

and

$$D_t = \ln 2/\mu. \quad (12)$$

These results are not shown since they were almost identical to those calculated in the first way. The only difference between the two methods is that the first assumes linear growth between sampling dates and the second assumes exponential growth.

Results

Annual distributions of temperature, oxygen, and sulfide are shown in Fig. 1 for the 2 yr of this study. The lake was mixed from October to February. Although slight temperature gradients could be found in January and February, they probably disappeared at night. During mixing the lake was completely anaerobic, with sulfide concentrations around 0.4 mM up to the surface. From March to September the lake was stratified, with a very sharp thermocline between 1 and 2 m. Maximal epilimnetic temperatures were around 23°C. During this period, the epilimnion was aerobic, with oxygen concentrations declining with depth, and the hypolimnion was anaerobic with uniformly high sulfide concentrations around 1.0 mM. In the metalimnion, there was a zone where sulfide and oxygen coexisted, albeit at very low concentrations.

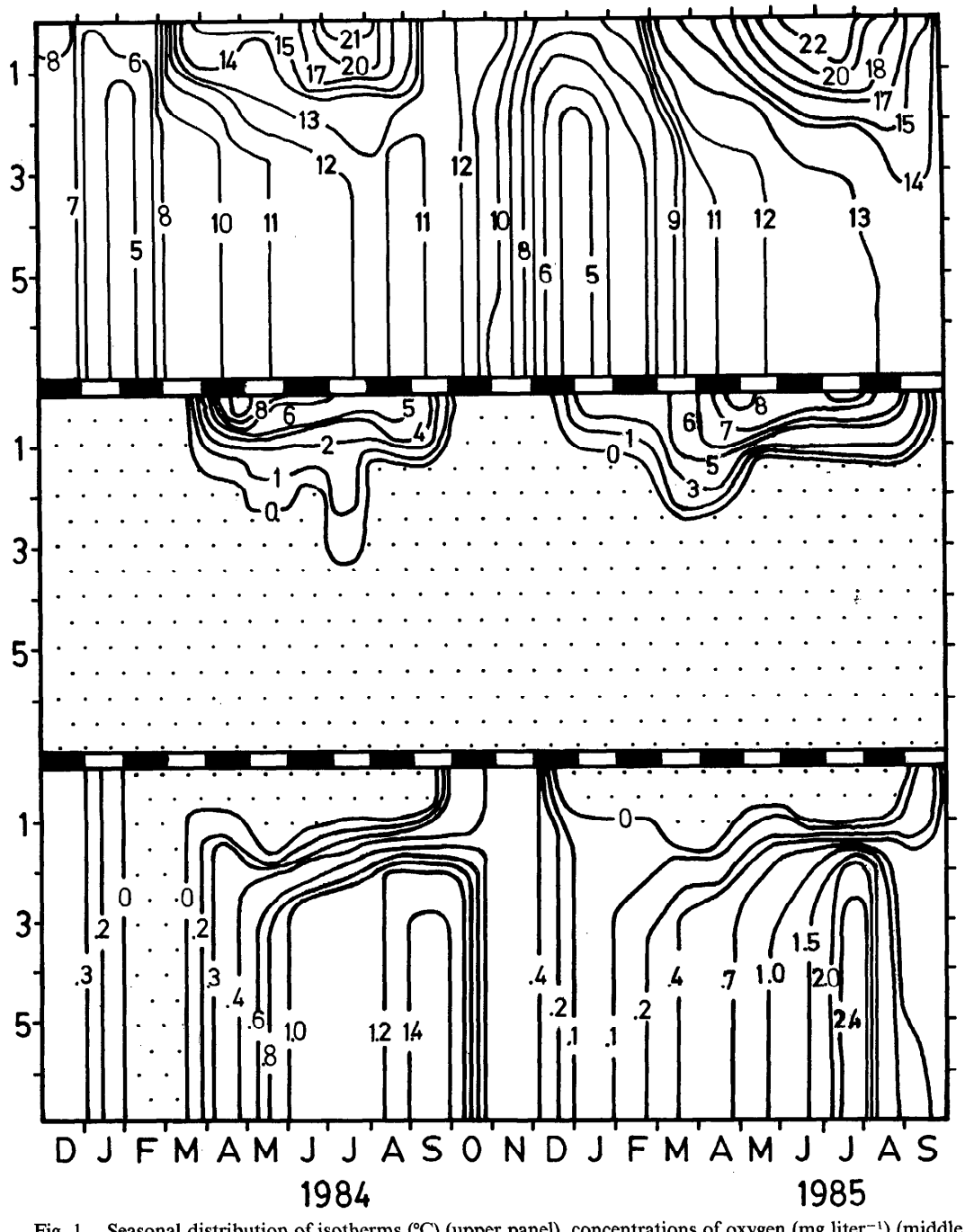

Fig. 1. Seasonal distribution of isotherms (°C) (upper panel), concentrations of oxygen (mg liter^{-1}) (middle panel), and of sulfide (mM) (lower panel) in Lake Cisó, 1984 and 1985. Stippled areas indicate absence of oxygen in middle panel and absence of sulfide in lower panel. Most of the lake was anaerobic most of the year. Also, oxygen and sulfide coexisted at low concentrations in the metalimnion.

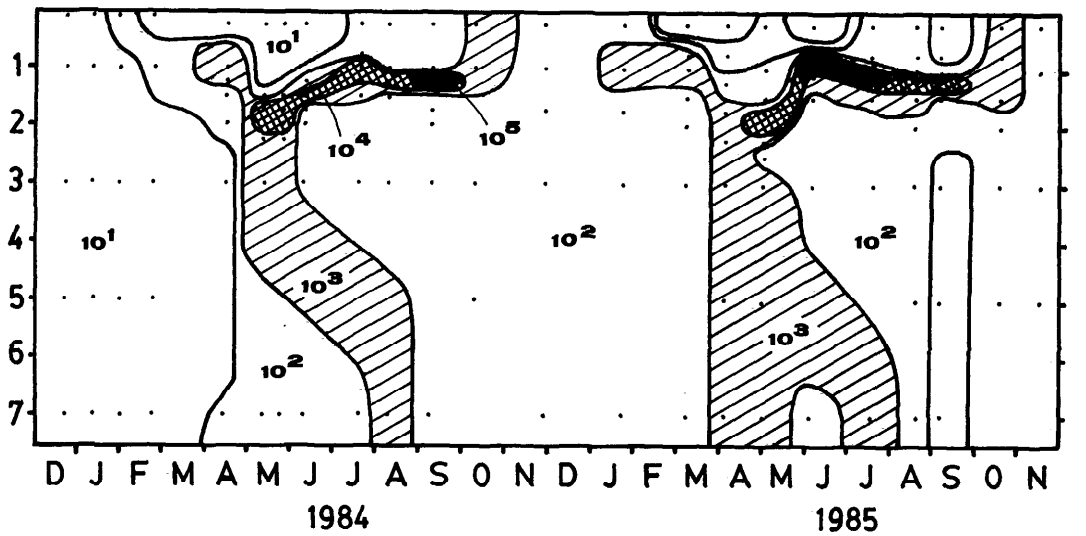

Fig. 2. Seasonal and depth distribution of *Cryptomonas phaseolus* (cells ml^{-1}) in Lake Cisó, 1984 and 1985.

It was precisely in this zone that *C. phaseolus* formed a bloom (Fig. 2), reaching concentrations as high as 10^5 cells ml^{-1}. Generally, *C. phaseolus* winter and hypolimnetic populations stayed between 10^2 and 10^3 cells ml^{-1} despite the presence of sulfide. Figure 2 shows that shortly after stratification, the population started to increase in numbers in the metalimnion, reaching maximal abundances by August or September. Volume of the cells changed substantially during the year, increasing during winter to a maximum at the beginning of stratification and diminishing thereafter (Fig. 3A). In Fig. 3A average cell volume is plotted together with integrated biovolume. Growth of the population, both in 1984 and 1985, always implied a reduction in cell volume, while periods without growth allowed increases in volume.

Figure 4 shows vertical profiles of physicochemical parameters and abundance of cells on selected winter and summer dates. The summer profile clearly showed the peak of the *C. phaseolus* population in a zone of oxygen and sulfide coexistence. The light profile showed a clear break in slope at this zone, due to absorption by the cells, which was not present in the winter profile (Fig. 4A, C). The extreme thinness of the algal layer can be seen in Fig. 4D. On this particular date the layer was 40 cm thick, but on some days it was only 20 cm thick.

In the laboratory (Fig. 5A) decomposition was very fast, especially in illuminated cultures, where 95% of the biomass disappeared in 1 d. In Fig. 5A three different slopes can be seen. The initial slope, lasting for about 1 d, is very steep and reduced the population by two orders of magnitude. Afterward, decomposition proceeded at a slower pace for about 14–40 d, causing decreases in the population down to 10^1–10^2 cells ml^{-1}. At this point, the number of cells counted in each experimental bottle was so low that estimates were very unreliable, and differences between 10^0 and 10^2 could not be distinguished. For this reason decomposition apparently ceased. In field samples, larger numbers of cells were counted every time, so that field concentrations were not subject to this type of error. Since deployment periods in the field lasted between 15 and 40 d, we used average decomposition rates including all points along the first two slopes of the decomposition curves.

Rates of decomposition were very similar at 3 and 7 m (Fig. 5B), which would be expected since both depths were at the hypolimnion (i.e. in the dark and at the same temperature). Thus, decomposition could be assumed to be a function of temperature

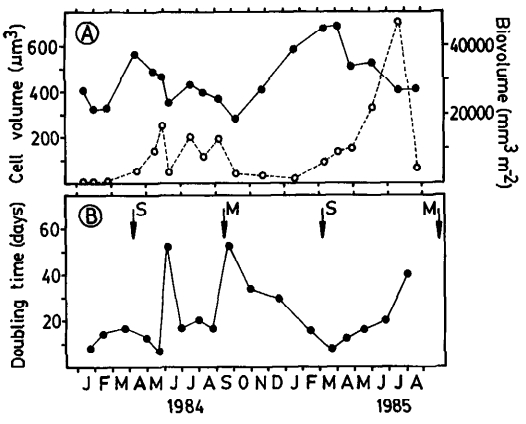

Fig. 3. A. Seasonal distribution of integrated biovolume (○) and average cell volume (●) in Lake Cisó, 1984 and 1985. B. Doubling time of the population (S—stratification; M—mixing).

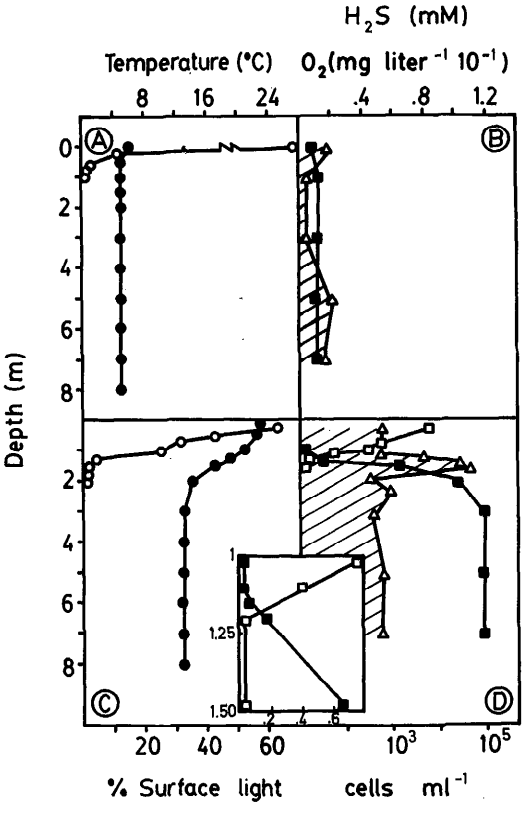

Fig. 4. A. Vertical profiles of temperature (●) and light penetration as percentage of surface light (○) for a typical winter day (11 January 1984) in Lake Cisó. B. Sulfide (■) and *Cryptomonas* (△) for 11 January 1984. No oxygen was present. C. As panel A, but for a typical summer day (19 August 1985). D. Oxygen (□), sulfide (■), and *Cryptomonas* (△) for 19 August 1985. Hatched areas in panels B and D mark the presence of *Cryptomonas*. Inset—enlarged view of oxygen and sulfide distributions in the thin layer of contact where *Cryptomonas* peaked. Symbols as above.

alone. Decomposition rate constants were calculated at the different temperatures, and a Q_{10} of 1.47 was obtained. This value was used to calculate decomposition throughout the year.

Sinking speeds of *C. phaseolus* at 7 m ranged from 0 to 55 cm d^{-1} (Fig. 6), showing peaks at the beginning of stratification when rapidly changing conditions might have affected the cells negatively. Total flux to the sediments was calculated with the 7-m traps. There were two peaks of sedimentation flux coinciding with the sinking speed peaks. Biomass diminished simultaneously (Fig. 3A), suggesting that sedimentation was important as a loss factor during these periods.

Although actual changes in biomass were either positive or negative, (minimal) net production was always relatively large in comparison to those changes (Fig. 7). Therefore, the population was apparently consuming most of its production in compensating losses rather than in growing. The lower part of Fig. 7 indicates the percentage of such losses accounted for by each factor. Outwash was relatively important in winter. Sedimentation was important in spring, and decomposition was clearly the most important loss factor.

Minimal net production was lower in 1984 than in 1985. During 1984 phototrophic bacterial populations were also smaller, especially in winter. Production was lower in winter than in summer for the 2 years of this study. Actual biomass increases were not tied to the absolute value of production and depended on the balance of production and loss factors. The year 1984 was characterized by short periods of increase alternating with short periods of decline (*see also Fig. 3A*), and total biovolume never reached the high values of 1985. In 1985, a steady increase was maintained by the population throughout spring and summer and, therefore, a high amount of biomass accumulated by the end of June. Afterward, the population started to decline. The length of such

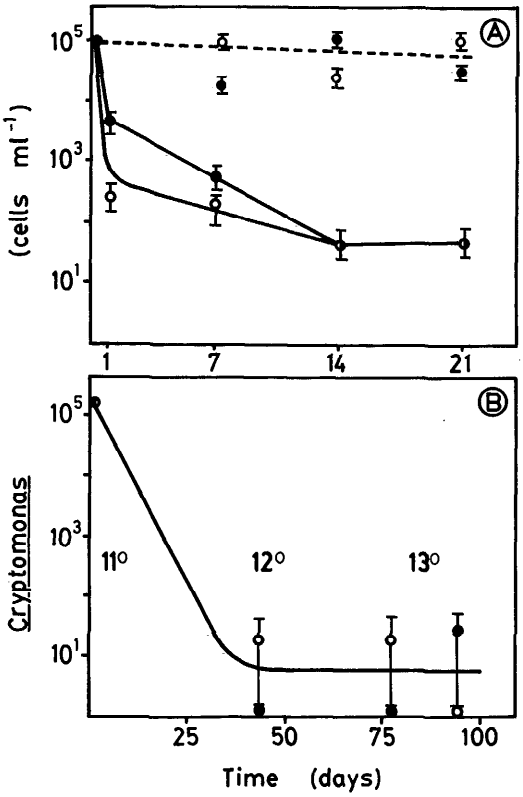

Fig. 5. Decomposition experiments. A. Number of cells remaining in samples incubated in the laboratory at 30°C in the light (O) and dark (●) (solid lines) and in the light (O) and dark (●) with Formalin (dashed line). B. Number of cells remaining in samples incubated in the field at 3 (O) and 7 m (●). Temperatures on each sampling date are indicated. Vertical bars indicate 95% C.I. for each count.

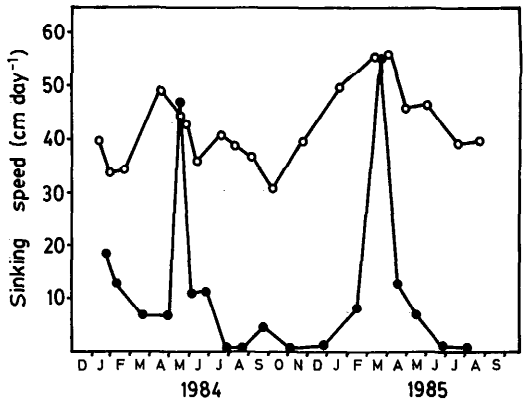

Fig. 6. Sinking speeds of *Cryptomonas* in Lake Cisó, 1984 and 1985, measured (●) and calculated with Stokes' law (O).

periods of increased biomass seems to be related to the amount of light reaching the metalimnion. When more than about 150 μEinst m^{-2} s^{-1} reached the layer, population growth occurred. If ≲100 μEinst m^{-2} s^{-1} reached the metalimnion, the population was unable to compensate for its loss factors and biomass decreased.

Calculated losses were highest during summer, due to increased decomposition at higher temperatures (*see Fig. 7, lower panel*). Thus, even though outwash accounted for 30–40% of losses in December 1985 and sedimentation up to 60% in March 1985, total losses were relatively low during such periods. Decomposition was, overall, the most important loss factor for the population.

With these estimated net production values and the measured biomass for each sampling date, doubling time of the population was calculated (Fig. 3B). Doubling times were minimal (7 d) in March at the onset of stratification and slowly increased through spring and summer, reaching a maximum just before mixing (around 50 d), and decreasing again through winter until the next March minimum.

Discussion

Usually, loss factors for algal populations are considered to be respiration, excretion, autolysis, parasitism, decomposition, sedimentation, washout, and grazing (e.g. Fallon and Brock 1980; Hecky and Fee 1981; Tilzer 1984; Forsberg 1985). Below we will discuss loss factors in decomposition, sedimentation, and outwash. We will then try to assess the possible importance of grazing and discuss questions related to production and doubling time.

Decomposition—In our experiments, death, autolysis, and parasitism would be pooled as decomposition. In the end, they would all result in decomposition in the water column. Decomposition rate constants varied between 0.090 and 0.150 d^{-1}—a similar range to that found by Fallon and Brock (1979). They also found a very fast disappearance of algal cell structure; 99% of

the *Aphanizomenon* cells disappeared in the first 8 d. It is easy to accept high decomposition rates for *Cryptomonas,* since it lacks a rigid cell wall, and, therefore, bacterial attack would cause morphology to change more quickly than in the case of diatoms, for example.

Sedimentation—Using a correction for decomposition in the traps, we found sinking speeds between 0 and 55 cm d^{-1}. If we consider a cell density of 1.100 g cm^{-3} for *C. phaseolus* (Guerrero et al. 1985*b*) and average volumes for each date, the sinking speed of *Cryptomonas* at 20°C according to Stokes' law is as shown in Fig. 6. The calculation is only approximate for several reasons. In the first place, density may be variable at different times of year. Second, temperature (which affects both density and viscosity of water) varies between 5° and 23°C in the lake. Third, volumes calculated from epifluorescence counts are underestimates of real volume (unpubl. results). And fourth, a *Cryptomonas* cell is not a perfect sphere, and thus some unknown correction factor should be applied. Nevertheless, it is striking that actual speed only reaches theoretical speed during the spring sedimentation periods. The rest of the year, actual speed is a small fraction of that expected according to Stokes' law.

In fact, with swimming speeds around 0.5 m h^{-1}, *Cryptomonas* could theoretically swim from the bottom to the top of the lake every day. This calculation suggests that *Cryptomonas* is able to resist sedimentation most of the year, either by actively swimming upward during stratification or through passive mixing during winter. When, at some times of the year, *Cryptomonas* cells sediment, they do so at velocities extremely close to theoretical ones for passive particles. Sedimentation periods are probably related to the physiological state of the cells. In this respect, diatoms have been found to sink only when senescent or dead (Wiseman et al. 1983).

The reason why most workers have not found significant sinking of Cryptophyceae may be because most studies are limited to a few isolated samplings and might miss the sedimentation peaks found here, or the cells might decompose in the unfixed traps, or

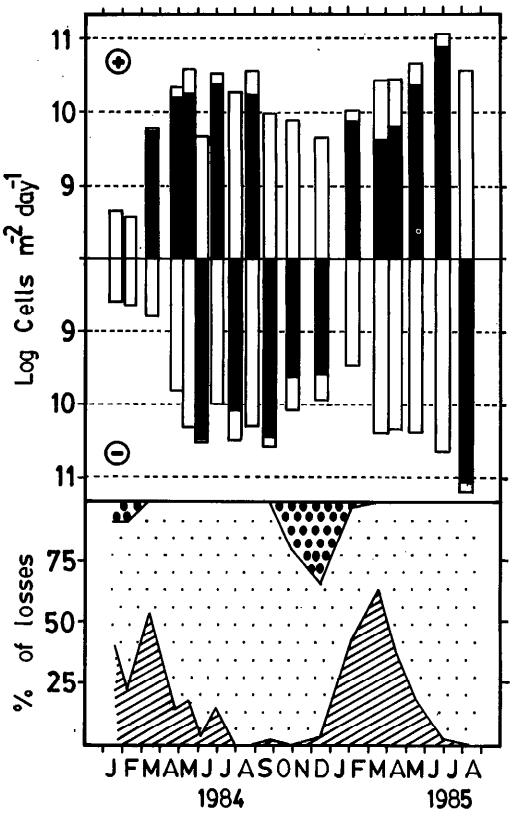

Fig. 7. Upper: Net primary production (positive white bars), biomass change (positive or negative black bars), and total loss factors (negative white bars) for the *Cryptomonas phaseolus* population throughout the study period. Vertical scale is logarithmic. Lower: Percentage of total losses accounted for by decomposition (stippled), sedimentation (hatched), and outwash (heavily stippled).

both (Livingstone and Reynolds 1981; Sommer 1984). In deep lakes, if only bottom-deployed traps are used, *Cryptomonas* may not be recovered because of complete decomposition in the water column.

Although two sedimentation peaks per year (one in spring and one in fall) have been found frequently (Fallon and Brock 1980), others have found sedimentation to be roughly proportional to phytoplankton biomass (Bloesch et al. 1977), and still a third group of researchers have found many short periods of high sedimentation for particular species throughout the year (Livingstone and Reynolds 1981; Sommer 1984). Sedimentation of phytoplankton seems to be related

more to the physiological state of the algae than to environmental parameters (Wiseman et al. 1983). These different behaviors in different lakes are probably based on the different species composition and the wax and wane of specific phytoplankton populations. In the case of *Cryptomonas* in Lake Cisó, one possibility is that many of the cells that are mixed throughout the water column during winter find themselves trapped in the hypolimnion right after stratification, and thus they become unable to swim upward due to lack of light. With the added handicap of increasing sulfide concentrations, they could die and sink. Low hypolimnetic temperatures slow decomposition, thus allowing sedimentation to be an important loss factor at this time of year.

Washout—Washout with the surface outflow draining lakes is usually of minor importance in large lakes (Jewson et al. 1981; Tilzer 1984). But in Lake Cisó, it may represent up to 30% of total *Cryptomonas* losses during certain periods. In winter, with low evaporation, there is a variable but substantial flux of water out of the lake (between 3 and 40 $m^3 d^{-1}$). Thus the lake has a turnover of about 54 d. Since doubling times of *Cryptomonas* may be of the same order of magnitude, it is understandable that outwash can be an important loss factor during winter. During summer, on the other hand, the water level of the lake drops below the outlet, and there is no outflow of water except for evaporation.

Grazing—Grazing has not been evaluated in this study. During mixing periods there are no possible predators in the lake. During stratification predation is probably not very important because doubling times calculated here are similar to doubling times for different *Cryptomonas* species found in the literature, suggesting that no important loss factors were ignored, and suitable predators in appropriate numbers are lacking. During stratification *Coleps hirtus* formed a layer with up to 10^4 individuals ml^{-1} at the same depth as *Cryptomonas* (Dyer et al. 1986). *Coleps hirtus* has been described as an eclectic feeder, grazing on algae but also eating bacteria and detritus (Bick and Kunze 1971). Moreover, many times it seems to have intracellular symbiotic algae, being then phototrophic (Christopher and Patterson 1985). When such large concentrations of ciliates have been found in the plankton of lakes, the organisms have always been associated with zoochlorellae (Hecky and Kling 1981). We do not think *Coleps* feeds significantly on *Cryptomonas* in Lake Cisó for three reasons: many of the *C. hirtus* in the lake contain symbiotic *Chlorella*-like algae which are completely different from *Cryptomonas* (Dyer et al. 1986); for *Coleps*-sized ciliates, maximum food particle diameter is around 10 μm and optimal diameter is between 0.2 and 5 μm (Fenchel 1980), obviously inadequate to handle *Cryptomonas* cells (18 × 10 μm); and, even if all the *Coleps* were eating *Cryptomonas,* the impact of such feeding would be not very important, since clearance rates of ciliates of the same size are around 0.1 $\mu l\ d^{-1}$ when feeding on bacteria (Fenchel 1980). With 10^6 bacteria ml^{-1} and an average volume of 0.5 μm^3 per bacterium, this means about 50 μm^3 of biovolume eaten per *Coleps* per day. With 10^4 *Coleps* ml^{-1}, this calculation implies that 5×10^5 μm^3 of *Cryptomonas* biovolume would be eaten per milliliter per day. The standing biovolume of *Cryptomonas* is 2×10^7 $\mu m^3\ ml^{-1}$. Thus, only about 2.5% of the biomass of *Cryptomonas* would be eaten daily. Altogether, it seems that *Coleps* cannot be an important predator of *Cryptomonas* in this lake.

The other candidate is the rotifer *Anuraeopsis fissa* which forms a layer with up to 10^4 individuals $liter^{-1}$ at the same depth in late summer. Although no studies have been performed with this organism, feeding studies that may be relevant have been done with *Keratella cochlearis* (Bogdan and Gilbert 1982, 1984). In view of such studies, a feeding rate of 100 *Cryptomonas* cells per rotifer per day seems reasonable. Then, 10^4 *A. fissa* $liter^{-1}$ would eat 10^6 *Cryptomonas* cells $liter^{-1}\ d^{-1}$. Since the population is about 10^8 *Cryptomonas* $liter^{-1}$, the impact of such predation would be small, also about 1%. In conclusion, grazing is nonexistent during the mixing periods and insignificant for a good part of the stratification periods in comparison to other loss factors, but it

could still become a significant loss factor during some periods if rotifers accumulated in the metalimnion in very large numbers.

Production and doubling time—For most of the study period, our calculated values of minimal net primary production are bound to be close to actual primary production. The growth rates and doubling times obtained from our estimates should then be comparable to growth rates in the literature for *Cryptomonas*. Unfortunately, no data are available about *C. phaseolus* itself, but the values found for other *Cryptomonas* can be used for comparison. Doubling times in laboratory cultures range between 1 and 65 d depending on light and nutrient conditions (Cloern 1977; Morgan and Kalff 1975; Lichtlé 1979), and a similar range has been found in the field (Morgan and Kalff 1979; Eloranta 1980; Braunwarth and Sommer 1985). When we consider that, if anything, our values are underestimates of growth rate (and overestimates of doubling time), the range found (7–50 d) fits well within ranges in the literature.

The balance between production and loss factors shows that almost all the biomass produced is consumed quickly, mostly by decomposition, but also by sedimentation, outwash, or grazing at certain times of year. This sort of dynamic equilibrium had been proposed for other algae forming metalimnetic blooms, such as *Chrysosphaerella longispina* in Jacks Lake, Ontario (Pick 1984), as well as for epilimnetic phytoplankton in tropical lakes (Hecky and Fee 1981). Other workers have suggested different situations for metalimnetic blooms. Fee (1976) proposed that the algae were actively growing in several Canadian lakes, while Kiefer et al. (1972), Pick et al. (1984), and others thought the algae had grown in the epilimnion and passively accumulated in the metalimnion. We think both the situation proposed by Fee (1976) and that proposed by Pick (1984) are true for the *C. phaseolus* population in Lake Cisó at different times of year, but the passive accumulation hypothesis is never applicable in this lake. In Fig. 8 exponential rate constants (k) for total losses and actual changes in biomass have been plotted against those for

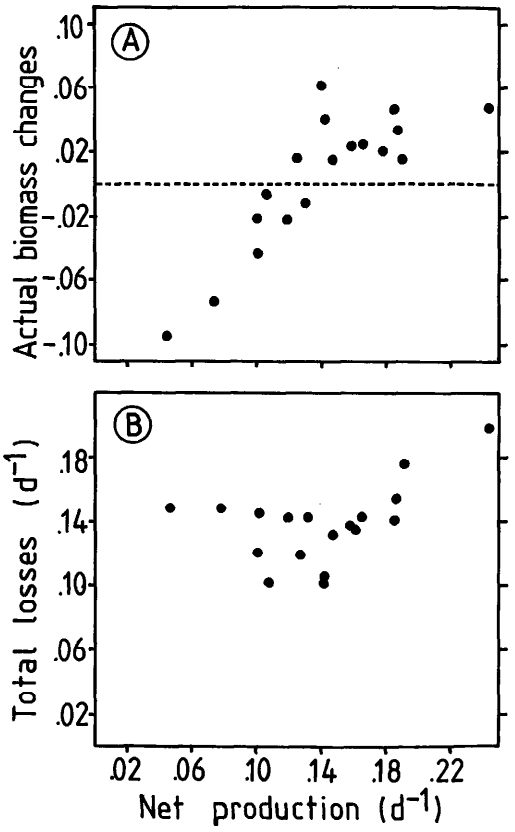

Fig. 8. Exponential rate constants for actual biomass changes between sampling dates, total losses, and net production. A. Rate constant for actual biomass changes vs. constant for net production. Dashed line separates biomass increases (above) from biomass decreases (below). B. Constant for total losses vs. constant for net production.

net production. The k for actual changes increased linearly with the k for net production (Fig. 8A). On the other hand, the k for all loss factors together does not show any relationship with production (Fig. 8B). This pattern means that rates of loss are high throughout the year, independent of production by the population. Rate of production, however, changes substantially during the year. Only when the rates of production are high is the population able to compensate losses and grow (above the dashed line in Fig. 8A).

In this analysis, we have considered the population to be uniform. In reality, the growth rates would be higher and decom-

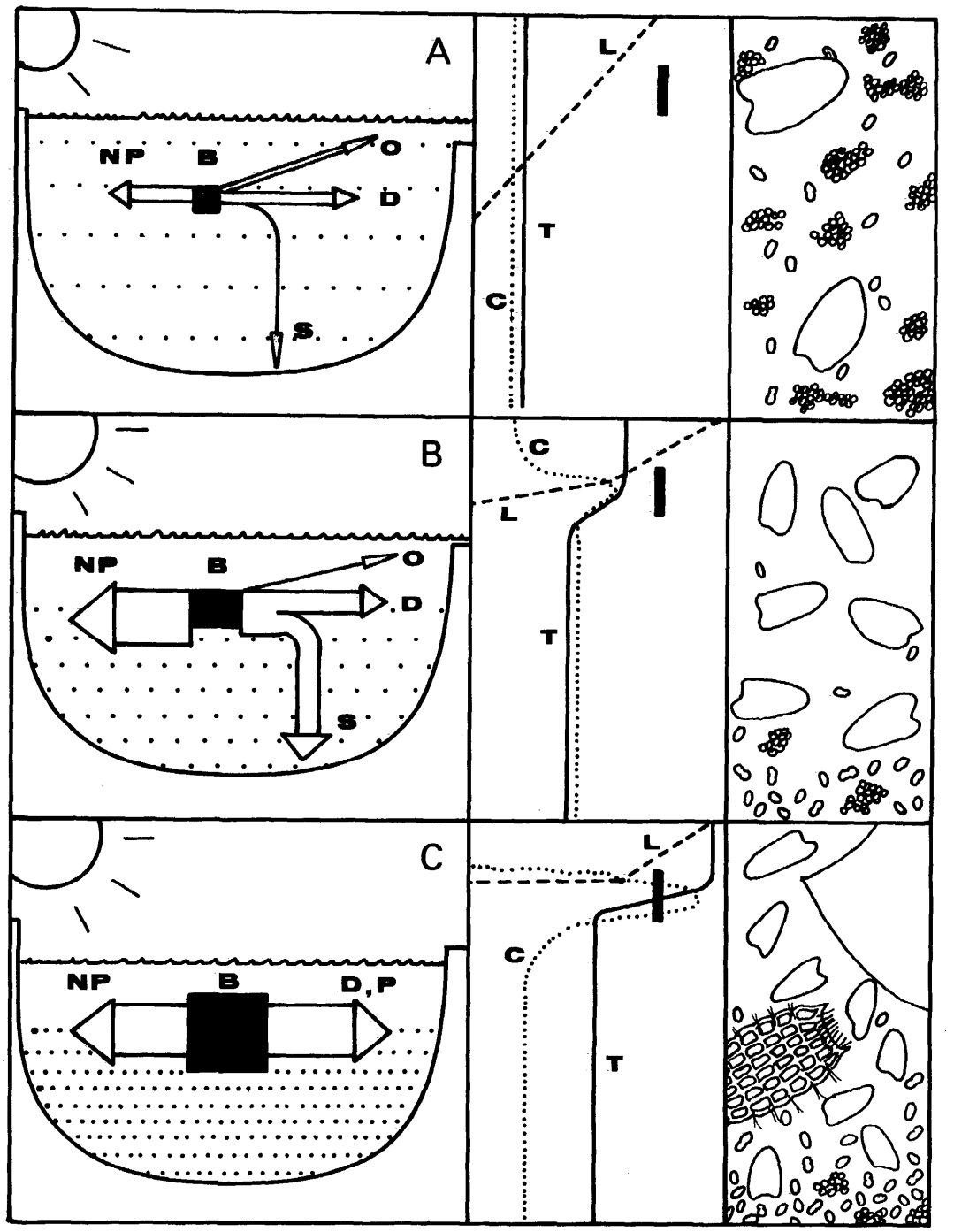

Fig. 9. Schematic representation of carbon flux through the population of *Cryptomonas phaseolus* in Lake Cisó during winter (A), spring (B), and summer (C). Left-hand panels show the main pathways of carbon. NP—Net production; B—biomass; O—outwash; D—decomposition; S—sedimentation; P—predation. Stippling is roughly proportional to sulfide concentration. Note differences in sunshine and outflow. Biomass only increases when NP is larger than total losses, which occurs mainly in spring. During summer the population is at steady state or dynamic equilibrium. Middle panels show vertical distributions of light (L), temperature (T), and *C. phaseolus* cells (C). Black stripes indicate the vertical transect "enlarged" in the right-hand panels. Right-hand

position lower for cells in the metalimnion than for cells in the hypolimnion. However, the data shown are valid for the population as a whole. Thus, from the data presented here, the following annual cycle can be deduced (Fig. 9). During the mixing period (Fig. 9A), thanks at least in part to its tolerance to low concentrations of sulfide, the population can remain alive in the lake. Mixing throughout the lake provides both nutrients and light, and the cells grow with slow generation times (55–20 d). The main loss factors are decomposition and outwash, and productivity is low. Since low temperatures inhibit cell division more than cell growth (Morgan and Kalff 1975), the average cell volume increases continuously (Fig. 9A). As temperature increases from February on, doubling times are progressively shorter, reaching 6 or 7 d in spring, around the beginning of stratification. At the same time, with increased temperature and light and access to nutrients due to mixing, the standing crop increases exponentially and sedimentation accounts for 40–60% of the losses (Fig. 9B).

With the onset of stratification, the population slows its growth rate, and cell volume starts to diminish immediately. At this point *C. phaseolus* enters a dynamic equilibrium which lasts through summer (Fig. 9C). Only 1–20% of surface light reaches the metalimnion, and the increases or decreases of standing crop depend on a delicate balance between the light reaching the metalimnion and occasionally important loss factors such as grazing—decomposition being very important but constant. When fall overturn occurs, the population returns to winter conditions.

Forsberg (1985), analyzing data from several lakes in the literature, concluded that phytoplankton communities are in a near steady state condition where carbon is lost almost as fast as it is produced. Our results indicate that assertion to be true for at least some metalimnetic populations. Both production and loss factors increase in summer and decrease in winter, following biomass trends. Actual growth of biomass, however, is only possible in spring, when production temporarily offsets loss factors. Therefore, carbon and energy fluxes seem to be more important than a mere assessment of the biomass would suggest. In this respect, metalimnetic populations do not differ from epilimnetic algae. The difference may lie in the outcome of a tradeoff between light and nutrient availability. Stability of vertical stratification allows development of metalimnetic algae preferring some hypolimnetic nutrient or vitamin at the expense of low light intensity. Currently, we are trying to test whether this hypothesis holds for the *Cryptomonas* population colonizing the metalimnion of Lake Cisó.

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panels indicate vertical distribution of the dominant organisms in the vertical transects shown by the black stripes in the middle panels. In A a uniform mixture of a few large cells of *Cryptomonas* with many Chromatiaceae (single-celled *Chromatium minus* and aggregate forming *Lamprocystis* M3) are found. In B the lake has started stratification. *Cryptomonas* cells are smaller and more abundant in the metalimnion than in A, and the Chromatiaceae are found below. Finally, in C, stratification is very advanced and marked, *Cryptomonas* cells are smaller and more abundant than in B, and both the ciliate *Coleps hirtus* (hairy organism with plates) and the rotifer *Anuraeopsis fissa* (upper left corner) are present. Organisms are drawn to scale.

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