Hydrobiologia **272**: 239–249, 1994.
H. J. Dumont, J. Green & H. Masundire (eds), Studies on the Ecology of Tropical Zooplankton.

Chlorophyll budget in a productive tropical pond: algal production, sedimentation, and grazing by microzooplankton and rotifers

Robert Arfi & Daniel Guiral
Centre de Recherches Océanographiques, BP V18 Abidjan, Côte d'Ivoire

Key words: chlorophyll budget, picoplankton, nanoplankton, grazing, rotifer

Abstract

Chlorophyll *a* and pheopigment standing stocks and fluxes were used during a two weeks colonization experiment in a productive tropical pond (Layo, Côte d'Ivoire) in order to establish a chlorophyll budget. The experiment started from an azoïc state (the pond was dried, limed and progressively filled with ground water). Algal production was the only input to the phytoplanktonic system, while grazing and algal sedimentation were the main outputs. Chlorophyll *a* reflected the algal biomass, and degradation pigments were considered as an index of grazing by zooplankton (here, protozoans and rotifers). An estimation of the input through the algal growth rate was performed for the two main biological events observed during the study. The first algal bloom, with a large picoplankton participation, was mainly regulated by microzooplankton (increase of the peak) and rotifers (decrease of the peak). The second bloom (exclusively nanoplankton) was regulated by rotifers (increase) and by sedimentation of living cells (decrease). This last process was related to a sudden exhaustion of ammonia in the water column. Because of the time-lag between algal proliferation and zooplanktonic bloom, the phytoplanktonic biomass was able to be adjusted according to the availability of nutrients. This self-regulation took the

pellets containing the degradation pigments (SooHoo & Kiefer, 1982a, b; Welschmeyer *et al.*, 1984; Welschmeyer & Lorenzen, 1985; Litaker *et al.*, 1988). But when such methods are used in open water, there is the complication of hydrodynamism and rapid changes of water. Working in closed shallow systems is a way of getting round this problem. From the chlorophyll standing stock variations, from these estimates of grazing and from the cell sedimentation rates

sedimentation, as opposed to through grazing activity. This budget would provide a basis for improving productivity in such aquaculture ponds by maximizing the utilization of natural resources, and limiting the artificial input of nutrients for plankton-eating fishes.

Outlines of ecological successions during the early colonization stages in the pond

system, less the outputs (grazing and cell sedimentation). This is expressed by the formula:

$$dCHL/dt = CHL\,[\mu - g - G - SED]$$

where CHL (mg m^{-2}) represents chlorophyll *a* concentration in water, and μ, g, G and SED (d^{-1}) respectively phytoplankton growth rate, grazing by microzooplankton (corrected from pheopigment photodegradation), grazing by rotifers and sedimentation rates. The coupled differential equations corresponding to this model (details in Welschmeyer & Lorenzen, 1985) give algal input (μ) and output rates (g, G and SED) for the pelagic system. Table 1 gives the solutions for the differential equation. During the present study, micrograzers and rotifers were the only grazers present in the pond; at the end of the study, neither copepod nor cladoceran was observed in the water.

Grazing parameters are thus approached through variations and differential distribution of pheopigment concentrations in the water column and at the bottom. This method rests on several assumptions:

Table 1. Solution set for the differential equation (abbreviations and units in the text).

Instantaneous rates are deduced from the equation

$$dCHL/dt = CHL * [\mu - g - G - SED]$$

using these particular solutions:

$$SED = \frac{[\text{CHL-flux} * \ln(CHL_1/CHL_0)]}{[\Delta t * (CHL_1 - CHL_0)]}$$

$$G = \frac{[\text{PHEO-flux} * \ln(CHL_1/CHL_0)]}{\Delta t * (CHL_1 - CHL_0)]}$$

$g = g_1 * g_2/g_3$ with:

$$g_1 = [k * I * \Delta t] + \ln(CHL_1/CHL_0)$$

$$g_2 = PHEO_1 - PHEO_0 * \exp^{(-k*I*\Delta t)}$$

$$g_3 = \Delta t * [CHL_1 - CHL_0 * \exp^{-k*I*\Delta t}]$$

$$\mu = g + G + SED + [1/\Delta t] * ln(CHL_1/CHL_0)$$

– the only way to produce pheopigments is an acid hydrolysis of chlorophyll (pheophytin when the acid liberates Mg from the porphyrin ring, pheophorbide after further loss of the phytol chain). Herbivorous grazing is the basis of these processes in aquatic environments (Vernet & Lorenzen, 1987).

– it is known that protozoans produce fecal particles which are not membrane bound and rapidly disintegrate once emitted in water (Stoecker, 1984); some studies reported an increase in pheopigment concentration in grazing experiments (Daley, 1973; Goldman & Caron, 1985), while some other were less conclusive (Burkill *et al.*, 1987; Barlow *et al.*, 1988). In open water, photodegradation of these compounds leads rapidly to colorless residues which are no longer fluorescent. Based on light intensity, this process is supposed to be attenuated in more turbid waters.

– digestion processes and fecal production in rotifers is poorly understood. Edible particles (bacteria, microphytes) are concentrated in the mouth by the movements of the rotatory apparatus, ingested, broken in the mastax and digested in the digestive tractus in acid conditions. Pigmented degradation products accumulate in 'pellets' filling progressively the gut. At the end of the digestive process, these merely bound pellets are ejected in the water (Grassé, 1965).

– the amount of degradation pigments in a sample can give an estimate of the quantity of chlorophyll ingested, even if a more or less significant fraction of the chlorophyll may be metabolized in the digestive tractus of grazers (Dam & Peterson, 1988). Direct assimilation is known to occur (in particular for large grazers) but shows high variations, both within and across species (Lopez *et al.*, 1988). No precise information is available for this process in rotifers, but their intense metabolism (here enhanced by high water temperature) induced a very short gut evacuation time (5 to 10 minutes for *Brachionus plicatilis*, Pagano, unpublished data), thus minimizing the direct assimilation phenomenon. In a first approach basis, the molar conversion efficiency of chlorophyll *a* to pheophorbide in an ejected fecal

pellet by herbivorous rotifers will be here estimated to 0.66 (average value proposed by Dam & Peterson, 1988, from several literature experiments made on copepods).

– when pheopigments are measured by fluorescence, it is not possible to distinguish between pheophorbide *a* and pheophytin *a*. Since pheophorbide *a* is the dominant degradation product of chlorophyll *a* (Jeffrey, 1974), pheopigments will be considered at this preliminary stage as pheophorbide *a*.

– degradation pigments in small particles remaining in the water column can be mainly attributed to microzooplankton grazing, while pigments in large particles sinking to the bottom will mainly correspond to the larger zooplankton grazing (SooHoo & Kieffer, 1982a, b; Welschmeyer & Lorenzen, 1985). Pheopigments are expressed as equivalent-chlorophyll following the 1:1 weight ratio proposed by Conover *et al.* (1986) for fluorometer measurements.

– chlorophyll *b* can cause an underestimate of chlorophyll *a* concentrations and an overestimate of pheopigments (Lorenzen, 1981; Parsons *et al.*, 1984; Herbland, 1988), but spectrophotometric measurements of Layo samples showed very low values of chlorophyll *b*.

Estimating pigments fluxes from chlorophyll and pheopigments concentrations is then based on discussed assumptions. Several hypothesis can be accepted for our particular environment, but other assumptions cannot be verified. But owing to the simplicity of the technique and the intensity of the ecological phenomenons in the pond, assessment of respective importance of the input and of the output ways is still very informative for the management of such systems. In order to solve the differential equation and to calculate the instantaneous rates, several parameters had to be known, which were derivated from biological measurements in the pond.

Pigments in the water column

Water was sampled daily at the center of the pond (10 cm under surface, representative of the water column in this shallow homogeneous environment) and immediately filtered on a 63 μm screen. Particulate matter $<63\ \mu$m was retained on Whatman GF/F filters. Chlorophyll (CHl_i) and pheopigments ($PHEO_i$) concentrations were measured with a Turner 111 fluorometer after methanol extraction (adapted from Yentsch & Menzel, 1963).

Algal sedimentation

Vertical pigment fluxes (CHL-flux and PHEO-flux) were estimated with a sediment trap (area: 0.036 m^2) deployed daily at the sediment-water interface (dark part of the water column). Throughout the study, sediments were recovered at 8 am, and the trap rapidly replaced, after rinsing and filling with filtered pond water. Chlorophyll and pheopigment concentrations in the sedimented material were measured as above. Dividing the sinking chlorophyll flux (difference between two 8 am measurements, mg $m^{-2}\ d^{-1}$) by the initial standing stock in the water column (value measured at 8 am each day, mg m^{-3}) gave an estimate of the phytoplankton sedimentation rate (m d^{-1}).

Grazing rate calculation

Pheopigment provided an estimate of the grazing activity of herbivorous zooplankton. But some differences resulted from variations in grazer size and the corresponding dimensions of the defecated particulate material:

– samples taken in the water column corresponded mainly to pigments contained in small size particles, with a very low sedimentation rate (essentially products from small organisms or broken parts of larger pellets).

– samples taken in the trap corresponded to pigments contained in large size particles (here, essentially pellets from rotifers since no other large grazer was present in the pond during the study) with high sedimentation rates.

Because of the high water turbidity (light attenuation coefficients comprised between 2 and 3 m^{-1}, Arfi *et al.*, 1991) a limited fraction of the pigments was subjected to photodegradation in the euphotic layer, depending on the sinking rate of the particles, and the light intensity (I) to which they were exposed. With a very limited residence

time in the water column, the pigments contained in the sedimented particles were not subjected to photodegradation. The global incident irradiance was measured with a LiCor 200 SB pyranometer in the site and integrated daily. Photosynthetic Active Radiation (PAR) under the air-water interface was estimated to around 38% of the irradiance (Morel, 1978; Coté & Platt, 1983). In order to determine the photodegradation coefficient of pheopigments (k), incubations of water filtered on 10 μm screens (zooplankton elimination) were carried out 10 cm below the pond surface. The coefficient was calculated on the basis of the difference in pheopigment concentrations related to the available PAR (SooHoo & Kiefer, 1982a, b; Welschmeyer & Lorenzen, 1985). For the present study, and calculated after the first colonization days (chlorophyll > 1 mg m^{-2}), k was estimated to 0.022 m^{-2} mol.

Zooplankton sampling

and concentrated; neutralized formaline (4% final concentration) was added for sample conservation. Biometrical measurements were done to estimate an average biovolume for the species.

Results

Chlorophyll pigments

Pigments in the water column

Chlorophyll *a* concentrations were close to 0 at the beginning of the study. They increased exponentially from D4 to D7 (Fig. 1), with a maximum of 14.3 mg m^{-2}. After that, values decreased until D10, when a second bloom started (maximum: 54.6 mg m^{-2} on D12). Then, values decreased until the last day of observation (D17: 5.7 mg m^{-2}).

Pheopigment values (expressed as chlorophyll-equivalent) were high throughout the first bloom (maximum on D7, 10.1 mg m^{-2}) with degraded

2.3 mg m^{-2}. At the end of the second bloom, an increase of these concentrations corresponding to the sharp chlorophyll decrease was noted (13.2 mg m^{-2}), but afterwards, values remained quite low, fluctuating around 5 mg m^{-2}.

Algal sedimentation

Hexarthra intermedia. Some nauplii of cyclopid were observed at the end of the survey.

The first *Brachionus plicatilis* appeared on D3. This species showed high abundance during two sequences (Fig. 3): between D8 and D11 (8.3 10^6 ind m^{-2}, D9) and between D13 and D15 (5.6 10^6 ind m^{-2}, D14). The first *Brachionus* with eggs

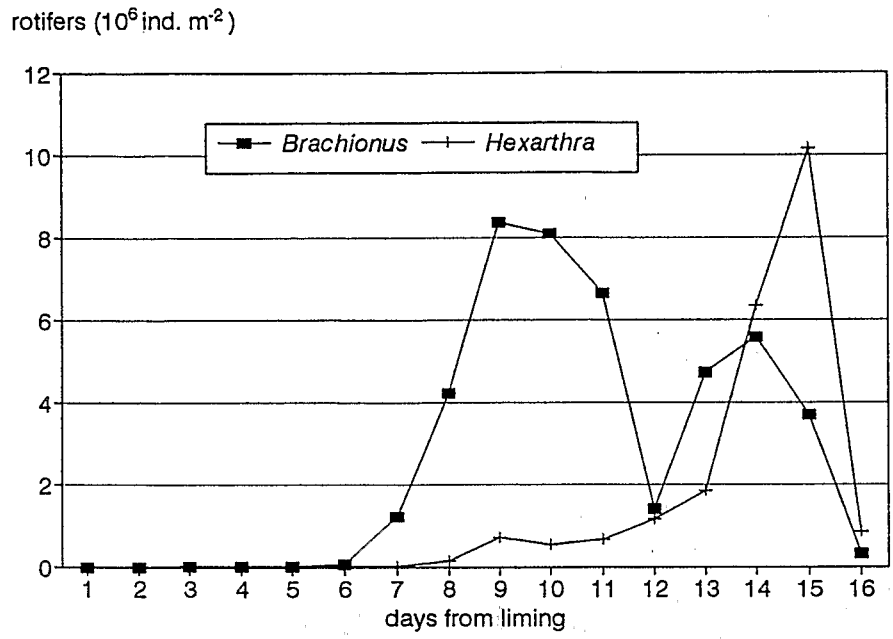

Fig. 3. Abundance of *Brachionus plicatilis* and *Hexarthra intermedia* in the water column.

For each of them, an increase and a decrease stage were considered. Parameters of the model were calculated from the relation between standing stocks and fluxes, giving instantaneous rates at the end of each stage (Table 1 and Fig. 4).

– The growth rate estimated for the increase stage of the first bloom (D4–D7) was high (4.16 d^{-1}, or 6 divisions per day), while the output components represented 72% of this input flux. The grazing activity of microzooplankton represented 44% of the output, completed for 34% by algal sedimentation. During the decrease

Table 2. Budget parameters during the four sequences studied.

	g micro-grazing rate (d^{-1})	G macro-grazing rate (d^{-1})	Sed chlorophyll sedimentation rate (d^{-1})	Total output (d^{-1})	μ growth rate (d^{-1})	Division per day	Doubling time (hours)	Output as percentage of input (%)
First algal bloom								
Increase sequence, D4-D7	1.30	0.67	1.01	2.98	4.16	6.0	4	72
Percentage of total output	43.6	22.5	33.9					
Decrease sequence, D8-D10	0.25	0.53	0.45	1.23	0.92	1.3	18	134
Percentage of total output	20.3	43.1	36.6					
Second algal bloom								
Increase sequence, D10-D12	0.03	0.57	0.35	0.95	2.08	3.0	8	46
Percentage of total output	3.2	60.0	36.8					
Decrease sequence, D13-D16	0.14	0.27	0.52	0.93	0.51	0.7	33	182
Percentage of total output	15.1	29.0	55.9					

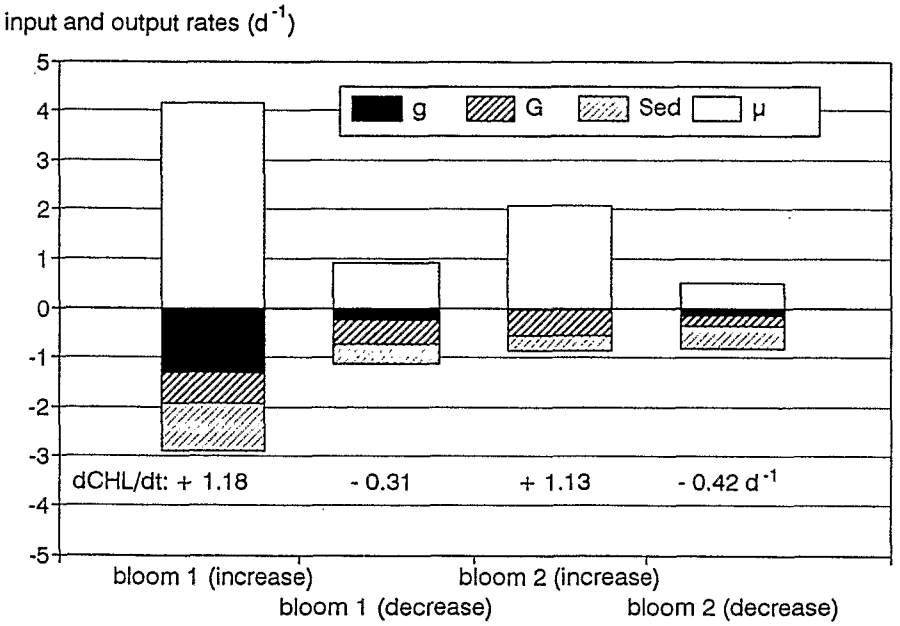

Fig. 4. Chlorophyll budget during the two algal blooms.

stage of this first phytoplankton bloom (D8–D10), output fluxes represented 134% of the input. The accumulation of microalgal materials observed from D4 to D7 was thus followed by a progressive decrease of the standing stock. Grazing by rotifers was the main output component (43% of the total output), and 37% resulted from cell sedimentation. During this sequence, the algal number of divisions per day was reduced to 1.3, and the growth rate to 0.92 d^{-1}.

– During the increase stage of the second algal bloom (D10–D12), the growth rate was 2.08 d^{-1} (3 divisions per day). Sedimentation of ungrazed cells represented more than a third of the whole output, and the algal system was mainly regulated by rotifers grazing (60% of output flux). The decrease stage of the second bloom (D13–D16) was characterized by outputs representing 182% of the input flux. Cell sedimentation was the main factor responsible for this biomass decrease (56% of the total output), along with rotifer grazing

Discussion

Phytoplankton growth rate

Variations of algal biomass in the pond during the 15 days following refilling featured two blooms, each made up of an exponential growth stage followed by a sharp decrease. The two periods of intensive development showed high growth rates (4.16 and 2.08 d^{-1} respectively). These values are in the top range of growth rates for several algae (Bonin *et al.*, 1986). They also correspond to high temperature situations (30 to 36 °C in the pond).

Control of phytoplankton development by sedimentation

Sinking rates of phytoplankton cells calculated during this study (0.11 to 0.36 m d^{-1}) come within the range noted in the literature for small

calculated for the two increase stages. However, the two algal communities were quite different, the first including 35% of picoplankton, the second quite exclusively comprised of nanoplankton (Arfi *et al.*, 1991). A preferential grazing of pico-

picoplanktonic cells represented a large part of the algal biomass. During the decrease stage of this first bloom, the proportion of outputs attributable to grazing by microzooplankton dropped from 44 to 20%. This decrease coincided with the

(Allan, 1976), might also contribute to this increased metabolic rate. The intensive grazing activity of *Brachionus plicatilis* led first to a reduction in the growth rate of phytoplankton, then to a sharp decrease in the algal biomass. The maximum abundance of *B. plicatilis* was observed on D9 (8.3×10^6 ind m^{-2}), while the chlorophyll biomass (17.1 mg m^{-2}) decreased by half compared to the maximum on D7. Related to the second algal bloom, a new development of zooplankton was noted from D12 (*B. plicatilis* and *Hexarthra intermedia*), with a maximum between D14 and D15. But, since the available algal biomass was suddenly reduced following a sharp decline in NH_4-N concentrations in the water (Arfi *et al.*, 1991), the rotifer abundance was drastically reduced.

Conclusion

Although based on discussed assumptions, the model used in the present paper produced rates in good agreement with the processes observed in to the protozoans elimination. Afterwards, the nanophytoplankton regulation resulted from the grazing by rotifers. Sedimentation of pellets was also a major mean of transfer toward the sediment for biological material produced in the water column.

Combined with intact cell sedimentation, grazing had drastic consequences in these shallow waters, and a major part of the material produced was removed from the planktonic system in a very short time. For optimal exploitation of the natural resources of an aquaculture pond, a study of the equilibrium nutrients-phytoplankton-zooplankton would provide a basis for artificial intervention, with a view to limiting the impact of this mode of regulation.

References

Allan, J. D., 1976. Life history patterns in zooplankton. Am Nat. 110: 165–180.

Arfi, R., D. Guiral & J. P. Torréton, 1991. Natural recolonization in a productive tropical pond, day to day variations

coid cyanobacteria in Lake Ontario, vertical and seasonal distributions during 1982. J. Phycol. 21: 171–175.

Conover, R. J., R. Durvasula, S. Roy & R. Wang, 1986. Probable loss of chlorophyll-derived pigments during passage through the gut of zooplankton, and some of the consequences. Limnol. Oceanogr. 3: 878–887.

Coté, B. & T. Platt, 1983. Day to day variations in the spring-summer photosynthetic parameters of coastal marine phytoplankton. Limnol. Oceanogr. 28: 320–344.

Daley, R. J., 1973. Experimental characterization of lacustrine chlorophyll diagenesis. II. Bacterial, viral and herbivore grazing effects. Mar. Ecol. Prog. Ser. 9: 35–42.

Dam, G. D. & W. T. Peterson, 1988. The effect of temperature on the gut clearance rate constant of planktonic copepods. J. exp. mar. Biol. Ecol. 123: 1–14.

Eppley, R. W., R. W. Holmes & J. D. H. Strickland, 1967. Sinking rates of marine phytoplankton measured with a fluorometer. J. exp. mar. Biol. Ecol. 1: 191–208.

Fahnenstiel, G. L., L. Sicko-Goad, D. Scavia & E. F. Stoermer, 1986. Importance of picoplankton in Lake Superior. Can. J. Fish. aquat. Sci. 43: 235–240.

Fenchel, T., 1982. Ecology of heterotrophic microflagellates. I. Some important forms and their functional morphology. Mar. Ecol. prog. Ser. 8: 211–223.

Fenchel, T., 1982. Ecology of heterotrophic microflagellates. II; Bioenergetics and growth. Mar. Ecol. prog. Ser. 8: 225–231.

Goldman, J. C. & D. A. Caron, 1985. Experimental studies on an omnivorous microflagellate: implications for grazing and nutrient regeneration in the marine microbial food chain. Deep Sea Res. 32: 889–915.

Grassé, P. P., 1965. Traité de zoologie, tome IV (III). Masson, Paris, 1496 pp.

Hem, S., M. Legendre, L. Trebaol, A. Cisse & Y. Moreau, 1993. Recherches sur les principales espèces d'intérêt aquacole en milieu lagunaire. Durand, J. R., P. Dufour & S. G. Zabi (eds), Environnement et ressources aquatiques de Côte d'Ivoire. 2 – Le milieu lagunaire. Editions de l'ORSTOM, in press.

Herbland, A., 1988. The deep phaeopigments maximum in the ocean: reality or illusion. In Rothschild, B. J. (ed.), Toward a Theory on biological-Physical Interaction in the World Ocean, Kluwer Academic Publishers: 157–172.

Jeffrey, S. W., 1974. Profiles of photosynthetic pigments in the ocean using the thin layer chromatography. Mar. Biol. 26: 101–110.

Lännergren, C., 1979. Buoyancy of natural populations of marine phytoplankton. Mar. Biol. 54: 1–10.

Legendre, M., M. Pagano & L. Saint-Jean, 1987. Peuplements et biomasse zooplanctonique dans des étangs de pisciculture lagunaire Layo, Côte d'Ivoire. Etude de la recolonisation après la mise en eau. Aquaculture 67: 321–341.

Litaker, W., C. S. Duke, B. E. Kenney & J. Ramus, 1988. Diel chl *a* and phaeopigment cycles in a shallow tidal estuary, potential role of microzooplankton grazing. Marine Ecol. prog. Ser. 47: 259–270.

Lopez, M. D. G., M. E. Huntley & P. F. Sykes, 1988. Pigment destruction by *Calanus pacificus*: impact on the estimation of water column fluxes. J. Plankton Res. 10: 715–734.

Lorenzen, C. J., 1981. Chlorophyll *b* in the ocean. Deep Sea Res. 28: 1049–1056.

Lorenzen, C. J. & N. A. Welschmeyer, 1983. The *in situ* sinking rates of herbivore fecal pellets. J. Plankton Res. 5: 929–933.

Lorenzen, C. J., N. A. Welschmeyer, A. E. Copping & M. Vernet, 1983. Sinking rates of organic particulates. Limnol. Oceanogr. 28: 766–769.

Morel, A., 1978. Available, usable and stored radiant energy in relation to marine photosynthesis. Deep Sea Res. 25: 673–688.

Parsons, T. R., Y. Maita & C. M. Lalli, 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York.

Pilarska, J., 1977. Ecophysiological studies on *Brachionus rubens* Ehrbg Rotatoria. I. Food selectivity and feeding rate. Pol. Arch. Hydrobiol. 24: 319–328.

Pourriot, R. & P. Champ, 1982. Ecologie du plancton des eaux continentales. Pourriot, R. ed., Masson, Paris, 198 pp.

Shuman, F. R. & C. J. Lorenzen, 1975. Quantitative degradation of chlorophyll by a marine herbivore. Limnol. Oceanogr. 20: 580–586.

SooHoo, J. B. & D. A. Kiefer, 1982a. Vertical distribution of phaeopigments. – I. A simple grazing and photooxidative scheme for small particles. Deep-Sea Res. 29: 1539–1551.

SooHoo, J. B. & D. A. Kiefer, 1982b. Vertical distribution of phaeopigments. – II. Rates of production and kinetics of photooxidation. Deep-Sea Res. 29: 1553–1563.

Stoecker, D. K., 1984. Particles production by phytoplanktonic ciliates. Limnol. Oceanogr. 29: 930–940.

Titman, D. & P. Kilham, 1976. Sinking in freshwater phytoplankton, some ecological implications of cell nutrient status and physical mixing processes. Limnol. Oceanol. 21: 409–417.

Vernet, M. & C. J. Lorenzen, 1987. The relative abundance of pheophorbide a and pheophytin a in temperate marine waters. Limnol. Oceanogr. 32: 352–358.

Waltz, N., 1983. Continuous culture of the pelagic rotifer *Keratella cochlearis* and *Brachionus angularis*. Arch. Hydrobiol. 98: 70–92.

Welschmeyer, N. A., A. E. Copping, M. Vernet & C. J. Lorenzen, 1984. Diel fluctuation in zooplankton grazing rate as determined from the downward vertical flux of pheopigments. Mar. Biol. 83: 263–270.

Welschmeyer, N. A. & C. J. Lorenzen, 1985. Chlorophyll budgets, zooplankton grazing and phytoplankton growth in a temperate fjord and the Central Pacific gyres. Limnol. Oceanogr. 30: 1–21.

Yentsch, S. & D. W. Menzel, 1963. A method for the determination of phytoplankton chlorophyll a and phaeophytin by fluorescence. Deep Sea Res. 10: 221–231.