Vol. 36: 61–72, 2004 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published June 24

Production and bioavailability of autochthonous dissolved organic carbon: effects of mesozooplankton

Theis Kragh*, Morten Søndergaard

Freshwater Biological Laboratory, University of Copenhagen, Helsingørsgade 51, 3400 Hillerød, Denmark

ABSTRACT: A phytoplankton bloom and decay sequence was created in 2 laboratory containers and mesozooplankton was added to one container before the peak of algal biomass. Each day for 22 d, the net production of autochthonous dissolved organic carbon (DOC) was measured and on 5 occasions the degradation kinetics and the total pool of biodegradable DOC (BDOC) were assayed in experiments lasting 230 d. Net accumulation of new DOC was 235 and 280 µM in the containers with and without zooplankton, respectively. The best description of microbial DOC degradation was a 2-pool model and 1st order exponential decay. Without mesozooplankton present, the degradation experiments showed accumulation of a large pool of labile BDOC characterised by decay coefficients >0.2 d^{-1}. The least labile pools in the 2 containers had similar coefficients (average 0.02 d^{-1}). The amount of newly produced recalcitrant DOC (RDOC) accounted for about 12% of new DOC. The differences observed with respect to degradation kinetics and net DOC production are explained by food web interactions and nutrient limitation. The presence of mesozooplankton resulted in high bacterial production keeping labile BDOC at low concentrations. In the container without mesozooplankton, the bacterial uptake capacity was reduced, probably by a combination of protist grazing and nutrient limitation. Consequently, about 75 µM BDOC with a half-life of less than 3 d accumulated during the experiment. Mineralisation of the accumulated dissolved organic matter (DOM) during microbial degradation in a nutrient replete environment was measured as the decrease in DOC and net mineralisation/immobilisation of inorganic N and P. The mineralisation of DOC was accompanied by low mineralisation of N and P and even immobilisation of phosphate during degradation of DOM produced in the container with mesozooplankton present. Bacterial production of DON and DOP is believed to result in a recalcitrant DOM pool enriched in N and P, and the activity of mesozooplankton seems to enhance this scenario.

KEY WORDS: Dissolved organic carbon · Dissolved organic matter · Autochthonous DOC · Mesozooplankton · Biodegradability · Mineralisation · N-rich recalcitrant DOM · P-rich recalcitrant DOM

INTRODUCTION

Dissolved organic carbon (DOC) in marine and freshwater ecosystems is a complex pool of compounds of autochthonous and allochthonous origin and the major proportion is refractory (RDOC) to degradation over time-scales of years. However, a variable but significant fraction can be degraded by micro-organisms and/or photochemically (e.g. Søndergaard & Middelboe 1995, Moran & Zepp 1997, del Giorgio & Davis 2003), and autochthonous DOC is considered more degradable than allochthonous DOC (Søndergaard & Middelboe 1995). Production and degradation of autochthonous DOC have been investigated in several previous studies (e.g. Norrman et al. 1995, Søndergaard et al. 2000a, Meon & Kirchman 2001), but only limited information is available on how food-web structures might affect DOC accumulation and subsequent degradation.

Multiple biotic processes occurring simultaneously explain the production of autochthonous DOC, which makes it difficult to identify the most important sources at any given time. No doubt, extracellular release from phytoplankton especially during nutrient deficiency

*Email: tkragh@bi.ku.dk

can be a major DOC source (Fogg 1966, Baines & Pace 1991, Søndergaard et al. 2000b). Cell lysis during 'natural' mortality, e.g. caused by viral attack, contributes to the continuous production of DOC and zooplankton grazing has also been suggested as a prominent DOC and dissolved organic nitrogen (DON) producing process (Lampert 1978, Olsen et al. 1986, Nagata 2000). With respect to grazing, it seems that the produced DOC is very labile, fuels instant bacterial production and does not accumulate to high concentrations (Olsen et al. 1986, Strom et al. 1997).

If DOC production and removal are in steady state, the concentration of each degradable compound or class of compounds is constant and inversely proportional to its lability. However, aquatic systems are seldom — if ever — in steady state and the concentration of biodegradable DOC (BDOC) is variable in time and space (Ogura 1972, Søndergaard et al. 1995, Hopkinson et al. 2002). Our knowledge about specific processes and mechanisms contributing to the accumulation of BDOC and production of autochthonous recalcitrant (RDOC) is rudimentary. Microbial foodweb processes with production of refractory components (Stoderegger & Herndl 1998, Ogawa et al. 2001), protection of proteins in submicron particles (Stoderegger & Herndl 1998), bacterial nutrient limitation (Williams 1995, Rivkin & Anderson 1997), bacterial uptake capacity controlled by protist grazing (Thingstad & Havskum 1999) as well as photochemical transformations (Tranvik & Kokalj 1998) are some of the processes used to explain DOC accumulation due to production of DOC with long turnover times (Søndergaard et al. 2000a,b).

To our knowledge, the studies by Park et al. (1997) and Olsen et al. (2002) are among the few directly addressing whether zooplankton grazing can enhance DOC accumulation including a large fraction of BDOC. Park et al. (1997) found a correlation between grazing and the accumulation of DOC and concluded that zooplankton excretion was the DOC producing process. Furthermore, in degradation experiments lasting 100 d, Park et al. (1997) found that between 60 and 80% of the new DOC was labile and the rest was refractory. In chemostat experiments carried out by Olsen et al. (2002) grazing did not increase the steady state concentration of DOC, but reduced the time to reach steady state indicating zooplankton to contribute to DOC production. The degradable DOC fraction during 15 d of bacterial degradation was between 15 and 25% of the total (Olsen et al. 2002). Thus, rather variable results concerning the effects of grazing on DOC production and accumulation have been reported.

The fate of new autochthonous DOC and allochthonous BDOC has ecological implications for the ecosystem metabolism and the cycling of elements (Wetzel 1995). BDOC with long turnover times can sustain bacterial production during periods of low primary production and mineralisation of DOC and dissolved organics (DON and dissolved organic phosphorus [DOP]) is delayed compared with the time of production. DOP and DON seem to be preferentially mineralised compared with DOC, which, in a diagenetic perspective, makes the C:N:P ratio of dissolved organic matter (DOM) carbon-rich (Williams 1995, Hopkinson et al. 2002).

In this study, we investigated how grazing by mesozooplankton might influence the production and accumulation of DOC during an experimental algal bloom and its decay. Accumulation of DOC was measured chemically and degradation experiments were used to quantify RDOC and characterise the lability of BDOC. Bacterial production was used to quantify the total DOC production. We found that microbial activity and the production and fate of DOM can be greatly impacted by the presence/absence of mesozooplankton and can have implications for the mineralisation of organic matter and nutrient dynamics.

MATERIALS AND METHODS

Experimental design. The experiment was carried out in two 100 l glass containers in the laboratory. Before use, the containers and all sampling equipment were acid washed. Commercially available spring water (Harilds Kildevand™) was used as medium, in order to keep the initial DOC background low and refractory (Søndergaard & Worm 2000). Twenty l filtered (100 µm to remove larger mesozooplankton) water from Lake Esrum (Søndergaard et al. 2000a) served as inoculum and was added to 80 l spring water. A water-cooling system was installed to keep the temperature constant at 14°C. Light was supplied by fluorescent tubes at a 12 h light:12 h dark cycle and a photon flux density of approximately 150 µmol m^{-2} s^{-1}. A 4-point aerating system with filtered atmospheric air provided circulation. Nutrients were added as KNO_3 (126 µM), KH_2PO_4 (8 µM) and $Na_2SiO_3 \cdot 9H_2O$ (200 µM) to promote a phytoplankton bloom. After 14 d, mesozooplankton > 200 µm was collected from approximately 200 l water from Lake Esrum and added to one container. The mesozooplankton was dominated by *Daphnia* and *Cyclops* species, but they were not quantified. After addition, the mesozooplankton was monitored to ensure their presence throughout the experiment.

Sampling procedures. Water for chemical and biological analyses was sampled daily. The measured variables included chlorophyll *a* (chl *a*), inorganic nutrients, DOC, DON and bacterial abundance and

production. Five experiments to measure DOC degradation and nutrient mineralisation were carried out over the course of the experiment.

Before sampling, the water was mixed carefully with a plexiglass stirrer. Water was drawn through a tube placed in mid-water into an acid rinsed bottle that previously had been flushed with sample water. Subsampling for all chemical and biological measurements was done from this bottle and in triplicates. For the chemical analyses of dissolved material, a 50 ml syringe was rinsed and filled with sample water and a 13 mm pre-combusted GF/F filter (Whatman) was mounted on the syringe. The first 5 ml filtrate was discarded and the next 5 ml was collected for DOC measurements in pre-combusted vials with 50 µl 2 M HCl added for conservation. The vials were stored in darkness at room temperature. Another 5 ml filtrate was collected in a cryo-vial and stored frozen for later nutrient analysis. Samples for chl *a* measurements and bacterial abundance and production were taken directly from the bottle. A similar sampling procedure was used to collect water for the degradation experiments.

DOC degradation experiments. Microbial DOC degradation was measured as the decrease in DOC over time in simple batch incubations after the addition of a bacterial inoculum to a filtered water sample (del Giorgio & Davis 2003). Samples were collected on Days 5, 12, 17, 20 and 22. A sample of about 2.5 l was filtered (GF/F), and inoculated with a GF/C filtrate from the respective containers (5%). The use of a GF/C filter allows the passage of flagellates, so the microbial community during degradation was composed of virus, bacteria and flagellates. Mineral nutrients (10 µM KNO_3 and 1.5 µM KH_2PO_4) were added to ensure carbon-limited bacterial growth. The bottles were darkened and incubated at room temperature (18 ± 1°C) for 230 d. At decreasing time intervals, subsamples were analysed for DOC and mineral nutrients.

Various decay models including 2 or 3 degradable DOC pools and a recalcitrant background have been used to describe DOC degradation kinetics (Ogura 1972, Søndergaard et al. 2000a, Hopkinson et al. 2002). A model with 2 degradable pools, each described by 1st order exponential decay, provided the best fit to our results with r^2 values > 0.8 for most regressions (see Table 1). The decision of pool size was made in each case from the maximal r^2 values reached in regressions of DOC versus time starting with 3 points and including more points until r^2 reached a maximum. By this approach, the degradable pools are described by the decay coefficients k_1 and k_2. We defined the recalcitrant endpoint as the concentration in the sample taken after 230 d of incubation, but realise that a 'true' endpoint is probably never obtained in most experiments (see also Fry et al. 1996). The BDOC pools described with k_1 and k_2 are called labile and semilabile, respectively.

Decay models and terminology are often linked to sampling frequency, experimental conditions and duration, and as these are extremely variable, comparisons among different studies are at best difficult (see del Giorgio & Davis 2003). In our study, the starting conditions for each degradation experiment are different with respect to abundance of bacteria; however, as we found that the fastest degradation of DOC in experiments added fewest bacteria, we assume that the differences are evened out within a period of 1 to 2 d, and that the observed differences in degradation kinetics are linked to the quality of DOC and not to the inoculum.

Analytical methods. ***Chl a:*** *Chl a* was extracted in ethanol and absorption was measured with a Hitachi U-1100 spectrophotometer (Jespersen & Christoffersen 1987).

Nutrients: Nitrite/nitrate, ammonium and phosphate were measured with standard methods with an Alpkem-RFA 300 autoanalyser (RFA manual). Silicate was measured with the acidic ammonium molybdate method (Anonymous 1985).

Bacterial abundance and production: Bacterial abundance was determined by flow cytometry (FACS Calibur, Becton Dickinson) after staining the fixed cells (2% glutaraldehyde) with the nucleic acid stain SYBR Green I (Molecular Probes) according to Marie et al. (1997). Briefly, 10 µl of a 100-times dilution of stock SYBR Green 1 was added to 1ml of sample, followed by 10 µl of a suspension of 2 µm fluorescent beads (Polyscience). Concentration of the beads was used for volume calibration and determined by epifluorescent microscopy after filtration onto 0.2 µm black membrane filters (Poretics). Bacterial production was measured with tritiated thymidine and converted to carbon production by 2×10^{18} cells mol^{-1} (Bell 1993) and 20 fg C $cell^{-1}$ (Lee & Fuhrman 1987).

DOC: DOC was measured with a Shimadzu TOC-5000. A 1.2% Pt catalyst on silica beads was used in the combustion column (Cauwet 1994). The calibration consisted of a 3-point calibration curve ($r^2 > 0.998$) and at least 3 injections with a maximum of 5 were made for each sample. Each sample run included standard and blank samples interspersed throughout the sample run. Deep ocean and blank samples provided by J. Sharp served as quality controls. The machine and the procedure had previously reached accepted standards in an intercalibration (J. Sharp pers. comm.).

Total nitrogen: Total nitrogen was measured with an integrated on-line system including a Shimadzu TOC-5000 and a Sievers NO analyzer. DON was calculated by subtraction of inorganic nitrogen. Dr. Gustave Cauwet kindly carried out the TN measurements.

Statistics. The experiment was carried out without replicate containers. Therefore, the comparative results cannot be evaluated statistically and the interpretations rely on the consistency and robustness of within container measurements. The slopes of the decay coefficients are tested for similarity by the method in Sokal & Rohlf (1981).

RESULTS

Nutrients, chl *a* and bacteria

The growth of algae depleted inorganic nutrients within 11 to 14 d and before mesozooplankton was added (Fig. 1). Phosphate was depleted slightly faster than silicate and inorganic nitrogen. All nutrients were depleted 1 to 2 d faster in the container where mesozooplankton was added at Day 14. The difference in chlorophyll *a* concentration was not known at the time of zooplankton addition. The difference between the containers was due to a faster development of phytoplankton despite identical inocula (Fig. 2A). Grazing by mesozooplankton reduced the algal peak biomass to 100 µg chl *a* l^{-1}, while the absence of mesozooplankton allowed the chl *a* to increase exponentially over 17 d and to peak at 148 µg chl *a* l^{-1} (Fig. 2A). From Day 17 to 22, the chl *a* concentrations decreased to about 25 µg chl *a* l^{-1} in both containers.

The diatoms *Fragilaria* sp. and *Asterionella* sp. dominated the phytoplankton in both containers until Day 16. From Day 16 onwards, chlorococalean green algae dominated in both containers, although diatoms were still present. The mesozooplankton did not change the composition of the algal community. The mesozooplankton was monitored for species present, but these were not quantified; *Daphnia* and *Cyclops* species dominated throughout the experiment.

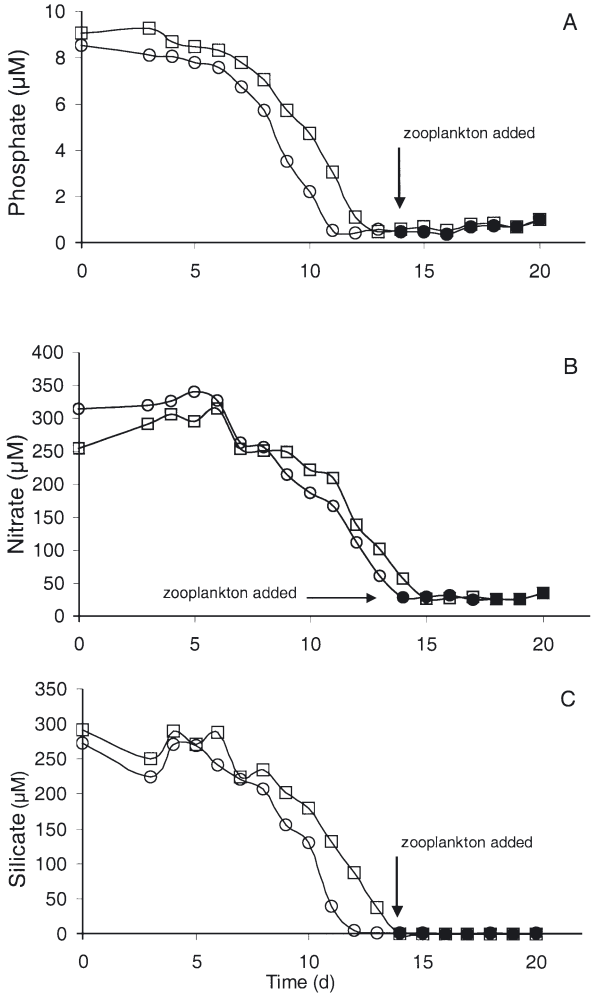

Fig. 1. Concentrations of inorganic nutrients in the 2 containers. (A) Phosphate, (B) nitrate, (C) silicate. (□,○) Without zooplankton present, (●) after addition of zooplankton

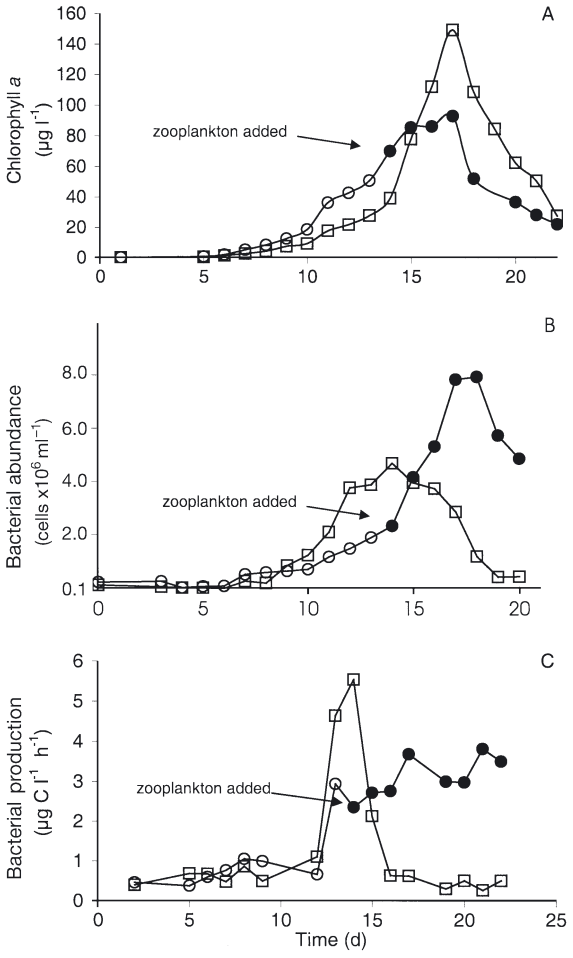

Fig. 2. Development of (A) chlorophyll *a* (chl *a*), (B) bacterial abundance and (C) bacterial production. (□,○) Without zooplankton, (●) with zooplankton

During Week 1 of the experiment, bacterial abundance was very low and ranged from 1 to 5×10^5 cells ml^{-1} (Fig. 2B). From Day 8, the bacteria responded to the growth of algae and the 2 containers started to deviate both with respect to bacterial abundance and production. In the container with the slower development of phytoplankton, the bacteria increased faster than in the other container and reached a peak of 4.8×10^6 ml^{-1} at Day 14, which was about double the abundance measured in the other container. The peak was followed by a decline to a low of 5×10^5 ml^{-1} at Day 20. In the container with added mesozooplankton, the abundance continued to increase to a peak of 8×10^6 ml^{-1} at Days 17 and 18 followed by a modest decline (Fig. 2B). Unfortunately, we lost the samples from Days 21 and 22. The time courses of bacterial production partly mirrored the abundances with low initial values and much higher peak values in the container that was later (by chance) selected to be without mesozooplankton. The peak production was followed by a fast decline to very low values (Fig. 2C). With mesozooplankton present, a high and prolonged production plateau developed and lasted until Day 22 (Fig. 2C). Bacterial production integrated from Days 14 to 22 was about 3-fold higher in the container with added mesozooplankton and about 6-fold higher from Days 16 to 22.

Dissolved organics

DOC increased over the course of the experiment from 110 to 345 and 390 µM for the containers with and without mesozooplankton, respectively (Fig. 3). Until Day 18, the DOC concentrations in the 2 containers were similar. Accordingly, the differences between the containers with respect to phytoplankton and bacteria did not have measurable effects on the net production of DOC. From Day 19 onwards, the concentrations of DOC in the container without mesozooplankton

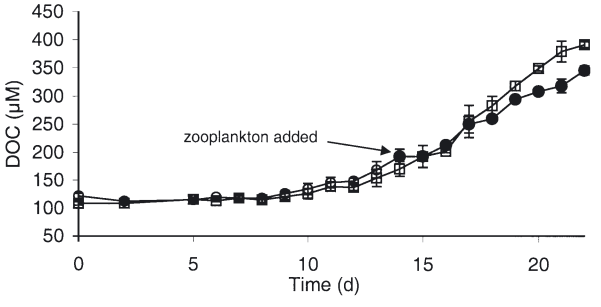

Fig. 3. Concentration of dissolved organic carbon (DOC) (mean ± SD, n = 3) in the 2 containers. (□,○) Without zooplankton, (●) with zooplankton

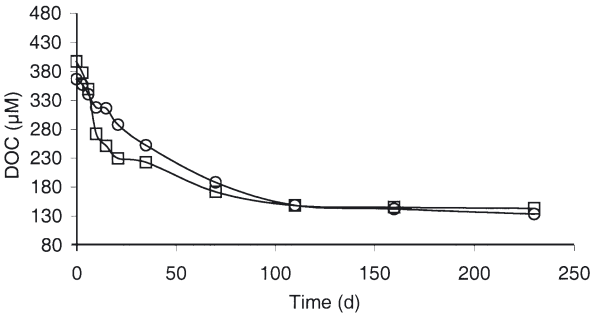

Fig. 4. Concentrations of dissolved organic carbon (DOC) in biodegradation experiments sampled at Day 22. (○) With zooplankton, (□) without zooplankton

appeared to be higher. During the first 18 d of the experiment, new DOC accumulated exponentially at about 0.1 d^{-1}, while linear accumulation rates of about 20 and 28 µM d^{-1} for + and – mesozooplankton, respectively, were measured during the decline of the algal communities. About 235 and 280 µM of newly produced DOC accumulated in the container with and without mesozooplankton, respectively.

Until Day 14, the calculation of DON from total nitrogen measurements were flawed by high nitrate concentrations (see Fig. 1B) and very low DON concentrations. DON could not be calculated with any confidence. However, low nitrate concentrations from Day 14 made it possible to calculate DON. At Days 14 and 15, the concentrations were close to zero (± 2 µM), but then DON increased at an apparent linear rate of about 2.7 µM d^{-1} (SD ± 1.1, $r^2 = 0.63$, n = 6) in both containers (data not shown). Thus, from Days 14 to 22, the C:N ratio of the accumulating DOM was between 7 and 10. The C:N ratio of new DOM during the entire experiment was between 13 and 16. The higher ratio values were calculated for the container without mesozooplankton due to higher DOC concentrations.

Biodegradability of new DOC

To measure the biodegradability of new DOC, it was assumed that the initial DOC is refractory. This assumption was tested on Day 5 in 2 degradation experiments showing utilisation of about 5 µM within 6 d and no further degradation over 225 d. This small amount of BDOC with a calculated half-life of 1.5 d was probably added with the inoculum and would be removed before the addition of mesozooplankton.

The results of a typical degradation experiment are shown in Fig. 4 with samples taken at Day 22. Two features are apparent: (1) the DOC concentrations reached after 230 d of degradation were not different; (2) the time courses of DOC utilisation differed

Table 1. Dissolved organic carbon (DOC) concentrations in the 2 degradable pools and 1 recalcitrant (R) pool at the indicated day of the experiment. Calculated decay coefficients are shown for the 2 biodegradable DOC pools. Zooplankton was added at Day 14 to the mesocosm indicated as +zoo. Break points (days) between the calculations of k_1 and k_2 are shown in parentheses

Day	k_1						k_2						R	
	+zoo			−zoo			+zoo			−zoo			+zoo	−zoo
	DOC (µM)	Decay coefficient	r^2	DOC (µM)	Decay coefficient	r^2	DOC (µM)	Decay coefficient	r^2	DOC (µM)	Decay coefficient	r^2	DOC (µM)	DOC (µM)
12	18	0.20 (15)	0.88	14	0.14 (15)	0.91	16	0.02	0.92	20	0.01	0.79	0	1
17	43	0.12 (35)	0.84	70	0.28 (15)	0.81	84	0.02	0.98	41	0.02	0.88	14	17
20	49	0.04 (110)	0.86	114	0.21 (21)	0.80	126	0.02	0.94	83	0.02	0.96	28	25
22	74	0.07 (70)	0.90	146	0.24 (15)	0.83	145	0.02	0.86	103	0.02	0.91	34	30

between the samples. A large fraction of the DOC produced in the container without mesozooplankton was decomposed much faster than the DOC produced with mesozooplankton present. This pattern was found in all 3 decomposition experiments carried out after mesozooplankton addition, i.e. Days 17, 20 and 22. The time courses of DOC utilisation and the concentration of RDOC were similar in the degradation experiments performed on Day 12 before the addition of mesozooplankton.

The exponential decay model identified rather distinct degradable DOC pools with decay coefficients ranging from 0.01 to 0.28 d^{-1} (Table 1). The degradation experiments from Day 12 were sampled before mesozooplankton addition and the decay coefficients were reasonably similar with k_1 and k_2 averaging 0.17 and 0.016 d^{-1}, respectively (Table 1). About 35 µM DOC had accumulated in both containers and 95% was biodegradable. In lability terms, the k_1 values for Day 12 were between those measured later in the experiment.

The k_1 coefficients for the 2 containers deviated significantly from Day 17 and onwards ($p < 0.01$, paired *t*-test). In the container without mesozooplankton, a large pool of DOC with k_1 values higher than 0.2 d^{-1} accumulated, while a labile pool with lower k_1 values (<0.12 d^{-1}) accumulated in the container with added mesozooplankton (Table 1). The initial decay coefficients were 2- to 5-fold lower for BDOC accumulating with mesozooplankton present than for BDOC without mesozooplankton. The half-lives of BDOC calculated from the k_1 values were less than 3 d in the absence of mesozooplankton and ranged between 6 and 16 d with mesozooplankton present. In all experiments, the semi-labile DOC pools had k_2 values of about 0.02 d^{-1} and half-lives of 35 d (Table 1). The k_2 values were significantly similar ($p < 0.05$, test for equality of slopes, Sokal & Rohlf 1981). Without mesozooplankton present, the k_2 coefficients described the decay after 15 to 20 d of incubation; however, with mesozooplankton present, the period for slow k_2 decomposition were more variable and the starting time ranged from 35 to 110 d. The break-point times between k_1 and k_2 are shown in Table 1.

RDOC was defined as DOC still remaining after 230 d of degradation. This 'endpoint' was not influenced by mesozooplankton. In the degradation experiments initiated at Day 12, RDOC was less than 5% of new DOC. In the samples harvested at Days 17, 20 and 22, the concentrations had increased and the relative fraction of RDOC had increased to between 9 and 12% (Table 1).

Nutrient mineralisation during degradation

In the degradation experiments, we added mineral nutrients to ensure that organic carbon was the limiting factor. Immobilisation and mineralisation of N and P were calculated from the measured inorganic nutrient concentrations during each degradation experiment. The results from the experiments started at Day 20 are used as an example.

After an initial bacterial uptake of added nitrate, ammonium was produced and subsequently nitrified. The net outcome was mineralisation of about 3.7 µM N (Fig. 5A) concomitant with a removal of between 183 and 212 µM DOC (Table 2). Assuming that the microbial biomass was very low after 230 d and most utilised DOC was mineralised, a C:N mineralisation ratio between 50 and 60 can be calculated. At Day 12, the high concentrations of NO_3 in the containers (see Fig. 1B) prevented the detection of a small amount of mineralised N. The samples from Day 17 were similar to Day 20 and showed a net mineralisation of NO_3. By contrast, a low N-immobilisation was measured in both samples from Day 22, while the mineralisation of DOC continued to increase (Table 2). Thus, the C:N mineralisation ratios moved from high but positive values at Days 17 and 20, to a negative value at Day 22. No systematic differences in N-mineralisation were observed between the 2 containers.

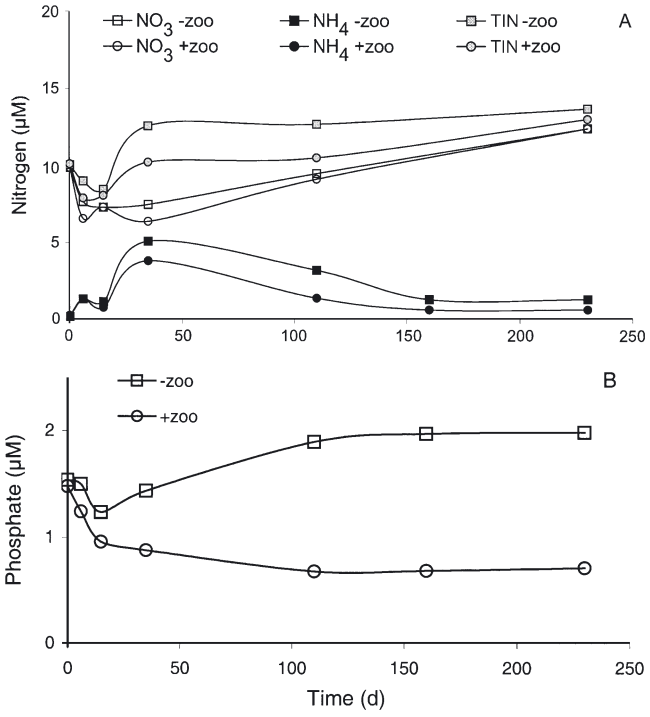

Fig. 5. Concentrations of (A) inorganic nitrogen and (B) phosphate during degradation of samples collected at Day 20. Total inorganic nitrogen (TIN) is presented. +zoo and –zoo: with and without zooplankton, respectively

After mesozooplankton was added, the containers started to deviate with respect to P-mineralisation. If no mesozooplankton was present, the net mineralisation of organic P was between 0.4 and 0.7 µM, and the C:P mineralisation ratio accordingly had a large range from 184 to 775 (Fig. 5B, Table 2). By contrast, all degradation experiments from the container with mesozooplankton resulted in a net immobilisation of phosphate (Fig. 5B, Table 2). Of the 1.5 µM phosphate present at the start of a degradation experiment, between 0.7 and 1.4 µM was immobilised within 230 d (Table 2). Most P was immobilised in the sample from Day 22, which also showed immobilisation of N.

Table 2. Δ mineralisation and Δ immobilisation (–) of carbon, nitrogen and phosphate after 230 d of degradation. All values are in µM. +zoo and –zoo: with and without zooplankton, respectively. nd: not detected

Day	Carbon		Nitrogen		Phosphate	
	–zoo	+zoo	–zoo	+zoo	–zoo	+zoo
12	34	34	nd	nd	–0.2	–0.01
17	111	127	4.8	6.3	0.7	–0.7
20	197	175	3.7	3.7	0.4	–0.8
22	248	219	–0.9	–0.7	0.4	–1.4

DISCUSSION

Production and degradation of autochthonous DOC

Accumulation of autochthonous and biodegradable DOC has previously been observed *in situ* over timescales of days (Ochiai et al. 1979, Billen & Fontigny 1987), seasons (Carlson et al. 1994), and during algal bloom and decay/sedimentation sequences in container experiments (Norrman et al. 1995, Fajon et al. 1999, Søndergaard et al. 2000a). In the present experiment, the production of new DOC after 22 d accounted for 35 to 43 % of the peak net accumulation of particulate carbon and is within the range observed in other studies encompassing nutrient limitation of phytoplankton (Norrman et al. 1995, Søndergaard et al. 2000a, Olsen et al. 2002). Accumulation of carbohydrates has often explained a substantial part of DOC accumulation, especially when diatoms are dominating (Ittekot et al. 1981, Biersmith & Benner 1998, Fajon et al. 1999). In the present experiment, combined neutral carbohydrates explained between 25 and 35 % of the new DOC and their relative contribution did not change over time (T. Kragh & N. H. Borch pers. comm.).

The ultimate reason for any DOC accumulation is a mismatch between the rate of production and utilisation. Low rates of bacterial utilisation might be due to an inherent biochemical resistance of the produced DOC towards fast bacterial uptake. Low bacterial uptake can also be caused by nutrient limitation and/or grazer control of the active biomass. All explanations have been analysed theoretically (Thingstad & Lignell 1997) and supported with data from container and chemostat experiments (Thingstad & Havskum 1999, Søndergaard et al. 2000b, Olsen et al. 2002). The explanations do not exclude each other. With respect to grazer control, the active bacterial biomass has to be reduced to a level not counteracted by an increase in cell-specific activity. Whatever reason(s) for the mismatch between production and uptake, the outcome is an accumulation of biodegradable DOC with different turnover times. Compounds with a high biochemical resistance are expected to accumulate under any environmental condition, while very labile compounds only can be expected to accumulate during nutrient limitation and/or grazer control of bacteria.

The concentrations of inorganic nutrients remained very low in the containers, even during algal decay. Therefore, the observed patterns with respect to DOC accumulation could be explained by a combination of the above-mentioned mechanisms. Before moving further into the discussion, we would like to emphasise that our experiment was not designed to establish a 'natural' plankton community, but only to have a high enough density of mesozooplankton to enable us to

detect signals relating their presence to new DOC production and its subsequent microbial degradation, if indeed any such effects were present. Thus, specific process rates cannot be directly compared with *in situ* values or those found in other experiments.

Some differences were found between the 2 containers before the addition of mesozooplankton. Phosphate, nitrate and silicate were depleted slightly faster in the container where mesozooplankton was to be added at Day 14 (+ container). In accordance, higher concentrations of chl *a* were measured in the + container before addition of mesozooplankton. There were no measurable differences between the 2 containers with regard to DOC concentrations before the addition of mesozooplankton. The water sampled for the degradation experiments at Day 12 showed that similar concentrations of DOC had accumulated, but that the k_1 decay coefficient for the + container was slightly higher (Table 1). The early depletion of nutrients in the 'to be' + container could have limited bacterial growth, which could result in the accumulation of labile DOC. We therefore argue that a bias caused by the faster utilisation of nutrients in the +container would be accumulation of labile DOC. When mesozooplankton was added, we found a distinct lowering of the k_1 decay coefficient and a shift from labile to semilabile DOC being dominant. This is contrary to what we would expect if the bacterial activity was limited by nutrients.

A lowering of the phytoplankton peak biomass, sustaining high bacterial abundance and production, and lower DOC concentrations were the obvious effects of the mesozooplankton (Fig. 2). It is known that zooplankton, especially cladocerans, release large amounts of biodegradable DOC during grazing (Olsen et al. 1986, Strom et al. 1997), enhance bacterial production (Hygum et al. 1997) and regenerate organically bound phosphorus and nitrogen (Vadstein et al. 1995). At the same time, they can effectively reduce protist grazing on bacteria with a consequential increase in bacterial abundance (Jürgens et al. 1994). We suggest that the high bacterial abundance and production in the mesozooplankton container during the latter phase of our experiment were due to these mesozooplankton attributes. The supply of regenerated P and possibly N to bacteria thus becomes the key to explain the absence of DOC with fast turnover in the mesozooplankton container and the lower DOC concentrations.

In the container without mesozooplankton, labile DOC started to accumulate at Day 17 and continued to do so over the following days (Table 1). At Day 22, labile DOC with a short turnover time accounted for about 52% of the BDOC. Bacterial nutrient limitation and/or a reduced uptake capacity by a low biomass could explain this event. At Day 17, bacterial production was very low although the abundance had not yet decreased substantially. The calculated growth rate at Day 17 based on cell abundance and production was a low 0.2 d^{-1}. At Day 20, the growth rate had increased 4-fold to 0.8 d^{-1}, which would indicate that a nutrient limited situation was followed by almost unlimited growth rates. The maximum bacterial growth rate for the entire experiment was 0.9 d^{-1}. Thus, the accumulation of labile DOC until Day 17 may be explained by nutrient limitation causing low growth rates, while the capacity of the bacterial community to remove labile DOC between Days 20 and 22 was kept low by high grazing, most probably by protists. Although the cell-specific activity increased 4-fold at this stage, it was not enough to prevent the continued accumulation of labile DOC.

Olsen et al. (2002) have shown that protist grazing on bacteria may effectively mineralise P. From Fig. 2B, it is evident that high bacterial grazing started at Day 12 in the container without mesozooplankton. The abundance was reduced to 5×10^5 ml^{-1} by Day 19. Thus, the grazing on bacteria at Day 17 should have allowed substantial nutrient recycling and a consequential removal of the labile DOC. However, phytoplankton peaked at the same time and can have competed successfully for the regenerated nutrients (Vadstein 2000, Olsen et al. 2002) and prevented high bacterial production. The low growth rates found at Days 16 and 17 with an ample supply of organic substrate are firm indications of nutrient limitation of the bacterial community.

The growth rates of the bacterial community in the mesozooplankton container at Days 17 and 20 were 0.44 and 0.53 d^{-1}, respectively, and thus between the extreme values for the other container. Growth rates at this level indicate no severe limitations, although growth was not at the calculated maximum. All growth rates are calculated with the assumptions of 20 fg C per bacterial cell and a thymidine conversion factor of 2×10^{18} cells mol^{-1}. The validation of these constants is supported by the fact that growth rates calculated from the increases in bacterial cell abundances during exponential growth between Days 10 and 17 for the mesozooplankton container and between Days 8 and 12 for the other container were between 0.4 and 0.5 d^{-1} (Fig. 2B) and in agreement with those calculated from production measurements and biomass.

The measured DOC accumulation and degradation kinetics can be interpreted to result from at least 3 BDOC pools with different lability. Labile BDOC with k_1 values >0.2 d^{-1} was only measured during a combination of severe suppression of bacterial uptake capacity and possibly nutrient limitation in the container without mesozooplankton. The k_1 values in the meso-

zooplankton container were lower and a pool with such decay characteristics was not detected in the container without mesozooplankton. We therefore speculate that the low k_1 for BDOC in the mesozooplankton container was related to some specific compounds produced by the mesozooplankton. Olsen et al. (2002) have shown a high DOC production related to zooplankton; however, no differences in degradation kinetics were obvious between their experiments with and without zooplankton.

Accumulation of bacterial cell remains and by-products like peptidoglucans with slow turnover (McCarthy et al. 1998, Nagata et al. 2003) could be possible candidates to explain the difference in k_1 values. The high bacterial biomass and production in the mesozooplankton container support such an explanation; however, the open question remains as to why the bacterial activity in the other container did not leave such a 'signal'? The simplest explanation for low k_1 values of BDOC in the mesozooplankton container is a direct link to the activity of mesozooplankton producing DOC with a unique affinity for bacterial utilisation.

The differences in DOC decay coefficients between the containers were eliminated after some 60 to 100 d of degradation, and the semi-labile BDOC in both containers was described by low and similar coefficients leading to the suggestion that these pools consisted of similar compounds. Furthermore, the relative amount of RDOC was not affected by the mesozooplankton treatment and was about 10 to 12% of new DOC. This value is lower than the 25 to 30% RDOC for autochthonous DOC found in other studies (Fry et al. 1996, Søndergaard et al. 2000a, Meon & Kirchman 2001). The conclusion is that autochthonous RDOC is produced both during the increase of a diatom bloom, and during its decay as observed by Søndergaard et al. (2000a), and that different communities of plankton and experimental conditions may control to what extent new DOC becomes recalcitrant to microbial degradation. This is also exemplified by the results of Meon & Kirchman (2001), who found that DOC produced in one experiment without addition of inorganic nutrients did not produce RDOM, while a nutrient-induced bloom resulted in RDOC production. In the chemostat experiment carried out by Olsen et al. (2002), the complexity of the food web apparently did not have any effect on short-term (15 d) biodegradation. Bacterial DOC degradation was in all their experiments surprisingly low at 15 to 25%. The variable results and lack of solid explanations for the variability show that our understanding of new DOC production and of the factors controlling the degradation kinetics by nutrient-replete microbial communities is still limited.

Very few studies are available for comparison with respect to degradation kinetics for BDOC. In large outdoor lake mesocosms with zooplankton, Søndergaard et al. (2000a) observed that BDOC with a half-life of between 12 and 17 d was dominating new autochthonous DOC during the decay of a diatom bloom. These decay rates resemble those found here for the labile pool accumulating in the mesozooplankton container. Hopkinson et al. (2002) measured the degradation of DOM in samples collected from the middle of the Atlantic Bight. Two degradable pools where k_1 averaged 0.22 d^{-1} and k_2 averaged 0.02 d^{-1} described the decomposition of DOC, however, with large spatial and temporal variations (2 extreme values were removed from the data in Table 4, Hopkinson et al. 2002). Their average k_1 is comparable to the values in the container without mesozooplankton and their k_2 value is similar to the coefficient for the semi-labile pool identified in our experiment. Hopkinson et al. (2002) presented no data on food web structure and we can only speculate whether the accumulation of very labile BDOC in their samples was controlled by nutrient limitation and/or food-web interactions.

DOC degradation and nutrient dynamics

Degradation of DOC is accompanied by microbial uptake of nitrogen and phosphorous from either organic or inorganic sources, and possibly net mineralisation of N and P during the course of a long incubation in a virus-bacteria-flagellate culture. The paradigm is that DOM during microbial degradation is carbon-enriched (Williams 1995, Hopkinson et al. 1997).

Nitrate and phosphate were added to the samples used for degradation and inorganic nutrients were never exhausted (see Fig. 5). Accordingly, access to energy from DOM must have been the factor limiting organic degradation. DON and DOP were not measured during the degradation experiments, so the mineralisation of DOM can only be evaluated from the inorganic nutrient measurements. In essence, we show how new autochthonous DOM is microbially degraded in a nutrient-replete situation where bacteria can utilise both organically bound and inorganic nutrients to sequester organic carbon. We do not know to what extent DOC was immobilised in microbial biomass; however, previous degradation experiments have shown that particulate carbon is of no quantitative importance after 7 to 10 d of incubation with flagellates present (Søndergaard & Theil Nielsen 1997). Therefore, we consider the removed DOC to be fully mineralised and that degradation over 230 d resulted in a very low particulate sequestration of N and P.

The time courses of nutrient concentrations showed an initial bacterial uptake of both nitrate and phos-

phate in all experiments. After about 20 d, the uptake was followed by mineralisation in some experiments, net immobilisation of N in the samples taken at Day 22 and net immobilisation of P in all samples taken from the mesozooplankton container (see Fig. 5). The general feature was that large amounts of DOC were mineralised either with a low net mineralisation of organic N and P or an immobilisation of inorganic N and P (Table 2). Recycling of N and P must have supported the removal of DOC as bacterial growth requirements for N and especially for P are high (Vadstein 2000). Furthermore, the immobilisation must have been due to the production of recalcitrant DON and DOP compounds by the microbial communities during degradation, which enriched the RDOM pool with both N and P compared with DOM at the start of an experiment. It is known that marine bacteria produce refractory DOC and DON (McCarthy et al. 1998, Ogawa et al. 2001) and that freshwater DON and DOP can be dominated by refractory components of heterotrophic origin (Lean 1973, Caraco & Cole 2003). During the sequestration of energy from autochthonous organic sources, the microbial community conserved N and P in recalcitrant DOM.

The newly produced DOM had C:N ratios between 13 and 16. Compared with the Redfield ratio of 6.6, the accumulated DOM was C-rich and within the range from 11 to 22 observed in other experiments (Norrman et al. 1995, Søndergaard et al. 2000b) and *in situ* (Williams 1995, Hopkinson et al. 1997). In an investigation of the degradation of DOM in samples collected from the middle Atlantic Bight, Hopkinson et al. (2002) found recalcitrant DOC and DON to account for 70 and 60% of the total DOC and DON, respectively. Thus, contrary to our finding, the RDOM became more C-rich during bacterial degradation. One part of an explanation for this difference is the high percentage of biodegradability of newly produced autochthonous DOC observed in our experiment. The reason for the high DOC availability is not known; however, compared with oceanic water, the DOC pool was very new and never exposed to UV radiation. UV radiation has been shown to decrease the biodegradability of algal DOC (Tranvik & Kokalj 1998, Tranvik & Bertilsson 2001).

With respect to DOP degradation, Hopkinson et al. (2002) found on average 80% to be degradable. This leads to higher relative organic C- and low P-content in the remaining DOM after degradation and is contrary to our findings. Although we do not have DOP measurements, the very high C:P mineralisation ratios and even immobilisation of P must have resulted in a P-enriched RDOM pool.

One unexpected result was that net immobilisation of phosphate was consistently measured in all degradation experiments with samples from the container with added mesozooplankton, but not in the other container. Using the Day 20 experiment as an example, the mineralisation of about 175 µM C immobilised 0.8 µM P (Fig. 5B, Table 2). This observation is unique and we have not been able to find comparable results in the literature. Phosphate must have been transformed to P-species not reacting in the molybdate method. Whether these P-species are organic or inorganic is not known. Bacteria are known to have the ability to accumulate polyphosphate in nutrient-replete environments (Vadstein 2000); however, poly-P is very labile (Vadstein 2002) and preservation over 230 d seems unlikely. An explanation based on poly-P production would also force an explanation of why it did not occur in the samples from the container without mesozooplankton; to this, we cannot produce an explanation. Most likely, the immobilisation was in the form of recalcitrant DOP compounds produced by bacteria and flagellates. Heterotrophic DOP production is a well-known process (Lean 1973, Olsen et al. 2002, Caraco & Cole 2003). Apparently, the presence of mesozooplankton via an unknown mechanism linked to DOM and its degradation resulted in a net production of DOP and immobilisation of phosphate. Future experiments must reveal whether this phenomenon is general and also whether the storage is organic or inorganic. To sum up, different biotic communities produced DOM resulting in either P-mineralisation or immobilisation during microbial degradation, and such a result has, to our knowledge, never been reported before.

CONCLUSIONS

We have shown, in accordance with previous studies, that biodegradable autochthonous DOC with short (days) and longer (weeks) half-lives can accumulate during the bloom and decay of freshwater phytoplankton. The presence of mesozooplankton resulted in a sustained high bacterial abundance and production possibly fuelled by mesozooplankton production of easily degraded DOC and nutrient mineralisation. Compared with the container without mesozooplankton, the presence of mesozooplankton resulted in lower DOC concentrations with initial DOC decay coefficients 2- to 5-fold lower than without mesozooplankton. The high concentrations of very labile DOC measured in the container without mesozooplankton were probably caused by a combination of a low bacterial uptake capacity due to protist grazing and nutrient limitation.

Microbial degradation of the accumulated DOC showed features with respect to mineralisation of N

and P not previously reported. During 230 d of degradation in a nutrient-replete environment, the refractory DOM pool was enriched with DON and DOP. Utilisation of a large degradable DOC pool by a microbial community resulted in mineralisation of low amounts of organic N and P compared with C and even immobilisation of nitrate and phosphate in some experiments. Immobilisation of phosphate was found in all degradations of DOM produced in the container with mesozooplankton present.

These findings do not change N and P budgets of lakes, but could have implications for the conceptual understanding of P-cycling in situations with high mesozooplankton grazing as well as on how organic matter is degraded. All the results are based on a single experiment, and therefore caution in interpretations must prevail until further studies provide evidence as to whether this is a special case created in an experimental environment or is a more general phenomenon.

Acknowledgements. This study was supported by the Danish Natural Sciences Research Council and the European Union (No. EVK3-CT-2000-00034). The technical assistance by Anne J. Jacobsen and Nils Willumsen is appreciated as are the valuable suggestions made by Niels Henrik Borch, Olav Vadstein and the reviewers. This is Eloise publication no. 452/42.

LITERATURE CITED

Anonymous (1985) Limnological methods. Akademisk Forlag, Copenhagen (in Danish)

Baines SB, Pace ML (1991) The production of dissolved organic matter by phytoplankton and its importance to bacteria — patterns across marine and fresh water systems. Limnol Oceanogr 36:1078–1090

Bell R (1993) Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 495–503

Biersmith A, Benner R (1998) Carbohydrates in phytoplankton and freshly produced dissolved organic matter. Mar Chem 63:131–144

Billen G, Fontigny A (1987) Dynamics of a *Phaeocystis*-dominated spring bloom in Belgian coastal waters. II. Bacterioplankton dynamics. Mar Ecol Prog Ser 37:249–257

Borch NH, Kirchmann DL (1997) Concentration and composition of dissolved combined neutral sugars (polysaccharides) in seawater determined by HPLC-PAD. Mar Chem 57:85–95

Caraco NF, Cole JJ (2003) The importance of organic nitrogen production in aquatic systems: a landscape perspective. In: Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press, Amsterdam, p 263–283

Carlson CA, Ducklow HW, Michaels AF (1994) Annual flux of dissolved organic-carbon from the euphotic zone in the northwestern Sargasso sea. Nature 371:405–408

Cauwet G (1994) HTCO method for dissolved organic carbon analysis in seawater: influence of catalyst on blank estimation. Mar Chem 47:55–64

Connolly JP (1992) Modeling carbon utilization by bacteria in natural water systems. In: Hurst CJ, Coffin RB, Landeck RE (eds) Modeling the metabolic and physiologic activities of microorganisms. Wiley-Liss, New York, p 249–279

del Giorgio PA, Davis J (2003) Patterns in dissolved organic matter lability and consumption across aquatic ecosystems. In: Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press, Amsterdam, p 399–424

Fajon C, Cauwet G, Lebaron P, Terzic S, Ahel M, Malej A, Mozetic P, Turk V (1999) The accumulation and release of polysaccharides by planktonic cells and the subsequent bacterial response during a controlled experiment. FEMS Microbiol Ecol 29:351–363

Fogg GE (1966) The extracelluar products of algae. Oceanogr Mar Biol Annu Rev 4:195–212

Fry B, Hopkinson CS, Nolin A (1996) Long-term decomposition of DOC from experimental diatom blooms. Limnol Oceanogr 41:1344–1347

Hopkinson CS, Fry B, Nolin AL (1997) Stoichiometry of dissolved organic matter dynamics on the continental shelf of the northeastern USA. Cont Shelf Res 17:473–489

Hopkinson CS, Vallino JJ, Nolin A (2002) Decomposition of dissolved organic matter from the continental margin. Deep-Sea Res Part II 49:4461–4478

Hygum BH, Petersen JW, Søndergaard M (1997) Disssolved organic carbon release by zooplankton grazing activity: a high-quality substrate pool for bacteria. J Plankton Res 19: 97–111

Ittekkot V, Brockman O, Michaelis W, Degens ET (1981) Dissolved free and combined carbohydrates during a phytoplankton bloom in the Northern North Sea. Mar Ecol Prog Ser 4:299–305

Jespersen AM, Christoffersen K (1987) Measurements of chlorophyll-a from phytoplankton using ethanol as extraction solvent. Arch Hydrobiol 109:445–454

Jurgens K, Gasol JM, Massana R, Pedrosalio C (1994) Control of heterotrophic bacteria and protozoans by *Daphnia pulex* in the epilimnion of Lake Ciso. Arch Hydrobiol 131: 55–78

Lampert W (1978) Release of dissolved organic carbon by grazing zooplankton. Limnol Oceanogr 23:831–834

Lean DRS (1973) Movements of phosphorus between its biologically important forms in lake water. J Fish Res Board Can 30:1525–1536

Lee S, Fuhrman JA (1987) Relationship between biovolume and biomass of natural derived bacterioplankton. Appl Environ Microbiol 53:1298–1303

Marie D, Partensky F, Jacquet S, Vaulot D (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Appl Environ Microb 63:186–193

McCarthy MD, Hedges JI, Benner R (1998) Major bacteria contribution to marine dissolved organic nitrogen. Science 281:231–234

Meon B, Kirchman DL (2001) Dynamics and molecular composition of dissolved organic material during experimental phytoplankton blooms. Mar Chem 75:185–199

Moran MA, Zepp RG (1997) Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnol Oceanogr 42:1307–1316

Nagata T (2000) Production mechanisms of dissolved organic carbon. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 121–153

Nagata T, Meon B, Kirchman DL (2003) Microbial degrada-

tion of peptidoglycan in seawater. Limnol Oceanogr 48: 745–754
Norrman B, Zweifel UL, Hopkinson CSJ, Fry B (1995) Production and utilization of dissolved organic carbon during an experimental diatom bloom. Limnol Oceanogr 40(5): 898–907
Ochiai M, Hanya T, Nakajima T (1979) Seasonal fluctuations of dissolved organic matter in Lake Nakanuma. The Japanese Limnology 40:185–190 (in Japanese with English Abstract)
Ogawa H, Amagai Y, Koike I, Kaiser K, Benner R (2001) Production of refractory dissolved organic matter by bacteria. Science 292:917–920
Ogura N (1972) Rate and extent of decomposition of dissolved organic matter in surface seawater. Mar Biol 13:89–93
Olsen LM, Reinertsen H, Vadstein O (2002) Can phosphorus limitation inhibit dissolved organic carbon consumption in aquatic microbial food webs? A study of three food web structures in microcosms. Microb Ecol 43:353–366
Olsen Y, Varum KM, Jensen A (1986) Some characteristics of the carbon compounds released by *Daphnia*. J Plankton Res 8:505–517
Park JC, Aizaki M, Fukushima T, Otsuki A (1997) Production of labile and refractory dissolved organic carbon by zooplankton excretion: an experimental study using large outdoor continuous flow through ponds. Can J Fish Aquat Sci 54:434–443
Rivkin RB, Anderson MR (1997) Inorganic nutrient limitation of oceanic bacterioplankton. Limnol Oceanogr 42:730–740
Sokal R, Rohlf F (1981) Linear regression. In: Sokal R, Rohlf F (eds) Biometry, 2nd edn. WH Freeman, New York, p 454–561
Søndergaard M, Middelboe M (1995) A cross-systems analysis of labile dissolved organic carbon. Mar Ecol Prog Ser 118:283–294
Søndergaard M, Theil Nielsen J (1997) Bacterial growth efficiency in lakewater cultures. Aquat Microb Ecol 12: 115–122
Søndergaard M, Worm J (2001) Measurement of biodegradable dissolved organic carbon (BDOC) in lake water with a bioreactor. Water Res 35:2505–2513
Søndergaard M, Hansen B, Markager S (1995) Dynamics of dissolved organic carbon lability in a eutrophic lake. Limnol Oceanogr 40:46–54
Søndergaard M, Borch NH, Riemann B (2000a) Dynamics of biodegradable DOC produced by freshwater plankton communities. Aquat Microb Ecol 23(1):73–83
Søndergaard M, Williams PJleB, Cauwet G, Riemann B, Robinson C, Terzic S, Woodward EMS, Worm J (2000b) Net accumulation and flux of dissolved organic carbon and dissolved organic nitrogen in marine plankton communities. Limnol Oceanogr 45:1097–1111
Stoderegger K, Herndl GJ (1998) Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. Limnol Oceanogr 43:877–884
Strom SL, Benner R, Ziegler S, Dagg MJ (1997) Planktonic grazers are a potentially important source of marine dissolved organic carbon. Limnol Oceanogr 42:1364–1374
Thingstad TF, Havskum H (1999) Bacteria-protist interactions and organic matter degradation under P-limited conditions: Analysis of an enclosure experiment using a simple model. Limnol Oceanogr 44:62–79
Thingstad TF, Lignell R (1997) Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquat Microb Ecol 13:19–27
Tranvik L, Bertilsson S (2001) Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. Ecol Lett 4:458–463
Tranvik L, Kokalj S, (1998) Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. Aquat Microb Ecol 14:301–307
Vadstein O (2000) Heterotrophic, planktonic bacteria and cycling of phosphorus – Phosphorus requirements, competitive ability and food web interactions. Adv Microb Ecol 16:115–167
Vadstein O, Brekke O, Andersen T, Olsen Y (1995) Estimation of phosphorus release rates from natural zooplankton feeding on planktonic algae and bacteria. Limnol Oceanogr 40:250–262
Wetzel RG (1995) Death, detritus, and energy flow in aquatic ecosystems. Freshw Biol 33:83–89
Williams PJLeB (1995) Evidence for the seasonal accumulation of carbon-rich dissolved organic material, its scale in comparison with changes in particulate material and the consequential effect on net C/N assimilation ratios. Mar Chem 51:17–29

Editorial responsibility: Karel Šimek, České Budějovice, Czech Republic

Submitted: August 9, 2003; Accepted: January 7, 2004
Proofs received from author(s): April 23, 2004