

# Carbon budgets of the microbial food web in estuarine enclosures\*

Bo Riemann<sup>1</sup>, Helene Munk Sørensen<sup>2</sup>, Peter Koefoed Bjørnsen<sup>3</sup>, Steen Jesper Horsted<sup>4</sup>, Lars Møller Jensen<sup>5</sup>, Torkel Gissel Nielsen<sup>3</sup>, Morten Søndergaard<sup>6</sup>

<sup>1</sup> The International Agency for <sup>14</sup>C Determination, Water Quality Institute, Agern Allé 15, DK-2970 Hørsholm, Denmark

<sup>2</sup> Århus Amtskommune, Lyseng Allé 1, DK-8270 Højbjerg, Denmark

<sup>3</sup> Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark

<sup>4</sup> Danish Institute for Fisheries and Marine Research, Charlottenlund Castle, DK-2920 Charlottenlund, Denmark

<sup>5</sup> Det Danske Hedeselskab, Klostermarken 12, DK-8800 Viborg, Denmark

<sup>6</sup> Institute of Biology and Chemistry, Roskilde University Center, DK-4000 Roskilde, Denmark

**ABSTRACT:** During 9 to 25 June 1987, carbon budgets were established for estuarine enclosures manipulated by additions of nutrients and suspension-feeding bivalves. An intensive sampling program and a detailed examination of autotrophic and heterotrophic microorganisms enabled construction of carbon budgets of the microbial food web and comparison of flow rates through a number of microbial components. Phytoplankton biomass and production covaried, and, as expected, lowest values were recorded in enclosures with added mussels, and highest values in enclosures with added nutrients. Bacteria and heterotrophic nanoflagellates peaked a few days after maxima in phytoplankton biomass and production. In enclosures with added mussels, biomasses were lower for bacteria and microzooplankton, and mesozooplankton, but slightly higher for heterotrophic nanoflagellates. Bacteria, flagellates, and microzooplankton, mostly ciliates, dominated heterotrophic processes, whereas larger mesozooplankton ingestion did not exceed 5% of phytoplankton primary production. Microzooplankton and flagellate clearances were higher in enclosures with added nutrients, whereas no such changes were found in the macrozooplankton, probably because the duration of the experiments did not allow full development of the macrozooplankton. The added mussels dominated heterotrophic consumption and controlled organisms > 20 µm. Exclusion of mussels induced a primary dominance of microzooplankton followed by a subsequent increase of mesozooplankton. Additions of nutrients and filtration by suspension-feeding bivalves caused qualitative and quantitative changes at all levels in the microbial food web. These changes were measured from a large number of microbial components and allowed balances of the carbon budgets to be made as well as identification of factors controlling the structure and function of the pelagic carbon cycle.

## INTRODUCTION

In pelagic environments, decomposition of phytoplankton production may follow several major routes, of which zooplankton grazing and bacterial degradation of dissolved organic compounds are considered the major ones (e.g. Williams 1981, Azam et al. 1983).

Since Pomeroy (1974) challenged the concept of the traditional phytoplankton-zooplankton food chain, the quantitative importance of these 2 routes of organic decomposition has been intensively discussed in the literature (see Fenchel 1988 for a review). Recent

methodological improvements in the field of microbial ecology have facilitated a number of direct studies on the role of bacteria in aquatic ecosystems. It has been demonstrated that (1) bacteria constitute a significant part of total microbial biomass (Azam & Ammerman 1984, Fuhrman et al. 1989), (2) a substantial part, and in some cases, the majority of phytoplankton primary production is channeled through bacteria, and (3) bacterioplankton is important not only in the recycling of nutrients but also in the transport of dissolved organic carbon (DOC) to higher trophic levels (Sherr & Sherr 1988).

Two suggestions were put forward to explain the new understanding of bacteria in an ecological context. The first, 'the cluster hypothesis', suggested a theoretic-

\* Contribution No. 525 from The Freshwater Biological Laboratory, University of Copenhagen

cal scenario for bacterial growth in a very dilute environment (the ocean) as well as a possible route for transport of bacterial particles to higher trophic levels (Azam & Ammerman 1984, Porter et al. 1988). The cluster hypothesis was further discussed by Mitchell et al. (1985), who suggested that clustering was most important in thermocline regions, where reduced turbulence and sinking rates of particles provided the necessary conditions for such microscale interactions.

Another more direct set of observations demonstrated that choanoflagellates can control the biomass of bacteria (Fenchel 1982a, b). Reports of clearance rates of heterotrophic flagellates from batch cultures (Fenchel 1982a) were within the same order of magnitude as those found from natural populations of flagellates (Andersen & Fenchel 1985, Andersen & Sørensen 1986, Bjørnsen et al. 1988). Thus, a carbon link connected the measured bacterial production rates with formation of new flagellate biomass.

The efficiency of this organic carbon transport through the 'microbial loop' compared to the respiration loss of carbon has not been well documented. Ducklow et al. (1986) demonstrated that most of the  $^{14}\text{C}$ -glucose added to marine enclosures was respired, suggesting that bacteria were not an important source of carbon for zooplankton. On the other hand, coupled oscillations between assemblages of bacteria, flagellates, and ciliates (Andersen & Sørensen 1986) indicate an important periodical transport of carbon up to the microzooplankton level. Whether such a transport exists between ciliates and mesozooplankton or directly between flagellates and mesozooplankton has not been documented. Moreover, direct comparisons between carbon transport from particulate phytoplankton production versus carbon from DOC via the microbial loop to higher trophic levels are not well described. It is possible that clustering of small-sized particles, like bacteria, is of greater importance in the nutrient-rich coastal environments than in oligotrophic oceanic waters. Such a phenomenon allows a trophic connection between large (mesozooplankton or ciliates) and small particles, e.g. bacteria and flagellates, and suggests new feedback mechanisms in the cycling of organic matter similar to those described in freshwater (Porter et al. 1988).

It was the aim of this study to describe the structure and function of the microbial food web in an estuarine environment. In order to follow pools and rates of organic matter within the same water body, we used large plastic enclosures and applied an intensive sampling program. The water in the enclosures was manipulated by additions of nutrients or benthic suspension feeders or combinations of the two, to create a range of photoautotrophic primary productivity and biomasses. From measurements of activities and bio-

masses of phytoplankton, bacteria, flagellates, microzooplankton, and mesozooplankton, we established budgets for the decomposition routes and rates of phytoplankton primary production and were able to identify and compare rates of heterotrophic production in natural microbial assemblages.

## MATERIALS AND METHODS

Enclosure experiments were carried out from 9 to 25 June 1987 in the Limfjord, Denmark, in the harbour of Rønbjerg. On 8 June, water from outside the harbour was filled into 8 transparent, cylindrical plastic enclosures (about 2.5 m deep, 1.5 m in diameter with 0.1 mm thick walls) by means of a motor-driven pump with a capacity of about  $1.4 \text{ l s}^{-1}$ . Water was pumped into each enclosure for periods of 10 min, in an attempt to achieve homogeneity between the 8 enclosures. The enclosures were fixed to a pontoon bridge.

The enclosures were manipulated in duplicates by additions of nutrients and mussels. Two enclosures were unmanipulated controls, 2 contained mussels *Mytilus edulis* distributed among 3 Japanese pearl nets (4.5 mm mesh) suspended in the center of the enclosures at 0.5, 1.5 and 2.5 m depth (see Riemann et al. 1988 for further details), 2 enclosures contained nutrients (see below) and 2 contained nutrients and mussels.

Nitrate and phosphate were added daily to avoid depletion. We added 210 mg  $\text{NO}_3\text{-N}$  per enclosure, corresponding to a final concentration of  $7 \mu\text{M NO}_3$ , and 30 mg  $\text{PO}_4\text{-P}$  per enclosure, corresponding to a final concentration of about  $0.4 \mu\text{M PO}_4\text{-P}$ . Water samples were taken every day between 10:00 and 11:00 h with a 3 l plastic sampler from 3 depths in the enclosures (0.5, 1 and 2 m) and mixed prior to analyses.

**Nutrients.** Inorganic phosphate, ammonium and nitrate were analyzed according to Murphy & Riley (1962), Solórzano (1969), and Crosby (1967). Samples for determinations of ammonia were preserved by adding reagents in situ immediately after sampling to avoid changes in the concentrations caused by storage (Riemann & Schierup 1978).

**Chlorophyll a.** Duplicate samples were filtered onto 25 mm Whatman GF/C filters and extracted in 96 % ethanol for 20 h without homogenization (Jespersen & Christoffersen 1987). The pigment extracts were measured fluorometrically (Aminco 2000) without correction for degradation products. Fluorometric emission values were calibrated to absorbance readings from a spectrophotometer (Beckman 240), using chlorophyll extracts from larger volumes of water from the same enclosures. Every second sampling day, a supplementary size fractionation procedure was carried out

through 20  $\mu\text{m}$  mesh nets and 2  $\mu\text{m}$  filters. A phytoplankton carbon to chlorophyll ratio of 30 was applied. The biomass of autotrophic picoplankton ( $< 2 \mu\text{m}$ ) was further evaluated by epifluorescence microscopy (green excitation) (Caron 1983).

**Phytoplankton primary production.** Equal volumes of water from the surface (0.1 m), and 1 and 2 m depths were collected every third or fourth day, mixed, and transported to the laboratory in borosilicate bottles placed in a cooling box.

In the laboratory, 25 ml subsamples were dispensed into Jena glass bottles and received additions of 74 kBq of a high grade  $\text{NaH}^{14}\text{CO}_3$  solution (Int. Agency for Carbon 14 Determination, Hørsholm, Denmark). Duplicate samples from each enclosure were incubated at irradiances of 0, 7.5, 37.5, 75, 250 and 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 2 h in an incubator at in situ temperature. Light was provided by cool white daylight tubes (Philips 35W, colour 33). After incubation, 8 ml from each sample was filtered under vacuum ( $< 10 \text{ cm Hg}$ ) through a 0.2  $\mu\text{m}$  Nuclepore polycarbonate filter. The filter was rinsed with 1 ml of 0.2  $\mu\text{m}$  filtered seawater, placed in a 20 ml glass scintillation vial and 100  $\mu\text{l}$  0.5 N HCl added. Seven ml of the filtrate was transferred to a 20 ml vial containing 100  $\mu\text{l}$  2 N HCl and bubbled with atmospheric air for 30 min. The flux of extracellular organic carbon (EOC) from algae to bacteria was estimated by serial filtration through 0.6 and 0.2  $\mu\text{m}$  filters and a recalculation of bacterial EOC uptake by the procedure of Søndergaard et al. (1985). The size distribution of bacterial activity was assessed by  $^3\text{H}$ -thymidine incorporation in the  $< 0.6 \mu\text{m}$  fraction compared with the total. The use of a 0.6  $\mu\text{m}$  pore size filter to separate all photoautotrophs from bacteria was necessitated by the presence of picoalgae. The light extinction coefficient for each enclosure and date was calculated from light measurements at 3 depths with a submersible quantum sensor (LiCor 192SA). Total flux of photosynthetic active radiation (PAR) from dawn to dusk was measured with an aerial quantum sensor coupled to an integrator. To calculate daily primary production from the 2 h incubations, it was necessary to calculate PAR at 2 h intervals through the day. This was done from integrated hourly measurements of global radiation at the Royal Airforce Station in Karup, ca 40 km from the laboratory. Daily integrated phytoplankton particulate and dissolved (EOC) primary production was calculated from the 2 h incubations by a computer program (Gargas & Hare 1976).

**Bacteria.** Cells were enumerated by epifluorescence microscopy using the standard acridine orange procedure (Hobbie et al. 1977). Cell volumes were measured from enlarged black and white photographs exposed to a screen (Lee & Fuhrman 1987). A total number of 50 cells per filter were measured and the sizing was cali-

brated to measurements of fluorescent microspheres (Polysciences Inc., Warrington, PA, USA), 0.22 to 1.0  $\mu\text{m}$  in diameter. Biovolumes were converted to carbon biomass by multiplying by 0.35  $\text{pg C } \mu\text{m}^{-3}$  (Bjørnsen 1986).

Bacterial net production was estimated from in situ measurements of  $^3\text{H}$ -thymidine incorporation into cold TCA precipitate (Fuhrman & Azam 1980). Samples of 10 ml were incubated with 10 nM (methyl- $^3\text{H}$ ) thymidine for 20 or 30 min. A conversion factor of  $1.1 \times 10^{18}$  cells  $\text{mol}^{-1}$  thymidine incorporated was used to calculate bacterial cell production (Riemann et al. 1987). Bacterioplankton grazing loss rates were calculated as average net production minus observed increase in bacterial biomass during 2 successive samplings (Bjørnsen 1988), assuming grazing to be the only cause of bacterial mortality.

**Flagellates** were enumerated from proflavine-stained preparations by epifluorescence microscopy (Haas 1982). Discrimination between autotrophic and heterotrophic flagellates was carried out by switching between blue and green excitation (for further details, see Bjørnsen et al. [1988]). Cell volumes were measured in the microscope from a subset of 1632 cells regularly distributed over the entire data set. Biovolumes were converted into carbon biomasses by multiplying by 0.12  $\text{pg C } \mu\text{m}^{-3}$  (Fenchel 1982a, Sherr et al. 1983). Flagellate ingestion and clearance rates on bacterioplankton were calculated by comparing estimates of daily bacterial grazing loss rates (see above) to biomasses of bacteria and flagellates. We assumed that heterotrophic flagellates were the only bacteriovores. Growth efficiency was assumed to be 30 % (Andersen & Sørensen 1986, Bjørnsen et al. 1988).

**Ciliates** were counted under phase-contrast microscope using Lugol-fixed samples. The entire content of the chamber (10 or 25 ml) or a minimum of 100 cells were counted (Utermöhl 1958). Biovolume was estimated from measurements of linear dimensions and assuming simple geometric organism shapes. A conversion factor of 0.073 from volume to carbon was used (Fenchel & Finlay 1983). Population clearance were calculated using  $10^5$  body volumes  $\text{h}^{-1}$  (Fenchel 1988). To calculate ingestion rates, clearance rates were multiplied with the biomass of the food items. Growth efficiency was assumed to be 30 % (Fenchel & Finlay 1983, Andersen & Sørensen 1986).

**Zooplankton**  $> 45 \mu\text{m}$  was sampled in triplicate every third day. Water was sampled from 5 depths, using a 5 l plexiglass water sampler. Water from the different depths was integrated and mixed, and volumes of 25 and 5 l were filtered through 140  $\mu\text{m}$  and 45  $\mu\text{m}$  mesh nets, respectively. Samples were fixed in buffered formalin (2 % final concentration). Zooplankton in the sample were counted, identified, and meas-

ured, using a settling chamber and an inverted microscope. Abundance data and length measurements (to the nearest 1  $\mu\text{m}$ ) were converted to biomasses according to values given in Berggreen et al. (1988) (*Acartia tonsa*), Rodhouse & Roden (1987) (*Balanus* sp.), Hernroth (1985) (*Podon* sp. and *Evadne* sp.), Johansson (pers. comm.) (polychaete larvae), Pechenik (1980) (gastropod larvae), and Jespersen & Olsen (1982) (bivalve larvae). Growth in carbon weight was converted to carbon consumption using a  $Q_{10}$  of 3.5 (Kjørboe et al. 1982) and a gross growth efficiency of 0.33 (Kjørboe et al. 1985a, b).

**Mussels.** *Mytilus edulis* were sampled from the Limfjord and sorted by size. Individuals of 31 to 39 mm (mean 34.7 mm) were acclimated under in situ conditions for 2 d before the experiment started. A subsample of 106 individuals was taken before mussels were added to the enclosure and shell length (maximum anterior-posterior dimension), dry weight and content of organic matter were measured. Fifty-one mussels were added to each enclosure, and their filtration rates per day, according to Winter (1973) and Møhlenberg & Riisgård (1979), corresponded to the volume of water in the enclosures (see also Riemann et al. 1988).

At the end of the experiment, shell length, dry weight (after 2 d at 60 °C), and content of organic matter (loss during 1.5 to 2 h at 500 °C) were measured. The carbon content was assumed to be 50% of organic weight. The carbon consumption was assumed to be 3 times the growth in carbon weight (Kjørboe et al. 1981).

## RESULTS

### Inorganic nutrients

Nitrate and phosphate remained low in all enclosures until 16/17 June (Fig. 1). During 13 and 14 June, nitrate values ranged from 0.0 to 0.9  $\mu\text{M}$  and phosphate 0.3 to 2.0  $\mu\text{M}$ , irrespective of additions of nutrients. The sum of nitrate + ammonium varied from 0.35 to 3.6  $\mu\text{M}$  during 14 and 15 June. After 16/17 June, both nitrate and phosphate increased, as expected, in enclosures with added nutrients.

### Phytoplankton

Qualitatively, the phytoplankton communities developed more or less similarly in the 8 enclosures. During the first 6 d of the period, *Skeletonema costatum* and *Nitzschia* sp. dominated but *Eutreptiella gymnastica* was also frequently found during the same period and dominated in enclosures with added mussels and nutrients. Irradiance rates were low during the

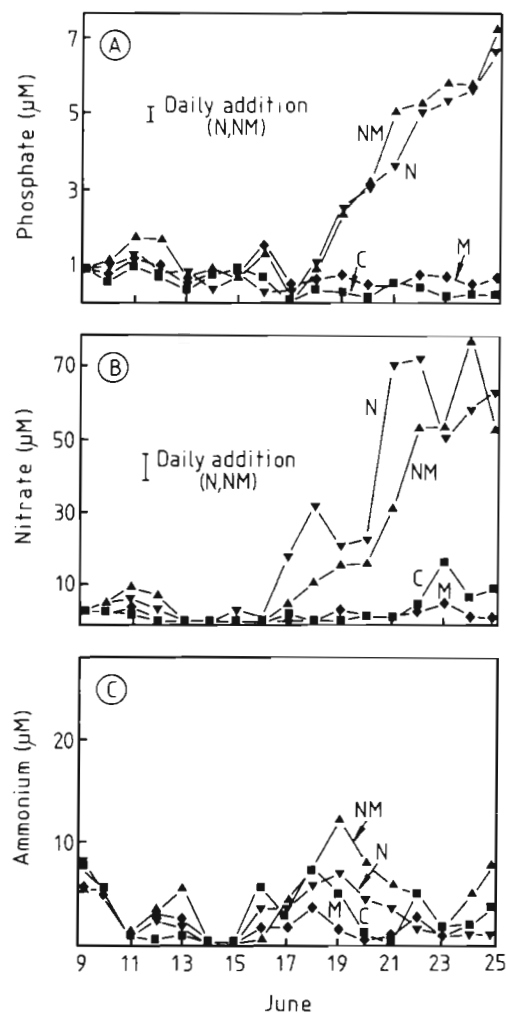


Fig. 1. Concentrations of (A) phosphate, (B) nitrate, and (C) ammonia in the enclosures. C: controls; N: added nutrients; M: added mussels; NM: added nutrients + mussels

entire period. Maximum values during 12 to 15 June ranged from 52 to 55  $\mu\text{mol m}^{-2} \text{d}^{-1}$ , and temperature for the period was about 15 °C.

Quantitatively, marked differences were observed in the development of phytoplankton biomass and production (Fig. 2) and in the development of different size classes of phytoplankton (Fig. 3). Generally, phytoplankton biomass and production covaried, with maximum values between 12 and 15 June (Fig. 2). Minor secondary increases, dominated by autotrophic nanoflagellates (mainly cryptophytes and prymnesiophytes), were found at the end of the period, but a distinct new bloom was found only in enclosures with added nutrients. Phytoplankton  $>20 \mu\text{m}$  was dominated by *Skeletonema costatum*, *Nitzschia* sp. and partly *Eutreptiella gymnastica*. Phytoplankton 2 to 20  $\mu\text{m}$  was dominated by autotrophic flagellates, and unicellular cyanobacteria dominated phytoplankton  $<2 \mu\text{m}$  (data



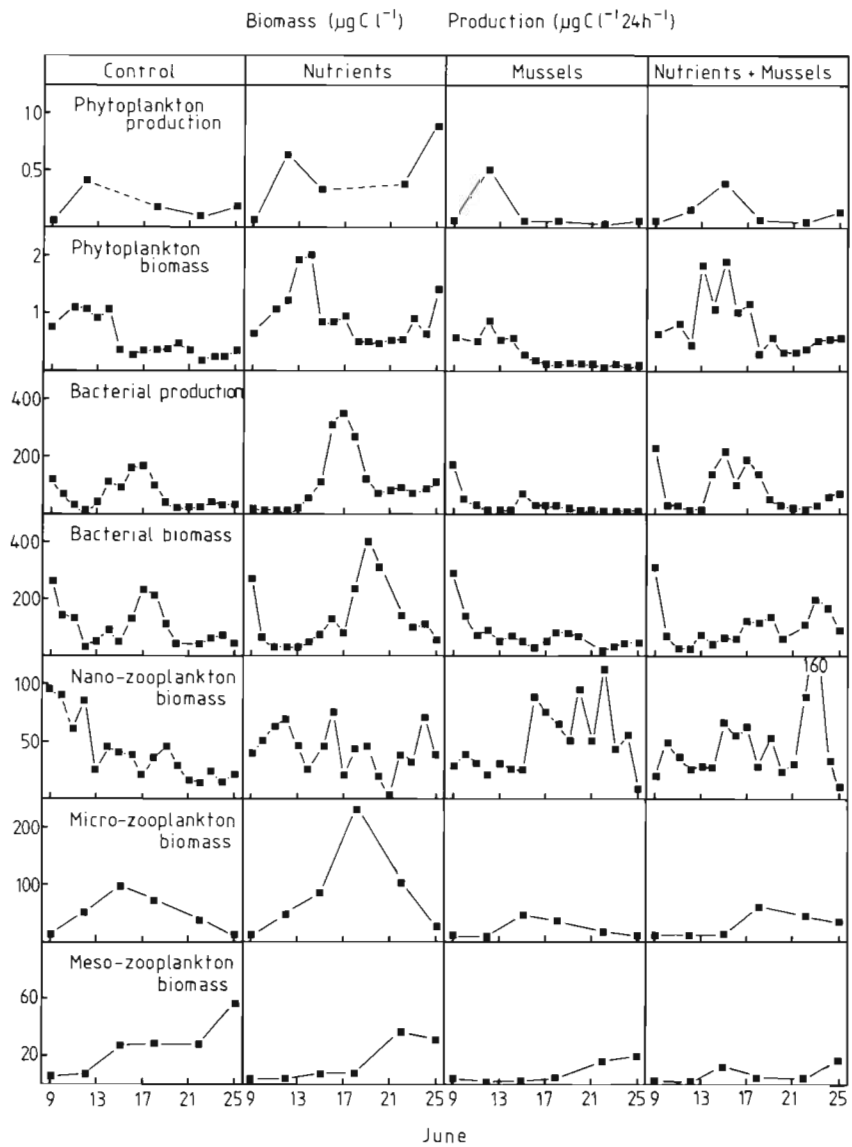


Fig. 2. Production rates and biomasses of phyto- and bacterioplankton, and biomasses of heterotrophic flagellates, micro- and macrozooplankton during 9 to 25 June

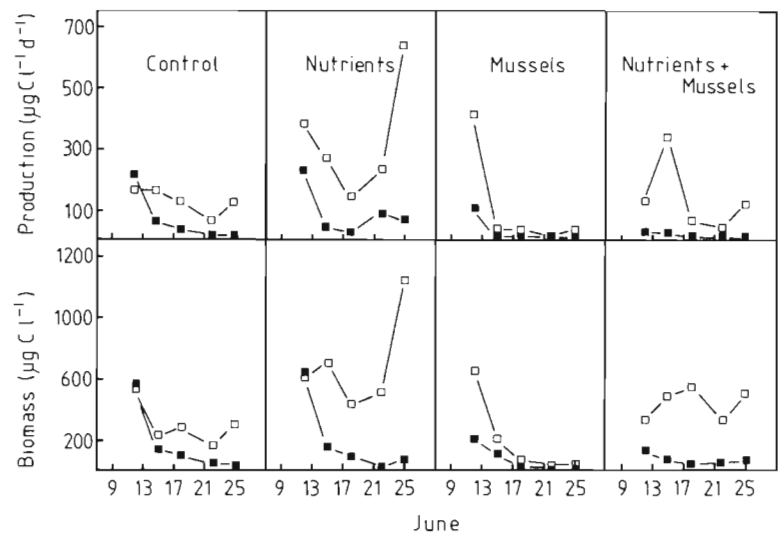


Fig. 3. Production and biomass of autotrophs  $> 20 \mu\text{m}$  (■) and  $< 20 \mu\text{m}$  (□) during 9 to 25 June

not shown). At the end of the experiment, the 2 to 20  $\mu\text{m}$  size fraction dominated biomass and production in enclosures without mussels (Fig. 3). In enclosures with added mussels, production rates of the 2 to 20  $\mu\text{m}$  fraction also dominated, but the biomass of the same fraction decreased.

Direct count of picoalgae gave biomass estimates for this size fraction of less than 4  $\mu\text{g C l}^{-1}$  (overall average 0.9  $\mu\text{g C l}^{-1}$ ). Even if we assume a fast turnover of this pool, the picoautotrophs remain quantitatively negligible compared to other autotrophs and to the picoheterotrophs (i.e. bacterioplankton). On the other hand, fractionated chlorophyll and  $^{14}\text{C}$ -incorporation measurements indicated biomasses of 40 to 180  $\mu\text{g C l}^{-1}$  and production rates of 10 to 20  $\mu\text{g C l}^{-1} \text{ d}^{-1}$  for the <2  $\mu\text{m}$  fraction (single enclosure averages during the experiment, data not shown). We suspect that particularly the high chlorophyll values for the picofraction may be caused by filtration artifacts, and we attach greater importance to the more reliable direct microscopic evidence. Consequently, measured values of chlorophyll and  $^{14}\text{C}$ -incorporation in the picofraction were added to the nanofraction, resulting in increases of 25 % at maximum.

Exudates released from phytoplankton constituted 28 to 35 % of primary production in all enclosures, corresponding to between 2.0 and 6.4 %  $\text{d}^{-1}$  of the phytoplankton carbon biomass (Table 1).

### Bacteria

Cell numbers varied from 1.9 to  $15 \times 10^6 \text{ ml}^{-1}$ . The initial decrease in cell number and cell production (Fig. 2) was due to sedimentation of particles introduced while filling the enclosures. Maximum values of cell number and cell production were observed 2 to 4 d after the peaks of phytoplankton biomass in all enclosures, and the highest values were found in enclosures with added nutrients, from which also the highest values of phytoplankton biomass and production were recorded. The average cell volume of bacteria decreased gradually throughout the study from 0.133  $\mu\text{m}^3$  on 9 June to 0.040  $\mu\text{m}^3$  on 25 June. There

were no significant differences (t-test,  $p > 0.01$ ) between the mean cell volume in the various enclosures calculated for the period as a whole. During 16 to 25 June, however, the mean cell volume from enclosures with added mussels were significantly lower (t-test,  $p < 0.05$ ) than those from other enclosures. Generation time of the bacterial assemblages ranged from 5 to 184 h, with lowest values a few days after the maximum phytoplankton biomass values. During 14 and 15 June, generation time ranged from 7 to 19 h in all enclosures. The average for the whole period was 52 to 67 h in enclosures with added mussels compared with 41 to 42 h in enclosures without mussels. The bacterial biomass ranged from 22 to 317  $\mu\text{g C l}^{-1}$ , with generally lower values in enclosures with added mussels and higher values in enclosures with added nutrients (Fig. 2).

### Nanoflagellates

Heterotrophic flagellates dominated the nanoflagellate biomass in all enclosures until 19/20 June. Bacterivory by heterotrophic nanoflagellates was quantified in terms of individual ingestion and clearance rates and as body volume equivalents cleared per hour (Table 2). The 2 former estimates showed higher values in enriched enclosures and lower values in enclosures with mussels. Clearance rates in terms of body volumes cleared per hour showed least variation between and within enclosures, with an overall mean value of  $1.0 \times 10^5$  body volumes  $\text{h}^{-1}$ . Clearance rates obtained from body volumes were used in the carbon budget (see below).

### Microzooplankton

Tintinnids, oligotrich ciliates and rotifers dominated the biomass (Fig. 4). Generally, the biomass was about 2 times higher in enclosures without mussels compared to their controls and slightly higher in enclosures with added nutrients. The biomass of oligotrich ciliates (dominated by *Lohmaniella oviformis*, *Strombidium vestitum* and *Strombidium* spp.) was about the same in

Table 1. Extracellular organic carbon released from phytoplankton: percentage of primary production, and percentage  $\text{d}^{-1}$  of phytoplankton carbon biomass from the various enclosures. C: controls; N: added nutrients; M: added mussels; NM: added nutrients + mussels. Values in brackets represent CV;  $n = 5$  or 6

	Enclosure			
	C	N	M	NM
% of primary production	28 (13)	35 (16)	32 (20)	28 (12)
% $\text{d}^{-1}$ of phytoplankton carbon biomass	4.6 (3.6)	6.4 (5.2)	3.7 (3.4)	2.0 (1.3)

Table 2. Ingestion and clearance rates of heterotrophic nanoflagellates on bacterioplankton. Means and 95 % confidence intervals of 16 successive estimates calculated from daily bacterial grazing loss rates (BGL)

Enclosure	Ingestion	Clearance	
	(pg C d <sup>-1</sup> ind <sup>-1</sup> ) <sup>a</sup>	(nl h <sup>-1</sup> ind. <sup>-1</sup> ) <sup>b</sup>	(10 <sup>5</sup> body vol. h <sup>-1</sup> ) <sup>c</sup>
Control	11.8 ± 4.1	5.4 ± 3.6	1.22 ± 0.42
Nutrients	36.7 ± 27.0	8.5 ± 4.0	1.24 ± 0.46
Mussels	6.7 ± 4.8	3.1 ± 1.6	0.87 ± 0.50
Nutr + Mus.	9.7 ± 7.6	3.4 ± 2.2	0.82 ± 0.25
Total	16.2 ± 7.6	5.1 ± 1.4	1.04 ± 0.24

<sup>a</sup> Estimated as BGL (# flagellates)<sup>-1</sup>  
<sup>b</sup> Estimated as BGL (# flagellates)<sup>-1</sup> (bacterial biomass)<sup>-1</sup>  
<sup>c</sup> Estimated as BGL (flagellate biovolume)<sup>-1</sup> (bacterial biomass)<sup>-1</sup>

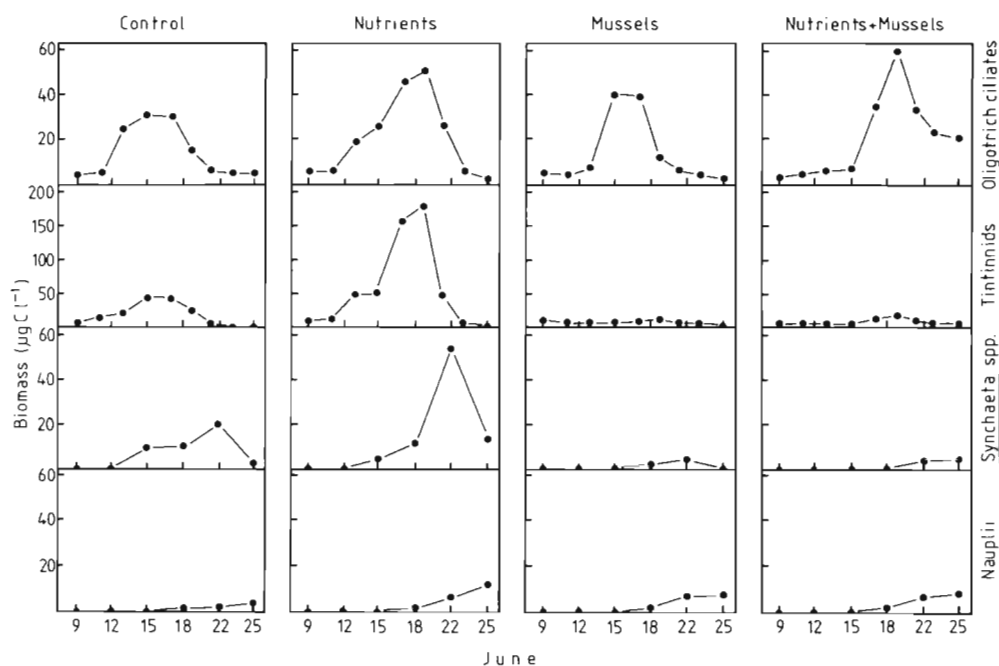


Fig. 4. Biomass of selected species in the microzooplankton fraction during 9 to 25 June

all enclosures, although the biomass peak was slightly higher in enclosures with added nutrients. Addition of mussels decreased the biomass of tintinnids (dominated by *Tintinnopsis labiancoi* and *T. minutus*) and rotifers (*Synchaeta* spp.), whereas additions of nutrients increased their biomasses compared with controls. The biomass of copepod nauplii was of minor importance during the first half period of the experiment (range 1.6 to 2.5 % of the biomass), but towards the end of the experiment, copepod nauplii made out 23 to 78 % (mean 42 %) of the total biomass, mostly because of the decline of ciliate and rotifer populations.

Microzooplankton grazing on nanoplankton was evaluated as described above for nanozooplankton bacteriivory. Grazing loss rates of auto- and heterotrophic nanoflagellates were estimated from produc-

tion rates and changes in biomass during 12 to 25 June. For this purpose, production rates of heterotrophic nanoflagellates were derived from biovolume data, assuming a clearance of 10<sup>5</sup> body volumes h<sup>-1</sup> (cf. above) and a carbon growth yield of 30 %. Only enclosures C and N were considered, to avoid interference from mussel filtration. Comparison of grazing losses to microzooplankton biovolume revealed an average clearance rate of 2 × 10<sup>4</sup> body volumes h<sup>-1</sup> and an average ingestion rate of 3.0 body weights d<sup>-1</sup>. Assuming a microzooplankton growth yield of 30 %, the estimated ingestion rate corresponds to a growth rate of about 1 doubling d<sup>-1</sup>. The rapid increases of ciliate biomass during the first week of the experiment (Fig. 2) confirmed that a growth rate of about 1 d<sup>-1</sup> was actually achieved by the microzooplankton.

### Mesozooplankton

The only copepods present were *Acartia tonsa* and *Eurytemora* sp., and *Podon* sp. and *Evadne* dominated the Cladocera. Larvae of bivalves and polychaetes dominated the meroplankton. The average biomasses of the mesozooplankton communities were 10 to 29  $\mu\text{g C l}^{-1}$  in enclosures without mussels and 7 to 8  $\mu\text{g C l}^{-1}$  in enclosures with added mussels.

### Carbon budget

During the period 12 to 25 June, a provisional carbon budget was established between phytoplankton, bacteria, nanoflagellates, microzooplankton, mesozooplankton, and mussels (Fig. 5). We omitted the first 3 d of the experiment because sand and detritus particles were settling in the enclosures.

In Fig. 5, numbers within the boxes indicate average biomasses ( $\mu\text{g C l}^{-1}$ ) during the period, whereas inward-going and outward-going arrows represent consumption and production rates, respectively. Production rates include observed changes in biomass

during the period, and thus represent what is available for consumption at the 'next' trophic level. Nanozooplankton consumption rates were estimated assuming a clearance of  $10^5$  body volumes  $\text{h}^{-1}$ , while the daily microzooplankton consumption was estimated as 3 times the biomass (see preceding section and 'Discussion'). Flux rates through the 'top predators' (i.e. mesozooplankton and mussels) were based on observed biomass increases. A carbon growth yield of 30% was assumed for all heterotrophs.

Phytoplankton primary production and biomass were highest in enclosures with added nutrients. The size fraction 2 to 20  $\mu\text{m}$  dominated in all enclosures. Bacterial net production balanced the calculated food consumption of heterotrophic flagellates, except in the enclosures with nutrients and mussels. The calculated food consumption of ciliates could be satisfied by auto- and heterotrophic nanoplankton production, except in enclosures with added nutrients without mussels. The biomass of ciliates was more than twice as high in control enclosures and those with added nutrient compared with enclosures with mussels. The calculated ciliate net production was much higher than the zooplankton food consumption in all enclosures. So, effects

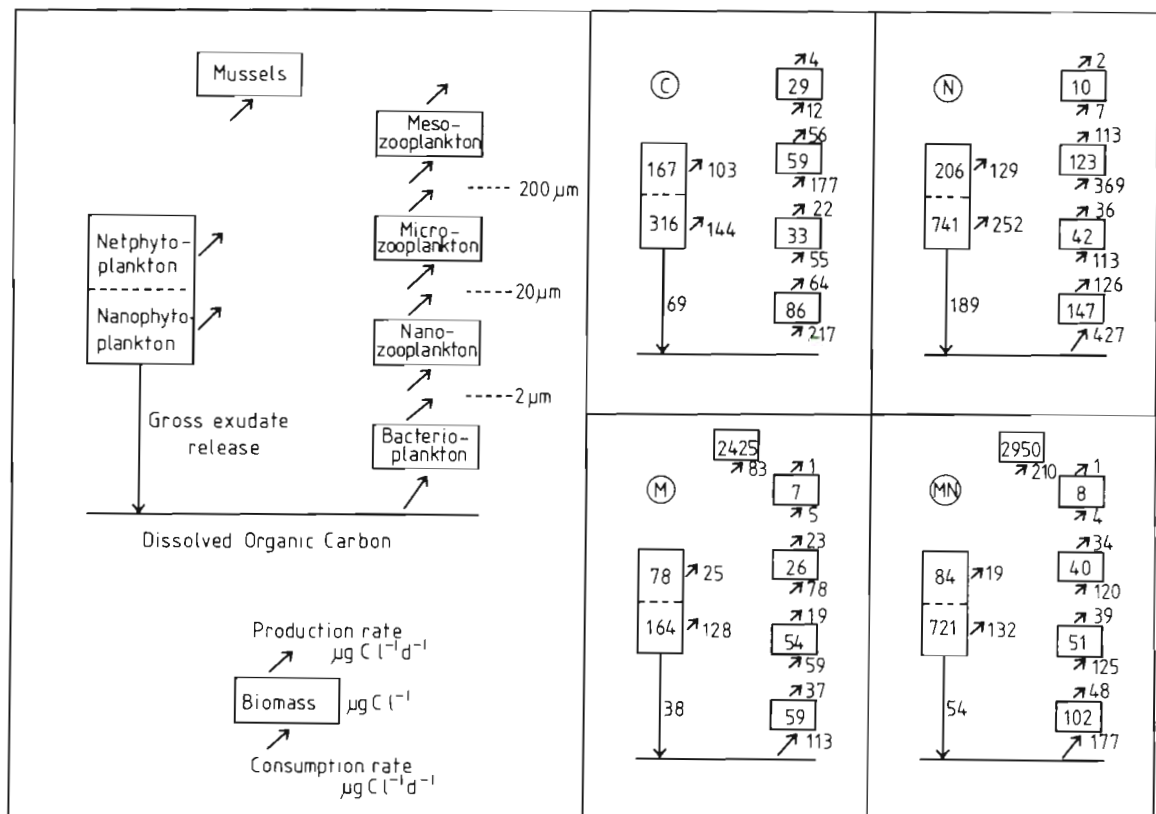


Fig. 5. Provisional carbon budgets for the microbial food web in the various enclosures. Numbers within boxes indicate average biomasses ( $\mu\text{g C l}^{-1}$ ) during the period. Inward and outward going arrows represent consumption and production rates, respectively ( $\mu\text{g C l}^{-1} \text{d}^{-1}$ )



of nutrient additions could be followed up to the ciliate level in the food chain, with most pronounced effect in enclosures without mussels. On the other hand, mesozooplankton biomass was almost the same in controls and in enclosures added nutrient, indicating that mesozooplankton was not limited by food or could not respond rapidly enough within the 17 d of the experimental period. Ingestion by mussels was 2.5 times higher in enclosures with added nutrients, indicating food limitations in enclosures without nutrients added.

Bacteria, flagellates, and ciliates dominated heterotrophic processes in all enclosures (Table 3), whereas larger zooplankton ingestion rates did not exceed 5 % of the total primary production.

Table 3. Total particulate autotrophic and heterotrophic production, and production of bacteria, and ingestion rates of heterotrophic nanoflagellates, ciliates, and mesozooplankton ( $\mu\text{g C l}^{-1} \text{ d}^{-1}$ ) from the various enclosures. See Table 1 for identification of enclosure symbols

	Enclosure			
	C	N	M	NM
Total autotrophic production	247	381	153	151
Total heterotrophic production	302	586	190	323
Autotrophic/heterotrophic prod.	0.82	0.65	0.81	0.47
Bacterial gross production	217	427	113	177
Flagellate ingestion	55	113	59	125
Microzooplankton ingestion	18	39	13	17
Mesozooplankton ingestion	12	7	5	4

### Enclosure variations

Deviations between duplicate enclosures were tested frequently for selected parameters (Table 4). Total variations of results obtained from duplicate enclosures were 7 to 37 % of the mean value, with highest values for mesozooplankton and lowest values for bacterial production and chlorophyll.

Table 4. Coefficients of variation (CV = SD % of mean) between duplicate enclosures presented from selected parameters. Means and range of variation and number of samples are given

	CV	Range	n
Nitrate	12	0–68	19
Phosphate	8	0–72	56
Ammonia	22	0–92	56
Chlorophyll	7	0–31	45
Bacterial production	7	0–30	64
Heterotrophic flagellates	9	2–38	12
Mesozooplankton	37	7–86	12

### DISCUSSION

The applied manipulations of the water in our enclosures induced the expected changes in phytoplankton production and biomass. Phytoplankton production and biomass were increased by addition of nutrients and decreased by additions of mussels. Addition of nutrients changed phytoplankton biomass rather than the pools of nutrients during the first half of the period, and inorganic nitrogen probably controlled phytoplankton primary production in all enclosures during 14 and 15 June. After 17 June, additions of nutrients changed the pools of nutrients rather than the phytoplankton biomass.

The bacterial net production followed the same pattern as phytoplankton production and biomass, with a lag period of 3 to 6 d (Fig. 2), suggesting phytoplankton excretion of organic matter to be one of the major substrate donors for bacterioplankton growth. The directly measured exudate release from phytoplankton, however, constituted only 31 to 44 % of the calculated bacterial gross production (assuming a 30 % growth efficiency of bacteria). Thus, a number of other sources of DOC must have contributed to the sustenance of the observed bacterial production. Such other sources of DOC may include lysis of organisms during sedimentation or grazing, and excretion from heterotrophic organisms. In any case, autotrophic extracellular release of organic matter alone could not sustain the observed bacterial carbon demand (assuming 30 % bacterial growth yield), unless the measured release was severely underestimated. A significant 're-fluxing' of DOC from the microbial loop must be assumed in order to balance the carbon budget at the bacterioplankton level. In fact, Jumars et al. (1989) demonstrated on a theoretical basis that large fluxes of DOC available to bacteria originate as byproducts of ingestion and digestion by animals rather than direct release from phytoplankton.

Phytoplankton release of exudates constituted 2.0 to 6.4 % of phytoplankton biomass per day (Table 1), close to theoretical values (Bjørnsen 1988) and results from phytoplankton cultures (Riemann unpubl.). Søndergaard et al. (1988) described a decrease in the relative release of exudates (% of primary production) from freshwater phytoplankton when nutrients were added, and a similar response was found by Lancelot (1983) in a marine environment. This phenomenon was not found in the enclosures, probably because succession of the algal community towards more nutrient-demanding species was delayed owing to low insolation rates.

The thymidine-based estimates of bacterial production were probably too low during periods with short generation time. Smits & Riemann (1988) demonstrated

that production rates of natural bacterial assemblages from freshwater environments could be severely underestimated when the generation time drops below 20 h. In fact, generation times ranged from 7 to 19 h in all enclosures during 14 and 15 June. Considering that the highest production rates were also found during this period, a possible underestimation can be of significance to the average values in the carbon budget (Fig. 5). To what degree bacterial net production rates may have been underestimated is impossible to say. Nevertheless, it seems unlikely that substrate sources would permit more than a doubling of the production, and moreover, generation times would then be unreasonably low compared to published values (Ducklow & Hill 1985a, b, Bjørnsen et al. 1988, Fuhrman et al. 1989).

The carbon budgets seem to balance well between bacterioplankton and heterotrophic nanoflagellates and between nanoplankton and microzooplankton (Fig. 5). An exception was Enclosure NM, where a concurrence of high biomasses of bacterioplankton and heterotrophic nanoflagellates (Fig. 2) gave rise to unrealistically high estimates of flagellate grazing. In rapidly fluctuating populations of microorganisms, the use of clearance rates from body volumes and ingestion rates based on the calculated clearance and the bacterial biomass produce overestimates. In the NM enclosures peaks of high biomasses of both bacteria and nanoflagellates were out of proportion to the average ingestion rate calculated for the period as a whole.

Clearance rate, in terms of body volumes per hour, seemed to be the best descriptor of nanoflagellate grazing (Table 2), whereas ingestion rate, normalized against biomass, appeared more useful in the case of microzooplankton. Theoretically, assuming Michaelis-Menten/Holling II functional response, clearance is the best descriptor at low food concentrations ( $< K_m$ ), and ingestion rate better at high food concentrations ( $> K_m$ ). Half-saturation constants ( $K_m$ ) for nanoflagellate and microzooplankton grazing at about  $400 \mu\text{g C l}^{-1}$  and  $50 \mu\text{g C l}^{-1}$ , respectively, have been published (Fenchel 1982a, Jonsson 1986), indicating that microzooplankton grazing, but not flagellate grazing, was saturated in our experiment. This saturation was due to high biomasses of autotrophic nanoflagellates, which also allowed microzooplankton to be the dominant part of the microbial food web.

Mesozooplankton played an almost insignificant role in the carbon budgets (Fig. 5) and were apparently unable to consume the available production in the microfraction (microzooplankton and phytoplankton  $> 20 \mu\text{m}$ ). The excess production may either sediment or enter the microzooplankton pool. By the end of the experiment, however, mesozooplankton biomass had increased considerably in the enclosures without mus-

sels (Fig. 2), so probably the duration of the experiment did not allow full appreciation of the grazing potential of the mesozooplankton. On the other hand, eutrophic estuaries seem to be characterized by fluctuating phytoplankton biomass (Boynton et al. 1982, Nixon & Pilson 1983), so the quantitative importance of mesozooplankton and their role in controlling phytoplankton biomass may be limited by their slow numerical response.

The added mussels reduced the biomass of microplankton (20 to  $200 \mu\text{m}$ ) by more than 50 %. The mussels had minor positive effects on nanozooplankton biomass, probably due to the reduced grazing pressure from the microzooplankton. Enclosures with mussels showed slightly lower bacterioplankton biomasses than enclosures without mussels, but this effect seemed to be mediated by a lower bacterioplankton production rather than via a change in grazing pressure (Fig. 5). The mussels in the nutrient-enriched enclosure showed a higher growth rate (data not shown) than the mussels in the unenriched enclosure, and their estimated carbon demand could not be met by the measured particulate primary production. Part of the observed mussel growth might have been based on detritus introduced during the filling of the enclosures, but this was also present in the unenriched enclosures, so the carbon imbalance in the NM enclosure rather points to a, perhaps general, underestimation of primary production. We assume that sedimentation was similar in all enclosures.

Our carbon budget allowed us to combine qualitative descriptions with quantitative measurements of carbon pools and fluxes. Such an approach is far from perfect and many assumptions are made. In general, the carbon budgets balanced reasonable well. Major imbalances occurred only in the NM enclosure at the mussel and nanozooplankton levels, and there was not sufficient primary production to support the heterotrophic rates. When ad hoc parameters were applied to estimate flux rates (mesozooplankton growth, microzooplankton ingestion, and nanozooplankton clearance), these parameters were in accordance with literature values and theoretical considerations.

The carbon conversion efficiency within the heterotrophic compartments is a crucial parameter for the establishment of accurate carbon budgets. We assumed a general value of 30 % as a compromise among the scarce and rather conflicting literature values. Especially for bacterioplankton, the published growth yields span a range from  $< 10$  to  $> 80$  % (Joint & Morris 1982, Bjørnsen 1986, Schwaerter et al. 1988). Since we measured bacterioplankton net production, a change of bacterial growth yield would only affect the estimates of bacterial carbon demand and thus the extent of required 're-fluxing' of DOC from the microbial food web.



The constructed carbon budgets were based on the model of Azam et al. (1983), by which pelagic organisms are divided into auto-/heterotrophs and according to their size: 0.2 to 2  $\mu\text{m}$  (pico), 2 to 20  $\mu\text{m}$  (nano), 20 to 200  $\mu\text{m}$  (micro) and > 200  $\mu\text{m}$  (meso) (Sieburth et al. 1979). Thus, organisms with cell volumes ranging 3 orders of magnitude are pooled together. This crude condensation of the pelagic variety of organisms is perhaps a major obstacle to reaching an adequate description of the carbon flow. The model of Azam et al. implies a predator-to-prey size ratio of 10. Although this may be the general case, an increasing number of studies report efficient grazing on smaller and larger prey (Rivier et al. 1985, Sherr & Sherr 1987, Fenchel 1988). The enclosure approach allowed us to derive ad hoc estimates of flux rates through the different zooplankton compartments. The application of these estimates to other situations, e.g. in situ, may be questionable, since even the application within the data set was not without problems. Finally, as noted above, too little is known about carbon conversion efficiencies of heterotrophic pelagic organisms.

In spite of these shortcomings, the carbon budgets did summarize the major food web characteristics of the enclosed pelagic systems. Suspension-feeding bivalves, when present, dominated heterotrophic consumption and exerted a strong regulatory control of organisms > 20  $\mu\text{m}$ . The fundamentally different development of enclosures with and without added mussels clearly demonstrated the necessity of including benthic suspension feeders in ecological studies of shallow estuaries. Exclusion of mussels from the enclosures induced a microzooplankton dominance, followed by a slowly emerging mesozooplankton importance. The relative superiority of the microzooplankton was probably facilitated by their fast numerical response and by the size distribution of the phytoplankton. Thus, in the absence of pico-autotrophs, bacterioplankton became the only food source for heterotrophic nanoflagellates, which remained a rather passive and quantitatively unimportant link from bacterioplankton to microzooplankton.

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