Carbon fluxes from the microbial food web to mesozooplankton. An approach in the surface layer of a pelagic area (NW Mediterranean Sea)

Microbial food web
Mesozooplankton
Carbon flux
NW Mediterranean

Réseau trophique microbien
Mésozooplancton
Flux de carbone
Méditerranée Nord-Ouest

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ABSTRACT

The microbial food web structure, the carbon flux between mesozooplankton and the microbial community, and the importance of heterotrophs in the diet of marine zooplankton were investigated in the surface layer of a pelagic area of the NW Mediterranean Sea in five 24-h shipboard experiments. Heterotrophic flagellates grazed 65 % to 91 % of the bacterial production. However, the protozoan community seems also to utilize alternative carbon sources, such as small autotrophs. In most of the experiments, the estimated production of the protozoan > 7 µm was sufficient to supply mesozooplankton carbon demand. Copepods stimulated bacterial growth, presumably through excretion and preference for heterotrophic flagellates. Variations of the experimentally estimated metabolic O/N ratio (oxygen consumption through respiration, relative to nitrogen excretion) indicated that the mesozooplankton nutrition varied with the type of food web found on each occasion. Mesozooplankton directly interacted with the microbial food web by grazing upon heterotrophic protozoa, especially when small phytoplankton dominated the autotrophic community or under oligotrophic conditions.

RÉSUMÉ

Flux de carbone du réseau trophique microbien au mésozooplancton. Une approche dans la couche de surface d'une aire pélagique (NW de la Mer Méditerranée).

La structure du réseau trophique microbien ainsi que l'importance des hétérotrophes dans la nutrition du zooplancton marin et les flux de carbone, ont été étudiées dans la couche de surface d'une région pélagique du NW de la Méditerranée lors de cinq expériences de 24 h faites à bord.
Des estimations de la production bactérienne couplées avec des mesures de broutage de bactéries par les protozoaires ont révélé que les flagellés hétérotrophes utilisaient 65 à 91% de la production bactérienne. Cependant, la communauté de protozoaires semble aussi utiliser d'autres sources de carbone, telles que les petits autotrophes. Dans la plupart des cas étudiés, la production estimée des protozoaires des fractions de taille > 7 µm était suffisante pour couvrir la demande en carbone du mésozooplancton. Les copépodes pourraient jouer un rôle important pour la croissance bactérienne, par le biais de leur utilisation préférentielle des flagellés hétérotrophes comme nourriture. De plus, les variations du rapport métabolique O/N (oxygène respiré sur azote excrété) montrent que la nutrition du mésozooplancton dépend du type de réseau alimentaire dominant

lors de chaque situation. Par conséquent, les copépodes interagissent directement avec le réseau microbien en se nourrissant de protozoaires hétérotrophes, et ce particulièrement lorsque le phytoplancton de petite taille domine la communauté autotrophe.

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INTRODUCTION

The microbial food web is recognized as an important component of the pelagic marine food web (Azam *et al.*, 1983; Pomeroy and Wiebe, 1988). At certain times and places, the microbial and classical food webs may be highly interdependent, whereas at others they may be substantially independent (Turner and Roff, 1993). The strength of the link between the microbial food web and the classical food chain depends on the structure of the planktonic community and on hydrological conditions (Nielsen and Richardson, 1989; Pace *et al.*, 1990; Rassoulzadegan, 1993).

The link between the microbial and the classical food web is thought to be through phagotrophic protozoa (Azam *et al.*, 1983). Protozoan grazing is believed to balance bacterioplankton production in both marine and freshwater environments (Rassoulzadegan and Sheldon, 1986; Coffin and Sharp, 1987; McManus and Fuhrman, 1988*a, b*; Sanders *et al.*, 1989; Pace *et al.*, 1990). As described through enclosure experiments, mesozooplankton may simultaneously control bacterial grazing pressure and bacterial nutrient resources: firstly by grazing on bacterivorous flagellates and ciliates (Wiadnyana and Rassoulzadegan, 1989); and secondly by 'sloppy feeding', excretion and defecation (Eppley *et al.*, 1981; Roman *et al.*, 1988; Peduzzi and Herndl, 1992).

The diel variations of bacteria, protozoa, phytoplankton and mesozooplankton, were followed simultaneously *in situ* during five diel cycles in the surface layer of a station in the North Mediterranean Sea. This approach was designed to describe the structure of the planktonic food web and to evaluate the influence of the migration of copepods on bacterial communities (described in detail in Christaki, 1995; Christaki *et al.*, 1996). The data obtained *in situ* were completed by experimental microcosms realized on board in order to follow in parallel and in semi-controlled conditions (size fractionation of populations) the short-term (24 h) trophic interactions between algae, microheterotrophs and copepods. Bacterial production was estimated and grazing on bacteria by heterotrophic nanoflagellates was investigated. The direct and indirect effect of copepods on the microbial food web was studied in microcosms containing 10 µm and 60 µm screened sea-water respectively. Furthermore, the nutritional strategy of copepods was evaluated from experiments on their physiology (atomic ratio of oxygen respired/nitrogen excreted, used as an indicator of the substrate metabolized). Results of these experiments were used to construct carbon budgets describing fluxes between mesozooplankton and the microbial community for spring, summer and autumn.

The aim of this study was thus to assess the link between the marine microbial food web and mesozooplankton in the surface layer of a station in the North Mediterranean Sea.

MATERIAL AND METHODS

Sampling strategy

Between June 1992 and June 1993, five 24-h experiments were conducted on a fixed station in different seasons and/or different hydrodynamic situations (14-15 June 1992, 17-18 June 1992, 10-11 October 1992, 1-2 May 1993 and 26-27 June 1993). The station was 17 miles from Marseilles (Mediterranean Sea: 43° 02 N, 05° 12 E), with water column depth of 1000 m. The hydrodynamic conditions, which are related to the position of the North Western Mediterranean Current, influence the structure of the planktonic communities (Christaki *et al.*, 1996). For *in situ* measurements, water samples were collected at three-hour intervals over 24 h, from water depths of 5 and 40 m. Zooplankton samples for identification and dry weight analysis were collected by vertical hauls (50-0 m) using a 200 µm WP-2 net. For all microcosm experiments realized onboard, water was collected from 40 m depth at 12.00 h with 8-litre Niskin bottles. Animals used for shipboard experimental studies were collected by one or two vertical hauls, gently pipetted, rinsed several times with 0.2 µm filtered seawater, and divided into experimental lots.

Enumeration, and biomass of phytoplankton, bacteria, protozoa and zooplankton

For determination of chlorophyll *a*, unfiltered, 10 and 3 µm (Nuclepore) filtrates from each depth were collected on Whatman GF/F filters and analysed by fluorometry (Strickland and Parsons, 1972) on a 'Turner designs' fluorometer. Samples for the enumeration of bacteria and protozoa were fixed with 0.2 µm filtered buffered formalin (final concentration 2 % v/v). Subsamples for bacteria and protozoa, of 5 ml and 20 ml respectively, were filtered (maximum 48 h after sampling) on black Nuclepore filters (0.2 µm for bacteria, 0.8 µm for nanoflagellates) and subsequently stained with DAPI (4,6-diamidino-2-phenylindole, final concentration 500 µg l^{-1}). For bacteria enumeration, epifluorescence microscopy coupled with an image analysis system was used (Van Wambeke, 1988); up to 40 fields containing 30-70 bacteria per field were counted and the coefficient of variation (CV) between fields was 6-16 %. Random filter transects were counted for flagellates. About 60-100 flagellates were counted per filter, average CV across strips ranged from 10 to 22 %. Heterotrophic nanoflagellates were considered all eucaryotic non-pigmented organisms < 20 µm, and distinguished from chlorophyll containing cells (phototrophic nanoflagellates) by their red autofluorescence under blue light excitation. Nanoflagellates were classified in different size categories with

an ocular micrometer. The average cell volumes, assuming a spherical form, for each of the < 5 µm, 5-7 µm and > 7 µm flagellate size classes, were 14, 65 and 382 μm^3, respectively. In order to estimate the abundance of ciliates, two to three 100 ml Lugol preserved samples were counted using the Utermöhl technique. Ciliates (mainly oligotrichous) were also divided into three size classes: < 20 µm, 20-50 µm and > 50 µm, according to Rassoulzadegan (1977).

To calculate bacterial biomass, we used the per-cell conversion factor of Lee and Fuhrman (1987) of 20 fg C $bacteria^{-1}$, because the current edge detection algorithm used in our image analysis system leads to a systematic overestimate of bacterial biovolume. Biomass conversion factors were 220 fg C μm^{-3} for nanoflagellates (Børsheim and Bratbak, 1987) and 190 fg C μm^{-3} for ciliates (Putt and Stoecker, 1989). The carbon/chlorophyll *a* ratio, which is used to estimate autotrophic biomass, was assumed to be 50 (Parsons *et al.*, 1984). Mesozooplankton biomass was estimated from dry weight values, which were converted into carbon units assuming a 40 % carbon content (Gorsky *et al.*, 1988).

Bacterial production

Bacterial production was estimated by the thymidine method (Fuhrman and Azam, 1982). Duplicate samples (20 ml) and a blank (TCA 5 % final concentration added immediately after label addition) were incubated with 20 nM [Methyl-^{3}H]-thymidine (^{3}H-Tdr, 46 Ci $mmol^{-1}$) at *in situ* temperatures (± 2 °C), for two hours. Time course experiments demonstrated that ^{3}H-Tdr incorporation was always linear over two hours in the two batches. The isotope saturation was also verified, by incubation of ^{3}H-Tdr at 5, 10, 20 and 40 nM final concentrations. Bacterial production was estimated from ^{3}H-Tdr incorporation rates by using two different conversion factors. The first conversion factor (1.3×10^{18} cells mole Tdr^{-1}) derived from microcosm experiments undertaken in the laboratory (Christaki and Van Wambeke, 1995) with water from the same region (Gulf of Marseilles). This conversion factor was close to those used in oligotrophic Mediterranean water (Hagström *et al.*, 1988; Zohary and Robarts, 1992). However, applying this conversion factor in the present study led us to inconsistent data (see 'Results'). Consequently, we calculated a second conversion factor from microcosm experiments undertaken on board (see below). This second coefficient was 5×10^{18} bacteria mole Tdr^{-1}. Bacterial carbon demand was estimated from the production rates assuming a growth yield of 50 % (Cole *et al.*, 1988).

Microcosm experiments

Five shipboard microcosm experiments (see "Sampling strategy" for dates) were performed to estimate short-term relationships between zooplankton (mainly copepods), protozoa and bacteria. Both direct and indirect effects of mesozooplankton on the pico- and nanoplankton community (organisms <10 µm) were studied. Copepods were added to water screened through 60 µm, containing pico-, nano- and microzooplankton as well as water screened through 10 µm containing only pico- and nanoplankton. Screened water samples without copepods added served as controls.

Water samples from 40 m depth were first gently prescreened through 60 µm mesh Nitex. Two carboys contained water screened through 60 µm, and two others contained water further screened through 10 µm (147 mm diameter Nuclepore polycarbonate filters) using inverse gravity filtration with a special siphon-filter apparatus (Sheldon and Rassoulzadegan, 1987). Zooplankton were added into two of the carboys (26 ± 12 ind. l^{-1}, biomass 0.1 ± 0.04 mg $C.l^{-1}$, mean ± sd, n = 10), containing 60 µm and 10 µm screened water, respectively. The carboys (4 acid-rinsed 20 l polyethylene) were maintained in attenuated light conditions (under a blue polyethylene screen transmitting 5 % of the incident light) in a circulating sea water bath during an incubation period of 24 hours. Samples for enumeration of bacteria (5 ml) and protozoa (25 ml) were taken every three hours. The copepods were collected at the end of the experiment for identification, counts and dry weight analysis. In the experiments carried out in May and June 1993, bacterial production in the microcosms was also measured every three hours and from the filtered microcosms without copepods we calculated the second thymidine-carbon conversion factor.

Grazing of bacteria, protozoan carbon requirement

Direct measurements of grazing on bacteria (BG) by flagellates were carried out on 1-2 May and 26-27 June 1993, using fluorescently labelled minicells (Pace *et al.*, 1990; Vaqué *et al.*, 1992). Minicells were added at a concentration of approximately 10 % of bacterial concentration into six acid cleaned (HCl) 2l polycarbonate bottles. Two bottles served as poisoned controls (1% final concentration formaldehyde). Subsamples (25 ml) were taken after 0, 15, 30, 60 and 120 min and were fixed with an equal volume of ice-cold 4 % glutaraldehyde (Sanders *et al.*, 1989; Pace *et al.*, 1990; Vaqué *et al.*, 1992). Minicells were counted in food vacuoles of protozoans; uptake of minicells was linear for the first 30 min. Ingestion (bacteria $protozoan^{-1}$ h^{-1}) and clearance (nl $protozoan^{-1}$ h^{-1}) rates were calculated from time courses uptake, multiplying the number of minicells ingested by the ratio of bacteria to added minicells (Pace *et al.*, 1990).

The gross growth efficiency of protozoa was assumed to be 40 % (Fenchel, 1987). With a maximum generation time of approximately four days, (*e.g.* Kuosa and Kivi, 1989) a minimum protozoan carbon requirement (CR) was estimated equivalent to 47.5 % of protozoan carbon d^{-1} for all protozoan categories. A maximum CR estimate was also calculated for comparison assuming a growth rate of 1 d^{-1}.

Zooplankton respiration, excretion and minimum carbon requirement

Mesozooplankton were incubated in 300 ml flasks in seawater containing natural particle assemblages and in seawater filtered through 0.2 µm (Sartorious cellulose acetate

filters); controls consisted of flasks without animals. The abundance of copepods in the flasks, chosen as a compromise between a significant signal and overcrowding (Omori and Ikeda, 1984), ranged from 230 to 430 individuals l^{-1}, mean: 300 individuals l^{-1}. The experimental and control flasks, prepared in triplicates, were installed on a vertical wheel rotating at 3.5 rpm and incubated at *in situ* temperature, in the dark for 24 h. The dissolved oxygen in the flasks was measured with a polarographic electrode (YSI 57 probe, 0.05 ppm precision). Ammonium excretion was measured in filtered sea-water (colorimetric method, Strickland and Parsons, 1972).

Metabolic rates, deduced from differences with controls, at the end of 24 h, were expressed per mg dry weight day^{-1} and the atomic ratio O/N calculated. The atomic O/N ratio (oxygen consumption through respiration, relative to nitrogen excretion) is an indicator of the type of substrate (carbohydrates or proteins) being oxidized through respiration (Gaudy and Boucher, 1983; Omori and Ikeda, 1984).The minimum carbon requirement (CR) for zooplankton was based on the respiration rates of starved animals and was calculated according to Omori and Ikeda (1984), as follows:

$$CR = R \times RQ \times 12/22.4 \ (\mu g\ C\ mg\ dw^{-1}\ d^{-1}) \quad (1)$$

where R ($\mu l\ O_2\ mg\ dw^{-1}\ d^{-1}$) is the Respiration rate and RQ (0.97: Gnaiger, 1983) is the Respiratory Quotient.

RESULTS

In situ measurements

The mean diel values of biological parameters from *in situ* measurements at 5 and 40 m are summarized in Table 1.

Table 1

Mean values of biological parameters for the five diel studies. Chl a = Chlorophyll a, PNAN = phototrophic nanoflagellates, HNAN = heterotrophic nanoflagellates, (number of samples: n = 9, except ciliates n = 3).

Valeurs moyennes des paramètres biologiques pour les cinq études de cycles journaliers. Chl *a* = Chlorophylle *a*, PNAN = nanoflagellés phototrophes, HNAN = nanoflagellés hétérotrophes. Nombre d'échantillons: n = 9, sauf pour les ciliés: n = 3.

Date of sampling		14 June 92	17 June 92	10 Oct. 92	1 May 93	26 June 93
	Depth (m)					
Chl *a* ($\mu g.l^{-1}$)	5	1.10	0.9	0.62	0.2	0.21
	40	1.25	0.47	0.7	0.3	0.11
Bacteria ($10^9.l^{-1}$)	5	0.68	0.53	0.46	0.95	0.63
	40	0.72	0.83	0.5	0.96	0.64
PNAN ($10^6.l^{-1}$)	5	1.16	0.79	0.85	1.6	0.70
	40	0.87	0.83	0.85	2.0	1.27
HNAN ($10^6.l^{-1}$)	5	1.23	1.11	1.9	1.15	1.70
	40	1.23	0.93	2.2	0.88	1.52
Ciliates ($10^3.l^{-1}$)	5	2.0	3.0	2.6	0.20	1.1
	40	1.8	2.8	2.7	0.24	1.2
Mesozooplankton ($ind.m^{-3}$)	0-50	911	629	347	3225	768
Copepods ($ind.m^{-3}$)	0-50	607	376	278	3093	546

With the exception of the 14-15 June 1992 experiment, when high concentrations (up to 3.3 10^3 cell ml^{-1}) of chain-forming diatoms (*Chaetoceros* sp., *Leptocylindrus danicus, Rhizosolenia fragilissima*, and *Skeletonema costatum*) were recorded, autotrophic biomass was generally dominated by phototrophic nanoflagellates (chlorophyll *a* < 10 µm, 53 to 80 %). The highest mesozooplankton concentration (mean: 3.2 ind. l^{-1}, biomass: 11.4 µg C l^{-1}) was observed in May and the lowest (mean: 0.35 ind. l^{-1}, biomass: 1.4 µg C l^{-1}) in October. Copepods numerically dominated the mesozooplankton population, from 60% in June 1992 to 96 % in May 1993. Other important mesozooplankton groups present were: Appendicularians (max 12 % in June 92) and Cladocerans (mainly *Evadne spinifera*, max 13 % in June 1993).

Microcosm experiments

The mesozooplankton added to the microcosms, or in the flasks for the estimation of respiration and excretion rates, were representative of the mesozooplankton community in the surrounding sea water. Four copepod genera - *Clausocalanus* spp. (mainly *C. pergens* and *C. paululus*), *Paracalanus* spp. (almost exclusively *P. parvus*), *Oithona* spp. (*O. plumifera* and *O. helgolandica*) and *Centropages* spp . (almost exclusively *C. typicus*) - prevailed in the experimental community (in abundance, from 70 % in June 1992 to 100 % in May 1993).

Bacteria

In all microcosms a lag phase of about 9-15 hours was followed by an exponential growth of bacteria (Fig. 1, Tab. 2).

Table 2

Bacterial growth rates (µ) calculated from exponential growth phase of bacterial abundance, after in transformation of data, 60: water screened through 60 µm, 10: water screened through 10 µm, 10 + cop: water screened through 10 µm + copepods added, 60 + cop: water screened through 60 µm + copepods added, n: number of samples. Test for equality of regression slopes: Student's t-test, p: significance level, df: degrees of freedom, ns: not significant, nd: no data.

Taux de croissance bactériens (µ) calculés à partir de la phase exponentielle de croissance des bactéries (transformation ln des données). 60: eau tamisée sur 60 µm; 10: eau tamisée sur 10 µm; 10 + cop: eau tamisée sur 10 µm avec ajout de copépodes; 60 + cop: eau tamisée sur 60 µm avec ajout de copépodes. n: nombre d'échantillons. Test pour l'égalité des pentes de regression: test-t de Student, p: niveau de probabilité, df: degré de liberté; ns: non significatif, nd: pas de données.

Date of experiment	Microcosms								Comparison of slopes			
	60		10		10 + cop		60 + cop		10 + cop × 10		60 + cop × 60	
	µ	n	µ	n	µ	n	µ	n	p	df	p	df
14 June 92		nd	0.031	3	0.057	5	0.041	4	< 0.01	4		nd
17 June 92		nd	0.031	5	0.044	5	0.038	8	< 0.01	6		nd
10 October 92	0.029	4	0.039	5	0.174	3	0.179	4	< 0.05	4	< 0.01	4
1 May 93	0.016	5	0.020	3	0.037	3	0.061	4	< 0.05	2	< 0.01	5
26 June 93	0.016	8	0.046	6	0.072	3	0.076	6	ns	5	< 0.01	10

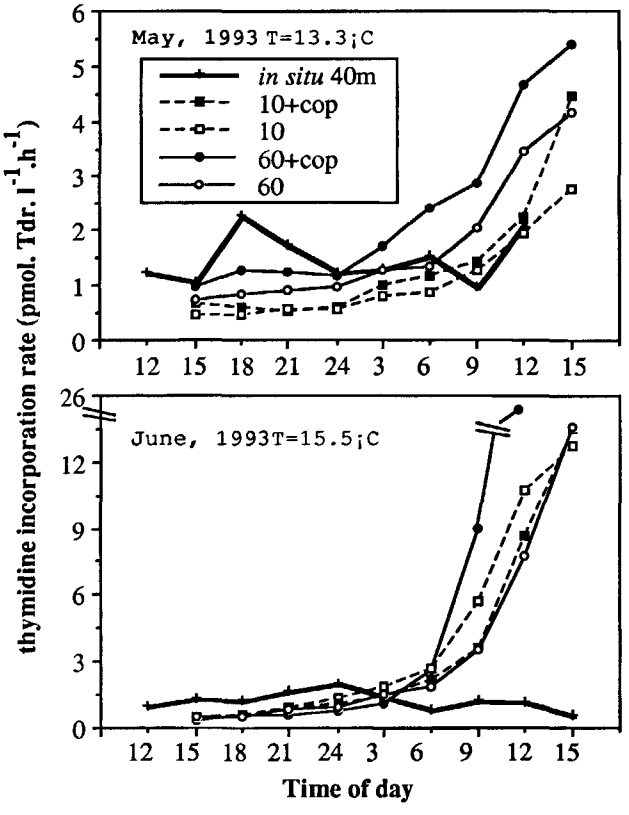

Figure 1

Thymidine incorporation rates in TCA precipitate in May and June 1993 experiments, in four microcosms and in situ, *at 40 m depth. Presented values are means of duplicate measurements (average variation between duplicates < 10 %) 60 : 60 µm screened seawater, 60 + cop: 60 µm screened seawater + copepods added, 10: 10 µm screened seawater, 10 + cop: 10 µm screened seawater + copepods added.*

Vitesses d'incorporation de thymidine dans le précipité TCA froid, lors des expériences de mai et juin 1993, dans les quatre microcosmes et *in situ* à 40 m de profondeur. Les valeurs présentées sont des moyennes de mesures dupliquées (coefficient de variation moyen entre les duplicats < 10 %). 60: eau de mer tamisée sur 60 µm; 60 + cop: eau de mer tamisée sur 60 µm avec ajout de copépodes; 10: eau de mer tamisée sur 10 µm; 10 + cop: eau de mer tamisée sur 10 µm avec ajout de copépodes.

The net growth rates were calculated from logarithm transformed data of the exponential growth phase. The slopes of the respective regression equations indicated that the net bacterial growth rates were higher in microcosms enriched with copepods. The general trend of the net growth rates (µ) was: (µ 60 µm) < (µ 10 µm) < (µ 10µm + copepods) or (µ 60µm + copepods). The net bacterial growth rates obtained in the batches containing copepods were in most cases statistically different from those without copepods (test for equality of regression slopes, Table 2). Integrated 24 hour thymidine-based production calculated from the 1.3×10^{18} bacteria mole Tdr^{-1} conversion factor was lower than the integrated cell production calculated directly from increase of bacterial numbers in the microcosms.

Nanoflagellates

Organisms < 7 µm dominated the nanoflagellate population (Fig. 2). To describe the trend of nanoflagellate abundance

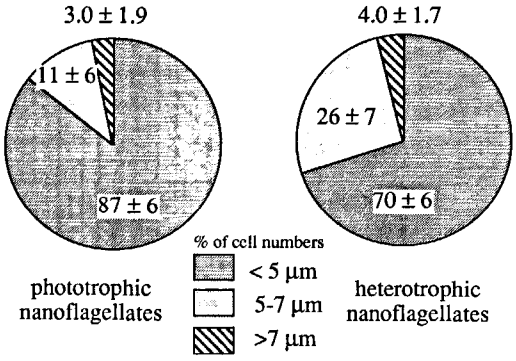

Figure 2

Relative abundance of different size classes of phototrophic (PNAN) and heterotrophic (HNAN) nanoflagellates. Values represent overall mean of the five diel sampling periods.

Abondance relative de nanoflagellés phototrophes (PNAN) et hétérotrophes (HNAN) de différentes classes de tailles. Les valeurs représentent la moyenne ± écart type de cinq expériences.

in experimental microcosms we used linear regression since the linear model provided the best empirical fit to the data. In the microcosms in which theoretically most of the nanoflagellate predators were removed (10 µm filtered water), significant growth was observed only once (PNAN, May 1993, Tab. 3), whereas in October 1992 a significant decrease was observed (HNAN). Except in the 17 June 1992 experiment, significant decreases of heterotrophic nanoflagellate abundance were observed in the microcosms containing copepods, whereas the changes of phototrophic flagellate numbers were not significant in most cases (Tab. 3).

Finally, the comparison of the ratios of final abundance/ initial abundance of microorganisms in treatments with copepods *vs.* treatments without copepods for the five experiments based on the Wilcoxon test (n = 10 pairs of values for each comparison), showed a significant decrease of heterotrophic nanoflagellate numbers ($z = -2.24$, $p < 0.05$) and a significant increase of bacterial numbers ($z = 2.5$, $p < 0.02$) in treatments with copepods. No significant differences were observed between treatments with or without copepods for the abundance of phototrophic flagellates.

Flagellate grazing experiments

Bacterial grazing by heterotrophic nanoflagellates was measured during the experiments of May and June 1993. Ingestion rates were low (5-6 bact fl^{-1} h^{-1}). Flagellates < 7 µm (body volume from 8.2 to 65.4 μm^3) numerically dominated the grazer community. The most abundant fraction (< 5 µm, Fig. 2) was almost exclusively represented by organisms of 2.0-3.5 µm diameter (average body volume 14 μm^3). The calculated clearance rates were 5.8 and 7.7 nl $flagellate^{-1}$ h^{-1} (specific rates of approximately $4\text{-}5 \times 10^5$ body volumes h^{-1}). The clearance extrapolation to the natural environment using the average *in situ* concentrations of nanoflagellates showed that 14 and 29 % (in May and June respectively) of the water was cleared of bacteria per day. Comparing the daily grazing with the simultaneously measured bacterial

Table 3

Results of linear regressions of phototrophic (PNAN) and heterotrophic (HNAN) flagellate abundance versus time *(24 hours) in experimental microcosms. n: number of samples ; –: significant decrease ; +: significant increase ; *: p ≤ 0.05 ; **: p ≤ 0.001 ; ns: no significant change ; nd: no data.*

Résultats des regressions linéaires entre l'abondance des flagellés phototrophes (PNAN) et hétérotrophes (HNAN) et le temps (jusqu'à 24 h) dans les microcosmes expérimentaux. n: nombre d'échantillons; -: décroissance significative; +: croissance significative; *: p < 0.005; ** p < 0.001; ns changement non significatif; nd: pas de données.

	Date	14 June 92			17 June 92			10 October 92			1 May 93			26 June 93		
	Treatment		n	slope		n	slope		n	slope		n	slope		n	slope
PNAM	10 µm	ns	10	0.013	ns	7	0.005	ns	10	–0.012	*+	9	0.039	ns	7	0.013
	10 µm + copepods	ns	10	–0.016	ns	6	–0.006	**-	10	–0.027	ns	8	0.004	ns	9	.0003
	60 µm	nd			nd			**-	10	–0.032	ns	7	0.023	ns	9	0.014
	60 µm + copepods	*+	10	0.017	ns	6	–0.009	ns	10	–0.013	ns	9	0.005	**+	9	0.034
HNAN	10 µm	ns	10	0.008	ns	7	–0.009	*-	10	–0.026	ns	9	0.009	ns	7	0.012
	10 µm + copepods	*-	10	–0.034	ns	6	0.005	**-	10	–0.053	**-	8	–0.024	*-	9	–0.015
	60 µm	nd			nd			*-	10	–0.041	ns	7	0.009	ns	9	–0.016
	60 µm + copepods	**-	9	–0.031	ns	6	–0.001	**-	10	–0.050	*-	9	–0.013	**-	9	–0.035

production showed that when using a conversion factor of 1.3×10^{18} bacteria mole Tdr^{-1}, grazing exceeded bacterial production by a factor 2.4 to 3.5. The same comparison using the conversion factor calculated from on-board incubated microcosms (5×10^{18} bacteria mole Tdr^{-1}) balanced reasonably with grazing. In that case heterotrophic flagellates removed 64 and 91 % of daily bacterial production in May and June, respectively.

Mesozooplankton respiration and excretion

The O/N ratio values (oxygen consumption through respiration, relative to nitrogen excretion), ranged from 9.4 to 28.4 (Tab. 4). When cells smaller than 10 µm dominated the phytoplankton community (October 1992, chlorophyll fraction < 10 µm = 80 %), and/or under oligotrophic conditions (May, June 1993), the O/N ratio was low (< 24). The O/N ratio was high (> 24) under higher concentrations of chlorophyll (14-15 June 1992, 1.88 µg Chl *a* l^{-1}), marked by the presence of chain forming diatoms (fraction >10 µm = 47 % of the total chlorophyll). The spearman correlation between O/N and chlorophyll *a* values was significant for total chlorophyll ($r = 0.99$, $p < 0.05$), but non significant for > 10 µm chlorophyll ($p > 0.05$).

DISCUSSION

To summarize our results we constructed carbon budgets for the plankton community (Fig. 3), using the following

Table 4

Mesozooplankton excretion and respiration rates, dry weight, number of zooplankton individuals (mean ± sd of triplicate samples), atomic O/N ratios, temperature and initial concentrations of chlorophyll a *in the experimental flasks, nd: no data.*

Taux d'excrétion et de respiration du mésozooplancton, poids sec, nombre d'individus du zooplancton (moyenne ± écart type d'échantillons triplicués), rapports atomiques O/N, température et concentrations initiales de chlorophylle *a* dans les flacons expérimentaux; nd: pas de données.

Parameters	14 June 92	17 June 92	10 October 92	1 May 93	26 June 93
Excretion rate (filtered water) µg N.mg dr $wt^{-1}.h^{-1}$	0.63 ± 0.1	1.18 ± 0.5	0.72 ± 0.3	0.62 ± 0.5	1.45 ± 0.9
Respiration rate (filtered water) µg O_2.mg dr $wt^{-1}.h^{-1}$	14.3 ± 0.3	12.1 ± 3.6	9.15 ± 4.8	5.5 ± 1.7	10.9 ± 1.8
Excretion rate (unfiltered water) µg O_2.mg dr $wt^{-1}.h^{-1}$	16.56 ± 4.9	16.0 ± 0.4	14.4 ± 2.4	7.5 ± 0.4	12.4 ± 2.2
Dry weight in experimental flasks $mg.l^{-1}$	1.49 ± 0.6	2.63 ± 0.6	1.96 ± 1.3	2.93 ± 0.6	1.16 ± 0.6
Animals.l^{-1} in experimental flasks	231 ± 40	238 ± 40	350 ± 112	429 ± 66	254 ± 46
Atomic Ration O/N	28.37	12.82	15.89	11.09	9.4
Temperature °C	18.0	18.0	17.3	13.3	15.5
Chl a ($\mu g.l^{-1}$)	1.88	0.28	0.62	0.2	0.11
% Chl *a* > 10 µm	47.3	31.0	20.9	nd	29.1
% Chl *a* 3 – 10 µm	11.8	30.4	9.7	nd	30.6
% Chl *a* < 3 µm	40.8	38.6	69.4	nd	40.3

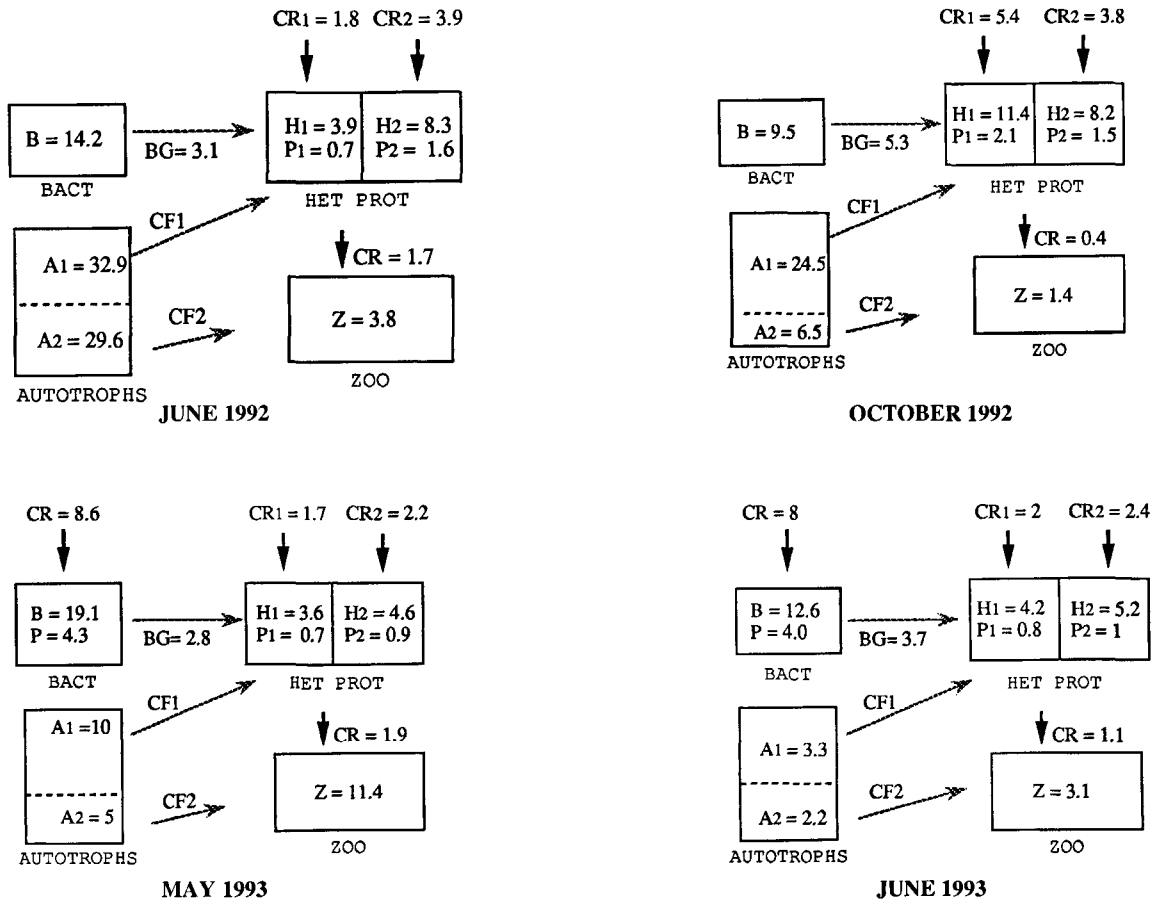

Figure 3

Carbon flow budgets for the 14 June 1992, October 1992, May 1993 and June 1993 experiments. Boxes : BACT (heterotrophic bacteria), AUTOTROPHS (autotrophic nanoplankton) A1= biomass of fraction < 10 µm and A2 = biomass of fraction >10 µm. HET PROT (heterotrophic protozoans), H1= biomass of fraction < 7 µm and H2 = biomass of fraction >7 µm. ZOO (mesozooplankton, mainly copepods), Symbol units: B, A, H, Z (biomass in µg C.l^{-1}), P (production in µg C.l^{-1}.d^{-1}), CR (carbon requirements in µg C.l^{-1}.d^{-1}), BG (grazing of bacteria by heterotrophic nanoflagellates < 7 µm in µg C l^{-1} d^{-1}). CF1 (Carbon flux from smallest size of autotrophic plankton to HET PROT.) and CF2: (Carbon flux from the >10 µm phytoplankton to ZOO), in µg C l^{-1} d^{-1}.

Bilan des flux de carbone pour les expériences du 14 juin 1992, d'octobre 1992, de mai 1993 et de juin 1993. Boites BACT (bactéries hétérotrophes) et AUTROPHS (nanoplancton autotrophe): A1= biomasse de la fraction <10 µm et A2 = biomasse de la fraction >10 µm. HET PROT (protozoaires hétérotrophes): H1= biomasse de la fraction <7 µm et H2 = biomasse de la fraction >7 µm. ZOO (mésozooplancton, surtout des copépodes), Z = biomasse du mésozooplancton. Toutes les biomasses sont exprimées en µg C l^{-1}. BG: Broutage des bactéries par les nanoflagellés < 7 µm (µg C l^{-1} d^{-1}), CR: demande en carbone, P: production, CF: flux de carbones provenant des autotrophes (µg C l^{-1} d^{-1}), Paramètres et unités: B1, B2 (biomasses µg C l^{-1}), P (production, µg C.l^{-1}.d^{-1}), CR (demande en carbone, µg C.l^{-1}.d^{-1}), BG (grazing des bactéries par les nanoflagellés hétérotrophes >7 µm, µg C.l^{-1}.d^{-1}), EF1: flux d'exportation depuis les plus petites tailles du plancton autotrophes jusqu'aux HET PROT. EF2: flux d'exportation depuis le phytoplancton >10 µm jusqu'au ZOO.

compartments: 1) Heterotrophic bacteria (BACT); 2) phytoplankton (AUTOTROPHS), divided into two categories (A1: fraction > 10 µm and A2: fraction < 10 µm), these two size fractions being determined from chlorophyll size fractionation in June 1992, October 1992 and June 1993, or from biovolume-biomass conversions of microscopic counts in May 1993 (chlorophyll fractionation data not available); 3) Protozoa (HET PROT), also divided into two size categories, heterotrophic nanoflagellates < 7 µm (H1: mainly bacterivores) and, heterotrophic nanoflagellates > 7 µm and ciliates (H2: capable of ingesting nanoplankton); and 4) Mesozooplankton (ZOO).

Such an approach involves some simplifying assumptions concerning a highly dynamic community. For example, the heterotrophic protozoan (HET PROT) section may include multiple trophic relationships (Rassoulzadegan and Sheldon, 1986; Wikner and Hagström, 1988; Weisse and Scheffel-Möser, 1991). Any given population may vary in abundance over short time scales. However, the values used are diel means of biological parameters measured (at 5 and 40 m depths for components of the microbial food web and in the 50-0 m layer for mesozooplankton), and so are representative of a short time and space scale (24 h, 0-50 m water column).

With the exception of a single diel study published by Hagström *et al.* (1988) based on a single 50 l microcosm, no similar study has been reported from the Mediterranean dealing with carbon flux in the microbial community. Generally our results concerning standing stocks of heterotrophic organisms and bacterial production are in good agreement with those of Hagström *et al.* (1988) from a coastal station (sampling point b, maximum depth 80 m,

Villefranche-sur-Mer, France, in October 1984). Moreover, the rate of predation of nanoflagellates upon bacteria are of the same range (6.0 µg bacterial C. l^{-1} .d^{-1} in Hagström *et al.* (1988), 3 and 5.6 µg bacterial C. l^{-1} .d^{-1} in this study). However, the nanoflagellate production was significantly higher (8.4 µg C. l^{-1} .d^{-1}) in Hagström *et al.* (1998) than in our study (from 0.7 to 2.1 µg C l^{-1} .d^{-1}).

Carbon budgets were constructed for four distinct periods (Fig. 3). In all four situations, bacterial grazing (BG, calculated from average measured specific ingestion rate) appeared to be sufficient to satisfy the minimum carbon requirements of < 7 µm heterotrophic protozoan (CR1). Nanoflagellates > 7 µm and ciliates could obtain their carbon demand (CR2) from either, bacteria, < 7 µm nanoflagellates or small autotrophs, suggesting that the carbon flux from small autotrophs (CF1, Fig. 3) may be an important energy pathway towards microheterotrophs as described by others (Pomeroy and Wiebe, 1988; Stoecker and Evans, 1985).

When a maximum carbon requirement of protozoans is used, (assuming one division per day), protozoan carbon requirements (CR1+CR2, Fig. 3) would range from 27 to 65 µg C l^{-1} d^{-1}. Measurements of total primary production in the surface layer of this station ranged from 12 to 34 µg C l^{-1} d^{-1} (1992-93, Conan, pers. comm.) suggesting that, in some cases, one division per day of protozoans could be supported by primary production. However, it should be noted that rapid reproduction of protozoa was not seen in our experiments.

The rates of ingestion (5-6 bacteria flagellate h^{-1}) obtained in this study are within the range (0.25 to 90 bacteria flagellate h^{-1}, mean 9.7 bacteria flagellate h^{-1}) reported by Vaqué *et al.* (1994) using similar methods. Small flagellates (2.0 to 3.5 µm diameter, average biovolume 14 μm^3) numerically dominated the community of bacterivores (Fig. 2). In previous studies it was been reported that the major grazers of bacteria are flagellates ranging in size from 1 to 3 µm (Rassoulzadegan and Sheldon, 1986; Coffin and Sharp, 1987; Wikner and Hagström, 1988), thus, the low ingestion rates could also be attributed to the small size of these bacterivores (Kuuppo-Leinikki, 1990). The calculated specific clearance (average 4-5 × 10^5 body volumes h^{-1}) which is probably a better parameter for comparison (McManus and Fuhrman, 1988a), compares well with the values of Fenchel (1987). Similar results have been reported by McManus and Fuhrman (1988*a*), and Kuuppo-Leinikki (1990). With the measured ingestion of 5-6 bacteria h^{-1} $flagellate^{-1}$, the flagellates would divide approximately every three days. Hagström *et al.* (1988) observed an increase of the nanoflagellate numbers during night hours (the population doubled), in our experiments we did not observe such a rapid increase either during *in situ* measurements or in the microcosm experiments. This result suggests that generation times of flagellates in the open sea station where our study was conducted were longer than in the microcosm containing water from the coastal station studied by Hagström *et al.* (1988). Thus, the calculations of protozoan carbon demand are made according the coefficient proposed by Kuosa and Kivi (1989).

Recent studies report that the percentages of bacterial production consumed by heterotrophic flagellates vary considerably. Thus, in some studies the estimates of bacterial production were substantially greater than grazing (Vaqué *et al.*, 1992), while in others the estimated heterotrophic flagellate carbon demand could not always be satisfied from bacteria (Kuosa and Kivi, 1989; Kuuppo-Leinikki, 1990; Van Wambeke, 1994) or else varied considerably (McManus and Fuhrman, 1988a). The use of a conversion factor of 1.3 × 10^{18} cell mole Tdr^{-1} resulted in estimates of bacterial grazing being much higher than bacterial production which could theoretically lead to the depletion of the bacteria in the water column within a few days. However, no decrease in bacterial numbers was observed, suggesting an underestimation of the conversion factor. Various authors in previous works have already addressed this problem (Sanders *et al.*, 1989; Pace *et al.*, 1990; Wikner *et al.*, 1990; Barcina *et al.*, 1992). Radiolabelled minicells (Wikner *et al.*, 1990) and fluorescently labelled bacterial techniques (Pace *et al.*, 1990) are not generally considered as apt to provide overestimates. Wikner *et al.* (1990) concluded that when daily grazing estimates exceed bacterial growth measured by the thymidine method, this is due to the bias of the conversion factor. The exponential increase of bacterial numbers in the 10 µm filtered microcosms permitted a rough calculation of an empirical conversion factor 5 × 10^{18} bacteria mole Tdr^{-1} (± 0.57 n = 4). Isotopic dilution may explain this high value (Moriarty, 1986), however, we checked by saturation kinetics that 20 nM were sufficient to reach Vmax conditions *in situ* and in the microcosms. Thus, other factors, such as non specific-labelling or the occurrence of bacteria unable to incorporate exogenous thymidine, could explain the high conversion factor obtained here (Bell, 1990). The main advantage of this empirical method is that it is not biased by the fact that some bacteria cannot utilize thymidine and avoids assumptions about the specific labelling of the DNA (Moriarty, 1986). The calculation of production on the basis of this empirical conversion resulted in a bacterial production of 4.3 and 4.0 µg C l^{-1} d^{-1} in May and June, respectively, which was in reasonable balance with the grazing estimates (65 % and 92 % of the bacterial production in May and June respectively, Fig. 3).

In this study, copepods enhanced bacterial growth in both 10 µm and 60 µm screened sea-water microcosms firstly by grazing on bacterivores and secondly by 'sloppy feeding', excretion and defecation. This observation is in good agreement with results from other studies (Eppley *et al.*, 1981; Güde, 1988; Roman *et al.*, 1988; Wiadnyana and Rassoulzadegan, 1989; Peduzzi and Herndl, 1992). Estimates of direct phytoplankton exudation (Coffin and Sharp, 1987) indicated that much of the carbon demand for bacteria may come from other carbon resources such as the decay of dead phytoplankton cells or zooplankton influences.

The changes of flagellate numbers in the experimental microcosms may be the result of complex trophic relations (Rassoulzadegan and Sheldon, 1986; Wikner and Hagström, 1988; Weisse and Scheffel-Möser, 1991). In microcosms where copepods were added, heterotrophic nanoflagellate numbers decreased, while numbers of phototrophic nanoflagellates did not show any significant decrease. These results indicate a preference of copepods for heterotrophic prey, as HNAN and PNAN size spectra

were about the same. The organic content and the quality of particulate food has been shown to influence the feeding rate of copepods (Mayzaud and Poulet, 1978; Cowles *et al.*, 1988). The preference for protozoa may be due to their enhanced nutritional quality (Wiadnyana and Rassoulzadegan, 1989; Stoecker and Capuzzo, 1990; Turner and Roff, 1993). Nielsen *et al.* (1993) showed that in the North Sea the estimated daily ciliate production was sufficient to meet copepod carbon demand. In the four situations presented (Fig. 3) in our carbon budgets, the production of the heterotrophic fraction > 7 µm was not always greater than the minimum mesozooplankton carbon requirement.

The O/N ratio (oxygen consumption through respiration, relative to nitrogen excretion), showed a herbivorous feeding behaviour (O/N > 24, *i.e.* predominance of fat and carbohydrate metabolism) when higher concentrations of the chlorophyll fraction > 10 µm occurred (14-15 June 1992). When small phytoplankton (< 10 µm) dominated the autotrophic community (October 1992), or under pronounced oligotrophic conditions (May and June 1993), the O/N ratio (< 24) indicated that copepods may be feeding on a nitrogen-rich prey, such as microzooplankton or detritus, the latter possibly harbouring attached bacteria and their protozoan predators (Omori and Ikeda, 1984). The O/N ratio differs according to the protein content of the substrate metabolized, and probably reflects the nutritional strategy of copepods (Gaudy and Boucher, 1983). Paffenhöfer and Knowles (1980) suggested that omnivory is inherent in most calanoid, and possibly many cyclopoid, species. The copepods can switch from herbivory to carnivory depending upon the availability and concentrations of 'plant' or 'animal' prey (Mayzaud and Poulet, 1978; Landry, 1981). The significant correlation of O/N values with total chlorophyll concentration deserves further attention. However, the range of chlorophyll *a* concentrations tested in this relationship is low (0 - 2 µg Chl *a* l^{-1}), and is established with five points; moreover the lack of correlation between O/N and chlorophyll > 10 µm suggests that the degree of herbivory may also depend on other factors such as the type (quality and size) of phytoplankton and mesozooplankton populations present.

As demonstrated in recent studies, the microbial food web may be an important link between primary production and the mesozooplankton, especially when pico- and nanoplankton dominate the food web (Nielsen and Richardson, 1989; Stoecker and Capuzzo, 1990; Nielsen *et al.*, 1993) and under oligotrophic conditions (Roman *et al.*, 1988). The lack of data for fractionated primary production did not permit us to estimate the potential importance of larger phytoplankton cells for copepod nutrition (CF2, Fig. 3).

The present study provides evidence, in the first place, that the copepods show a flexible feeding behaviour, relying on the type of food web prevailing at the time. Thus, they were directly interacting with the microbial food web by grazing upon heterotrophic protozoa, especially when small phytoplankton dominated the autotrophic community or under oligotrophic conditions. At times, perhaps only a small percentage of bacterial production reaches mesozooplankton, however it may be of crucial importance, especially in oligotrophic systems, such as the NW Mediterranean. Secondly, the study shows that the activity of copepods can be a key factor for bacterial growth by providing organic substrate or by eliminating bacterial predators.

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