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Trophic relationships between planktonic microorganisms in the river Meuse (Belgium): a carbon budget

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With 9 figures and 3 tables

Abstract: During the period of plankton development (April to October 1996), the trophic relationships among phytoplankton, metazooplankton, protozooplankton and bacterioplankton were quantitatively studied at one location in the Belgian part of the river Meuse. Biomass fluctuations of phytoplankton, metazooplankton, bacteria and protozoa were monitored fortnightly. On the basis of in situ measurements of the fluxes of primary production, metazooplankton grazing, organic matter consumption by bacteria and grazing of bacteria by protozoa, the carbon fluxes between the different compartments of the first trophic levels were estimated on eleven sampling dates. Net primary production was measured from incubations with ^{14}C bicarbonate and grazing of algae by metazooplankton was determined by in situ incubations with labelled algae in a grazing chamber. The algal biomass ingested was corrected for the edible phytoplankton and the carbon assimilated by metazooplankton was calculated taking into account zooplankton assimilation yield estimated from experimental data and from literature values. Bacterial production was evaluated by ^{3}H-thymidine and ^{3}H-leucine incorporation rates, and bacterial carbon demand was calculated taking into account a growth yield of 30 %. Measurements of mortality and grazing of bacteria showed that grazing by protozooplankton was the major loss process indicating the main role of this compartment in the control of bacterial biomass. In the studied stretch of the river Meuse, autochthonous production exceeded, in most situations, the allochthonous inputs to the river. On average over the studied period, carbon consumption by metazooplankton and bacteria balanced organic carbon inputs from primary production and external loading. The large part of primary production used by

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bacteria and indirectly by protozooplankton points to the potential importance of the microbial food web in this river system.

Key words: Phytoplankton, metazooplankton, bacteria, protozooplankton, carbon budget, river Meuse.

Introduction

The view of a linear food chain in aquatic systems has prevailed for a long time. According to this simple concept, herbivorous metazooplankton plays a central role by consuming most of the phytoplankton production and constituting the major food resource for pelagic fishes. In the 1980s, studies conducted mainly in marine systems (Williams 1981) but also in lakes (Cole et al. 1988) have substantially modified this view of the planktonic food web. The concept of "microbial loop" (Azam et al. 1983) has been introduced, based on experimental evidence that a large fraction of primary production flows through the pool of dissolved organic matter (DOM). The processes involved in DOM production may be either extracellular release by phytoplankton, lysis of dead algal cells, or sloppy feeding. This part of primary production, unavailable to herbivorous metazooplankton, is mainly utilised by bacteria. As grazing by heterotrophic protozooplankton (planktonic protozoa) is considered as the principal loss process of bacteria in aquatic habitats (Sanders et al. 1992), a part of the energy "lost" by production of dissolved organic carbon becomes available to metazooplankton through the microbial food web.

The relative contribution of direct grazing by metazooplankton (i.e. planktonic metazoans) and of the microbial food web in the utilisation of primary production has been studied in various marine and lacustrine systems (Cole et al. 1988), demonstrating in many cases the quantitative importance of the microbial loop. In large rivers, information concerning the planktonic food web is rather scarce but various elements can be gathered from the literature.

On the one hand, there is evidence that significant trophic relationships can develop within the river plankton, involving primarily consumption of phytoplankton by small-bodied grazers. Declines of potamoplankton biomass have been observed in several large rivers (Admiraal et al. 1994, Köhler 1995, Garnier et al. 1995). These declines may be explained by a decrease of net algal production due to light limitation as turbidity and depth increase, but algal losses through grazing and sedimentation may also occur (Descy et al. 1994, Descy & Gosselain 1994, Gosselain et al. 1994, Reynolds & Descy 1996). However, the extent to which metazooplankton may play a role seems to vary among rivers, depending on opportunities for metazooplankton development. For instance, studies in North American and Canadian rivers have mostly reported that metazooplankton numbers are too low to exert any effective control on phytoplankton (Pace et al. 1992, Basu & Pick 1997a). By contrast, most large eutrophic European rivers have been shown to support high numbers and biomass of small zooplankters, mainly rotifers (Pourriot et al. 1982, Van Zanten & Van Dijck 1994, Marneffe et al. 1996, Lair & Reyes-Marchant 1997, Lair et al. 1998). In those rivers, phytoplankton abundance and increased retention times due to river regulation seem to favour metazooplankton development (Viroux 1997). In a few studies, the role of metazooplankton in carbon transfer within large rivers has been estimated by calculation, mainly by running simulation models (Descy et al. 1987, Admiraal et al. 1993, Billen et al. 1994, Gosselain et al. 1994, Garnier et al. 1995). Measurements of phytoplankton grazing by metazooplankton have been carried out in a few cases (Gosselain et al. 1998a and b, Kobayashi et al. 1996) and have confirmed the trophic role of herbivorous metazooplankton in large river systems, where and when conditions are met for the development of these organisms (i.e. hydrology-related factors, temperature, availability of edible resources).

On the other hand, studies of the bacterioplankton compartment in rivers, aiming to assess the role of bacteria in ecosystem functioning (Edwards & Meyer 1986, Servais 1989, Garnier et al. 1992, Servais & Garnier 1993) have provided some indications about trophic relationships involving bacteria and protozooplankton. Indeed, in some large rivers, grazing by flagellates and ciliates has been shown to be a major loss process of bacterial biomass, like in other aquatic systems (Servais et al. 1989). Accordingly, detailed studies on microzooplankton have demonstrated the existence of large and varied protist communities in large European rivers. In the river Loire, Lair et al. (1998, 1999) have observed abundant (maximum biomass reaching 0.1 mg C L^{-1}) and diverse ciliate communities, mostly composed of small forms. Reports from the Hungarian part of the river Danube mentioned a biomass of heterotrophic nanoflagellates exceeding the biomass of cladocerans by a factor of 10 (V.-Balogh et al. 1994). Other authors reported numbers of heterotrophic flagellates in rivers which correlated with bacterial abundance, but not with zooplankton abundance (Basu & Pick 1997b), and concluded that there presumably was little carbon transfer from the microbial to the planktonic food web.

To date, few attempts have been made to establish a comprehensive carbon budget in rivers. These few studies were based on primary production measurements and on estimates of loss processes (Descy et al. 1987, Admiraal et al. 1993, Descy & Gosselain 1994) or on measurements of "dark carbon losses" (Köhler 1995). In addition, several studies were conducted in estuaries for investigating carbon fluxes and trophic relationships (e.g. Dolan & Gallegos 1991, Sherr et al. 1986).

In this study we aimed to establish a detailed budget of the organic carbon in a productive stretch of a large river, the upper middle river Meuse (Belgium), which receives a limited amount of degradable allochthonous organic matter. We conducted parallel studies of the key communities (phytoplankton, protozooplankton, metazooplankton and bacteria), and of the main processes involved in organic carbon transfer: net primary production, metazooplankton grazing, carbon utilisation by bacteria and grazing of bacteria by protozoa.

Material and methods

Study site and samples collection

The river Meuse (Fig. 1) rises in the East of France and flows through Belgium and The Netherlands, where it meets the lower Rhine, forming the Dutch Delta, which opens into the North Sea. The total length of the river is 885 km and its catchment is about 36,000 km^2, 40 % of it in Belgium. In all its Belgian course the river Meuse has been regulated for navigation, with weirs and locks distributed along its length. The study site, "La Plante", is situated 537 km from the source. At this site, the mean depth is 3.95 m and the mean width is 100 m. Discharge and temperature of the river in 1996 are presented in Fig. 2. The river Meuse has alkaline, nutrient-rich waters. Some variations in the nutrient content occur over an annual cycle, due to inputs from the drainage area (N, Si), from sewage (mostly P) and to uptake by primary producers. However, in this stretch of the river, nutrients are not usually depleted to levels where they may be limiting for phytoplankton growth (Descy et al. 1987). Various environmental parameters were directly measured in the field: temperature, surface irradiance and vertical light attenuation in the water column. Water samples were collected from the surface in the middle of the river with a 3 L opaque Van Dorn bottle. Measurements of activities (primary production, metazooplankton grazing, bacterial production and mortality) were performed on eleven dates on the same river water sample, between May and October 1996, while estimations of abundance and biomass were made more frequently between January and November 1996.

Phytoplankton abundance and biomass

Phytoplankton biomass was estimated through chlorophyll-a (Chl-a) measurements by HPLC analysis (Descy & Métens 1996) and by conversion to carbon biomass using a C/Chl-a ratio of 37 (Descy & Gosselain 1994). The ratio of edible/inedible phytoplankton items was determined by microscopic examination of the phytoplankton samples. After sedimentation of a 1 L Lugol-preserved sample, examination under a standard Leitz Laborlux D microscope (12.5 × 40 magnification) was conducted on subsamples mounted in a Bürker cell. Algal units were counted and their dimensions (± 1 µm) were measured in every sample. Data were computed using modified Hamilton's software (Hamilton 1990) which records numbers and calculates unit biovolumes from recorded dimensions, using the closest simple geometric shape as reference. Conversion to carbon (pg C) was then done by means of the Eppley equations (Smayda 1978). Phytoplankton cells or colonies were considered as inedible when their greatest axial linear dimension (GALD) exceeded 20 µm (Reynolds 1986) and the ratio edible/inedible algal biomass was applied to phytoplankton carbon biomass estimated from Chl-a

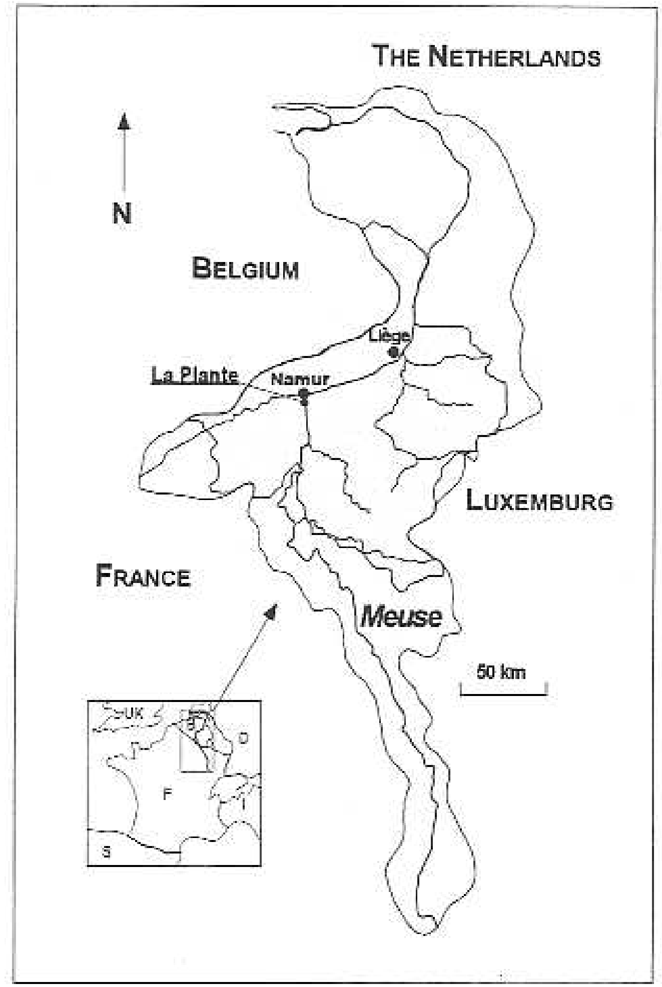


Fig. 1. Map of the studied station (La Plante) on the river Meuse.

This size limit is somewhat arbitrary, as some rotifers have a more specialised diet, including sometimes larger algae. However, the most abundant rotifers in the river plankton (*Brachionus calyciflorus, B. angularis, Keratella cochlearis*) are considered as generalist species (Pourriot 1977, Rothhaupt 1990).

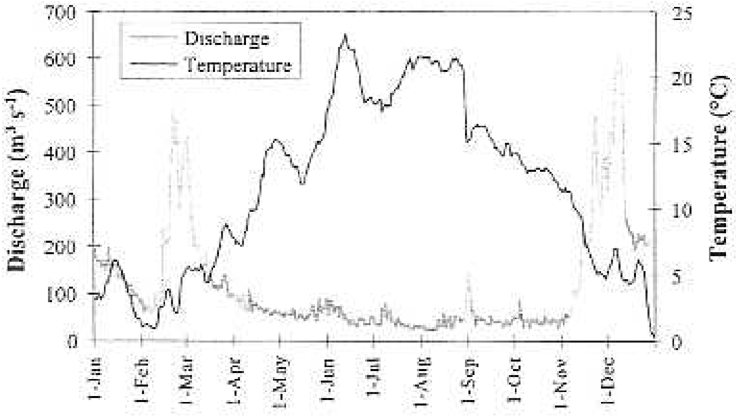


Fig. 2. Discharge and temperature in the river Meuse in 1996 measured at Tailfer (10 km upstream of the studied site, La Plante).

Metazooplankton abundance and biomass

Metazooplankton samples were collected by repeated sampling with the Van Dorn bottle to obtain a minimum of 9 L of water which was sieved through a 37 or 63 µm-mesh Nitex plankton net. Qualitative and quantitative analysis of metazooplankton samples was done using a dissecting microscope at 35× magnification, on samples fixed with acid Lugol after concentration by a factor ranging from 250 to 1000. Rotifers were determined to the genus or species level while crustaceans were counted as either cladocerans or copepods, with no further level of identification. Rotifer carbon biomass was estimated considering 50% carbon in the dry biomass, which was calculated for the individual populations from various literature data for the main rotifer taxa observed in the river Meuse. For crustaceans, the following individual carbon content were used: 1.1 µgC for bosmina, 1.75 µgC for the other cladocerans, 0.125 µgC for the nauplii and 1.87 µgC for the copepodites.

Bacterial abundance and biomass

Bacterial abundance was determined using epifluorescence microscopy at 1000× magnification, following the procedure proposed by Porter & Feig (1980). After fixation with buffered formalin (2.5% final concentration) and DAPI (4,6 diamidino-2-phenylindole) (1 µg mL^{-1}, final concentration) staining, at least 500 cells were counted in each sample. During microscopic observation bacteria were classified among 24 size classes by comparison with an eyepiece graticule and cell volume (V: μm^3 $cell^{-1}$) in each class was calculated. Biomass was estimated from abundance and biovolume distribution using the relationship relating carbon content per cell (C: Fg C $cell^{-1}$) to biovolume ($C = 92 \times V^{0.598}$) which was determined from Simon & Azam's (1989) data.

Protozoa abundance and biomass

The abundance and biomass of protozoa was determined by epifluorescence microscopy after DAPI staining. Twenty mL of glutaraldehyde-preserved (0.5 °C final concentration) water samples were stained with DAPI (10 µg mL^{-1}, final concentration) for 15 min. Stained protists were collected by filtration on a 0.8 µm Nuclepore black filter. Filters were mounted on microscope slides and stored at −20 °C until examination for a maximum of one month prior to observation. Nano-sized (2–20 µm diameter) protists were identified, counted and measured at a magnification of 1000× and 625× while micro-sized (20–200 µm diameter) organisms were analysed at a magnification of 125×. A minimum of 100 organisms per filter were counted. Autotrophic forms were distinguished from heterotrophic ones by the red autofluorescence of Chl-a observed under blue light excitation. To distinguish mixotrophic organisms from strictly heterotrophic or autotrophic organisms, protozoa were incubated with fluorescently labelled bacteria as in the procedure used to estimate bacterial ingestion by protozoa (Servais et al. 1998). The experimental procedure for FLB ingestion is fully described in Servais et al. (1998). Protozoa were considered as mixotrophic when they combined the presence of chloroplasts and ingested fluorescently labelled bacteria. Cell biovolumes were calculated from cell dimensions and shapes. Recommended ratios to convert biovolume to biomass range between 0.08 and 0.22 pg C μm^{-3} according to the taxon and the preservation and staining procedure (Choi & Stoecker 1989) In this study, the conversion factor of 0.19 pgC μm^{-3} suggested by Putt & Stoecker (1989) for estimating the carbon biomass of ciliates was chosen. Biovolumes of flagellates and dinoflagellates were converted to organic carbon using the equation established by Smayda (1978), log B = 0.94 (Log V) − 0.6, in which B is flagellate or dinoflagellate biomass and V flagellate or dinoflagellate biovolume.

Gross and net primary production and estimation of allochthonous inputs

Primary production was measured by a short subsurface incubation (between ½ and 2 h) of river water inoculated with $NaH^{14}CO_3$ (25 µCi L^{-1} of sodium bicarbonate, prepared from Amersham CFA3, 50–60 mCi $mmole^{-1}$); water subsamples were poured into 9 different 100 mL glass bottles exposed each to 0–100% relative irradiance, in a field incubator immersed just beneath the water surface. Absolute irradiance was monitored simultaneously by a data logger. The photosynthesis–light curve was established after measurement of the radioactivity of the algae collected on Millipore HA 0.45 µm filters, and photosynthesis parameters were obtained by fitting the data to Smith's (1936) equation. Subsequent calculations of daily gross primary production (GPP) were performed as in Descy et al. (1987). Algal respiration, calculated according to Descy et al. (1994), was subtracted from daily GPP to obtain daily net primary production (NPP). Phytoplankton growth rates were calculated as in Harris (1986), from the ratio NPP ($g\,C\,m^{-2}\,d^{-1}$)/ algal biomass ($g\,C\,m^{-2}$).

As direct measurement of inputs of degradable carbon to the river were not available, allochthonous organic matter inputs were obtained by running a non-stationary version of the PEGASE model (Smitz et al. 1997). The model takes into account inputs of industrial, agricultural and urban waste water, as well as inputs from tributaries and from soil leaching in the watershed. To estimate the allochthonous carbon flux upstream of our sampling station, we performed a calculation for a 45 km stretch, from March to October. The simulation gave a total input of degradable organic matter (particulate and dissolved), which was averaged over the surface area of the river stretch. This average value was considered as a realistic estimate of the organic matter loading for the studied reach of the river.

Metazooplankton grazing and assimilation yield

In situ grazing measurements were carried out using a 6.5 L grazing chamber designed following Gawler & Chapuis (1987). Cultures of *Dictyosphaerium ehrenbergianum* Näg. (grown as unicells of 4.4 µm mean diameter and labelled at least for 24 h by adding 1 µCi mL^{-1} of $NaH^{14}CO_3$, prepared from Amersham CFA3, 50–60 mCi mmole^{-1}, in the culture medium) were used as tracer food; suspensions of ^{14}C-labelled algae (maximum 10 % of the phytoplankton biomass trapped in the grazing chamber) were added to the natural sample. The carbon content of *Dictyosphaerium ehrenbergianum* was measured using a NA 1500 Carlo Erba nitrogen–carbon analyser while that of natural phytoplankton was calculated on the basis of a carbon/Chl-a ratio of 37 (Descy & Gosselain 1994). Incubations in the river lasted between 6 and 14 min (time shorter than the known gut passage time for most zooplankters: Downing & Rigler 1984), from the closure of the grazing chamber to the end of sieving of the chamber content through a 37 µm mesh (or rarely a 63 µm mesh) Nitex screen. The animals were then narcotised in soda water. Before freezing in liquid nitrogen, they were either collected on 25 mm Whatmann GF/C filters (samples for total community measurements) or suspended in a small amount of water (samples for subsequent sorting). A small volume of the water collected from the sieving through the Nitex screen was preserved for determining the radioactivity of the algal suspension. Zooplankton samples collected on GF/C filters were treated for the measurement of radioactivity by dissolving animals in 1 mL Lumasolve (Lumac LSC). For all series of measurements, five replicates were run through the entire procedure. The radioactivity was measured with a LS 600 Beckman scintillation counter. Background corrections were made by running control in situ incubations with the grazing chamber without addition of labelled algae. Community filtration rate was calculated according to Haney (1971) and was expressed in % d^{-1}, i.e. the percentage of water volume filtered per day. Rates of phytoplankton loss or zooplankton ingestion rate were calculated by multiplying the daily filtration rate by the algal biomass in the river water, corrected for the proportion of edible phytoplankton.

It is to be noted that on the 10th of June and the 12th and 26th of August, too many labelled algae were injected in the Haney chamber, so that the amount of algal carbon added was much higher than the admissible limit of 10 % of the edible algal biomass. In consequence, these radioactive algae did not act as a tracer but more probably fed the zooplankton community. Furthermore, because of the addition of these amounts of labelled algae, the algal biomass may have exceeded the ILL (incipient limiting level), which was not reached at this time in the river. Therefore, it may have reduced the filtration activity of the zooplankton. In these conditions, it is very likely that community filtration rate calculated from zooplankton numbers (see below) was closer to the actual grazing rate (see Gosselain 1998). For the three dates mentioned above, these estimates were used, instead of measured grazing, to establish the carbon budget of the river (see Gosselain et al. 1998b and Gosselain 1998 for measured values and details of discussion); that is the reason why no error bars were associated to these values in Fig. 6.

The amount of carbon assimilated by zooplankters, i.e. the amount of carbon that was absorbed through the gut lining, was obtained when multiplying the carbon ingested by the assimilation yield. The assimilated material can either contribute to tissue or egg production or to respiration. A community assimilation yield was calculated for each sample by weighting the specific assimilation yield of the most abundant taxa according to their grazing rates using the following equation:

$$Ac = \frac{\sum_{i=1}^{n} A_i G_i}{\sum_{i=1}^{n} G_i}$$

where Ac = Community assimilation yield
A_i = Assimilation yield of taxon i
G_i = Grazing rate of taxon i (% d^{-1})

Assimilation yields of the main taxa were determined on the basis of laboratory measurements on the rotifer *Brachionus calyciflorus* (Joaquim-Justo & Thomé, unpubl.) and from data collected in the literature (Leimeroth 1980, Lair & Oulad Ali 1990, Lair 1991 and 1992; Table 1). The assimilation yields used for calculation were

Table 1. Specific assimilation yields of metazooplankton.

Species	Food	Specific assimilation yield (%)	Reference
Brachionus calyciflorus	*Kirchneriella lunaris*:		Leimeroth (1980)
	9.2 mg DW L^{-1}	49	
	23 mg DW L^{-1}	33	
	115 mg DW L^{-1}	20	
	Dictyosphaerium ehrenbergianum:		Joaquim-Justo & Thomé
	4.8 mg DW L^{-1}	64	(pers. comm.)
	8 mg DW L^{-1}	60	
Keratella cochlearis	Natural plankton	6–32	Lair & Oulad Ali (1990)
Keratella quadrata	(eutrophic lake, Aydat, France)	3–30	
Bosmina longirostris	Natural plankton (eutrophic lake, Aydat, France)	2–50	Lair (1991)
Cyclops vicinus (copepodites and adults)	Natural plankton (eutrophic lake, Aydat, France)	2–44	Lair (1992)

those measured with food concentrations comparable to the edible phytoplankton biomass present in each sample. For specific assimilation yields of *Keratella* spp., *Bosmina longirostris* and copepods only the maximum values measured by LAIR et al. (1998) were retained, since minimum values were obtained during episodes of dominance by cyanobacteria which may have reduced zooplankton grazing rates.

Specific grazing rates of the main taxa were calculated from specific ingestion rate parameters, metazooplankton abundances, edible algal biomass, and temperature. Ingestion rate parameters of a rectilinear ingestion equation were obtained from in situ measurements, normalised to 20 °C, for the most abundant taxa (i.e. *Brachionus* spp. [mainly *B. calyciflorus*], *Keratella* spp. [mainly *K. cochlearis*], and *Bosmina longirostris*). For less abundant taxa (Synchaetidae, nauplii, copepods and crustaceans other than bosminids) these parameters were selected according to the values found in the literature. Details of the calculation can be found in GOSSELAIN (1998).

Bacterial production and bacterial carbon demand

Bacterial production was estimated from both tritiated thymidine (FUHRMAN & AZAM 1982) and leucine incorporation rates (KIRCHMAN et al. 1985).

Ten mL samples were incubated with ^{3}H-thymidine (Amersham, 45 Ci mmole^{-1}) for one hour in the dark at field temperature, at a saturating concentration of 20 nM (SERVAIS 1989). After incubation, cold trichloracetic acid (TCA) was added (final concentration 5 %) and the samples were filtered through a 0.2 µm pore size cellulose nitrate membrane. Radioactivity associated with the filters was estimated by liquid scintillation. Cell production was calculated from the thymidine incorporation rate using the conversion factor 0.5×10^{18} cells produced per mole of thymidine incorporated into DNA, experimentally determined by SERVAIS (1989) for the river Meuse. Cellular production was multiplied by the average carbon content per bacterial cell in order to get the bacterial production based on thymidine incorporation (BPT) expressed in µg C L^{-1}h^{-1}.

Incorporation of ^{3}H-leucine (Amersham 165 Ci mmole^{-1}) was measured at six leucine concentrations which ranged from 2 to 77 nM (2 nM of tritiated leucine in each case with 0–150 nM non-radioactive leucine). Six 5-ml samples were incubated with the different concentrations of leucine for 40 min to 1 hour in the dark at field temperature. After incubation, TCA was added (final concentration 5 %) and the samples were filtered through a 0.2 µm pore size cellulose nitrate membrane. Radioactivity associated with the filters was estimated by liquid scintillation. The maximum incorporation rates were estimated by fitting a hyperbolic function to the experimental data, using a software based on the least squares criterion (SERVAIS 1995). Maximum incorporation rate into TCA insoluble material was multiplied by 0.93 to obtain the maximum incorporation into protein; this factor was established experimentally for river Meuse samples by SERVAIS (1995). Bacterial production estimated from leucine incorporation (BPL) was calculated by multiplying the maximum incorporation rate into proteins by the conversion factor of 1100 g C mole^{-1}, determined experimentally (SERVAIS 1995).

Bacterial carbon demand (BCD) was calculated by dividing bacterial production by the growth yield of 0.3 obtained from batch experiments performed with river Meuse water (SERVAIS 1989).

Bacterial mortality and grazing by protozoa

Measurement of bacterial mortality and contribution of grazing in the mortality process was measured using the method based on the disappearance of the radioactivity from the genetic material of bacteria previously labelled with ^{3}H-thymidine (SERVAIS et al. 1985, 1989). A 200 mL water sample was inoculated with (methyl-^{3}H)-thymidine (Amersham, 40–50 Ci mmole^{-1}) at a concentration of 4 nM and incubated in the dark at field temperature for 20 hours. This incubation period was sufficient for thymidine depletion from the medium. Then the sample was divided into two subsamples, one of the subsamples was left unmodified while the other was filtered through a 2 µm pore-size filter (Nuclepore membrane) to retain most of the eukaryotic micro-organisms. A mixture of cycloheximide–colchicine at respective concentrations of 200 and 100 mg/L was added to the filtered subsample. This mixture has been reported as an efficient inhibitor of protozoan reproduction and feeding while having no direct effect on bacterial growth (SHERR et al. 1986). The radioactivity was measured twice a day for 2–3 days on 5 mL aliquots from both subsamples. TCA (final concentration 5 %) was added to the 5 mL which were then filtered on a 0.2 µm pore size cellulose nitrate membrane and the radioactivity associated with the filter was measured by liquid scintillation. A linear decrease of the radioactivity in both subsamples was usually observed on semi-log plots of radioactivity versus time. The slope of this decrease in the unmodified subsample gave the first order rate of overall mortality (k_t) expressed in h^{-1}; the slope of radioactivity versus time in the treated subsample allowed the calculation of the mortality rate not attributable to grazing. The difference between the two rates gave the mortality rate resulting from grazing (k_g).

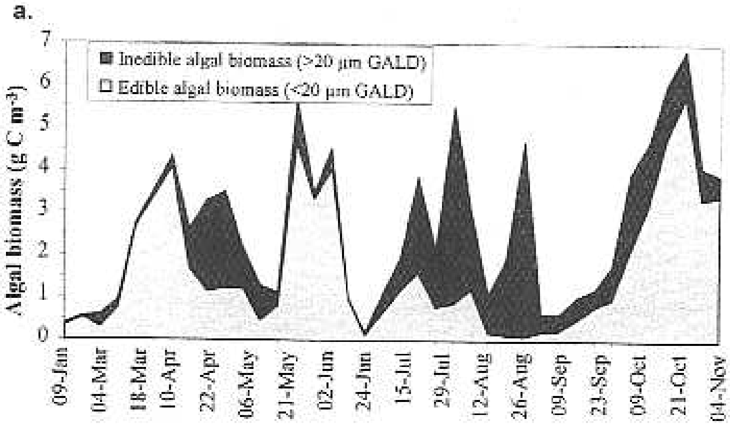
Fluxes of bacterial mortality and of grazing were obtained by multiplying the first order rates by bacterial biomass.

Results and discussion

Seasonal biomass fluctuations of plankton organism

Phytoplankton

The details of phytoplankton dynamics in the river Meuse in 1996 have been published elsewhere (GOSSELAIN et al. 1998 b); given the focus of the present paper on the carbon budget, we present here only a general account on the changes in the algal community. As usually observed (DESCY et al 1987, GOSSELAIN et al. 1994), phytoplankton of the upper middle Belgian river Meuse was dominated by diatoms and green algae, which represented, on average over the 1996 growing season, 34 % and 62 % of total algal numbers, respectively. In terms of total algal biomass, diatoms represented on average 79 %



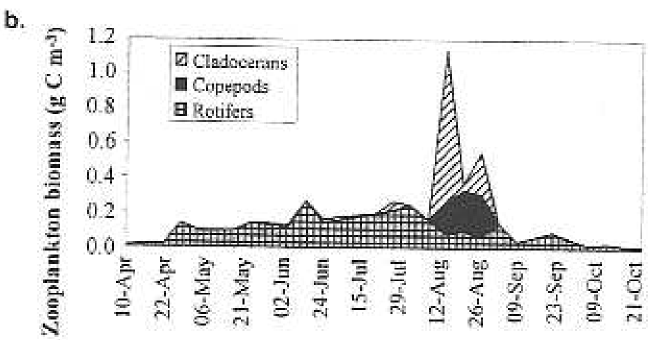
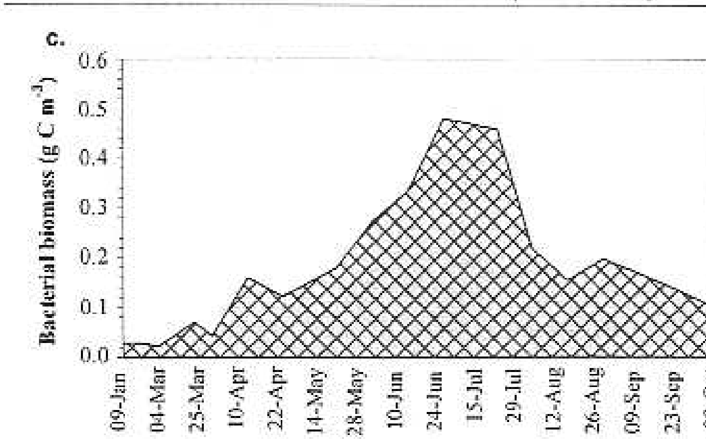


Fig. 3 a & b.

and green algae 12 %. Other algal groups were Cryptophyceae, Cyanobacteria and Dinophyceae; Euglenophyceae and Chrysophyceae were rarely present. Small centric diatoms, mostly *Stephanodiscus hantzschii* Grun., developed a first bloom in early March, as a result of favourable light conditions associated with low winter flow. Other algal groups appeared when temperature increased and light conditions improved. After a drastic biomass decline occurring in mid-June, non-siliceous algae developed more significant communities in summer. Small centric diatoms again represented the main part of the phytoplankton biomass in autumn. The maximum algal biomass observed was



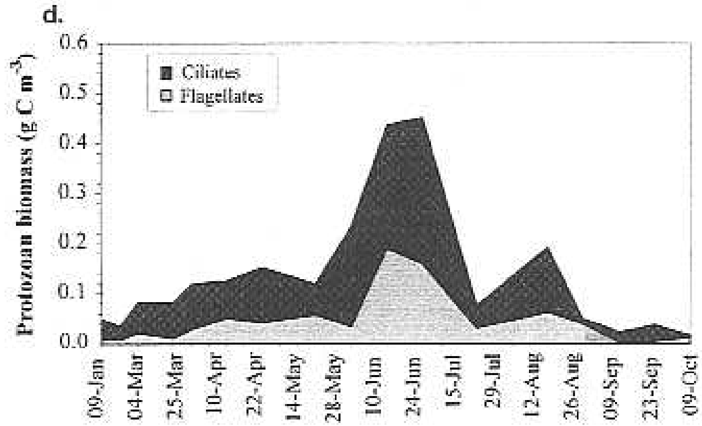


Fig. 3. Biomass of phytoplankton (**a**), metazooplankton (**b**), bacterioplankton (**c**) and protozooplankton (**d**) in the river Meuse at La Plante in 1996. GALD = greater axial linear dimension.

about 6 $g\,C\,m^{-3}$ (more than 160 mg Chl-a m^{-3}; Fig. 3 a). In Fig. 3 a, total algal biomass is divided in two broad size categories, the smaller algae (< 20 µm GALD) being considered as edible by small zooplankters. Inedible algae were mostly composed of filamentous *Aulacoseira* species and of large unicellular centric diatoms. Edible cells comprised small centric diatoms (various taxa of *Stephanodiscus, Cyclotella, Thalassiosira*,...), green algae and Cryptophyceae. Most of the time, the edible biomass was at levels which can be considered as saturating for zooplankton grazing, i.e. between 0.5 and 1.5 mg C L^{-1} for the dominant zooplankton taxa encountered in this study (Rothhaupt 1990, Gosselain et al. 1996). A notable exception is the late summer period, where a low biomass of edible algae, ranging between 0.1 and 0.2 g C m^{-3}, was maintained for several weeks.

Metazooplankton

Metazooplankton presented abundances higher than 1000 ind L^{-1} by the end of April and was mainly composed of rotifers with *Brachionus* spp., *Keratella* spp. and Synchaetidae as dominant taxa. Microcrustaceans developed significant numbers throughout August, with a peak of 800 *Bosmina longirostris* per litre recorded on the 12th, along with adult copepods and nauplii. Maximum metazooplankton biomass (up to 1.1 g C m^{-3}) was observed during this period. In August, cladocerans and copepods constituted the major part of metazooplankton biomass while during the other months, rotifers dominated largely with biomass up to 0.27 g C m^{-3} (Fig. 3 b). Metazooplankton dynamics followed a pattern similar to that previously reported for the river Meuse as well as for other European rivers. Nevertheless, rotifer densities greatly exceeded values previously reported for the river Meuse (Viroux 1997), as well as those usually reported for river systems (Pace et al. 1992).

Bacteria

During the study, bacterial biomass ranged between 0.02 and 0.5 g C m^{-3} (Fig. 3 c). These values are in the same range as those reported previously for an annual cycle in two different stations in the river Meuse by Servais (1989). Seasonal fluctuations showed minimum values in winter and maximum values in the period from end of June to mid-July. The maximum biomass appeared several weeks later than the second and higher spring peak of phytoplankton biomass. The first peak of phytoplankton in spring did not induce a large increase in bacterial biomass probably because of lower temperatures. A lag between bacterial and phytoplankton maximum biomass has already been observed in the river (Servais 1989) as well as in other aquatic ecosystems such as lakes (Garnier & Benest 1991) and marine waters (Billen & Becquevort 1991). This suggests a coupling during the spring period between phytoplanton and heterotrophic bacteria in this large eutrophic river.

Protozooplankton

Seasonal fluctuations of protozooplankton biomass are reported in Fig. 3 d. The taxonomic groups identified were ciliates and flagellates including dinoflagellates and naked nanoflagellates. Protozooplankton biomass was characterised by low values during winter (around 0.05 g C m^{-3}) and maximum values between mid-June and end of June (about 0.45 g C m^{-3}). Ciliates largely dominated total protozoan biomass and accounted for 21 to 89 % (average 67 %) of it. They represented around 61 % of protozoan biomass at its maximum. Flagellates represented between 11 and 79 % (average 33 %) of total protozoan biomass and dominated the protozoan community at two sampling dates (26 August and 9 October). The maximum of protozoan biomass co-occurred with maximum of bacterial biomass. Protozoans tend to be the major bacterivores in marine systems (Sherr & Sherr 1988 and references therein) and lakes (Sanders et al. 1989) and by analogy, are assumed to be responsible for the control of bacterial numbers in freshwaters (Carlough & Meyer 1990, Basu & Pick 1997 b), although the supply of organic matter may have a prominent role in determining bacterial production (bottom-up control) (Billen et al. 1990). In freshwaters, rotifers and cladocerans may also be significant consumers of bacteria (Wylie & Currie 1991, Vaqué et al. 1992).

The protozooplankton community was composed of mixotrophic and strictly heterotrophic organisms (Fig. 4). Phagotrophic mixotrophs, i.e. combining autotrophic and heterotrophic nutrition modes (Gaines & Elbrächter 1987, Sanders 1991, Jones 1994), were recorded during the whole period of investigation. Mixotroph, defined experimentally as chloroplast-bearing protozoa with FLB within food vacuoles, accounted for 12 to 80 % (average 54 %) of total protozooplankton biomass. At the period of maximum development of protozooplankton, mixotrophs represented 78 % of total phagotrophic protozooplankton biomass. Recent research has demonstrated the widespread oc-

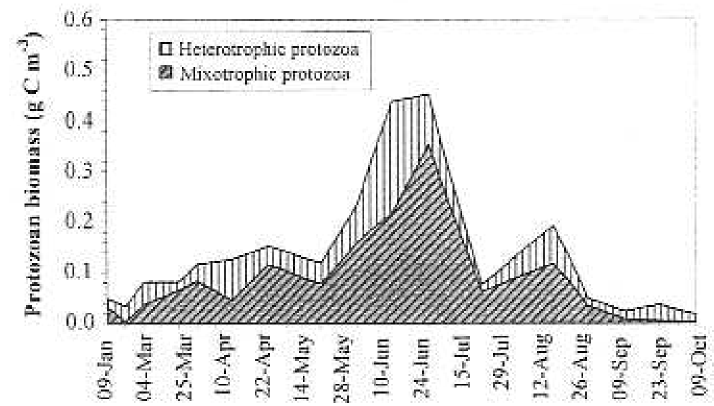


Fig. 4. Biomass of heterotrophic and mixotrophic protozooplankton at La Plante in 1996.

currence of mixotrophic protozoa in both freshwater and marine plankton (JONES 1994). It is quite possible that part of the mixotrophic flagellates were also counted as phytoplankton cells, most probably among Cryptophyceae or Dinophyceae. However the contribution of mixotrophic flagellates to phytoplankton biomass was low (maximum 8 % of phytoplankton biomass and 1.8 % in average) during our study.

Carbon sources

Net primary production

For the sampling dates studied in 1996, daily gross primary production (GPP) varied between 1.1 and 6.7 g C m^{-2} d^{-1} and net primary production (NPP) between 0.2 and 5.3 g C m^{-2} d^{-1} (Fig. 5). Growth rates in the range 0.03 to 0.85 d^{-1} were calculated from the values of net primary production and biomass.

Allochthonous input

A part of the carbon available in the river for heterotrophic organisms is due to the input of biodegradable organic carbon of allochthonous origin. This input was estimated at 0.375 g C m^{-2} d^{-1} for the river Meuse upstream from our sampling station. For all the eleven studied situations except one (23[th] September), allochthonous inputs were lower than the autochthonous organic matter from

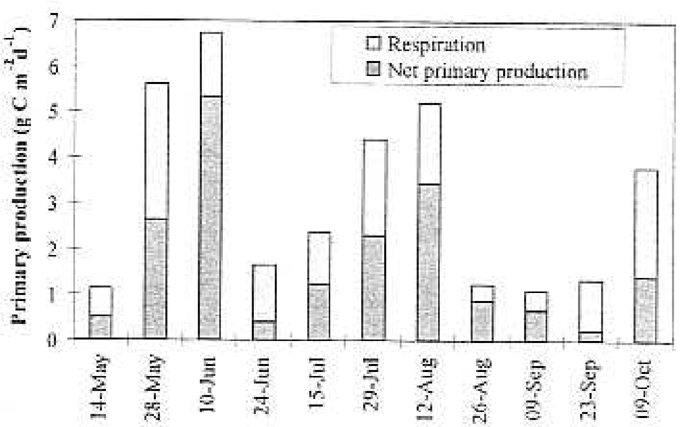


Fig. 5. Fluctuations of gross primary production divided in net primary production and phytoplankton respiration in the river Meuse at La Plante in 1996.

net primary production (cf. Fig. 9). This clearly results from river eutrophication combined with good opportunities for phytoplankton development in the upstream stretches of the river.

Carbon consumption

Metazooplankton grazing

A detailed account of the variations of community filtration rates (CFR) can be found in GOSSELAIN et al. (1998 b). In 1996, due to the large development of zooplankton in the river Meuse, the grazing pressure exerted on algae by the dense rotifer community reached high values several times, particularly from May through July. The planktonic grazers filtered between 2 and 113 % of the water daily: the estimated contribution of the dominant taxa is presented in Table 2. In August, the contribution of the cladoceran *Bosmina longirostris* to community grazing may have been significant, and it is probably better rendered by the calculated CFR than by the measured grazing rates, due to technical problems (see Material and methods section). As observed in previous years (GOSSELAIN et al. 1998 b), a shift to dominance by large inedible units in the phytoplankton (see Fig. 3 a) followed episodes of intense grazing. The estimated grazing losses in 1996 ranged between 0.04 and 5.40 g C m^{-2} d^{-1} (Fig. 6). The assimilation yields of the zooplankton community and the values used for their calculation are presented in Table 2; they varied between 33 and 52 % with a mean of 44 ± 6 %. Taking into account these values, algal carbon assim-

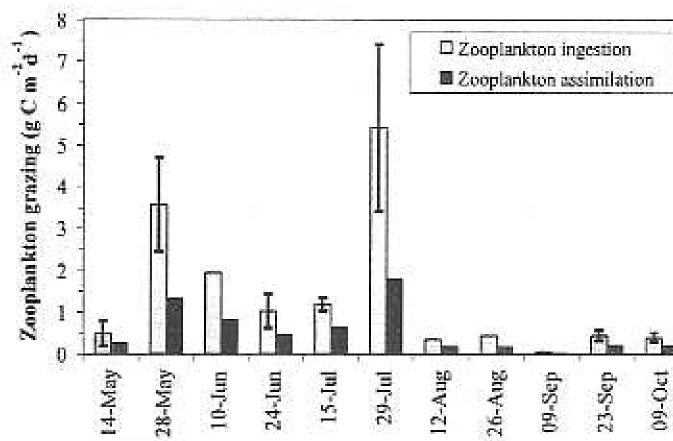


Fig. 6. Fluctuations of metazooplankton ingestion and metazooplankton assimilation taking into account the assimilation yield in the river Meuse at La Plante in 1996.

Table 2. Community assimilation yields of the dominant species of metazooplankton for each sampling date and data used for the estimation of the community assimilation yields.

Sampling date	Taxa	G (% d^{-1})	Edible biomass (mg DW L^{-1})	Specific assimilation yields (%)	Community assimilation yields (%)
14-05-96	*Brachionus calyciflorus*	8.96	1.62	64	51
	Keratella cochlearis	6.4		32	
28-05-96	*Brachionus calyciflorus*	3.43	6.7	60	37
	Keratella cochlearis	16.77		32	
10-06-96	*Brachionus calyciflorus*	16.33	1.78	64	42
	Keratella cochlearis	36.24		32	
	Bosmina longirostris	0.62		50	
24-06-96	*Brachionus calyciflorus*	11.55	2.34	64	42
	Keratella cochlearis	26.51		32	
	Bosmina longirostris	0.56		50	
15-07-96	*Brachionus calyciflorus*	24.2	1.6	64	52
	Keratella cochlearis	14.63		32	
29-07-96	*Brachionus calyciflorus*	3.13	2.42	64	33
	Keratella cochlearis	86.91		32	
12-08-96	*Brachionus calyciflorus*	6.68	0.28	64	47
	Keratella cochlearis	10.34		32	
	Bosmina longirostris	29.82		50	
	Copepods	10.5		44	
26-08-96	*Brachionus calyciflorus*	1.18	0.02	64	39
	Keratella cochlearis	22.81		32	
	Bosmina longirostris	7.54		50	
	Copepods	13.19		44	
9-09-96	*Brachionus calyciflorus*	3.52	1.02	64	48
	Keratella cochlearis	3.66		32	
23-09-96	*Brachionus calyciflorus*	7.18	1.96	64	45
	Keratella cochlearis	10.26		32	
	Copepods	0.07		44	
9-10-96	*Brachionus calyciflorus*	0.51	6.36	62	44
	Keratella cochlearis	0.77		32	
Average					44±6

ilation by zooplankton was in the range of 0.02 to 1.78 g C $m^{-2}d^{-1}$. The difference between algal carbon ingested and carbon assimilated by zooplankton was considered as available to bacteria.

Bacterial carbon demand

Two methods based on labelled substrate incorporation were used to estimate bacterial production in the present study. The ^{3}H-thymidine incorporation method (FUHRMAN & AZAM 1982) is based on the proportionality between DNA synthesis and the rate of bacterial division; it has been the most widely used method to estimate bacterial production in various types of aquatic environments: oceans (DUCKLOW & CARLSON 1992), lakes (RIEMANN et al. 1982) and rivers (SERVAIS & GARNIER 1993). The second technique, ^{3}H-leucine incorporation into bacterial proteins, allows the estimation of the protein synthesis rate (KIRCHMAN et al. 1985). Theoretically, both methods reflect two basic processes within a bacterial cell: thymidine incorporation is linked to an increase in cell number while leucine incorporation measures an increase in bacterial biomass. Fig. 7a shows that results from both methods were well correlated in our study ($r^2 = 0.84$; n = 15; $p<0.0001$). The average ratio between bacterial production calculated from leucine incorporation (BPL) and from thymidine incorporation (BPT) was not significantly different from 1 (average BPL/BPT = 0.91 ± 0.24), showing a good agreement between both estimates of bacterial production. Several studies have already compared bacterial production calculated from thymidine and from leucine incorporation rates and found satisfying agreement between the two estimations (SIMON & AZAM 1989, SERVAIS 1992), but some authors have mentioned that modifications of environmental conditions (temperature, nutrient level, bacterial growth rate) may induce large fluctuations between both methods as a result of unbalanced bacterial growth (CHIN-LEO & KIRCHMAN 1988).

Fig. 7b presents the fluctuations of bacterial production (average of estimates based on leucine and thymidine incorporation) expressed in g C $m^{-2}d^{-1}$ in the river Meuse in 1996. Production was low during the first part of the year and reached a maximum at mid-June (0.67 g C $m^{-2}d^{-1}$). During the summer period, bacterial production ranged between 0.33 and 0.42 g C $m^{-2}d^{-1}$ and decreased from mid-September. A quite similar pattern and range of bacterial production data were reported by SERVAIS (1989) for a station in the river Meuse located about 30 kilometres upstream of our sampling site. Bacterial carbon demand (BCD) calculated from these production values ranged between 0.30 and 2.24 g C $m^{-2}d^{-1}$.

Fate of bacterial biomass

First order mortality rates ranged between 0.002 h^{-1} and 0.022 h^{-1} in the river Meuse (Table 3). These values are in the range usually reported in the literature for natural aquatic systems (SERVAIS et al. 1992). Mortality rates approached growth rates of bacteria at our sampling station, indicating a balance between growth and disappearance of bacteria in the studied reach of the river. Grazing represented on average 71 % (range 40 % to 93 %) of total bacterial mortality rates. A part of the bacterial mortality not due to grazing probably resulted from viral lysis, as it has been shown viruses can be sometimes as ef-

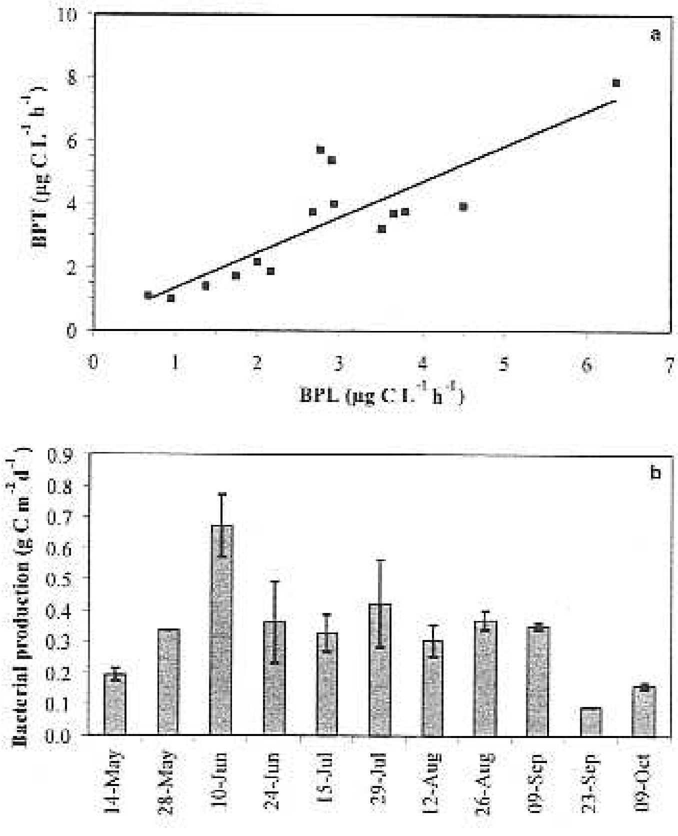


Fig. 7. (a) Relationship between bacterial production calculated from thymidine incorporation rate (BPT) and bacterial production calculated from leucine incorporation rate (BPL). Regression: BPT = 1.12 BPL + 0.21 (r^2 = 0.85; n = 15; p<0.0001). (b) Bacterial production (average of BPT and BPL) in the river Meuse at La Plante in 1996.

ficient as protozoa to control bacterial biomass in aquatic systems (Fuhrman & Noble 1995).

The fluctuations of the fluxes of total bacterial mortality and mortality due to grazing are presented in Fig. 8. A good correlation was found between grazing fluxes estimated as described in this paper and data obtained by the fluorescently labelled bacteria (FLB) method on the same samples (Servais et al. 1998). Using the FLB method, Servais et al. (1998) have shown that protozoa

Table 3. Total mortality rates of bacteria (k_t) and mortality rates due to grazing (k_g) for each sampling date.

Sampling date	k_t (h^{-1})	k_g (h^{-1})	k_g/k_t
14-05-96	0.0086	0.0070	0.81
28-05-96	0.0092	0.0086	0.93
10-06-96	0.0109	0.0084	0.77
24-06-96	0.0114	0.0080	0.70
15-07-96	0.0135	0.0091	0.67
29-07-96	n. d.	n. d.	n. d.
12-08-96	0.0182	0.0110	0.60
26-08-96	0.0220	0.0158	0.72
09-09-96	0.0148	0.0104	0.70
23-09-96	0.0132	0.0105	0.80
09-10-96	0.0128	0.0052	0.41
Average			0.71±0.14

n. d. = not determined.

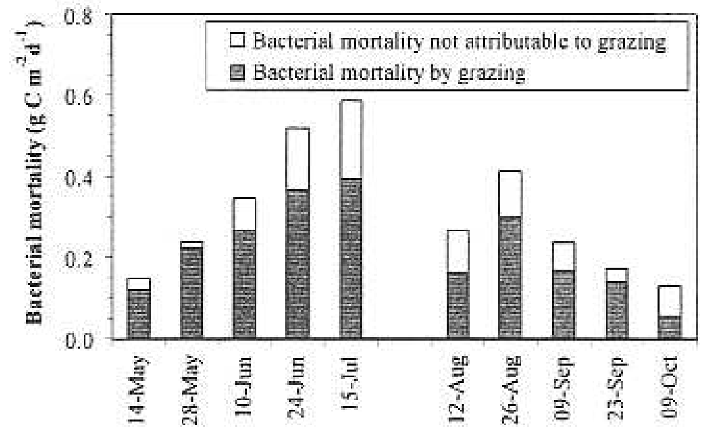


Fig. 8. Fluctuations of fluxes of total bacterial mortality and mortality due to grazing in the river Meuse at La Plante 1996.

(flagellates and ciliates) were the main grazers of bacteria and that the contribution of mixotrophic protozoa was significant (between 10 and 40 % of bacterial grazing). Bacterial mortality increased during the course of the year to reach a maximum in July and then decreased. This kind of seasonal fluctuation with a summer maximum has also been described for other aquatic ecosystems (Servais et al. 1989, 1992).

Carbon budget

The main objective of this research was to establish a carbon budget through the study of the planktonic food web in the river Meuse. Fig. 9 presents the comparison of carbon sources (net primary production and allochthonous input) and carbon consumption (assimilation by metazooplankton and bacterial carbon demand) at La Plante for the eleven sampling campaigns during which the different activities were measured. As already mentioned, the studied stretch of the river was dominated by autochthonous carbon production: on average for the eleven studied dates, NPP (1.73 g C m^{-2} d^{-1}) was 4.6 times higher than the estimated allochthonous input of biodegradable organic carbon (0.38 g C m^{-2} d^{-1}). However, large variations of the ratio NPP/allochthonous input were observed during the studied period depending on the intensity of NPP; from 0.22 when NPP was the lowest (23th September) to 14.2 when NPP reached its maximum (10th June). Concerning carbon consumption, in most cases (9 out of 11) bacterial carbon demand exceeded algal assimilation by metazooplankton. On average, BCD (1.09 g C m^{-2} d^{-1}) was twice as high as algal carbon assimilation by metazooplankton (0.55 g C m^{-2} d^{-1}). The ratio BCD/metazooplankton assimilation presented large fluctuations from 0.78 to a value exceptionally high (58.5) when there was almost no assimilation of al-

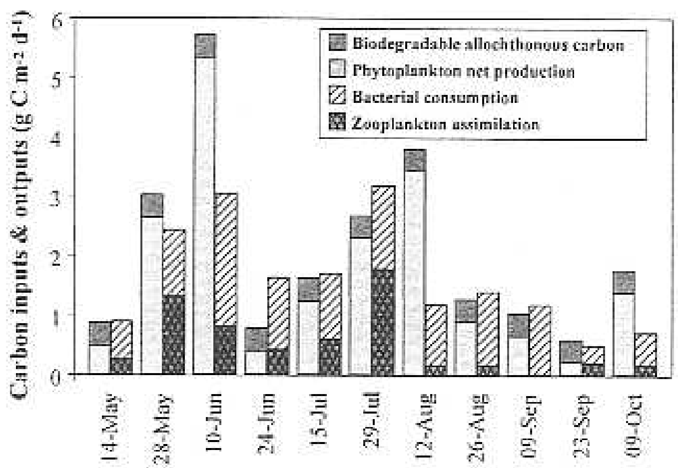


Fig. 9. Carbon budget for the eleven studied situations in the river Meuse at La Plante in 1996. Comparison of carbon inputs (net primary production and allochthonous input) and carbon consumption (metazooplankton assimilation and bacterial carbon demand).

gae by metazooplankton. The large contribution of bacteria to carbon utilisation indicates the potential importance of the microbial loop in energy transfer to higher trophic levels. The part of the carbon consumed by protozoa was estimated at 0.23 g C m^{-2} d^{-1} on average when considering that 71 % of bacterial production was grazed by protozoa (mean of our measurements). A part of this carbon can reach the metazooplankton compartment through grazing of protozoa.

If we compare the carbon sources and losses, the ratio input/consumption ranged between 3.23 and 0.48. On five occasions, it was higher than 1, indicating an export of organic matter from the studied stretch to the river downstream. In the other six situations, carbon consumption exceeded the inputs, indicating, most probably, utilisation of organic matter produced upstream in the river. The mean input/consumption ratio for the studied situations was 1.29. This value indicates a globally consistent carbon budget. Overall, for the studied period, we observed a balance between input and losses of organic carbon, with probably some export of organic matter to the downstream part of the river during periods of high primary production.

Conclusions

This study of organic carbon transfer within river plankton has focused on phytoplankton and bacterioplankton production and losses, in order to explore the possible trophic link between these two compartments.

On the one hand, herbivore consumption of particulate primary production has been found to be very effective, provided that the conditions are met for large developments of the small-bodied grazers (i.e. mostly rotifers and sometimes small cladocerans), the major components of river metazooplankton. According to our data, as much as 100 % of the phytoplankton production on one occasion (24th June) and 32 % on average for the situations studied can be transferred through metazooplankton assimilation to higher trophic levels during the growing season in the river Meuse. This is in agreement with estimates in earlier work (Descy et al. 1987). On the other hand, most of the time in the studied stretch of the river Meuse, the carbon consumed by heterotrophic bacteria exceeded the assimilation by metazooplankton showing the importance of the microbial loop in carbon utilisation. As carbon is mainly from autochthonous origin this indicates that algal excretion and mortality followed by lysis are major carbon sources for heterotrophic bacteria in large river systems, as already suggested from other studies (Servais 1989, Servais & Garnier 1993) and thus that NPP is a main factor controlling bacterial activity.

In addition, we found that the fate of bacterioplankton was heavily dependent on protozooplankton grazing. This means that part of the algal production resulted indirectly in protozoan production. Then, the existence of an effective microbial food web in the river Meuse depends on whether or not rotifers and small cladocerans feed on protozoans and/or on bacteria. The literature contains ample evidence about the ability of "herbivorous" metazooplankton to feed on heterotrophs (WYLIE & CURRIE 1991, VAQUÉ et al. 1992). More particularly, effective feeding on heterotrophic nanoflagellates has been demonstrated for filter-feeders (rotifers and cladocerans, among which *Bosmina*), which may be abundant in lowland rivers, and for some calanoid copepods (JÜRGENS et al. 1996) The question that remains to be answered is basically: do they consume heterotrophs when autotrophs are abundant? Existing studies for estuaries (e.g. HOLST et al. 1998) indicate that a rotifer community may be able to switch from organic and heterotrophic resources to autotrophic plankton, with changes within the metazooplankton community related to the contrasting food sources. In large rivers, more research on plankton food webs is needed to answer this question, with detailed studies on metazooplankton feeding on different resources.

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