RESEARCH ARTICLE

Fate of heterotrophic bacteria in Lake Tanganyika (East Africa)

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Abstract

Bacterial mortality was studied using two complementary methods between 2002 and 2004 in the two main basins (north and south) of Lake Tanganyika. The disappearance of radioactivity from the DNA of natural assemblages of bacteria previously labeled with tritiated thymidine was used to estimate the mortality due to grazing by predators (72%) and due to the cell lysis (28%). Measurements of ingestion rate of bacteria by protozoa using fluorescent micro-particles yielded protozoan grazing rates similar to those provided by the thymidine method, and showed that heterotrophic nano-flagellates were responsible for most of the grazing pressure on the bacterial community of the pelagic zone (92–99%). Bacterial cell lysis was the second process involved in bacterial mortality, ranking before ciliate grazing. Overall, bacterial mortality was balanced with bacterial production. With regard to the assessment of the trophic role of bacteria, it was estimated that *c.* 5–8% of the organic carbon taken up by bacteria was converted into protozoan biomass and was thus available for metazoans.

Introduction

In Lake Tanganyika as in other oligotrophic lakes, bacterial biomass is the less variable component of the plankton compartments (del Giorgio *et al.*, 1996; Pirlot *et al.*, 2005, 2006). Large variations in phytoplankton biomass and production are accompanied by only small fluctuations in the bacterial compartment (Ducklow & Carlson, 1992; del Giorgio & Gasol, 1995; Pirlot, 2006). This presumably results from a tight regulation of growth and mortality of bacteria (Wright, 1988; Sanders *et al.*, 1992). Two factors mainly control bacterial communities in aquatic systems: availability of resources (bottom-up control) and predation by bacterial consumers (top-down control) and other mortality factors as e.g., viral lysis.

Lake Tanganyika is meromictic and permanently stratified, but the depth of the upper mixed layer varies at various spatial and temporal scales. Particularly, during the dry season (May–September), deeper vertical mixing occurs in the oxic layer and an upwelling takes place at the southern end (Coulter, 1991; Plisnier *et al.*, 1999). In October, with the return of the rainy season, the north–south tilting of the thermocline and water movements generate internal waves (Plisnier & Coenen, 2001). All these water movements favor the input of nutrients from water below the thermocline (Coulter, 1991). Observations indicate that primary producers take advantage of these events and the phytoplankton biomass increases (Descy *et al.*, 2005) while bacterial biomass does not vary significantly although large amounts of nutrients are supplied to surface waters during upwelling events (Pirlot, 2006).

Pirlot (2006) found no direct correlation between particulate primary production and bacterial production in Lake Tanganyika. As the sole source of organic matter is from autotrophic production in the pelagic zone, this apparent uncoupling may result from a slow response to the availability of DOC, due to low growth rates of bacteria or from hypothetical rapid consumption of bacterial production by grazers (Pirlot, 2006). In the present study, the hypothesis of top-down control of the bacterial community was tested, in order to assess whether or not losses due to grazing and lysis control bacterial abundance in Lake Tanganyika.

In Lake Tanganyika, protozooplankton is abundant and may represent a significant food source for mesozooplankton. The high ciliate numbers, most of them bacterivorous, were already pointed out by Hecky & Kling (1981). Pirlot *et al.* (2005, 2006) have recently confirmed these observations and they also found high biomass of heterotrophic

nanoflagellates (HNF) and of bacteria. In these papers, morphotypes and sizes of heterotrophic bacteria were characterized, in order to obtain accurate estimation of cell biovolumes. Regarding bacterial community composition, a parallel study was conducted by De Wever *et al.* (2005) using denaturing gradient gel electrophoresis of PCR amplified 16S RNA fragments, followed by sequencing of dominant bands. Grazing-resistant forms of heterotrophic bacteria were not observed in the 0–100 m water layer, although filamentous forms were observed by De Wever *et al.* (2005) but at depths >200 m. On average for several observations at contrasted seasons at two distant sites of the lake, HNF (2–20 μm in size) and ciliates represented, respectively, *c.* 20% and $<1\%$ of autotrophic biomass. Copepods, which represent the bulk of metazoan plankton in Lake Tanganyika, are known to be selective feeders with a preference for larger prey, and are thus not likely to be significant bacterial grazers. Accordingly, Hecky & Kling (1981) suggested that grazing by protozoa should be the main fate of bacteria and that a substantial amount of carbon could be transferred through the microbial food web to upper trophic levels.

For around two decades, the role of protozoa, in particular HNF, in controlling bacterial production in various types of aquatic systems has been demonstrated by different authors (Azam *et al.*, 1983; Bloem *et al.*, 1989; Servais *et al.*, 2000). By efficiently feeding on bacteria, protozoa can channel up bacterial production to upper trophic levels, provided that metazooplankton actually prey upon them. In Lake Tanganyika, the trophic efficiency of the food web, from autotrophs to fish, is considered to be very high (Hecky *et al.*, 1981). A microbial food web could elongate and multiply trophic pathways, but, given the losses at each step even though a large amount of carbon is produced by bacteria, the final amount of matter reaching fish could be reduced to a trickle. Hence, estimating the amount of carbon transiting through the microbial food web by measurements of actual protozoan grazing rates is of primary importance to understand Lake Tanganyika food web functioning, and to assess whether or not the microbial loop can contribute to the high trophic efficiency of this large tropical lake.

The aim of this work was (1) to estimate the bacterial mortality by lysis and by protozoan predation and (2) to quantify the flux of organic carbon transiting through the microbial food web. Two methods were used to estimate bacterial mortality and grazing by protozoa. The first method follows the disappearance of radioactivity from the DNA of natural assemblages of bacteria previously labeled with tritiated thymidine (Servais *et al.*, 1985, 1989; Menon *et al.*, 2003). The rate of decrease of radioactivity can be measured, with and without predator activity using a combination of selective filtration and addition of inhibitors, to estimate the part of mortality due to grazing. Grazing rates by flagellates and ciliates were also estimated by measurements of ingestion of fluorescent micro-particles (FMPs) by protozoa (Børsheim, 1984; Pace & Bailiff, 1987).

Materials and methods

Study site and sampling strategy

Lake Tanganyika is located in the Rift Valley in East Africa and is bordered by four countries (Burundi, Tanzania, Zambia and D.R. Congo). The lake area is about 32 900 km^2 and it can be divided into two main deep basins (north and south) (Fig. 1 and Coulter, 1991). The maximum depth (1470 m) is located in the south basin. The lake is permanently stratified with a thermocline located between 40 and 60 m depth. The anoxic zone is located below 150–200 m.

The experiments described in this paper were performed in two main stations, one off Kigoma (Tanzania) in the north basin and one off Mpulungu (Zambia) in the south basin, during 3 years (2002–2004). Sampling campaigns were carried out in January–February during the wet season and in July–August during the dry season. Sites between the two main stations were sampled during three cruises in both north (TK1–TK3) and south (TK5–TK11) basins. The cruises

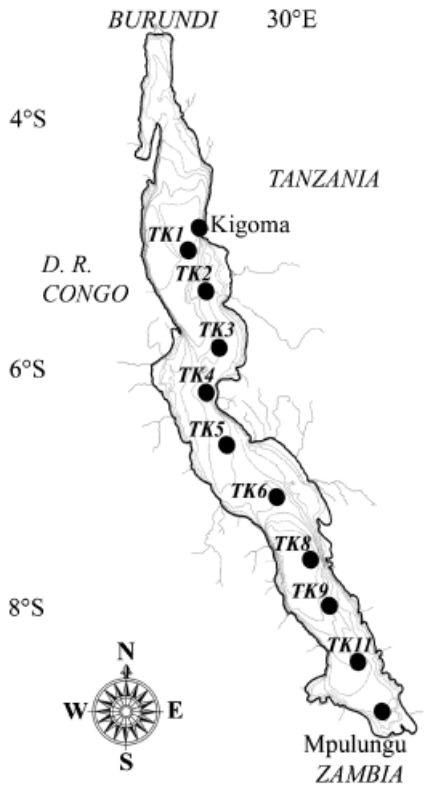

Fig. 1. Map of Lake Tanganyika with the location of the sampling stations.

of 2002 and 2003 were performed during the dry season while the cruise of 2004 took place during the rainy season.

Water column samples were collected with Hydrobios sampling bottles (Kiel, Germany) (5-L) from 0, 10, 20, 30 and 40 m, and were pooled together to obtain a single sample representative of the mixed layer. Samples were also collected at 50–60 and at 80–100 m; on these samples, bacterial and protozoan biomass were estimated (see Pirlot *et al.*, 2005, 2006), and ingestion rates of flagellates and ciliates were determined by the FMP technique.

Estimate of mortality and grazing rates

The total mortality rate was determined using the method developed by Servais *et al.* (1985, 1989). This method measures mortality as the loss of DNA integrity and is thus a minimum estimate of the mortality rate, as a bacterium is considered to be dead when its DNA has been hydrolyzed. Mortality due to grazing by protozoa was determined by a modification of this procedure (Menon *et al.*, 2003). A total of six experiments were realized with epilimnetic water samples from both the north and the south basin and during wet (2002 and 2003) and dry (2002) seasons. A 1.2 L water sample was inoculated at each occasion with methyl-^{3}H-thymidine (80 Ci mmol^{-1}, Amersham Corp.) at a final concentration of 4 nM and incubated under *in situ* temperature and light conditions. After tritiated thymidine was depleted from the medium (no more incorporation), usually after around 24 h, the sample was divided into two subsamples (600 mL). One of the subsamples was not modified and the other was filtered through a 2-μm pore-size filter (Nuclepore membrane) to retain most of the eukaryotic microorganisms. A mixture of cycloheximide–colchicine at respective concentrations of 200 and 100 mg L^{-1} was added to the latter subsample. This mixture was reported to be an effective inhibitor of protozoan reproduction and feeding while having no direct effect on bacterial growth (Sherr *et al.*, 1986). The disappearance of radioactivity was followed twice a day for around 5 days on three replicates of 15-mL aliquots collected in both subsamples incubated at *in situ* temperature. Cold trichloroacetic acid (TCA) (final concentration 5%) was added to the 15 mL, which were then filtered on a 0.2-μm pore-size Sartorius membrane. Filters were dried, transferred to scintillation vials, conserved in the dark at $-20\,^{\circ}$C and brought back to Belgium. The radioactivity associated with the filters was estimated by liquid scintillation. A linear decrease of the radioactivity in both subsamples was usually observed when plotted in semi-log plot vs. time (Fig. 2). The slope of this decrease in the unmodified subsample gave the first-order rate of overall mortality expressed in h^{-1}. The slope of the decrease of radioactivity in the other subsample allowed calculating the lysis rate of bacteria; the difference between the two rates (total minus lysis) was considered bacterial mortality due to grazing. On the basis of a series of experiments performed in triplicate, Menon *et al.* (2003) showed that the average relative error on mortality and grazing rates estimated as described above was 15%.

Ingestion of bacteria by protozoa estimated by the FMP technique

The uptake of FMPs (Børsheim, 1984; Pace & Bailiff, 1987) was used for estimating the ingestion rates of flagellates and ciliates. Ingestion rates were measured in 43 samples (27 from the epilimnion, eight from the metalimnion and eight from the hypolimnion) in parallel with estimates of bacterial biomass and of flagellates and ciliates abundance.

Bacterial and protozoan abundance and biomass were determined by epifluorescence microscopy (Zeiss) at $\times 1000$ magnification, following the procedure proposed by Porter & Feig (1980). After fixation and preservation (the same procedure as described below for the ingestion rate method), organisms were stained with 4,6 diamidino-2-phenyl-indole (10 μg mL^{-1}, final concentration), and 500–2000 bacteria, 50–230 flagellates and 0–90 ciliates were enumerated. Bacteria were sized using digital image analysis (Zeiss KS300) and the relationship $C_{bact} = 92 \times V^{0.598}$, determined from the data of Simon & Azam (1989) relating carbon content per cell (C_{bact}: fg C cell^{-1}) to biovolume (V: μm^3), was used to calculate bacterial biomass. Protozoa biovolumes were calculated considering different categories of size for both flagellates and ciliates (Pirlot *et al.*, 2006). Biomass was estimated from abundance and the distribution of biovolume using the following carbon content per biovolume unit: 190×10^{-15} g C μm^{-3} for ciliates (Putt & Stoecker, 1989) and 220×10^{-15} g C μm^{-3} for flagellates (Børsheim & Bratbak, 1987).

For ingestion rate estimates, FMPs of 0.5 μm diameter (Fluoresbryte®, Polysciences) were used in this study as the average bacterial diameter in Lake Tanganyika was close to 0.5 μm. Water samples (500 mL) were incubated in the presence of FMPs at a concentration corresponding to 5–15% of the incubation bacterial abundance. After 0, 5, 15 and 30 min, aliquots of 40 mL were collected and fixed with 40 mL glutaraldehyde 4% (final concentration 2%). Aliquots were preserved in the dark at 4 °C. Microscope slides were made once back in Belgium. FMPs ingested within flagellates and ciliates were enumerated by epifluorescence microscopy. Ingestion rates were estimated globally for flagellates and ciliates; specific ingestion rates within these communities of protozoa were not estimated. The numbers of FMPs ingested by flagellates and by ciliates were plotted against time of incubation. The initial slopes (between 0 and 5 min) of ingested FMPs by flagellates and ciliates were taken as a measure of FMPs ingestion rate (I_{FMP}). The *in situ* ingestion

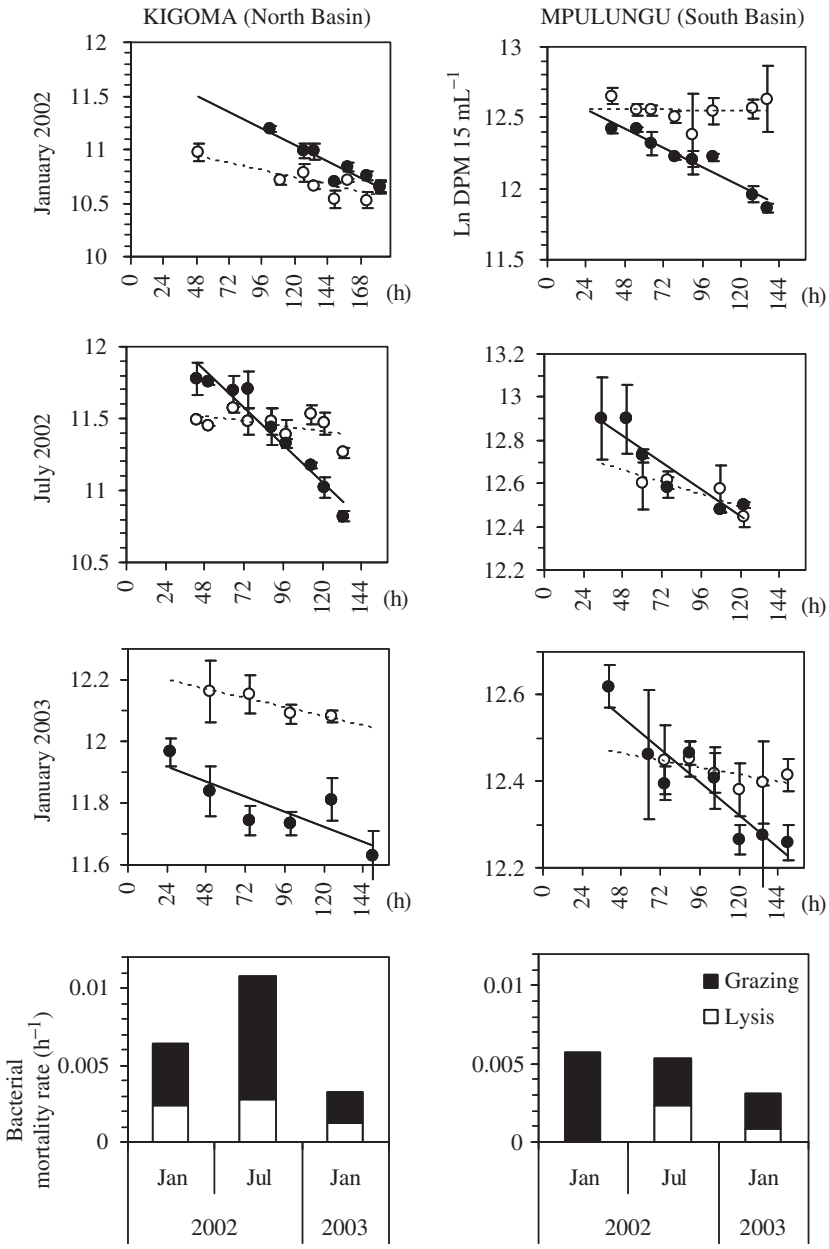

Fig. 2. Decrease of radioactively labeled DNA in heterotrophic bacteria in the mixed layer of Lake Tanganyika off Kigoma (Tanzania) and Mpulungu (Zambia). Empty circles correspond to predator-free incubations and black dots correspond to unfiltered water incubations.

rate of bacteria (I, bacteria cell^{-1} h^{-1}) was estimated for flagellates and ciliates taking into account the FMPs concentration (N_{FMP}) counted at 0 min (2.0 mL filtered on a 0.2 µm pore size filter) and bacteria (N_{BACT}) concentration using the following relationship: $I = I_{FMP}\ (N_{FMP}+N_{BACT})/N_{FMP}$. This calculation assumes that bacteria and FMPs were ingested at the same rate. Replicates of ingestion rates determination showed that the average variation coefficient (CV) was around 25% (data not shown). The clearance rates (nL cell^{-1} h^{-1}) for both flagellates and ciliates were calculated by dividing the ingestion rates of bacteria (I) by the bacterial abundance. Grazing of bacteria expressed in carbon unit per volume unit (g C_{bact} m^{-2} day^{-1}) was calculated by multiplying the ingestion rate of bacteria by the average bacterial carbon per cell and dividing by the abundance of protozoa; this calculation was performed for both flagellates and ciliates. Grazing rates (h^{-1}) were calculated by dividing the ingestion rate of bacteria (bacteria mL^{-1} h^{-1}) by the bacterial concentration (bacteria mL^{-1}).

Results

Bacterial mortality

The decrease of radioactively labeled DNA was followed for a maximum of 182 h in two parallel incubation conditions.

The first batch with epilimnetic unfiltered water allowed to estimate the overall bacterial mortality while the other one, predator-free, was dedicated to cell lysis measurement. When radioactivity in TCA insoluble material in the first batch is plotted against time in a semi-log plot, the slope corresponds to the total mortality rate while in the second batch it corresponds to the lysis rate of bacteria (Fig. 2).

The total bacterial mortality rates ranged between 0.0031 and 0.0108 h^{-1} (i.e. 0.074 and 0.259 day^{-1}) (Fig. 2). The mortality rates were always higher off Kigoma than off Mpulungu, although the means were not significantly different (Student's *t*-test, $P < 0.95$). The maximum was observed off Kigoma during the dry season (July 2002). Cell lysis corresponded to a variable fraction of the total mortality. Lysis rates varied from 1% to 43% of the total mortality rates (with an average of 28%). The flux of lysed cells was calculated for each sample by multiplying the lysis rate by the corresponding bacterial abundance: this flux ranged between 0.04×10^5 and 1.79×10^5 bacteria mL^{-1} day^{-1}. In terms of carbon, this corresponds to carbon loss ranging from 0.03 to 2.85 mg C m^{-3} day^{-1}.

Grazing rates were calculated as the difference between total mortality rates and lysis rates. Grazing was always the main cause of mortality for bacteria (Fig. 2). Grazing rates ranged between 0.0020 and 0.0080 h^{-1} (i.e. 0.048 and 0.192 day^{-1}). The flux of bacterial carbon grazed by protozoa per day ranged from 1.36 to 8.15 mg C m^{-3} day^{-1} in the epilimnion of Lake Tanganyika.

Ingestion of bacteria by protozoa

The FMP technique was used in this work to study the grazing of bacteria by protozoa. FMPs were added to natural samples (5.6–15.7% of the *in situ* bacterial abundance, which ranged between 0.6×10^6 and 3.7×10^6 mL^{-1}), and ingestion was estimated by counting FMPs within protozoa. The average ingestion rates of bacteria by flagellates and ciliates are presented in Table 1 for three layers of the lake, as no significant difference was found for each layer between sites and seasons. On average, ingestion rates of ciliates were about one order of magnitude higher than those of flagellates (Table 1). In deeper layers, grazing per ciliate drastically decreased; in fact, no ingestion of FMPs by ciliates was observed in the hypolimnion. Maximum grazing rates by HNF reached 29 bacteria HNF^{-1} h^{-1} in the hypolimnion. HNF ingestion rate remained in the same order of magnitude in the three studied layers. The pattern for clearance rates showed increasing values with depth for flagellates (Table 1).

Ciliates were less abundant (maximum 3250 cells L^{-1}) than flagellates that ranged between 0.2×10^6 and 1.5×10^6 L^{-1}. More details on plankton abundance can be found in Pirlot *et al.* (2005). The fluxes of carbon ingested by flagellates and ciliates were calculated by multiplying the ingestion rates of bacteria by the average bacterial carbon per cell and dividing by the abundance of flagellates and ciliates, respectively (Fig. 3). Flagellates accounted for 92–99% of the total flux of carbon grazed by protozoa. Flagellates always grazed 10 times more in terms of carbon than ciliates. The daily carbon flux grazed by flagellates ranged between 1.18 and 5.79 mg C m^{-3} day^{-1} (with an average 2.63 mg C m^{-3} day^{-1}) whereas the flux grazed by ciliates never exceeded 0.14 mg C m^{-3} day^{-1} (Fig. 3).

The data obtained by the two methods allowing estimation of grazing used in this study were compared. From the data obtained by the FMP technique, grazing rate was calculated by dividing the measured ingestion rate of bacteria by the bacterial concentration; this calculation was made for flagellates and ciliates and both rates were summed to obtain the total grazing rate. The grazing rates estimated by both methods on the same samples are presented in Fig. 4. The grazing rates by protozoa (flagellates and ciliates) ranged between 0.0018 and 0.0057 h^{-1}. Grazing rates by protozoa estimated by the ingestion of FMPs were not significantly different (Student's *t*-test, $P < 0.95$) from those estimated from the decrease of the labeled DNA technique (Fig. 4).

Protozoan grazing rates were measured not only off Kigoma and Mpulungu in the epilimnion but also at other

Table 1. Average ingestion rates and clearance of protozoa in the pelagic zone of Lake Tanganyika

Layer	Bacteria (L^{-1}) Average	Protozoa (L^{-1}) Average	Ingestion rate (bacteria $cell^{-1}$ h^{-1}) Min–Max	Average	Clearance rate (nL $cell^{-1}$ h^{-1}) Average	*n*
Ciliates						
Epilimnion	2.3×10^9	1652	32–405	92	40.2	27
Metalimnion	1.6×10^9	1012	0–123	25	15.4	8
Hypolimnion	1.0×10^9	118	0–0	0	0.0	8
Flagellates						
Epilimnion	2.3×10^9	1.3×10^6	< 1–18	6	2.8	27
Metalimnion	1.6×10^9	8.1×10^5	4–16	9	5.6	8
Hypolimnion	1.0×10^9	3.0×10^5	3–29	10	10.3	8

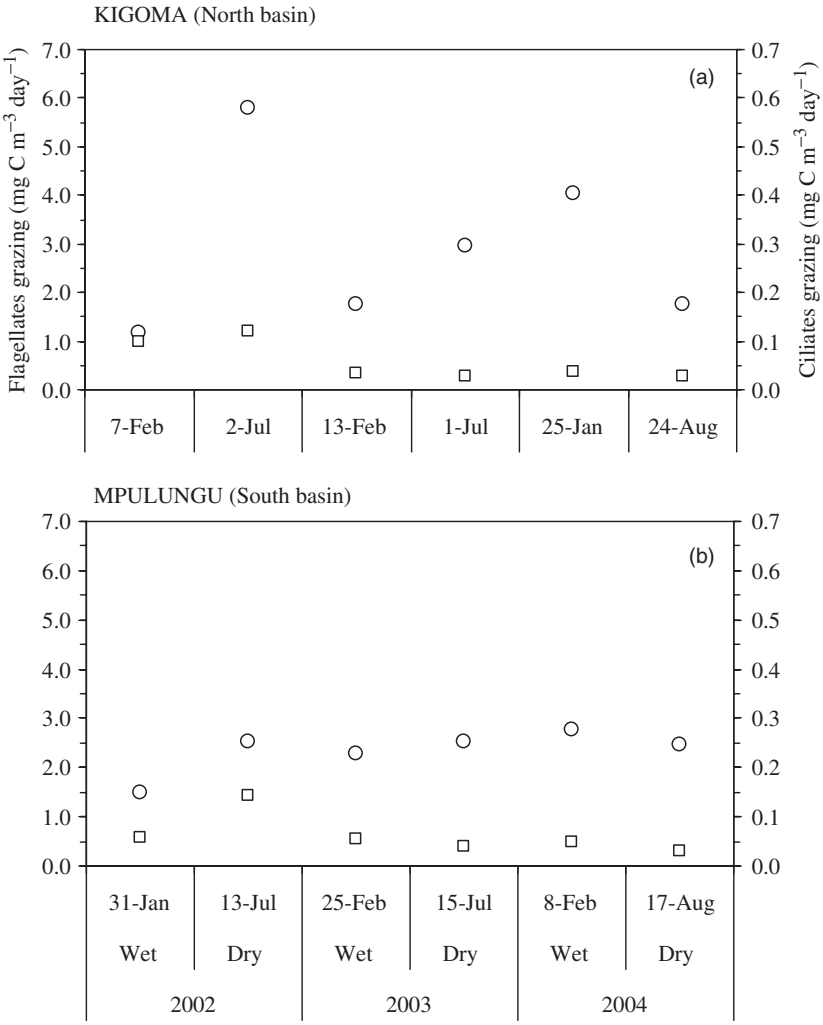

Fig. 3. Heterotrophic flagellates (○) and ciliates (□) grazing on bacteria in carbon units in Lake Tanganyika off Kigoma (a) and Mpulungu (b).

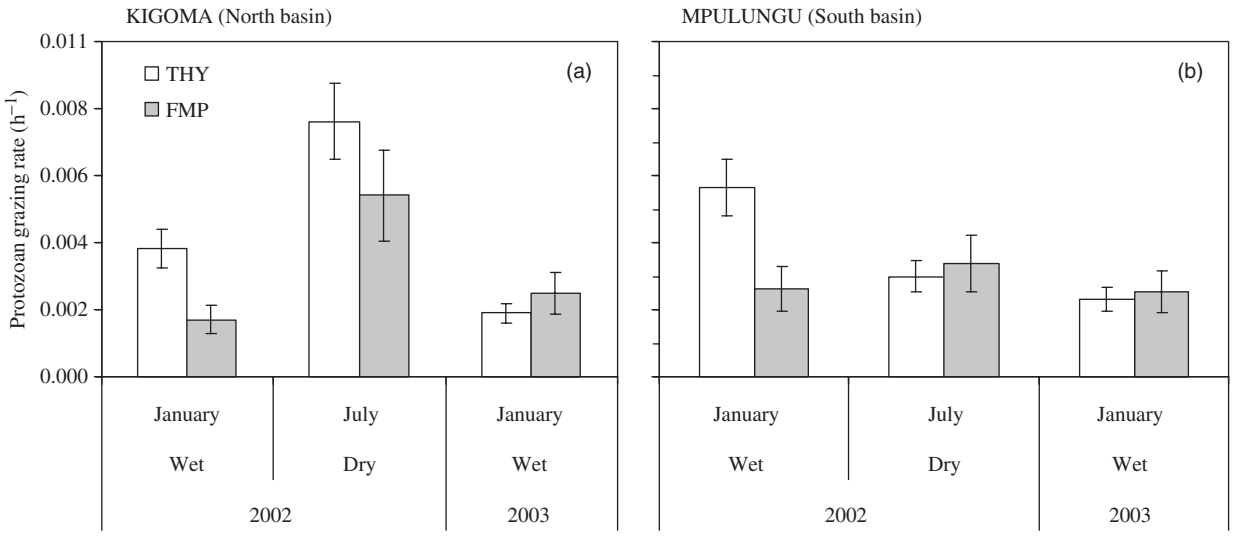

Fig. 4. Comparison of protozoan grazing rates in Lake Tanganyika off Kigoma (a) and Mpulungu (b) obtained with both methods used: decrease of labeled bacterial DNA (THY) and ingestion of FMP by protozoans (FMP).

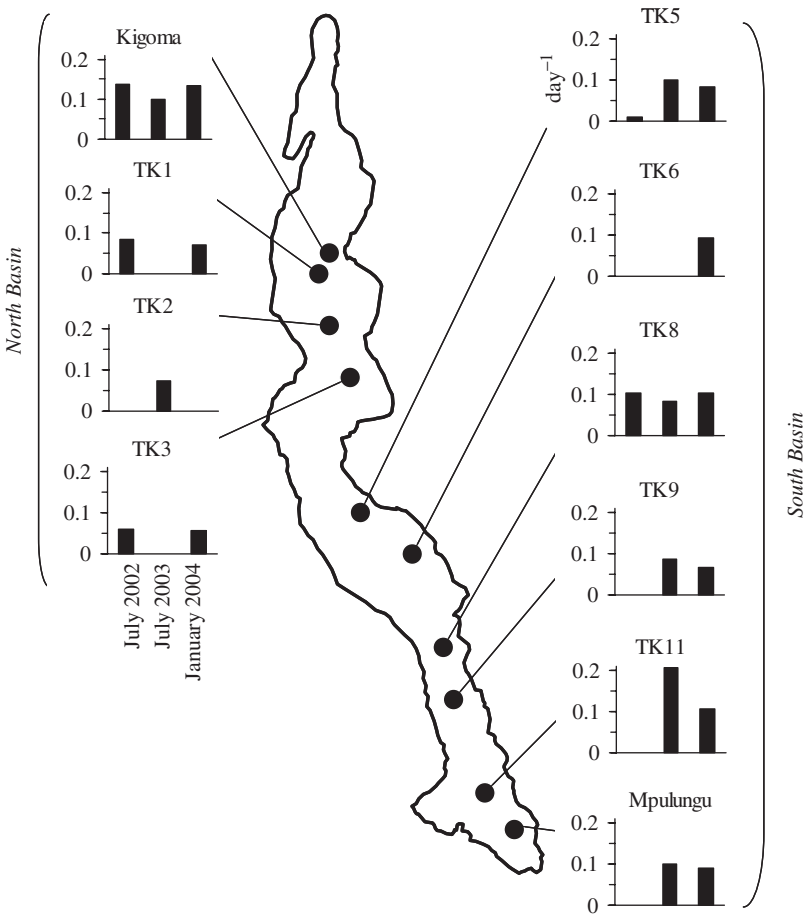

Fig. 5. Grazing rates by protozoan on bacteria per day, from FMP ingestion, at different sites in both main basins of Lake Tanganyika.

sites (presented in Fig. 5) during three cruises. The results showed grazing rates in the same range in both north and south basins.

Measurements of tritiated thymidine incorporation rates allowed us to estimate bacterial production and growth rates of heterotrophic bacteria in Lake Tanganyika during the same periods and at the same sites (Pirlot, 2006). These results provide the opportunity to compare the growth and mortality of heterotrophic bacteria. The net increase rate of bacteria (bacterial growth rates minus bacterial mortality rates) ranged between -0.19 and $0.17\ day^{-1}$ (averages $= -0.01\ day^{-1}$ in the epilimnion, $-0.05\ day^{-1}$ in the metalimnion and $-0.02\ day^{-1}$ in the hypolimnion) (Fig. 6). As no lysis rate estimates were performed in the metalimnion and the hypolimion, these estimates are based on the assumption that the contribution of bacterial cell lysis to total mortality was similar all over the three strata (28.5% as measured in the epilimnion).

Carbon fluxes through bacteria

An evaluation of the organic carbon flux transiting from the bacterial compartment to protozoa was performed for the 0–100 m upper water column in the pelagic zone of Lake Tanganyika (Table 2). If the average bacterial production was slightly higher during the wet season than during the dry season (323 and 217 mg C m^{-2} day^{-1}, respectively), the mortality remained fairly constant all year round. Once integrated in the top 100 m water column, bacterial total mortality corresponded to 275 and 279 mg C m^{-2} day^{-1} during the wet and dry season, respectively. These results represented 85% and 128% of the bacterial production. Protozoan growth efficiency from the literature of 0.4 was used to estimate protozoan production from grazing rates of about 75 and 77 mg C m^{-2} day^{-1}. This represents a production rate for protozoa of more than 0.1 day^{-1}.

Discussion

Several studies demonstrated the importance of bacterial biomass and/or production in the pelagic zone of Lake Tanganyika with respect to phytoplankton biomass and production (Van Meel, 1954; Hecky *et al.*, 1981; Sarvala *et al.*, 1999; Pirlot, 2006). In this paper, it was attempted to evaluate the fate of bacterial production, with special attention to grazing by protozoa, which are known to be

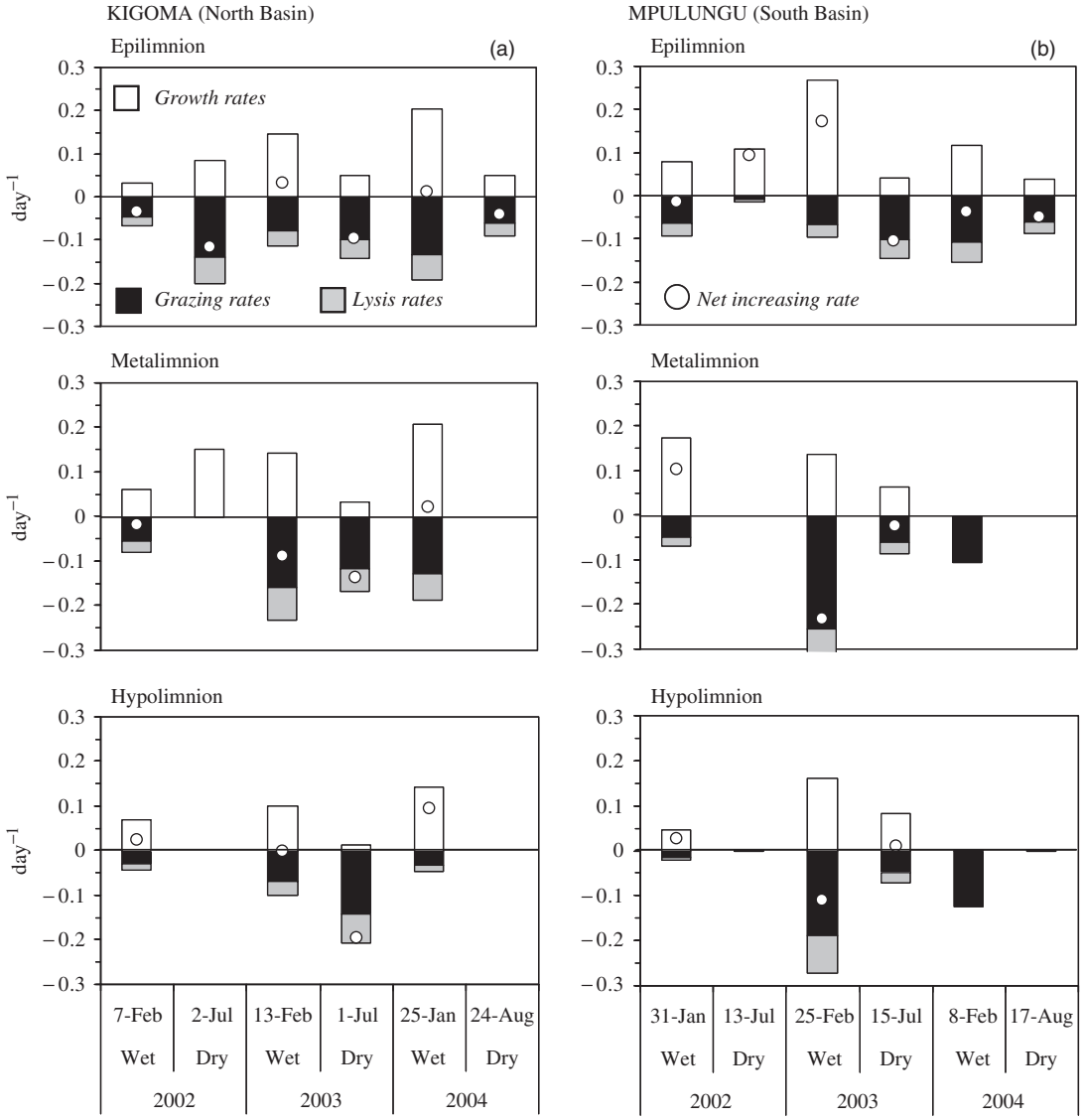

Fig. 6. Bacterial growth rates against grazing rates in Lake Tanganyika off Kigoma (a) and Mpulungu (b). Dots represent the net growth of the population (growth rate minus grazing rate). Bacterial productions are from Pirlot (2006). Lysis rates are only estimations based on Fig. 2 results.

Table 2. Average carbon fluxes transiting from phytoplankton to protozoans through heterotrophic bacteria in the pelagic Lake Tanganyika during the wet season and the dry season

	Abbreviation	Wet season	Dry Season	Note
Primary production	PP	500	1400	*
Bacterial carbon demand	BCD	1617	1087	†
Bacterial respiration	R	1293	870	†
Bacterial production	BP	323	217	*
Bacterial total mortality	M	275	279	
Mortality (M) induced by lysis	L	85	86	‡
M induced by flagellates ingestion	FI	188	192	
M induced by ciliates ingestion	CI	2	1	

Values presented in mg C m^{-2} day^{-1} are averages from 2002, 2003 and 2004.
*From Pirlot (2006).
†Estimated from the growth efficiency (BGE) of 0.2 found in e.g., del Giorgio *et al*. (1997).
‡Estimated from the proportion of M measured in the epilimnion (28.5%, Fig. 2).

abundant in this environment (Hecky & Kling, 1981; De Wever, 2006; Pirlot, 2006).

Bacterial total mortality was measured using the DNA of natural assemblages of bacteria previously labeled with tritiated thymidine (Servais *et al.*, 1985, 1989). This method allows simultaneous estimates of cell lysis (Furhman & Noble, 1995) and grazing losses (Menon *et al.*, 2003).

Alternatively, protozoan grazing was also measured using ingestion of FMPs by bacterial predators. The first method allowed to demonstrate that predation by protozoa was the main process involved in the mortality of heterotrophic bacteria in Lake Tanganyika. The second method gave protozoan grazing rates very similar to the first one, and showed that HNF accounted for most of the total grazing of bacteria. From a methodological point of view, the agreement of both methods based on different principles strengthens the validity of our results. Comparisons of grazing rate measurements by the decrease of the labeled DNA method and by the ingestion method [fluorescently labeled bacteria (FLB) method] have already shown similar estimates (Servais *et al.*, 1998; Menon *et al.*, 2003). Fuhrman and Noble (1995) have used the disappearance of bacterial DNA to estimate virus-induced mortality; they compared this method with others and showed that the tested methods resulted in similar estimates of virus-induced lysis.

Both methods have advantages and drawbacks. Grazing underestimations, due to the use of synthetic beads instead of labeled bacteria, is known to be observed sometimes (Sherr *et al.*, 1987; Sanders *et al.*, 1989). By contrast, a recent study underlined the absence of any selectivity between inert particles or living bacteria by HNF and even the advantages of nondigestible beads for enumeration into protozoa (Carrias *et al.*, 1996; Thouvenot *et al.*, 1999; Boenigk *et al.*, 2002). Preliminary experiments in Lake Tanganyika indicated that HNF preferentially grazed on FLB, whereas they actively discriminated against artificial preys (FMP) in the same range described in Sherr *et al.* (1987). The results presented here should thus be considered as the lower range for HNF grazing in Lake Tanganyika. The method based on the decrease of labeled DNA offers the advantage to allow simultaneous determination of the grazing rate, lysis rate and total mortality rate. The disadvantage of this method is the quite long-term incubation that it involves. The main problem due to this incubation is not the changes in bacterial diversity (even if they may occur) but the possible change in the composition of the protozoan community. The change in bacterial diversity probably affects the mortality rate estimate weakly as the method measures the mortality of the bacteria that have incorporated thymidine during the first hours of the incubation (active bacteria in the initial sample). The modification in protozoan community during the incubation can affect the determination of the grazing rate, but the fact that good linear regressions were observed in the semi Log plot of radioactivity vs. time (Fig. 2) suggests the weak influence of this factor on bacterial mortality rate estimate.

Data on HNF ingestion rate obtained in this study are in agreement with those found in the literature, between < 1 and 25 bacteria cell h^{-1} (Andersen & Sørensen, 1986; Bloem *et al.*, 1989). The highest values (up to 29 bacteria cell h^{-1}) were found in hypolimnetic waters, where protozoans can only prey on bacteria, whereas, in the surface waters, they may also prey preferably on picocyanobacteria, as in other oligotrophic systems (Hagström *et al.*, 1988). The clearance rates of flagellates in Lake Tanganyika compare well with those found in other large lakes, as, for instance, Lake Michigan (Carrick & Fahnenstiel, 1991). The fact that the clearance rate of HNF increased with depth may simply result from a decrease in prey density as bacterial concentration tends to decline with depth (Pirlot, 2006). HNF grazing integrated in the top 100 m water column showed remarkably low variations between years, basins and seasons. The constancy of grazing pressure associated with a high HNF biomass stability leads to assume a constant bacterial mortality due to grazing all year round.

High HNF grazing pressure on the bacterial community could explain the small cell size of the heterotrophic bacteria in the offshore waters of Lake Tanganyika (Pirlot *et al.*, 2005). It has been shown that flagellates graze preferably large bacterial cells (Chrzanowski & Simek, 1990; Jürgens *et al.*, 1999). Size-selective ingestion is probably the main factor determining the selective removal of certain types of bacteria (Jürgens & Güde, 1994).

Cell lysis was the second process in importance responsible for bacterial mortality, ranking before grazing by ciliates. Cell lysis can be caused by viruses or senescence. Recently, virioplankton has been shown to possibly play a major role in bacterial mortality in freshwater systems (Bettarel *et al.*, 2004; Jacquet *et al.*, 2005). Virus-induced bacterial mortality varies, in some European lakes, from 0.1% to 74% (see the review in Jacquet *et al.*, 2005). Hence, it is conceivable that viruses in Lake Tanganyika could be responsible for the mortality due to lysis measured in this study (28.5% of the total bacterial mortality).

Ranking in third position among the factors involved in bacterial losses, ciliate grazing on bacteria was responsible, in the present measurements, for $<$ 1–8% of protozoan grazing. Nevertheless, it is likely that at times grazing by ciliates on bacteria may be higher at some periods, as these microorganisms may occasionally reach large numbers in Lake Tanganyika (De Wever, 2006). However, they also feed heavily on picocyanobacteria, which may constitute a major carbon source for heterotrophic protists.

Metazoan grazing on bacteria was not quantified in the present study but this process is probably negligible as in other similar systems (Calbet & Landry, 1999). However,

grazing on picoplankton (< 2 μm) by juvenile copepods remains to be investigated further, as nauplii having ingested FMPs were observed. Indeed, preliminary results showed evidence of ingestion of *Synechococcus* (and probably also of bacteria) by nauplii.

Bacterial productions measured in Lake Tanganyika by Pirlot (2006) were in the same range as bacterial mortalities, indicating that bacterial growth and mortalities were balanced. Although periods of higher grazing by protozoans alternated with higher production periods (Table 2), bacterial biomass remained almost stable all year round (Pirlot *et al.*, 2006).

In this study, for the first time, to the authors' knowledge, the bacterial organic carbon flux passing through the microbial food web was quantified in a large tropical lake. It was estimated that *c.* 5–8% of the carbon taken up by bacteria was converted into protozoan production and was available for metazoans. Even if this figure may seem small, this represents a significant fraction of the organic carbon production by autotrophs that is transformed into protozoan biomass.

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