Size-Fractionated Phytoplankton and Relationships with Metazooplankton in a Newly Flooded Reservoir

key words: phytoplankton, zooplankton, production, microheterotrophs, new reservoirs

Abstract

In order to yield some insights into the planktonic food web structure of new reservoirs, size-fractionated biomass and productivity of phytoplankton were examined from 1996 to 1997 (following the 1995 flooding of the Sep Reservoir, Puy-de-Dôme, France), in relation to nutrients (P, N) and metazooplankton (Rotifers, Cladocera, Copepods). Autotrophic nanoplanckton (ANP, size class 3–45 µm) dominated the phytoplankton biomass (as Chlorophyll a) and production, while autotrophic picoplankton (APP, 0.7–3 µm) exhibited the lowest and relatively constant biomass and production. Cells of the autotrophic microplankton (AMP, >45 µm) were considered inedible for planktonic herbivores. The production-biomass diagram for the different size classes and the positive correlation between APP production and ANP + AMP production suggested that grazing was potentially more important than nutrients in shaping the phytoplankton size structure. Metazooplankton biomass was low compared to other newly flooded reservoirs or to natural lakes with phytoplankton biomass similar to that of the Sep Reservoir. This resulted in low ratios (metazooplankton to edible phytoplankton) both in terms of production (average 0.43% in 1996 and 0.76% in 1997) and biomass, suggesting that only a small fraction of phytoplankton was directly consumed by metazooplankton. We suggest that the observed low ratios in the Sep Reservoir, reflect possible low metazooplankton inputs in the main influents, changes in hydrologic conditions and a high potential role of microheterotrophs. The latter role was supported by (i) the positive inter-annual correlation between ciliates and phytoplankton, (ii) the significant and negative correlations between ciliates and metazooplankton, and (iii) the significant and negative correlations between total metazooplankton biomass and total phosphorus (TP), whereas neither TP nor total metazooplankton biomass was correlated with phytoplankton variables.

1. Introduction

Numerous studies of phytoplankton in both natural (oceanic, coastal and fresh waters) and laboratory conditions show that small-size cells generally outcompete large cells in nutrient-poor environments (MALONE, 1971; TAKAHASHI et al., 1982; STOLTE and RIEGMAN, 1995), indicating the role of nutrients in shaping phytoplankton size structure. Size-dependent loss
processes are also major regulating factors for the size distribution within natural phytoplankton assemblages. For example, herbivorous microzooplankton and most of the metazooplankton preferentially graze on small algae (generally <35 µm) (PORTER, 1973; LEHMAN, 1991; WATERHOUSE and WELSCHMEYER, 1995). Sinking of algae, in relation to hydrodynamic processes in the water column, is also well known to affect the size structure of natural phytoplankton (LEGENDRE and LE FÈVRE, 1989), and the flows of carbon through the ecosystem (LEGENDRE and LE FÈVRE, 1995). The study of phytoplankton size structure in relation to the above three factors (i.e. nutrient status, water column stability and the grazing environment) can thus provide valuable information on the food web structure of aquatic systems. The prominence of small cells in the plankton is generally indicative of a situation where the multistep microbial food web is prevalent in the overall metabolism of pelagic systems (POMEROY, 1974; AZAM et al., 1983), whereas the dominance of larger cells is often indicative of the presence of a more direct two-step carbon transfer to fish based on the activity of larger herbivores (e.g. LEGENDRE and LE FÈVRE, 1995).

Few studies in freshwaters have reported size fractionated algal production and biomass, including those from autotrophic picoplankton (e.g. FRENETTE et al., 1994; MALINSKY-RUSHANSKY et al., 1997). The picoplankton form an important food source for small grazers (STOCKNER, 1988; SMEK et al., 1995). This is particularly true for newly formed reservoirs where few attempts have been made to analyze the size distribution of phytoplankton communities. Because of the submersion of lands, these ecosystems are expected to receive large amounts of nutrients and terrestrial organic matter during the first years of flooding (PINEL-ALLOUL et al., 1989; PATTERSON et al., 1997; CAMPBELL et al., 1998). These allochthonous materials may play an important role in shaping the structure of the planktonic communities in newly formed reservoirs. Several studies have reported significant changes in the trophic status of these ecosystems (e.g. OSTROFSKY, 1978; GRIMARD and JONES, 1982; ROBARTS et al., 1992), but none has examined whether such trophic evolution is accompanied by changes in phytoplankton size structure. On the other hand, the coupling between phytoplankton and their potential grazers, i.e. micro-and metazooplankton, have rarely been examined in this type of ecosystems. To our knowledge, only PATTERSON et al., (1997) have conducted a size fractionation study in a newly flooded reservoir (the experimental Reservoir 979, Ontario, Canada). However these authors dealt only with phytoplankton biomass and did not address the fate of algal cells from different size classes. They hypothesized, from their zooplankton and phytoplankton dynamics, that microorganisms might play an important role in matter transfer in new artificial lakes.

Many of the world’s rivers are either impounded or facing impoundment in the near future. Large reservoirs (>0.5 km³), for example, represent about 20% of the global mean runoff (KALFF, 2002). For these reasons, an understanding of ecological changes in reservoirs, especially in new ones, is important. As part of a comprehensive study intended to understand the food web structure in the newly flooded Sep Reservoir, we analyzed the size distribution of phytoplankton communities and tested their relationships with metazooplankton in this ecosystem. For this purpose, we (i) determined the relative importance of various size classes of phytoplankton (including picoplankton) to total phytoplankton biomass (as chlorophyll a) and production, in relation to nutrients and metazooplankton (rotifers, cladocera and copepods) biomass, (ii) estimated the metazooplankton:phytoplankton production ratio, and (iii) examined the potential fate of different algal size classes, using an approach based on the establishment of a diagram model that combines production and biomass data (P-B diagram). The P-B diagram offers an operational framework for reporting and interpreting data on the size-fractionated production and biomass of phytoplankton (TREMBLAY and LEGENDRE, 1994).
2. Methods

2.1. Study Site and Sampling

The Sep Reservoir was formed in 1994 by damming the Sep stream, for the summer irrigation of an agricultural region known as the ‘Haute-Morge’ located in the French Massif Central (ca 46° N, 3° E). At its full supply level, the reservoir contains about 5 million m$^3$ of water, has an area of 33 ha, a mean depth of 14 m and a maximum depth of 37 m. The reservoir was first flooded in May 1995. It was drawn down in summer, usually from July to September, in 1995, 1996 and 1997, for downstream irrigation and to prevent deoxygenation of bottom waters. The reservoir was generally empty by late October. During the present study, conducted in 1996 and 1997, the sluices of the reservoir were opened on July 3 each year. During both years, the water column of the reservoir was thermally stratified, starting from early April. For each year, the spring corresponds to the period that lasted from the beginning of the study to June 21 while the summer corresponds to the rest of the study. Additional details on the site can be found in TADONLÉKÉ et al. (2000) and TADONLÉKÉ and SIME-NGANDO (2000).

Water samples were collected from April to September 1996 and from March to August 1997, at two stations chosen partly because of accessibility. However, because the data from the two stations were not significantly different (TADONLÉKÉ, 1999), we herein present only results from station 2 located in the deepest area of the reservoir, at about 150 m from the dam. In 1996, samples for phytoplankton size-fractionation were collected once in April, every two weeks in May and weekly from June to September, while in 1997 they were collected every two weeks. Samples for metazooplankton analyses were collected in 1996 at the same frequency as phytoplankton samples, except for the period lasting from June to September 1996 when they were collected every two weeks. In 1997, metazooplankton was collected weekly. These differences in the sampling frequency for zooplankton and phytoplankton were mainly due to logistic problems. Phytoplankton samples were taken with a Van-Dorn bottle, at three depths in the epilimnion (0, 1, 4 m), and at one depth in the metalimnion (7 m) and the hypolimnion (15 m), while metazooplankton organisms were caught from three vertical hauls from the bottom to the surface of the water column, using a Juday type net of 55 µm mesh size.

2.2. Size Fractionation

During our study, we examined the phytoplankton biomass and production in three size classes: <3, 3–45 and >45 µm. For the biomass (i.e. chlorophyll $a$), the three size classes were obtained from the following fractionations: (1) for the whole community, algae in lake water were caught on Whatman GF/F glass fibre filters (nominal pore size ~0.7 µm), (2) for the 0.7–45 µm size fraction, lake water was prefiltered through a 45 µm Nitex netting and algae collected on GF/F filters, (3) for the 0.7–3 µm size fraction, lake water was prefiltered through 3 µm pore-size filters (Durieux filters for chlorophyll or Sartorius filters for primary production) and algae collected on GF/F filters. The <3 µm size fraction was obtained directly from step 3, whereas the 3–45 µm size fraction was obtained by subtracting the result of step (3) from that of step (2), and the >45 µm size fraction by subtracting the result of step (2) from that of step (1). For primary production (determined from $^{14}$C uptake), the same size fractionations as for the biomass were done but we replaced the GF/F filters by 0.45 µm pore-size Millipore filters. Comparison of results obtained with GF/F filters with those obtained with 0.45 µm Millipore filters showed no significant difference, both for algal biomass and production measurements (Mann-Whitney U test, $p > 0.05$). Since the cells with size <20 µm largely dominated the 3–45 µm size fraction in terms of both abundance and biomass (93 and 91% in 1996 and 97 and 95% in 1997; TADONLÉKÉ, 1999), this size class is considered in this study as representative of the autotrophic nanoplanктon (ANP). The <3 µm size fraction corresponds to the autotrophic picoplankton (APP), and the >45 µm size fraction to the autotrophic microplankton (AMP).

2.3. Analytic Methods

In a previous paper (TADONLÉKÉ et al., 2000), we presented preliminary data on phytoplankton composition and total primary production related to light, dissolved nutrients and water column stability in the
Sep Reservoir. In this work, which deals mainly with trophic interactions, total nitrogen (TN) and total phosphorus (TP) are presented. Organic nitrogen (i.e. Kjeldhal nitrogen) was analyzed on unfiltered lake water by the indophenol blue method and used in the calculation of TN. TP was also analyzed on unfiltered lake water, by the molybdate-ascorbic acid method, after conversion into orthophosphates with a persulfate digestion. TN and TP were assayed spectrophotometrically using AFNOR (1990) standard methods.

Chlorophyll a (Chl) from the studied size classes was extracted in 90% acetone at 4 °C overnight in the dark and concentrations measured spectrophotometrically (SCOR-UNESCO, 1966). Primary production was measured in situ, usually between 11:00 and 14:00 (local time), from 14C incorporation according to STEEMANN-NIELSEN (1952). Detail of the procedure can be found in TADONLÈKE et al. (2000). The biomass and the production of each size class were integrated over the euphotic zone.

The production-biomass (P-B) diagram proposed by TREMBLAY and LEGENDRE (1994) was applied to our data in order to estimate the potential fate of the algal size classes under study. The approach consists in plotting biomass and production data of size-fractionated phytoplankton in a diagram where $P_S : P_T$ (biomass of $S$ : biomass of total phytoplankton or the $P_S$%) is a function of $B_S : B_T$ (biomass of $S$ : biomass of total phytoplankton or the $B_S$%). In developing their model, TREMBLAY and LEGENDRE (1994) assessed the relationship between $C_L : C_T$ and $\text{Chl}_L : \text{Chl}_T$ (i.e. contribution of large cells to total phytoplankton carbon vs their contribution to total chlorophyll a concentration) and found good agreement between the two sets of data. They concluded that the P-B diagram can be analyzed using chlorophyll a concentrations as an estimate of algal biomass. We checked this for the large size class (AMP) in the Sep Reservoir during our study and found good correlations between $B_{\text{AMP}} : B_T$ and $\text{Chl}_{\text{AMP}} : \text{Chl}_T$, where $B_v$ is the algal biovolume ($r = 0.71$, $n = 72$ in 1996 and $r = 0.83$, $n = 60$ in 1997).

TREMBLAY and LEGENDRE (1994) interpreted the P-B diagram in terms of export (grazing, sedimentation and advection), the main diagonal of the diagram (i.e. the zero axis) corresponding to the situation in which $P_S – B_S = 0$, implying that there is a dynamic balance between production and export of small and large phytoplankton cells. Location of data points below the diagonal (i.e. $P_S – B_S < 0$) means that the export of cells from the target size class is lower than their share of total production, hence their higher accumulation in the water column, compared to the other size class. In contrast, location of data points above the diagonal (i.e. $P_S – B_S > 0$) indicates that the export of cells from the studied size class (relative to the other) is higher than their share of total production. In our P-B diagrams, euphotic zone-integrated data were used in order to account for processes such as self-shading and surface photoinhibition (if they occurred), which fundamentally influence the balance between production and export. Phytoplankton cells forming the APP and ANP size classes were considered as edible and those in the AMP size fraction as inedible, for the metazooplankton.

Metazooplankton species composition and dry weight biomass and the methods used for their analysis are presented in THOUVENOT et al. (1999a, b). In the present study metazooplankton wet weight is shown, mainly for comparison purpose. The production of each metazooplankton group was estimated for each sampling date, using the model proposed by SHUTER and ING (1997) and assuming a carbon content of 44% of the dry weight. The model predicts a daily metazooplankton production : biomass ratio ($P/B$) from the mean daily temperature ($T$) of the water column as $P/B = 10^{0.66(T-10)}$, where $T$ is equal to $-1.748$, $-1.725$, $-2.458$, and $-1.766$, and $\beta = 0.052$, 0.044, 0.05 and 0.04, for rotifers, cladocera, calanoids, and cyclopoids, respectively (SHUTER and ING, 1997). This model has the advantage of generating rapid estimation of metazooplankton production and has been found to provide reliable results at the community level, when compared to other methods such as the egg ratio method or the body size based methods (STOCKWELL and JOHANNSSON, 1997). In our study, the total metazooplankton production ($P_{\text{PTZ}}$, mg C · m$^{-3}$ · d$^{-1}$) was obtained by summing the production rates estimated for the different taxonomic groups. The metazooplankton:phytoplankton production ratio (%) was then calculated as $P_{\text{PTZ}} : P_E (\times 100)$, where $P_E$ is the production from the edible phytoplankton size classes (i.e. APP + ANP). $P_E$ was obtained by multiplying the primary production values of interest (i.e. in the euphotic zone) by the ratio of the daily incident light to the incident light recorded during the in situ incubations of primary production samples. This $P_E$ value was then divided by the thickness of the euphotic zone to obtain $P_E$ in mg C · m$^{-3}$ · d$^{-1}$.

### 2.4. Statistical Analysis

For the main variables under study, between-year and between-season comparisons were undertaken using the non-parametric Mann-Whitney U test, since our data were not normally distributed. Pearson correlation analyses with log$_{10}$-transformed data, the Durbin-Watson D test and the first order autocor-
relation coefficient ($AC_C$) were used to establish empirical relationships between variables. For significant Pearson’s $r$, when $D$ was <1.33 and $AC_C$ far from zero, the correlation was considered an autocorrelation (Berk and Steagall, 1994). $D$ and $AC_C$ were not calculated for correlations between changes in the water level in the reservoir and both nutrients and biological variables.

3. Results

3.1. Physical and Chemical Environment

From March to late June, for both years, the water level in the reservoir slightly increased (Figs. 1A, B). During these periods, the rate of water flow at the outlet of the reservoir was quite constant, with the exception of peaks registered between mid-May and mid-June in 1996 and in early May 1997. The sluices of the reservoir were opened in early July for sum-
Figure 2. Absolute and relative (%) concentrations of chlorophyll $a$ in the euphotic zone (ChlZeu) of the Sep Reservoir during a two-year survey, for three size fractions: autotrophic picoplankton (APP, 0.7–3 $\mu$m), nanoplankton (ANP, 3–45 $\mu$m), and microplankton (AMP, >45 $\mu$m).
Table 1. Mean values and coefficients of variation (in brackets) for the chlorophyll $a$ concentrations (mg · m$^{-2}$) and primary production (mg C · m$^{-2}$ · h$^{-1}$) associated with each of the three size classes, and for their importance relative (%) to total chlorophyll $a$ and primary production in the euphotic zone of Sep Reservoir. Superscript numbers indicate interannual comparison, whereas letters indicate, for each year, the seasonal comparison when there was a significant difference. Values with $^1$ are significantly (Mann-Whitney U test, $p < 0.05$) higher than those with $^2$ and values values with $^a$ are significantly higher than those with $^b$. For each size class, comparisons concern comparable variables (for example relative importance vs relative importance). For seasonal mean, only absolute values are given. Note that in 1996, size fractionation was done from April to September and that the mean values of total chlorophyll $a$ and primary production for that year are for the whole study, i.e. February to September. Sp = spring, Su = Summer.

<table>
<thead>
<tr>
<th>Year</th>
<th>Size class (as in the text)</th>
<th>Chl</th>
<th>Primary production (PP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>absolute</td>
<td>percent of total Chl</td>
<td>Seasonal mean in $Z_{eu}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>APP</td>
<td>5.51 (49)$^1$</td>
<td>12 (48)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ANP</td>
<td>37.14 (54)$^1$</td>
<td>68 (29)</td>
</tr>
<tr>
<td>(3–45 µm)</td>
<td></td>
<td></td>
<td>47.73 (31)$^a$ (Su)</td>
</tr>
<tr>
<td></td>
<td>AMP</td>
<td>7.94 (59)$^1$</td>
<td>20 (83)</td>
</tr>
<tr>
<td>(&gt;45 µm)</td>
<td></td>
<td></td>
<td>6.07 (61)$^b$ (Su)</td>
</tr>
<tr>
<td></td>
<td>Total Chl (range)</td>
<td>46.88 (45)$^1$</td>
<td>9.16–80.51</td>
</tr>
<tr>
<td>1997</td>
<td>APP</td>
<td>2.78 (86)$^2$</td>
<td>10 (57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.57 (96) (Su)</td>
</tr>
<tr>
<td></td>
<td>ANP</td>
<td>19.54 (61)$^2$</td>
<td>74 (15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24.75 (65) (Su)</td>
</tr>
<tr>
<td></td>
<td>AMP</td>
<td>5.23 (122)$^2$</td>
<td>17 (49)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.75 (140) (Su)</td>
</tr>
<tr>
<td></td>
<td>Total Chl (range)</td>
<td>28.7 (66)$^2$</td>
<td>10.1–77.52</td>
</tr>
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</table>
mer irrigation of agricultural fields. Consequently, the outflow of water increased, and the water level at the deepest area of the reservoir decreased, between July and September, from 37 to 16 m in 1996 and from 27 to 16 m in 1997 (Fig. 1A, B).

The average concentration of total nitrogen (TN) for all discrete depths in 1996 [2.93 mg N · l⁻¹, range 1.03–5.9, coefficient of variation (CV) = 38%] was similar to that in 1997 (3.44 mg N · l⁻¹, range 1.32–6.1, CV = 26%). In contrast, the mean total phosphorus (TP) concentration significantly decreased from 79.4 µg P · l⁻¹ (range 54–135, CV = 18%) in 1996 to 57.8 µg P · l⁻¹ (range 2.5–221.8, CV = 74%). These concentrations are characteristic of relatively productive waters (WETZEL, 1983). However, in 1996, we did not detect orthophosphates in the epilimnion in May and early June, and the overvall relative contribution of this directly assimilable P to TP was substantially lower (~10%) than in 1997 (50%) (TADONLÉKÉ, 1999).

3.2. Size-Fractionated Phytoplankton Biomass

Total chlorophyll a concentrations (Chl) as well as concentrations from each size class significantly decreased from 1996 to 1997 in the euphotic zone of the reservoir. In contrast, the importance of each Chl size class relative to the total Chl remained about the same during the two study years (Table 1). Most of the Chl was associated with ANP during the two years, while APP had the lowest biomass (<10 mg · m⁻²) and contribution to total biomass (generally <20%) (Table 1, Fig. 2). Both APP biomass and contribution to total Chl remained relatively constant with seasons for both years, whereas significant seasonal differences occurred for ANP and AMP, but only in 1996. During 1996, ANP biomass and contribution to total Chl was lowest between May and June and increased significantly in summer. In contrast to ANP, AMP biomass and contribution to total Chl were highest in May and early June, and significantly decreased in summer (Fig. 2A, B). In 1997, no clear temporal trend was observed. Highest concentrations for all the three Chl size classes were measured at the beginning (March–April) and at the end (late July–August) of the sampling period (Fig. 2C, D).

3.3. Size-Fractionated Phytoplankton Production

No significant difference was observed between the two years for the total production in the euphotic zone (PPZ eu), for the production associated with each of the size classes, and for the contribution of APP to PPZ eu. In contrast, ANP and AMP contributions to the total PPZ eu significantly increased and decreased, respectively, from 1996 to 1997 (Table 1). As for the biomass, the bulk of PPZ eu was from ANP, whereas APP had the lowest production rates and contributions to PPZ eu (generally <7 mg C · m⁻² · h⁻¹ and 15%, respectively) (Figs. 3A, B, C, D). Moreover, similar to the pattern in biomass, seasonal differences occurred only for ANP and AMP in 1996, with higher production and/or relative contribution to PPZ eu in summer for ANP, and in spring for AMP (Table 1, Fig. 3A, B). In 1997, ANP production peaked on April 9, May 21, July 30 and August 27, which, at times, coincided with peaks in their contribution to total PPZ eu or biomass. AMP highest production was noted in late July during this second study year (Figs. 2C, D; 3C, D).

3.4. Production-Biomass Diagram

The values of P%–B% for edible phytoplankton and ANP alone are plotted in Figure 4 as a function of sampling dates. The highest differences of the P%–B% values from the zero axis generally occurred in spring time and at the end of each study year. Marked negative P%–B% appeared in April-May (for edible algae, due to APP), in late August and mid-Sep-
Figure 3. Same as in Figure 2 but for primary production (PPZeu).

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September 1996. Substantial positive $P\% - B\%$ were noted in May–June and late July 1996 and in April–May and late August 1997, especially for ANP alone (Fig. 4). Note that opposite curves to those shown for edible algae will correspond to AMP curves.

3.5. Metazooplankton Production and Comparison with Phytoplankton

The mean total metazooplankton wet weight biomass in 1996 (68.33 µg ww · L$^{-1}$) was similar to that in 1997 (75.88 µg ww · L$^{-1}$) (Mann-Whitney U test, $p > 0.05$). This contrasts...
Figure 5. Seasonal changes in the biomass of metazoan zooplankton groups (A, B) and in the metazooplankton to edible phytoplankton production ratio (C, D) in 1996 and 1997 in the Sep Reservoir.
with the significant decrease in phytoplankton biomass from the first to the second study year (Table 1). In 1996, Cladocera, usually the most important metazooplankton group in terms of biomass in spring, were replaced in summer by Rotifers. The latter represented, on average 78% of total metazooplankton biomass (Fig. 5A). The proportion of Cladocera and copepods increased from 1996 to 1997 (from 28 to 49% for Cladocera and from 15 to 31% for copepods), and Cladocera were the most important group in both spring and summer 1997 (Fig. 5B).

As for metazooplankton total biomass, no significant interannual difference (Mann-Whitney U test, p > 0.05) was observed for the total metazooplankton production, which averaged 0.237 mg C · m⁻³ · d⁻¹ (range 0.015–0.651 mg C · m⁻³ · d⁻¹) in 1996 and 0.39 mg C · m⁻³ · d⁻¹ (range 0.12–1.56 mg C · m⁻³ · d⁻¹) in 1997. Similarly, the mean ratio of metazooplankton to edible phytoplankton production in 1996 (0.43%, range 0.06–1.13%) did not differ significantly from that in 1997 (0.76%, range 0.15–2.3%), although the relative quantitative importance of the different metazooplankton groups showed interannual changes. No consistent seasonal trend was observed in the temporal changes of this ratio. For the two study years, highest values were noted in spring, specifically during the April-May period (Fig. 5C, D).

3.6. Correlations between Variables

Strong negative relationships were found between the flushing of water at the outlet of the reservoir and the water level in the reservoir for the two study years ($r = -0.748$, $p = 0.0002$ in 1996 and $r = -0.755$, $p = 0.005$ in 1997). For this reason, only one of these variables (the water level) was used to examine relationships with nutrients and biological variables. Significant relationships with water level were found only for TN, ANP, AMP (in 1996 and 1997) and cladoceran biomass (in 1996 only) (Table 2). TN increased with water level during the two years. A similar trend was observed for cladoceran biomass (in 1996). In contrast to TN, ANP increased as the water level decreased during the two years. The relationship between AMP and the water level changed from positive in 1996 to negative in 1997.

### Table 2. Significant correlations (Pearson’s $r$) between changes in the water level and both nutrients and biological variables, and between the main phytoplankton variables and both nutrients ($n = 18$ in 1996 and 12 in 1997) and metazooplankton ($n = 12$ in 1996 and 1997) in the Sep Reservoir. Acronyms as in the text. For each correlation, except for those with water level, the Durbin-Watson statistic is given in the first brackets and the first order autocorrelation coefficient in the second (see text for explanations); $p < 0.05$.  

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<tbody>
<tr>
<td>ANP</td>
<td>$-0.748$</td>
<td>$-0.685$</td>
<td>$-0.779$</td>
<td>$-0.760$</td>
<td></td>
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<tr>
<td></td>
<td>$-0.663$</td>
<td>(1.57) (0.16)</td>
<td>(1.77) (0.02)</td>
<td>(1.36) (0.134)</td>
<td></td>
</tr>
<tr>
<td>Production/ biomass (ANP)</td>
<td>$0.721$</td>
<td></td>
<td></td>
<td></td>
<td>(2.13) ($-0.12$)</td>
</tr>
<tr>
<td>AMP</td>
<td>$0.678$</td>
<td>$0.668$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$-0.716$</td>
<td>(1.89) (0.018)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>$0.757$</td>
<td>$0.663$</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cladoceran biomass</td>
<td>$0.66$</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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The number of significant correlations between the main phytoplankton variables and TN, TP and metazooplankton was low. All these correlations were found in 1996 and involved only ANP and AMP (Table 2). ANP was negatively correlated with TN and both total cladoceran and *Daphnia longispina* biomass, while AMP was positively correlated with TN. The production to biomass ratio of ANP was significantly and positively correlated with cladoceran biomass. Among all the relationships that showed significant Pearson’s $r$, one (the negative correlation between AMP and rotifer in 1996, $r = -0.786$) exhibited $D (0.813)$ and $ACc (0.551)$ indicating autocorrelation. This relationship was not interpreted, since AMP was considered inedible for zooplankton.

4. Discussion

4.1. General Aspects and the Fate of AMP and APP

Autotrophic nanoplankton dominated the phytoplankton community during the two years of sampling, followed by microplankton, and by picoplankton which had the lowest and relatively constant contribution to total phytoplankton biomass and production.

The results presented here showed that the absolute value and the contribution of AMP to total phytoplankton were higher in spring than in summer 1996. During the latter period, the water column stability strongly decreased due to increased outflow of water from the reservoir (Fig. 1A). Although there was no seasonality in 1997, the marked negative values of $P%–B%$ for AMP in May–June 1996 and in March–May 1997 (opposite curves to those of edible algae, Fig. 4A, B) indicate that during spring for the two study years, the export of AMP relative to total phytoplankton was lower than their share of the primary production. Their accumulation (relative to edible algae) in the euphotic zone was thus greater. In May–June 1996, a bloom of the AMP species *Dinobryon divergens* and *Volvox aureus*, known to be resistant to zooplankton grazing, occurred in the euphotic zone of Sep Reservoir. In March–May 1997 the mucilaginous and colonial chlorophyte *Dictyosphaerium pulchellum* dominated the AMP community (TADONLÉKÉ, 1999; TADONLÉKÉ et al., 2000). These observations are consistent with the knowledge that unstratified or weakly stratified water columns are favorable for larger cells, whereas strong thermal stratification and/or decrease in water column stability are unfavorable for them (LEGENDRE and LE FÈVRE, 1989). The apparent contradiction to this, observed in August 1997 (Fig. 4B), is probably due the fact that the dominant AMP species at that time (*Botryococcus brauni*, a colony of small spherical cells of ~5 $\mu$m) is mucilaginous and positively buoyant. In general, we consider, according to their size and morphology (TADONLÉKÉ, 1999), that AMP cells were inedible for planktonic grazers and that their fate during this study was mainly through transitory accumulation in the surface waters and sedimentation.

During this study, we examined the biomass and the production of APP on a relatively long time scale and in the entire euphotic zone. Few studies dealing with APP in freshwaters have been undertaken with such spatial and temporal resolutions, although they are essential in understanding their importance and ecological role (STOCKNER et al., 2000). Both APP biomass and production were low and constant from one season to another and from the first to the second study year (Table 1). These results agree well with those from several nutrient-rich freshwater ecosystems (review in STOCKNER et al., 2000). Our APP data are also consistent with those reported by PINEL-ALLOUL et al. (1996) in 6 lakes of varying trophy in Quebec (Canada). These authors found that picophytoplankton outcompete larger cells at TP concentrations typically <10 $\mu g \cdot l^{-1}$.

The low relative importance of APP in nutrient-rich waters compared to oligotrophic waters has been partly related to the fact that when nutrients are abundant, APP cells lose their competitive advantage over larger cells for nutrient assimilation (TAKAHASHI et al., 1982). In the Sep
Reservoir, although nutrient concentrations were relatively high, APP absolute production was correlated, positively, with ANP + AMP absolute production (Fig. 6). A similar pattern has recently been reported by Agawin et al. (2000) for pelagic data sets from various geographical areas encompassing a wide range of nutrient conditions. These results support the idea that the low importance of APP in productive waters is only partly due to the fact that these cells lose their competitive advantage for nutrient assimilation in nutrient-rich systems. In warm productive waters, it has been suggested that the reduced importance of APP is, to a large extent, a result of grazing by protists (Weisse, 1993; Simek et al., 1995) or cell lysis (Heldal and Bratbak, 1991). Although APP biomass and production were relatively constant during our study, there were considerable variations from one sampling date to another in the absolute values of these variables (Table 1). Furthermore, most (6 out of 11) of the highest values [>4 mg C · (mg Chl)]⁻¹ · h⁻¹] of production to biomass ratio were from APP (June 12, August 7 and 28 in 1996 and May 21, July 16 and August 13 in 1997). This ratio, for APP, was positively correlated to ciliate abundance (data in Thouvenot et al., 1999a, b) in 1996 (r = 0.644, p < 0.05) and 1997 (r = 0.591, p < 0.05), but not to nutrients and incident light. These results suggest that the grazing processes were likely important in the fate of APP.

4.2. ANP Dynamics, and Implications for the Food Web Structure

P-B diagram (Fig. 4) suggests that, (i) the export of ANP cells markedly exceeded their share of primary production for the periods May–June and late July 1996 and March–May and August 1997, (ii) the rest of the time, the values of P%–B% were, in general, close to zero or the relative accumulation of ANP tended to be greater (late August and September 1996). We suspect that the loss and export of ANP during spring for the two study years, and in August 1997, were mainly through grazing. ANP biomass decreased obviously from

Figure 6. Relationships between the production of autotrophic picoplankton (APP) and that of nano (ANP) + microphytoplankton (AMP) in the Sep Reservoir (Y = 0.9516X – 0.953, r = 0.697, n = 30, Durbin-Watson D = 1.36, ACc = 0.148). Even when the two outliers (indicated by arrows) were removed, the relation remained significant (Y = 0.852X – 0.779, r = 0.513, n = 28, Durbin-Watson D = 1.47, ACc = 0.189; see text for explanations).

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April to mid-June 1996 (Figs. 1A, B), coincident with the replacement of Rotifers by Cladocera. ANP biomass was negatively correlated with the biomass of total Cladocera and the dominant *D. longispina* (Table 2). During this period (April to mid-June 1996), the highest production to biomass ratio [9.14 mg C/(mg Chl a)⁻¹ · h⁻¹] was recorded from ANP cells. The fact that the production to biomass ratio of ANP was correlated to cladoceran biomass (Table 2) and not to other potential controlling factors such as nutrients and light supported the idea that cladocera were important in determining grazing losses of ANP during this study. In the spring of the two study years and in August 1997, ANP communities mainly comprised cryptomonads (*TADONLÉKÉ et al., 2000*), which are known to be suitable food items for herbivorous zooplankton (review in *KLAVENESS, 1991*). The genus *Polyarthra*, which represented an important fraction of the rotifer biomass at the beginning of the study during the two years (*THOUVENOT et al., 1999a, b*), is also well known to preferentially feed on cryptomonads (*POURRIOT, 1977; GILBERT and BOGDAN, 1984*). The contention that ANP losses during these periods were through grazing is consistent with the occurrence of the clear-water phases in May 1996 and in April 1997 (*TADONLÉKÉ et al., 2000*).

Location of ANP data points around or largely below the zero axis during the rest of the study suggests that the relative export of ANP during these times was, in general, substantially lower than in spring (Fig. 4), even though during certain periods (summer 1996 for example), ANP comprised small edible algae such as *Cyclotella meneghiniana*, *Stephanodiscus* sp., and cryptomonads (*TADONLÉKÉ et al., 2000*). In summer 1996, Rotifers dominated metazooplankton in terms of biomass (Fig. 5A). These small metazoans, however, are generally less efficient than daphnids in controlling algal biomass (*LEHMAN, 1991*).

The grazing pressure on ANP was likely also low from mid-May to mid-July 1997, when ANP community was dominated by the chlorophytes *Golenkinia* sp. and *Neochloris* sp. (*TADONLÉKÉ et al., 2000*), which typically are surrounded by mucilage layers known to be an effective physical defense against grazers (*KLAVENESS, 1991*). The preponderance of these species coincided with a strong decrease in ANP absolute production (Fig. 3C) and the lowest values of total metazooplankton biomass (*THOUVENOT et al., 1999b*). These chlorophytes were later found in high abundance in sediment traps (unpubl. data), supporting the idea that they were not grazed.

Although the importance of Cladocera and Copepoda (which are thought to exert a stronger grazing pressure on algae than rotifers) relative to total metazooplankton increased from 1996 to 1997 during this study, the mean metazooplankton to edible phytoplankton production ratio (Pz : Pe) was similar for both years. These ratios (0.43% and 0.76% in 1996 and 1997, respectively) were at the low end of the values (0.1–27.4%) generally reported for freshwater ecosystems (*LE GREN and LOWE-MCCONNELL, 1980; MAKAREWICZ, 1985*). Following the impoundment of the experimental Lake 979 (Ontario, Canada), *PATERSON et al. (1997)* reported zooplankton to phytoplankton ratio values exceeding 100% and concluded that zooplankton could not obtain all their energetic needs from phytoplankton. From data presented by *ROBARTS et al. (1992)* for Rhenosterkop Reservoir two years after its flooding, we estimated the metazooplankton to phytoplankton production ratio in this South African reservoir at ~6.2%. To our knowledge, no other studies in newly flooded reservoirs are available for further comparisons. Values of euphotic zone primary production (PP) measured every three hours (and summed for calculation of the daily PP) during a 48 h cycle in July 1997 were not significantly different from those obtained during the same cycle by multiplying data from a single 3 h (10 h–13 h or 14 h–17 h) measurement by the ratio of the daily incident light to the incident light recorded during the *in situ* incubations of PP samples (data not shown). This and the fact that the daily incident light was relatively high during most of the sampling dates (*TADONLÉKÉ et al., 2000*) suggest that the daily euphotic zone PP as calculated for the present work did not strongly affect our Pz : Pe. Our low Pz : Pe suggest that overall, only a small proportion of edible phytoplankton was consumed by metazooplankton. These values are characteristic of systems where most of the phytoplankton pro-
Phytoplankton and Zooplankton in New Reservoirs

Table 3. Comparison of metazooplankton biomass in the Sep Reservoir with data reported in rivers, in other newly flooded reservoirs and in natural lakes with similar phytoplankton biomass. *exceptional value.

<table>
<thead>
<tr>
<th>Ecosystems</th>
<th>Periods</th>
<th>Phytoplankton biomass</th>
<th>Metazooplankton biomass</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chl a (µg · L⁻¹)</td>
<td>Wet weight (µg · L⁻¹)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(lake 979) (Canada)</td>
<td>After flooding (1993)</td>
<td>140</td>
<td>1980</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After flooding (1994)</td>
<td>610</td>
<td>2170</td>
<td></td>
</tr>
<tr>
<td>Rhenosterkop Reservoir</td>
<td>2 years after flooding</td>
<td>1.1–27</td>
<td>3333–26667*</td>
<td>ROBARTS et al., 1992</td>
</tr>
<tr>
<td>(South Africa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Natural lakes (U.S)</td>
<td></td>
<td>0.8–9.9</td>
<td>487–8247ab</td>
<td>CYR and PACE, 1992</td>
</tr>
<tr>
<td>31 rivers (Canada)</td>
<td></td>
<td>1.77–27.62</td>
<td>0.11–1368a</td>
<td>BASU and PICK, 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>75.06c</td>
<td></td>
</tr>
<tr>
<td>River Danube (Hungary)</td>
<td></td>
<td>50–170</td>
<td>8–80a</td>
<td>BOTHAR and KISS, 1990</td>
</tr>
<tr>
<td>Sep Reservoir (France)</td>
<td>1996 (1 year after the first flooding)</td>
<td>0.5–22.5</td>
<td>1401.4d</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>0.29–17.3*</td>
<td>457.5d</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Calculated from dry weight assuming that dry weight/wet weight = 0.15

b 82% of these values were >650

c Mean value

d From TADONLÉKÉ (1999)

Production is stored as non-desirable algal blooms (e.g. ADALSTEINSSON, 1979) or where high predation is exerted on zooplankton.

The values of total metazooplankton biomass in the Sep Reservoir were generally lower than those reported in lake systems with chlorophyll $a$ biomass similar to ours and in other newly flooded reservoirs, but similar to or higher than values reported in rivers (Table 3). We did not quantify fish abundance or biomass in the Sep Reservoir. Few fishes (mainly perch) were observed in the water column during our study. It is possible that predation by fishes partly explains the reduced metazooplankton biomass. However, it is unlikely that this predation was strong, as the reservoir was drawn down in 1995 and 1996, a situation which caused fish mortality. The increase in biomass and in the relative importance of large bodied zooplankton (Cladocera and Copepoda) in 1997, when the water column was physically more stable (compared to 1996) supports this view.

Indices of P limitation of phytoplankton were found in the Sep Reservoir in 1996 (TADONLÉKÉ et al., 2000). However the fact that the average number of eggs per ovigerous female of the dominant Daphnia longispina was significantly higher in 1996, when nutrients were less abundant in the reservoir, than in 1997 (THOUVENOT, 1999), suggests that phytoplankton-
Figure 7. Relationships between metazooplankton total biomass and total phosphorus in the Sep Reservoir in 1996 and 1997 (Durbin-Watson D = 2.51 and 2.28 while ACc = –0.04 and –0.26 in 1996 and 1997, respectively; see text for explanations).

One possible explanation for the observed low zooplankton to phytoplankton ratio in the Sep Reservoir is the change in hydrologic conditions. Development times of Cladocera and especially Copepoda are higher than for rotifers, phytoplankton and protists. These crustaceans might have been exported out of the reservoir before they have completed their development, as a consequence of the increase in the flushing rates from early July. Cladoceran biomass, for example, was significantly and positively correlated with changes in the water level (Table 2), and Rotifers dominated metazooplankton during the filling of the reservoir (i.e. at the beginning of the study each year, THOUVENOT et al., 1999a, b) and in summer 1996, when the rate of water outflow strongly increased (Figs. 1A, 5A). Zooplankton with longer generation times seem more susceptible than do phytoplankton to advective loss (PACE et al., 1992). However, as metazooplankton biomass was also low when the water level in the reservoir was relatively constant (April–July), we suspect that metazooplankton
inputs from the main reservoir influents might have been also low; unfortunately, we were not able to check this.

Another possible explanation for the observed low metazooplankton : phytoplankton ratio may be that the link between phytoplankton and metazooplankton was not direct and that microheterotrophs (e.g., ciliates) were important as phytoplankton grazers or direct food sources for metazooplankton in this reservoir. This is supported by at least three observations. First, both ciliate and phytoplankton biomass decreased from 1996 to 1997 and increased from

Figure 8. Relationships between ciliate abundance and (A) the biomass of the dominant daphnid *Daphnia longispina* \( (Y = -0.363X + 0.293, \ r = 0.797; \) Durbin-Watson \( D = 1.52, \ ACc = 0.168) \), (B) Copepod biomass \( (Y = -1.47X - 0.764, \ r = 0.866; \) Durbin-Watson \( D = 2.88, \ ACc = -0.447) \) in the Sep Reservoir in 1996 and 1997. See text for explanations. For Figure 8B, the two outliers indicated by arrow were not included in the regression. Ciliate data are from *THOUVENOT et al.*, (1999a, b).
1997 to 1998 (this study, Thouvenot et al., 1999a, b; Tadonléké and Sime-ngando, 2000), suggesting a trophic link between these two compartments. These empirical observations were supported by an experimental study in 1998, which showed that microzooplankton, largely dominated by ciliates, recycled an important fraction of nanophytoplankton production in the water column through grazing (Tadonléké and Sime-ngando, 2000). Second, for the two study years, metazooplankton biomass was significantly correlated, negatively, with total phosphorus (Fig. 7). If this indicates metazooplankton grazing or a high proportional P storage in metazooplankton biomass, the non significant relationship between phytoplankton and both total phosphorus and total metazooplankton biomass suggests that a significant fraction of the stored P was not from autotrophs. Third, the dominance of Cladocera in May–June 1996 also coincided with a strong decrease in ciliate abundance (Thouvenot et al., 1999a), and a significant negative relationship, slightly stronger than in the case of phytoplankton, was obtained between D. longispina and ciliates in 1996 (Fig. 8A). In addition, copepod (mainly Eudiaptomus gracilis followed by Cyclops vicinus) biomass was negatively correlated with ciliates in 1997 (Fig. 8B), when diatoms, which are known to generally have a dominant role in the nutrition of copepods (Kiorboe and Nielsen, 1994; Irgoien et al., 2000), were scarce in both the water column and the sediment traps (Tadonléké et al., 2000 and unpubl. data). Another support for this contention is that ciliate community comprised a high proportion of species such as Pela-gohalteria viridis and Strobilidium spp. (Thouvenot et al., 1999a, b). These species are reported to have the ability to jump very quickly, generally considered as an effective escape response against metazoan predators (Tamar, 1979; Jack and Gilbert, 1993).

Finally, our results showed that APP absolute biomass and production and their contribution to total phytoplankton were low and relatively constant from one season to another and from the first to the second study year. Our empirical evidence suggests that (i) generally a small proportion of edible phytoplankton was directly consumed by metazooplankton in 1996 and 1997 in the Sep Reservoir and (ii) the fate of the dominant nanophytoplankton was probably influenced by its species composition and by the relatively high discharge. Changes in hydrologic conditions in the reservoir, the possible low inputs of metazooplankton in the main influents of the reservoir, and the high potential role of microheterotrophs, are suggested as main explanations for the observed low metazooplankton to phytoplankton ratio.

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