Potential grazing impact of the mixotrophic flagellate *Ochromonas* sp. (Chrysophyceae) on bacteria in an extremely acidic lake

ANDREA SCHMIDTKE*, ELANOR M. BELL AND GUNTRAM WEITHOFF

DEPARTMENT OF ECOLOGY AND ECOSYSTEM MODELLING, INSTITUTE FOR BIOCHEMISTRY AND BIOLOGY, UNIVERSITY OF POTSDAM, MAULBEERALLE 2, D-14469 POTSDAM, GERMANY

*CORRESPONDING AUTHOR: andrschm@uni-potsdam.de

Received February 24, 2006; accepted in principle March 22, 2006; accepted for publication August 10, 2006; published online August 17, 2006

Communicating editor: A. Mitra

Flagellates are important bacterial grazers in most planktonic food webs. The prey-size preference of the mixotrophic flagellate, *Ochromonas* sp. (Chrysophyceae), isolated from an extremely acidic lake, Lake 111 (pH 2.6), was determined using fluorescently labelled microspheres (beads). According to grazing experiments with cultured bacteria, also isolated from Lake 111, the potential grazing impact on Lake 111’s single-celled bacterial production was calculated. *Ochromonas* sp. ingested the smallest beads offered (0.5 μm diameter) at the highest rate. Ingestion rate declined with increasing bead size. The highest prey volume-specific ingestion was measured for *Ochromonas* sp. feeding on intermediate-sized beads (1.9 μm). Ingestion rates were low due in part to the large fraction of inactive flagellates observed. According to the bacterial ingestion rate, a mean of 88% (epilimnion) and 68% (hypolimnion) of in situ single-celled bacterial production is potentially grazed daily by *Ochromonas* sp. In the epilimnion of Lake 111, the heterotrophic carbon gain is three times higher than the autotrophic production. Alongside carbon uptake, *Ochromonas* sp. also benefits from ingesting bacteria through the uptake of phosphorus. A biovolume minimum corresponding to the prey size at which *Ochromonas* sp. feeds most efficiently occurred in the Lake 111 epilimnetic bacterial community, implying top-down control of the bacterial community by *Ochromonas* sp.

INTRODUCTION

Mixotrophy, the ability of organisms to gain carbon from both photosynthesis and the uptake of organic matter, is a widespread phenomenon in protists (Jones, 2000). Pigmented flagellates, in particular, are able to combine photosynthesis with either the uptake of dissolved organic carbon (DOC) (osmo-mixotrophy) or the ingestion of particulate carbon (phago-mixotrophy). The ingestion of prey can be a means of obtaining nutrients (Caron *et al*., 1990) and specific growth factors (Skovgaard, 2000) or acquiring carbon during periods of low photon flux density or short photoperiods. The relative contribution of mixotrophic flagellates to the total phytoplankton biomass tends to increase when environmental conditions are unfavourable for their specialist competitors (Laybourn-Parry *et al*., 2000; Bell and Laybourn-Parry, 2003; Tittel *et al*., 2003).

In the extremely acidic lakes (pH < 3) of Lusatia, eastern Germany, the food webs are extremely species poor, dominated by bacteria, protists (mainly mixotrophic flagellates) and a few Metazoa. In these lakes, the mixotroph, *Ochromonas* sp., dominates the plankton, contributing between 45 and 80% to plankton biomass (Wollmann *et al*., 2000; Kamjunke *et al*., 2004). Autotrophic production in extremely acidic mining lakes including the study lake (Lake 111, pH 2.6, doi:10.1093/plankt/fbl034, available online at www.plankt.oxfordjournals.org
© The Author 2006. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org
Lake 111 (51°29’N, 13°38’E) was found to be low (Kamjunke et al., 2005). However, the measured heterotrophic contribution of Ochromonas sp. to its total production and the impact on the natural bacterial community of Lake 111 remained unknown.

In general, when a consumer dominates the community and few potential prey organisms are present, as is the case in the truncated food web of Lake 111, a pronounced impact of the consumer on its prey can be expected (Tittel et al., 1998). Phagotrophic phytoflagellates, in particular, often shape the size structure of natural bacterial communities, shifting the size spectrum towards larger, filamentous cells (Pace et al., 1990; Jürgens, 1994; Jürgens and Stolpe, 1995); they can also influence the taxonomic composition of bacterial communities (Langenheder and Jürgens, 2001; Jürgens and Matz, 2002).

To address the potential grazing impact of Ochromonas sp. on the bacterial production of Lake 111, we measured the ingestion rate using cultured bacteria. In order to estimate the size selectivity of Ochromonas sp., we used fluorescently labelled microspheres (beads). Special emphasis was put on determining the fraction of inactive, relative to actively grazing Ochromonas individuals. Subsequently, their total quantitative impact on the natural bacterial production and its size structure in Lake 111 were estimated.

**METHOD**

**Study site**

Lake 111 (51°29’N, 13°38’E) is situated in the Lusatian mining district of eastern Germany. The lake has a surface area of 10 ha and a mean depth of 4.6 m. The lake is dimictic (Wollmann et al., 2000). However, a monimolimnion exists at the deepest point of the lake at 9 m (Karakas et al., 2003). Lake 111 is characterized by its extremely low pH (2.6–2.9) and extremely high concentrations of iron, sulphate and aluminium. The high iron concentration pigments the water (red) and consequently modifies the vertical light spectrum throughout the water column (Koschorreck and Tittel, 2002). Moreover, DOC and total inorganic carbon (TIC) concentrations are low in the epilimnion (Packroff, 2000). In Lake 111, the total nitrogen (TN) concentration was 200–330 μM, and the total phosphorus (TP) concentration was 0.26–0.52 μM (Packroff, 2000). The planktonic food web is truncated (Wollmann et al., 2000; Kamjunke et al., 2004) consisting of only bacteria, two mixotrophic flagellates (the osmoxenotrophic chlorophyte Chlamydomonas acidophila Negro and the phago-mixotrophic chrysophyte Ochromonas sp.), protozoans (the Heliozoa Actinophrys sol Ehrenberg and very low concentrations of ciliates, chiefly Oxytricha sp.) and two rotifer species (Cephalodella sp. and Elosa worallii Lord). The mixotrophic flagellates dominate the biomass in the pelagic zone; Ochromonas sp. is the dominant flagellate in the epilimnion and does not display a diel vertical migration (Tittel et al., 2003), and C. acidophila dominates in the hypolimnion (Kamjunke et al., 2004). Heterotrophic flagellates and crustaceans are absent.

**Culture conditions**

Bacteria (strain 99P5, University of Potsdam) and Ochromonas sp. (strain 1B3, University of Potsdam) were isolated from Lake 111 and cultured in a medium that reflects the chemical environment of the lake (Bissinger et al., 2000). The medium is rich in iron (2.6 mM), aluminium (0.74 mM) and sulphate (13.74 mM) and has a pH of 2.65. The dissolved inorganic nitrogen concentration is 180 μM, and the dissolved inorganic phosphate concentration is 50 μM (Bissinger et al., 2000). The organisms were cultured at 20 ± 1°C in the dark (bacteria) or under a 16:8 h light : dark cycle at an irradiance of 73 μmole photons m⁻² s⁻¹ measured in air of the climate chamber (Ochromonas sp.). This is the photon flux density in the epilimnion on a bright summer’s day at a depth of 4.3 m, assuming an attenuation coefficient of 0.77 m⁻¹ (Kamjunke et al., 2004). The bacteria were maintained with glucose as a carbon substrate (20 mg C L⁻¹). Ochromonas sp. was grown mainly phototrophically without glucose addition, but a bacterial concentration of ~2 × 10⁵ mL⁻¹ was necessary to supplement the photosynthesis of the flagellate.

**Grazing experiments with beads**

In grazing experiments, it is desirable to use prey items that resemble the natural prey of the consumer. A common method is to stain bacteria, provide them as food particles and count the number of ingested bacteria per consumer using fluorescence microscopy (Sherr and Sherr, 1993). A major disadvantage is that common stains do not fluoresce under extremely acidic conditions. Modifying the procedure by Sherr and Sherr (Sherr and Sherr, 1993), we attempted to alkalize our samples after each grazing experiment to induce prey fluorescence, but no ingested bacteria could be detected in the Ochromonas cells. Therefore, we performed our grazing experiments with live, unstained, cultured bacteria for the estimation of the grazing impact, and we used fluorescently labelled microspheres (beads; Polysciences, Inc., Washington, PA, USA) to determine the prey-size preference of Ochromonas sp. Our experimental design for the estimation of the size preference was a modification of that...
described by Sherr and Sherr (Sherr and Sherr, 1993). Stock solutions of beads were pre-soaked in bovine serum albumin (1 mg mL\(^{-1}\); Pace and Bailiff, 1987) to prevent clumping and to improve palatability. The stock solutions were subsequently stored at \(-20^\circ\)C to discourage bacterial growth. Before a grazing experiment commenced, the stock solution was defrosted and sonicated to disperse the beads. Between 80 and 100 mL of the \(Ochromonas\) sp. culture (~1 \(\times\) \(10^4\) flagellates mL\(^{-1}\)) was pipetted into sterile Erlenmeyer flasks (300 mL) and allowed to recover from handling for 30 min. Subsequently, beads were added to the required concentration. Each feeding experiment was run in triplicate in the light (73 ± 3 \(\mu\)mole photons m\(^{-2}\) s\(^{-1}\) measured in air) and at a temperature of 20\(^\circ\)C, which falls within the \textit{in situ} epilimnetic summer temperature range. Sub-samples of 3 mL were taken after 0, 10, 20, 30, 40 and 50 min. Before sub-sampling, the Erlenmeyer flasks were gently mixed to achieve a homogeneous distribution of the beads. The sub-samples were added to pre-cooled sample bottles and fixed by adding 25% ice-cold glutaraldehyde to a final concentration of 2%. The sub-samples were then filtered onto 0.2 \(\mu\)m polycarbonate membrane filters (Whatman, Nucleopore) using low-pressure vacuum (<150 mbar). The flagellates on the filter were stained with 1 mL Proflavin (0.033 weight : volume) for 3 min, and the filter was mounted on a glass microscope slide. The slides were kept in the dark at \(-20^\circ\)C for a maximum of 3 days until they were analysed. For each sample, at least 200 cells were inspected under UV excitation using an epifluorescence microscope (Zeiss, Axioskop2, mercury vapour lamp, HBO 100W). The numbers of ingested beads per flagellate were counted and averaged over active and inactive \textit{Ochromonas} cells, that is, cells that had not ingested beads. If an \textit{Ochromonas} cell was observed to have ingested more than ten beads, an occurrence limited to our experiments with 0.5 \(\mu\)m beads, it became difficult to distinguish individual beads, and the number of ingested beads was designated as 11 beads cell\(^{-1}\). The fraction of flagellates ingesting >10 beads never exceeded 2.6\% of the total number of flagellates in any one sample.

In order to determine the functional response for ingestion, we used beads with diameters of 0.5 and 0.9 \(\mu\)m, representing the natural size of single-celled bacteria in lakes. According to Sherr and Sherr (Sherr and Sherr, 1993), the optimal concentration of labelled food particles is \(~30\%\) of the total natural bacteria abundance but should be \(~1-2 \times 10^6\) mL\(^{-1}\) for short-term uptake experiments with flagellates. Owing to the low mean bacterial concentration of ~0.5 \(\times\) \(10^6\) mL\(^{-1}\) in Lake 111 (Kamjunke \textit{et al}., 2004), the 30\% of natural population rule suggested by Sherr and Sherr (Sherr and Sherr, 1993) had to be disregarded for the three highest bead concentrations. We chose instead to employ concentrations of \(1 \times 10^5\), \(5 \times 10^5\), \(1 \times 10^6\) and \(5 \times 10^6\) mL\(^{-1}\) in our grazing experiments. To determine the effect of bead size on the ingestion rate of \textit{Ochromonas} sp., we used beads of 0.5, 0.9, 1.9 and 3.0 \(\mu\)m in diameter and measured the ingestion rates of \textit{Ochromonas} sp. on each bead size at a fixed bead concentration of \(1 \times 10^6\) mL\(^{-1}\).

### Grazing experiments with bacteria

It is known that protists can and do discriminate between beads and bacteria during grazing experiments (Pace and Bailiff, 1987; Nygaard \textit{et al}., 1988). Thus, experiments with bacteria (strain 99P5, University of Potsdam) were performed to estimate the grazing impact of \textit{Ochromonas} sp. on natural bacterial production in Lake 111. An \textit{Ochromonas} sp. culture was filtered onto 2-\(\mu\)m pore membrane filters (Whatman, Nucleopore) using a hand pump (Nalgene, Nityvac) with a low-pressure vacuum (<100 mbar) and washed twice with medium to reduce the bacterial concentration in the flagellate culture. The single-celled bacteria employed in the experiment were harvested at the saturation phase of cultivation. Erlenmeyer flasks containing ~80 mL of the glucose-free medium were inoculated with a bacterial abundance of \(5 \times 10^7\) mL\(^{-1}\), a concentration that falls within the range of the natural, single-celled bacteria abundance in Lake 111 (Kamjunke \textit{et al}., 2004). The flagellate abundance was ~1 \(\times\) \(10^4\) mL\(^{-1}\) equivalent to the concentration employed in the bead experiments. In order to measure the growth rate of bacteria in glucose-free medium, we also established flagellate-free controls. All treatments were run with three replicates at 20 ± 1\(^\circ\)C under a 16:8 h light–dark regime at a photon flux density of 73 ± 3 \(\mu\)mole photons m\(^{-2}\) s\(^{-1}\) measured in air. Samples were taken after 24 h and fixed with Lugol's iodine and sulphuric acid (to prevent iron precipitation). After filtering onto 0.2 \(\mu\)m Nucleopore filters (Whatman) and staining with acridine orange (Hobbie \textit{et al}., 1977), bacteria were enumerated and measured using an epifluorescence microscope (Axioskop2, Zeiss) and an eyepiece micrometer (New Porton grid G12). The flagellates were counted under an inverted microscope (Thallheim, Germany). At least 600 bacterial cells and 600 flagellates were counted for each replicate.

### Calculations and statistical analysis

The ingestion rate of beads was calculated from the linear regression of the change in the number of ingested beads (beads cell\(^{-1}\) over time (min\(^{-1}\)), with the assumption that \textit{Ochromonas} sp. ingests beads at the same rate as it takes up natural prey. We calculated ingestion rates in...
two ways: the particle-based ingestion rate (pIR) and volume-based ingestion rate (vIR). The first rate represents the number of ingested beads per flagellate and time (beads flagellate\(^{-1}\) h\(^{-1}\)), and the latter provides an estimate of the aggregate volume of the ingested beads per flagellate and time (\(\mu m^3\) flagellate\(^{-1}\) h\(^{-1}\)). All ingestion rates were calculated with respect to the ratio of beads to the total particle concentration in the samples (beads plus background bacteria).

The experimentally observed fraction of inactive *Ochromonas* cells was compared with the expected fraction, according to the calculations of González (González, 1999). Assuming a Poisson distribution of the number of ingested bacteria per flagellate, it is possible to calculate the ingestion rates by counting only the number of flagellates that had ingested beads. However, we reversed this approach and calculated the fraction of inactive flagellates from the measured ingestion rates, assuming a Poisson distribution. As the number of ingested particles per protist followed a negative binomial distribution rather than a Poisson distribution (McManus and Okubo, 1991; Cleven and Weisse, 2001), we also calculated the fraction of inactive grazer according to a negative binomial distribution. A chi-square test was used to compare the observed number of ingested beads per flagellate with the two expected data sets. If the expected values were less than one in a single category, values were amalgamated and categorized: ‘x beads per cell and more’ (Dytham, 2003).

The bacterial ingestion rate \((I)\) was calculated according the ‘presence–absence’ method of Peters (Peters, 1984), whereby \(I = FR \cdot C\). The bacterial concentration \((C)\) was calculated for the time of exponential increase:

\[
C = \frac{(N_t - N_0)}{(\ln N_t - \ln N_0)}
\]

where \(N_0\) and \(N_t\) referred to the bacterial concentration at the beginning and the end of the grazing time, respectively. The filtration rate (FR) was calculated as

\[
FR = V \cdot \frac{(\ln N_t - \ln N_0)(\ln N_{ak} - N_{bk})}{F \cdot \Delta t}
\]

where \(F\) represented the grazer abundance for the time of exponential increase \([F = \frac{(F_t - F_0)}{(\ln F_t - \ln F_0)}]\), where \(F_0\) and \(F_t\) referred to the concentration of flagellates at the beginning and the end of the grazing experiment, respectively and \(\Delta t\) the duration of the experiment. \(N_{ak}\) and \(N_{bk}\) were the bacterial concentrations at the beginning and the end of the treatment without grazing. The carbon content of the bacteria was calculated as a function of cell volume, according to Simon and Azam (Simon and Azam, 1989), and the bacterial ingestion rates were converted into units of carbon.

In order to estimate the potential grazing impact of *Ochromonas* sp. on the natural single-celled bacterial production in Lake 111, we calculated the carbon-based ingestion rate (pg C flagellate\(^{-1}\) day\(^{-1}\)) as a function of food particle volume. Our estimated carbon-based ingestion rate for *Ochromonas* sp. grazing on cultured bacteria likely represents an overestimation because their equivalent spherical diameter (ESD) was 0.93 \(\mu m\) compared with only 0.56 \(\mu m\) for the single-celled bacteria in Lake 111. According to our bead-size preference results (see Results), the vIR of *Ochromonas* sp. grazing on the 0.5 \(\mu m\) beads was 2.3 times lower than for the 0.9 \(\mu m\) beads. As the single-celled bacteria in Lake 111 and our cultured bacteria had the same ESD as these two bead sizes, respectively, we corrected our vIR for *Ochromonas* sp. grazing on cultured bacteria to minimize the overestimation. We divided the bacterial vIR by the same factor of 2.3 as we observed in the size-preference experiments. The corrected carbon-based ingestion rate was calculated with the mean per cell content of cultured bacteria (45 fg C cell\(^{-1}\)). The accurate per cell ingestion rates (pg C cell\(^{-1}\) day\(^{-1}\)) were subsequently calculated relative to the actual concentrations of *Ochromonas* sp. in the epilimnion (0.3, 2 and 4 m depth) and hypolimnion (6.5 and 8 m depth) of Lake 111. The lower temperature in the hypolimnion was not taken into account.

The concentration, size, and the per cell carbon content of the bacteria in Lake 111 were provided by N. Kamjunke (University of Potsdam, Germany); after staining with acridine orange, the bacteria were enumerated under a fluorescent microscope (Axioskop2, Zeiss), and the size of 200 bacterial cells was measured with an eyepiece micrometer (New Porton grid G12). The carbon content of the bacteria was calculated according to Simon and Azam (Simon and Azam, 1989). The single-celled bacterial production (\(\mu g C L^{-1} day^{-1}\)) was estimated using the bacterial biomass and seasonal mean of the bacterial production-to-biomass ratios presented in the work of Gaedke et al. (Gaedke et al., 2004), where the bacterial production was measured in *situ* using \(^{14}\)C-leucine, according to Simon and Azam (Simon and Azam, 1989). Subsequently, the daily single-celled bacterial losses (%) due to *Ochromonas* sp. grazing were computed for the period May to September for the years 2001 and 2002. The prey-size preference of *Ochromonas* sp. was compared with the bacterial cell size in Lake 111.

All statistical analyses were performed using SPSS software (version 12). One-way ANOVAs were used to test the influence of bead size on the ingestion rates, the
differences between the ingestion rates of the two smallest beads and bacteria and the differences between the grazing impacts within a year in the two water strata. The Tukey test was applied to make post hoc comparisons of the means. Differences between the ingestion rates on the two smallest bead sizes at different bead concentrations were tested using the t-test and differences between the grazing impact in the epilimnion and hypolimnion using the U-test.

RESULTS

Feeding time
The number of ingested beads per flagellate increased linearly from the start of the experiment until a plateau was reached. Depending on the bead size, this plateau was reached after about 30–50 min (Fig. 1).

Fraction of inactive grazers
In each experiment, the fraction of actively grazing Ochromonas sp. increased with increasing grazing time until saturation was achieved (data not shown). However, inactive Ochromonas cells were dominant in all experiments, their fraction ranging from 80 to 96% of total mixotrophs (Table I). In all experiments, the observed fraction of inactive Ochromonas sp. cells was higher than expected values based on calculations assuming a Poisson distribution of particle uptake. The observed frequency of 1.9 µm beads ingested per flagellate followed a Poisson distribution (P = 0.32); the observed frequency of ingested 3.0 µm beads differed significantly from a Poisson distribution (P = 0.02); the observed data from the two smallest bead sizes were highly significantly different from a Poisson distribution (P ranged from $5.9 \times 10^{-3}$ to $2.9 \times 10^{-288}$). The differences between the observed and expected values according to the Poisson distribution were dependent on bead size and density: the larger the bead size, the better the match between observed and expected values of inactive flagellates, and the higher the particle concentration of small beads (0.5 and 0.9 µm), the greater the discrepancy between observed and expected. Our observed frequencies of ingested beads per flagellate better fit a negative binomial distribution ($P > 0.05$), with the exception of the 0.5 µm beads at particle concentrations of $0.1 \times 10^6$ ($P = 0.004$) and $1 \times 10^6$ ($P = 6.1 \times 10^{-7}$) beads mL$^{-1}$. Consequently, the discrepancy between the observed and the expected values of inactive grazer was lower when predicted by the negative binomial distribution than by the Poisson distribution.

Functional response
Our data set did not allow us to observe a clear functional response of ingestion to the particle density range applied during the study (Fig. 2), suggesting that the food uptake of Ochromonas sp. was saturated at low food concentrations. The small 0.5 µm beads were ingested at a higher pIR than that of the 0.9 µm beads: the maximum uptake capacity of Ochromonas sp. for the 0.5 µm beads was 1.7 beads flagellate$^{-1}$ h$^{-1}$, compared with 0.7 beads flagellate$^{-1}$ h$^{-1}$ for the 0.9 µm beads.

Particle size-specific ingestion
The pIR of Ochromonas sp. decreased with increasing bead diameter of the beads (Fig. 3). The highest pIR was observed for the 0.5 µm beads and was 2.4–2.8 times higher than for the 0.9 and 1.9 µm beads. Ochromonas sp. exhibited the lowest pIR when provided with 3.0 µm beads. However, the vIR exhibited a different pattern. Very low vIR was estimated for the two smallest bead sizes: the ingestion rates of the 0.5 and 0.9 µm beads were significantly ($P < 0.05$) lower than for the 1.9 µm beads. Although Ochromonas sp. reached a high pIR when fed with 0.5 µm beads, the uptake of 1.9 µm beads resulted in the highest vIR for the flagellate. Despite the low ingestion rate of Ochromonas sp. on the 3 µm beads, their volume-specific ingestion was 5–10 times higher than those for the two smallest bead sizes.

Bacterial ingestion rates
The estimated bacterial ingestion rate of Ochromonas sp. was 1.17 (±0.62) bacteria cell$^{-1}$ h$^{-1}$ at the concentration of $5 \times 10^5$ bacteria mL$^{-1}$. The ingestion rates of the two

---

**Fig. 1.** Time-dependent ingestion of beads by Ochromonas sp. Error bars show standard deviation (SD), n = 3.
The data shown are from different bead sizes and bead concentrations. 1.9 and 3.0 µm bead experiments were only performed at one bead concentration, 1 × 10^6 beads mL⁻¹.

**Table 1**: Fraction (%) of the experimentally observed and expected inactive grazers after a 40 min grazing experiment according to two different distribution models [Poisson distribution (Gonzalez, 1999) and negative binomial distribution].

<table>
<thead>
<tr>
<th>Bead concentration (10^6 mL⁻¹)</th>
<th>0.1</th>
<th>0.5</th>
<th>1.0</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 µm beads</td>
<td>95.8</td>
<td>90.6</td>
<td>86.7</td>
<td>74.3</td>
</tr>
<tr>
<td>0.9 µm beads</td>
<td>96.4</td>
<td>90.6</td>
<td>86.7</td>
<td>90.6</td>
</tr>
<tr>
<td>1.9 µm beads</td>
<td>90.6</td>
<td>90.6</td>
<td>86.7</td>
<td>86.7</td>
</tr>
<tr>
<td>3.0 µm beads</td>
<td>96.4</td>
<td>90.6</td>
<td>86.7</td>
<td>86.7</td>
</tr>
</tbody>
</table>

Observ: observed; Poiss: Poisson distribution; NBD: negative binomial distribution.

Fig. 2. Density dependent (0.5–5 × 10^6 beads mL⁻¹) ingestion rate of *Ochromonas* sp. feeding on bacteria and on beads of 0.5 and 0.9 µm in diameter. Error bars show standard deviation (SD), n = 3.

Fig. 3. Particle-based (pIR, beads flagellate⁻¹ h⁻¹) and volume-based (vIR, µm³ cell⁻¹ h⁻¹) ingestion rates of *Ochromonas* sp. for different bead sizes at a bead concentration of 1 × 10^6 beads mL⁻¹. Error bars show standard deviation (SD), n = 3.

The smallest bead sizes (0.5 and 0.9 µm beads) at a bead concentration of ~5 × 10^5 beads mL⁻¹ and the bacterial ingestion rate were not significantly different. According to the mean carbon content of single-celled bacteria in culture (44.59 fg C cell⁻¹), the carbon-based ingestion rate was 1.25 pg C cell⁻¹ day⁻¹.

**Grazing impact**

According to the experimentally determined bacterial ingestion rate, the estimated percentage of *in situ* single-celled bacterial production potentially ingested daily by the *in situ Ochromonas* sp. population was calculated. The estimated grazing losses due to flagellate grazing varied from 24 to 225% (mean 88 ± 63% SD) in the epilimnion and from 22 to 168% (mean 68 ± 44% SD) in the hypolimnion during the summers of 2001 and 2002 (Fig. 4). In both years, the estimated percentage of
bacterial production ingested was not significantly different between the epilimnion and the hypolimnion, although both the abundance of *Ochromonas* sp. and single-celled bacterial production were higher in the epilimnion than in the hypolimnion (Table II). Between the years 2001 and 2002, no significant differences in the grazing impact were detected, but within a year significant seasonal changes in the grazing impact of the flagellate were detected in the epilimnion (*P* < 0.05). When the bacterial size spectrum of the natural bacterial community in the epilimnion of Lake 111 was considered, the biovolume of bacteria in the size classes between the 0.9 μm beads (volume 0.4 μm$^3$) and 1.9 μm beads (volume 3.6 μm$^3$) was lower in comparison with the adjacent size classes (Fig. 5). These size classes are close to the experimentally derived size preference of *Ochromonas* sp. This pattern was less pronounced in the hypolimnion where *Ochromonas* sp. was found in lower numbers.

**DISCUSSION**

**Grazing rates and the fraction of inactive grazers**

The ingestion rates of *Ochromonas* sp. determined using beads as food particles were in the same range as rates determined in other studies of *Ochromonas* sp. employing similar bead concentrations, with the exception of Pfandl et al. (Pfandl et al., 2004) (Table III). In general, the use of fluorescently labelled microspheres (beads) tends to underestimate the ingestion rates of flagellates because of their lower uptake affinity for beads compared with bacteria, faster egestion because of indigestibility (Pace and Baliff, 1987; Nygaard et al., 1988; Jurgens and DeMott, 1995) or lower cell flexibility (Wu et al., 2004). Our measured bacterial ingestion rates for *Ochromonas* sp. are still considered to be low compared with literature values (Table III). The *in situ* abundance of bacteria in Lake 111 was also low (Kamjunke et al., 2004), as is common for oligotrophic systems (Sommer et al., 2002). Thus, the saturation of bead uptake by *Ochromonas* sp. at low food densities can be considered an adaptation of this mixotroph to its low food environment.

Kamjunke et al. (Kamjunke et al., 2004) calculated the carbon flux within the food web of Lake 111 and found that *Ochromonas* sp. had a very low production-to-biomass ratio. The low ingestion rates measured during the present study coupled with the low *in situ* primary production previously determined (Kamjunke et al., 2005) support this. Such a low production-to-biomass ratio can only be maintained when population losses, for example, through predation, are also low. This is indeed the case in Lake 111; *Ochromonas* sp. supports neither the growth of the two rotifer species *Cephalodella* sp. and *E. worallii* (Weithoff, 2004, 2005) nor of the Heliozoa *A. sol* (Bell et al. 2006), their only potential predators in Lake 111.

Remarkably, a large fraction of the *Ochromonas* individuals observed in our experiments did not ingest any beads during the experimental period. González (González, 1999) observed a very wide range of inactive flagellates ranging from 0 to 93% in natural assemblages. We calculated the fraction of inactive flagellates from the measured ingestion rates assuming a Poisson distribution, the reverse approach to that of González’s (González, 1999). Our results demonstrated that most ingested beads per flagellate frequencies did not follow a Poisson
distribution; the observed fraction of inactive flagellates was higher than predicted in all experiments. In previous studies (McManus and Okubo, 1991; Cleven and Weisse, 2001), the number of ingested particles per grazer followed a negative binomial distribution. Our estimations of observed inactive grazer fraction were in the same range as these expected values, and thus, the negative binomial distribution proved a better approach for the calculation of inactive grazer. However, some of our ingested beads per flagellate frequencies followed neither a Poisson distribution nor a negative binomial distribution: the frequencies of ingested 0.5 µm beads per flagellate at two particle concentrations. Consequently, some actively feeding flagellates ingested disproportionately more beads than predicted by both equations. In fact, there was a large fraction of inactive cells and only a small fraction of active cells, but these few active cells ingested more 0.5 µm beads than predicted. The active Ochromonas cells ingested as many 0.5 µm beads as possible until a plateau was reached which was much lower for the larger bead sizes than for the 0.5 µm beads (Fig. 1). An Ochromonas cell can ingest fewer larger sized beads, and thus, the frequencies of the ingested larger sized beads better fit a negative binomial distribution. It should be noted that the frequency of ingested particles per protist is dependent on the particle size employed in the grazing experiments. The large fraction of phagotrophically inactive flagellates coupled with the few very active cells suggest that individual Ochromonas cells in general, or at least temporarily, favour one mode of nutrition over the other. This view is supported by the increasing difference between observed and expected fraction of inactive flagellates (according to the Poisson distribution) with increasing bead concentration. A similar discrepancy between the observed and expected fraction of inactive flagellates was recorded by McManus and Okubo (McManus and Okubo, 1991) for flagellates from oligotrophic systems.

**Size selectivity and grazing impact on the natural bacterial community**

Most flagellates are known to be size-selective feeders (Chrzanowski and Šimek, 1990; Hahn et al., 1999; Hammer et al., 1999; Jürgens and Matz, 2002). In general, the pIR of Ochromonas sp. from Lake 111 decreased with increasing bead diameter, and thus the flagellate exhibited the highest pIR for the 0.5 µm beads, the smallest food item offered. Compared with other Ochromonas strains, the Ochromonas sp. from Lake 111 has a lower prey size optimum (Posch et al., 1999; Jürgens and Matz, 2002). Food size selection or preference is purported to be a passive rather than an active selection mechanism because increasing the size of the prey

### Table II: Mean abundance (±SD) of Ochromonas sp. (Kamjunke et al., 2004) and single-celled bacterial production in Lake 111 from May to September in 2001 and 2002 (Gaedke et al., 2004)

<table>
<thead>
<tr>
<th>Year</th>
<th>Ochromonas sp. (cells L⁻¹)</th>
<th>Single-celled bacterial production (µCl⁻¹ day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epilimnion</td>
<td>Hypolimnion</td>
</tr>
<tr>
<td>2001</td>
<td>5.0 × 10⁶ (± 2.5)</td>
<td>2.2 × 10⁶ (± 1.2)</td>
</tr>
<tr>
<td>2002</td>
<td>6.8 × 10⁶ (± 3.4)</td>
<td>4.8 × 10⁶ (± 2.8)</td>
</tr>
</tbody>
</table>

**Fig. 5.** Biovolume-size spectrum of the natural bacterial population in the epilimnion (above) and the hypolimnion (below) of Lake 111. The cell volumes of the specific size classes were summed and then averaged over depth for the period May to September 2001 and 2002. Vertical lines represent the corresponding volume of the 0.9 µm beads (left) and 1.9 µm beads (right).
Table III: Ingestion rates of different Ochromonas species feeding on (1) similarly sized beads to those employed in our experiments and (2) bacteria

<table>
<thead>
<tr>
<th>Ochromonas sp. (Lake 111)</th>
<th>Food concentration (particle mL⁻¹)</th>
<th>Ingestion rate (particle cell⁻¹ h⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–5 × 10⁶ beads</td>
<td>0.5–1.5 beads</td>
<td>This study, size preference</td>
</tr>
<tr>
<td>Ochromonas sp. (CCMP 583)</td>
<td>1 × 10⁶ beads</td>
<td>0.09 beads</td>
<td>(Keller et al., 1994)</td>
</tr>
<tr>
<td>Ochromonas minuta</td>
<td>5 × 10⁶ beads</td>
<td>0.8 beads</td>
<td>(Sanders and Porter, 1988)</td>
</tr>
<tr>
<td>Ochromonas danica</td>
<td>7.7 × 10⁶ beads</td>
<td>1 bead</td>
<td>(Sanders and Porter, 1988)</td>
</tr>
<tr>
<td>Ochromonas sp. DS</td>
<td>4.2 × 10⁶ beads</td>
<td>26.8 beads</td>
<td>(Pfandl et al., 2004)</td>
</tr>
<tr>
<td>Ochromonas sp. (Lake 111)</td>
<td>0.5 × 10⁶ bacteria</td>
<td>1.2 bacteria</td>
<td>This study, grazing impact</td>
</tr>
<tr>
<td>Ochromonas sp.</td>
<td>2.5 × 10⁶ bacteria</td>
<td>11.2 bacteria</td>
<td>(Boenigk et al., 2002)</td>
</tr>
<tr>
<td>Ochromonas sp.</td>
<td>2–10 × 10⁶ bacteria</td>
<td>10–120 bacteria</td>
<td>(Zubkov et al., 2001)</td>
</tr>
<tr>
<td>Ochromonas sp. DS</td>
<td>20.4 × 10⁶ bacteria</td>
<td>27.8 bacteria</td>
<td>(Wu et al., 2004)</td>
</tr>
</tbody>
</table>

particle increases the encounter rate between predator and prey (Boenigk et al., 2001, 2002). In our experiments, all bead sizes were offered with the same bead concentration, but the highest vIR of Ochromonas sp. was observed for the 1.9 µm beads, that is, per unit of time Ochromonas sp. ingested the greatest volume of food when these larger beads were offered. Therefore, the mechanism found by Boenigk et al. (Boenigk et al., 2001, 2002) might be of lesser importance in our study. In agreement with the highest estimated vIR for the 1.9 µm beads, the biovolume of the natural bacteria in the epilimnion of Lake 111 in the size classes between the 0.9 and 1.9 µm beads was lower when compared with adjacent size classes in Lake 111. This suggests a strong, top-down grazing impact of Ochromonas sp. on bacteria within this size range. The size classes of the natural bacteria were expressed in terms of volume, but their shape is different from the beads. The larger, natural bacterial size classes in Lake 111 also comprise short filamentous bacteria that might be handled less efficiently than spheres, leading to a preference for smaller particle sizes than suggested by our grazing experiments. Indeed, in the hypolimnion, where Ochromonas sp. densities are significantly lower than in the epilimnion, the bacterial biovolume minimum is less pronounced and slightly shifted towards smaller bacterial cell sizes. In Lake 111, Ochromonas sp. is the dominant bacterivore; therefore, a pronounced top-down control of the bacterial community size spectrum is to be expected (Jurgens and Matz, 2002). However, such an effect is not restricted to flagellate–bacteria relationships; it also occurs at higher trophic levels. For example, a similar scenario was observed during the clear-water phase in neutral lakes in which cladocera are the dominant consumers of phytoplankton: Tittel et al. (Tittel et al., 1998) found that high Daphnia densities cause a significant decline in biomass in the size classes of the phytoplankton of their preferred prey-size range.

In Lake 111, the estimated daily in situ grazing losses of single-celled bacterial production due to the grazing by Ochromonas sp. ranged from 22 to 225%, depending on season and lake stratum. From May to September, an average of 88% of the daily bacterial production in the epilimnion is consumed per day by Ochromonas sp. This equals a heterotrophic carbon gain of ~6 µg C L⁻¹ day⁻¹ and is three times higher than autotrophic production (Kamjunke et al., 2004). The high heterotrophic carbon uptake relative to the autotrophic production, along with the high phosphorus content of bacteria relative to that of flagellates (Brathak and Thingstad, 1985), suggests that Ochromonas sp. covers its phosphorus demand through the ingestion of bacteria. In the hypolimnion, the grazing impact is likely to have been overestimated; as Ochromonas sp. is the dominant flagellate in the epilimnion, the lower temperature in the hypolimnion was not taken into account during our grazing experiments. The temperature in our experiments was higher than the summer hypolimnetic temperatures in Lake 111 (Karakaş et al., 2005), and it is therefore likely that the physiological activity of Ochromonas sp. and consequently its ingestion rate are lower in situ than we estimated. Furthermore, there is evidence that Ochromonas sp. also feeds on Chlamydomonas acidophila in Lake 111, although C. acidophila is similar in size to Ochromonas sp. and, thus, much larger than the experimentally derived preferred prey size (Kamjunke et al., 2004). The ingestion of a single C. acidophila cell offers Ochromonas sp. huge gains in terms of carbon/energy; thus, C. acidophila can be considered a suitable carbon subsidy for Ochromonas sp. even when ingested at very low rates. Nevertheless, food web analyses suggest that this trophic relationship is quantitatively of lesser importance than the Ochromonas–bacteria link
ACKNOWLEDGEMENTS

We thank Norbert Kamjunke and Jörg Tittel for providing data on bacteria and flagellates from Lake 111. Alexander Wacker, Elly Spijkerman, Jörg Tittel and Norbert Kamjunke offered valuable comments on an earlier version of the manuscript. A.S. conducted this work as part of an M.Sc. project funded by The University of Potsdam. E.M.B. was funded by a European Union Marie-Curie Host Fellowship (EVK1-CT-2000-56006) and the Deutscher Akademischer Austauschdienst: International Quality Network. Part of this work was funded by the German Ministry of Education and Research, Grant no. 0339746.

REFERENCES


