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Exploring strategies for nanoflagellates living in a 'wet desert'

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ABSTRACT: We investigated the survival strategies and the competitive strength of pigmented and colourless bacterivorous flagellates using 2 model organisms, the bacterivorous chrysophytes *Poterioochromonas malhamensis* and *Spumella* sp., fed small ultramicrobacteria (0.044 µm^3) and large bacteria (0.38 µm^3). The numerical responses indicated that small bacteria are not such a good food source as large bacteria, even when bacterial cell volume was taken into account. The growth rates of the heterotrophic *Spumella* sp. exceeded those of the mixotrophic *P. malhamensis* at high bacterial concentrations of both sizes of bacteria. At very low prey abundances, *P. malhamensis* grew better than *Spumella* sp., mainly because the mortality rate of the latter was very high. At bacterial biovolumes encountered in oligotrophic/mesotrophic lakes, the growth curves of the 2 flagellates intersected. Although *P. malhamensis* possesses a chloroplast, growth of cultures incubated in the light and the dark did not differ even at very low prey concentrations, indicating that photosynthesis plays a minor role for this chrysophyte. Experiments carried out with mixed flagellate cultures showed that *P. malhamensis* ingested *Spumella* sp. cells. Applying our data to natural abundances of small and large bacteria as well as pico/nanoplankton we could demonstrate that *P. malhamensis* is a *K*-strategist. Large bacteria made up the major portion of its carbon uptake, but small bacteria, pico-nanoplankton and (to a minor extent) photosynthesis also contributed to its gross carbon uptake, indicating a broad use of different resources by this species. *Spumella* sp. is more of an *r*-strategist and relies mainly on large bacteria for growth although small bacteria can contribute significantly to its diet.

KEY WORDS: Chrysophytes · Microbial food web · Numerical response · Starvation · Light · pH

INTRODUCTION

Bacterivorous flagellates comprise a large number of taxonomically different organisms with various growth and survival strategies ranging from mainly photosynthetic flagellates (e.g. *Uroglena*) that only take up very small numbers of bacteria, probably as a vitamin source, to entirely heterotrophic flagellates (e.g. *Spumella* spp.). Between these 2 extremes are organisms that utilise both photosynthesis and phagotrophy to different extents depending on the environmental conditions experienced (e.g. Jones 1994, Stoecker 1998). Growth and survival strategies are therefore highly diverse, but in many cases have not been studied in detail. Despite the importance of the flagellates, our knowledge on their ecology is limited, and they are often lumped together in large functional groups, i.e. heterotrophic nanoflagellates, and autotrophic nanoflagellates (Azam et al. 1983, Sherr & Sherr 2002, Weisse 2004). Furthermore, our knowledge of flagellate survival strategies is sparse and even contradictory, mainly due to basic methodological limitations and conceptual problems. Specifically, the coexistence of taxa with similar modes of nutrition (i.e. taxa that should be reciprocally exclusive according to the niche separation concept) cannot be explained.

Some basic knowledge on the growth and feeding rates of bacterivorous flagellates has been gained by laboratory studies, but many of these have been carried out under conditions far removed from those the flagel-

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lates experience in nature. For instance, planktonic bacterivorous flagellates can achieve very high growth rates when grown in the laboratory with excess food, and doubling times of 4 to 6 h are not unusual at temperatures of 15 to 20°C (Hansen et al. 1997). Such high growth rates are, however, never or rarely achieved in nature, mainly because of food limitation related to bacterial abundance and/or size. The great majority of natural bacteria in oligotrophic/mesotrophic marine and freshwater habitats (i.e. 'ultramicrobacteria' between 0.01 and 0.05 μm^3 in size: Hahn et al. 2003, Cottrel & Kirchman 2004, are much smaller than the bacteria commonly used as prey for flagellates in laboratory experiments (0.25 to 0.5 μm^3; e.g. Rothhaupt 1996b, Pålsson & Daniel 2004). Thus, even though bacterial abundances in laboratory experiments are close to those in nature, bacterial biovolume usually exceeds natural conditions many-fold because experiments are typically performed with cultured bacteria that are larger than those found in plankton. In contrast to laboratory conditions, flagellates in the field can be assumed to be severely food-limited, i.e. to live in a 'wet desert'. Except for a few studies, strategies to cope with these food-limited conditions have been largely unexplored. The general ability of heterotrophic nanoflagellates to prey on ultramicrobacteria was recently demonstrated in laboratory experiments by Boenigk et al. (2004), but numerical response data on flagellates preying on ultramicrobacteria are lacking. Until recently, the typical field situation (low bacterial biovolume despite fairly high bacterial abundance) could not be created in laboratory studies. In many cases this probably led to biased estimates of grazing rates and parameters of functional and numerical responses. Specifically the ability of flagellates to cope with starvation has received very little attention. How do the flagellates react when prey concentrations become so low that their ingestion rates do not meet their maintenance requirements? Their typical response to starvation seems to be continuation of cell division for 1 or 2 generations but with smaller than normal cells, a phenomenon often referred to as residual divisions. In addition, they tend to decrease their respiration rates to values that are only 2 to 5% of the rate for growing cells (e.g. Fenchel 1982).

Using a model system of the pigmented nanoflagellate *Poterioochromonas malhamensi* and its closely related abundant heterotrophic analog *Spumella* sp. (Boenigk 2005) we have (1) compared survival strategies of flagellate taxa coexisting in the field, (2) estimated the contribution of different food sources to the nutrition of these flagellates and (3) demonstrated to what extent biased expectations and the current limitations in mimicking natural conditions in laboratory studies lead to misinterpretation of flagellate survival strategies. We have further demonstrated the potential of recent methodological advances to tackle these current problems. The growth and starvation responses of the 2 flagellates were studied alone and in mixed cultures. For this purpose we established axenic cultures of these organisms, both of which originated from oligotrophic/mesotrophic lakes. We then studied (1) the numerical response of *P. malhamensis* and *Spumella* sp. fed either an ultramicrobacterium or a large bacterium in the dark, (2) competition between *P. malhamensis* and *Spumella* sp. in the light and in the dark when fed either the ultramicrobacterium or the larger bacterium, and (3) their growth and survival when subjected to starvation.

MATERIALS AND METHODS

Origin of strains and culture conditions. The flagellate *Poterioochromonas malhamensis* Strain DS originated from mesotrophic Lake Constance, Germany. This strain has been used in a variety of laboratory studies under the name *Ochromonas* sp. (e.g. Hahn & Höfle 1998, Boenigk & Arndt 2000) and under the name *Ochromonas* sp. Strain DS (Boenigk et al. 2002, 2004, Hahn et al. 2003). The *Spumella* sp. Strain JBM10 (GenBank Accession No. AY651074) was isolated from oligomesotrophic Lake Mondsee, Austria. The *Spumella* sp. Strain JBM10 clusters with the Chrysophycean Subcluster C3 which comprises flagellates of the *Spumella* morphotype from aquatic environments (Boenigk et al. 2005). Despite a high molecular variation in this taxon (see present 'Discussion', Boenigk 2005, K. Pfandl & J. Boenigk unpubl. data) this strain can be considered typical for flagellates affiliated with the *Spumella* morphotype with respect to the ecophysiological factors investigated, i.e. feeding characteristics and starvation tolerance.

We used 2 laboratory-grown bacterial strains as food organisms, an ultramicrobacterial strain and a large bacterial strain: the ultramicroobacterium Strain MWH-NR1 affiliates with the monophyletic D subcluster of the *Polynucleobacter* group and originates from Lake Mondsee (Hahn & Höfle 1998; Hahn 2003); the gammaproteobacterium *Listonella pelagia* Strain CB5 (GenBank synonym *Vibrio pelagius*) originates from Lake Constance. All flagellate and bacterial strains originate from oligotrophic/mesotrophic lakes and represent abundant freshwater organisms (Happey-Wood 1976, Bird & Kalff 1989, Bennet et al. 1990, Hahn 2003, Boenigk 2005, Hahn et al. 2005).

Flagellate size (n > 50 cells) was determined from Lugol-fixed cells at 1000× magnification from a video screen connected to an inverted video microscope. Bacterial size (n > 100 cells) was measured after DAPI

staining at 1250× from a video screen using the Lucia image-analysis software package (Lucia 4.51; resolution, 750 × 520 pixels; 256 grey levels; Laboratory Imaging). At the start of the experiments the cell volume of the flagellates *Poterioochromonas malhamensis* and *Spumella* sp. was 254 ± 120 and 100 ± 41 μm^3 (after Lugol fixation), respectively. Cell volume of the ultramicrobacterium and the large bacterium was 0.044 ± 0.017 and 0.38 ± 0.2 μm^3, respectively.

The bacteria were grown in NSY medium (3 g organic matter l^{-1}; Hahn et al. 2003) prior to the experiments. During permanent culture both flagellate strains were grown axenically on heat-killed large bacteria (*Listonella pelagia* Strain CB5) at 16°C but transferred to organic NSY medium (Hahn et al. 2003) 1 wk before the experiments to remove particulate food. Flagellates were adjusted to the experimental conditions (specifically temperature and illumination) 48 h before the experiments were started.

Experimental media. For the experiments we used 3 different media. For estimation of growth and competitive strength under conditions comparable to the field situation we used sterile filtered lake water (pH 8.2) from Lake Mondsee (the origin of 2 of the 4 organisms used in this study) which was prepared as follows: immediately after collection the lake water was filtered (0.2 µm) into 1 l Schott flasks using a vacuum pump. The water was heated (microwave 900 W) for 7 min 30 s, cooled in an ice bath, reheated, and finally cooled to the experimental temperature (16°C).

In some cases artificial media were used for control experiments, comprising NSY inorganic basal medium (Hahn et al. 2003) at different pHs (see subsection on effect of pH below) and a WC medium (Guillard & Lorenzen 1972) modified as follows: 42.5 mg l^{-1} $NaNO_3$; 4.35 mg l^{-1} K_2HPO_4; 31.5 mg l^{-1} $NaHCO_3$; 0.13 mg l^{-1} H_3BO_3; 0.115 g l^{-1} TES buffer.

Fixatives and enumeration of organisms. As fixatives we used either PBS-buffered Lugol's solution (2.3 ml PBS to 97.7 ml Lugol; 100 µl per 5 ml subsample) or formaldehyde (2% final concentration) mixed with PBS-buffered Lugol's solution. Bacteria were counted after formaldehyde fixation, DAPI staining and filtration onto 0.2 µm, black, Nuclepore filters under an epifluorescence microscope. Flagellates were counted either after Lugol fixation in Sedgewick Rafter chambers at 200× magnification under an inverted microscope, or after Lugol/formaldehyde fixation under an epifluorescence microscope. Prior to filtration, 500 µl thiosulfate was added to exclude interference of the Lugol with chlorophyll autofluorescence. In the experiments where both flagellates were present, chlorophyll autofluorescence was used to differentiate *Poterioochromonas malhamensis* and *Spumella* sp.

Experimental set-up. ***Growth of Poterioochromonas malhamensis and Spumella sp. feeding on different food sources:*** In the first set of trials (Expt 1) the effects of light, bacterial food size and concentration were studied in uniflagellate and mixed cultures. All organisms were collected by centrifugation (5000 × *g* for 8 min) and subsequently washed with sterile medium 4 to 5 h before the experiments. Centrifugation resulted in a pellet, but the flagellates easily resuspended and swam away. We found no indications of increased cell damage or disturbed behaviour. The flagellates were washed 3 times with sterile inorganic medium to remove dissolved organic substances. Experiments were carried out in which the 2 flagellates were fed the ultramicrobacterium and the large bacterium separately. Total flagellate abundance at the start of the experiment was around 1000 flagellates ml^{-1}. We further tested a mixed culture of the flagellates (~700 cells of each species ml^{-1}) fed the ultramicrobacterium and the large bacterium. Bacterial abundance was adjusted to values representing low and high field abundances (0.8×10^6 and 8×10^6 bacteria ml^{-1}, respectively). All experiments were conducted using sterile lake water both in the light and in the dark, except for the treatments without *P. malhamensis* (the treatments without photosynthetically active organisms). This set-up thus yielded 20 different treatments, i.e. a total of 60 (3 replicate treatments each). In addition, experiments without bacterial food ('no food treatment') were set up for both flagellates in triplicate. The initial experimental volume was 50 ml in 100 ml Schott flasks. Experimental conditions were 16°C and continuous light (200 µE m^{-2} s^{-1}) without shaking. Dark treatments were kept in aluminium foil; 5 ml subsamples were taken every 3 h for 21 h and fixed for subsequent analysis. The 'no food' treatments were further sampled every 3rd to 7th day for 2 mo (see next subsection for details).

In the second set of trials (Expt 2), the growth of both flagellates using 2 different types of bacterial prey were studied for a wider prey concentration range. Growth of the flagellates was tested at different bacterial abundances between 0.1 and 200 × 10^6 bacteria ml^{-1} and for a 'no food' control. Flagellates were adapted to the respective food conditions 24 h before the experiment started. All experiments were run in NSY inorganic basal medium at 16°C in the dark. Initial experimental volume was 50 ml in 100 ml Schott flasks. Experiments were set up 3 h prior to the first sampling and food concentrations were adjusted by either dilution or food addition. The initial abundance of flagellates was adjusted to $1–2 \times 10^3$ flagellates ml^{-1}. Subsamples were taken every 3 h for 15 h, fixed and counted by epifluorescence microscopy or by an ordinary inverted microscope using a Sedgewick Rafter

chamber. Growth rates were calculated from the exponential growth phase onwards.

Effects of pH, medium and dissolved organic components on starvation and mortality: Unexpected results on the growth and survival of *Poterioochomonas malhamensis* in both the light and dark in lake water led us to run some additional experiments using artificial media to help interpretation of the data. Starvation experiments were carried out on *Spumella* sp. in the dark and on *P. malhamensis* in both the light and the dark. The flagellate cultures were grown on organically enriched NSY medium, and 24 h before initiation of the experiments we washed the culture 3 times in the medium used in the respective experiments in order to remove all food sources. Flagellate abundance at the start of the experiment was adjusted to 1000 flagellates ml^{-1}. All experiments were run in triplicate at 16°C in the dark as well as in the light. Initial experimental volume was 70 ml in 100 ml Schott flasks.

We used WC medium (organic buffer, 119.36 mg l^{-1}; pH 6.9 and 8.8) and NSY medium (organic buffer, 4.4 mg l^{-1}; pH 6.8 and 8.5). Further, NSY inorganic basal medium supplemented with 0, 20 and 200 mg l^{-1} of organic matter (equal amounts of nutrient broth, soyotone, peptone and yeast extract [Difco]) was used to assess the effects of dissolved organic carbon. These experiments were sampled every day during the initial phase and every 3rd to 7th day for 2 mo thereafter. Experiments were regularly checked for bacterial contamination by either (1) fixing small samples, staining with DAPI and subsequently checking by epifluorescence microscopy after filtration onto 0.2 µm polycarbonate filters, or (2) adding small samples to bacterial growth medium and visually checking for bacterial growth. In no cases were the cultures contaminated. Flagellates were fixed and counted as described above.

Non-linear regression and statistics. Data from the 2 growth experiments in which the flagellates were tested separately were pooled. Data corresponding to prey biovolumes in the range of 0 to 2.5 and 8×10^6 μm^3 ml^{-1} for the small and large bacterium, respectively, were then used to calculate the numerical response using Michaelis-Menten kinetics:

$$\mu = \mu_{max} (P - x')/(K_m + P - x')$$

where μ = growth rate d^{-1}, μ_{max} = maximum growth rate d^{-1}, P = prey biovolume in 10^6 μm^3 ml^{-1}, K_m = half-saturation constant for growth, and x' = threshold concentration for growth. Ingestion rates of *Poterioochromonas malhamensis* feeding on *Spumella* sp. were calculated from growth rates of *Spumella* sp. in the mixed treatments compared to the control treatment (*Spumella* sp. only) according to the formula:

$$I = (\mu_{control} - \mu_{mixed}) \times N_{prey}/N_{predator}$$

where $\mu_{control}$ = the growth rate of *Spumella* sp. without *P. malhamesis*, μ_{mixed} = the growth rate of *Spumella* sp. with *P. malhamensis* and N_{prey} and $N_{predator}$ = the respective geometric mean abundance of predator and prey. All statistical tests, specifically the ANOVA and Student's *t*-tests for comparison of growth rates, mortality rates and grasing rates, were performed using the software package SigmaStat 2.03.

Calculations of carbon uptake from different sources. The potential contribution of different carbon sources at natural levels of irradiance, concentrations of bacteria and nanoflagellates for the 2 flagellates was calculated using a number of conversion factors and relationships. Ingestion rates were calculated from growth rates as:

$$I = \mu \times P_{car} / Y$$

where μ = the exponential growth rate (d^{-1}), P_{car} = predator carbon content and Y = growth yield. A growth yield of 0.3 was used (i.e. 30% of carbon taken up is used for growth; Hansen et al. 2000). The amount of carbon uptake required to balance mortality rate was estimated from exponential mortality rates when starved as: M = mortality rate (d^{-1}) × predator carbon content. Carbon content of *Poterioochromonas malhamensis* and *Spumella* sp. used in the calculations were 50.4 and 20 pg C $cell^{-1}$, respectively, assuming a conversion factor of 20 fg C μm^{-3}. We used the conversion factor from Wetzel & Likens (1991) as this factor has been applied by other investigators working on *P. malhamensis* (e.g. Pålsson & Daniel 2004) and closely matches recently suggested carbon conversion factors (e.g. Menden-Deuer & Lessard 2000).

RESULTS

Expt 1: Growth of *Poterioochromonas malhamensis* and *Spumella* sp. in light and dark

The growth rates of both flagellates were lower when feeding on the ultramicrobacteria than when feeding on the large bacteria (Table 1, Fig. 1). This difference was largely due to the difference in bacterial cell volumes, i.e. to the available bacterial biovolume. Nevertheless for comparable ingested biovolumes, growth rate was always higher for flagellates feeding on the large bacterium (Table 1, Fig. 1). Light, however, had no effect on the growth of *Poterioochromonas malhamensis* in our experiments (ANOVA, Tukey's post-hoc test, $p >> 0.05$ for pairwise light/dark comparisons under all food conditions, Table 1). For further analyses, light and dark treatments were there-

Table 1. *Poterioochromonas malhamensis* and *Spumella* sp. Expt 1: Growth rates (μ, d^{-1}; mean ± 1 SE; n = 3) of *P. malhamensis* in the light and in dark and of *Spumella* sp. feeding on different biovolumes of bacteria, and significance of pairwise comparison (Tukey's test)

Food type	Bacterial biovolume (μm^3 ml^{-1})	*P. malhamensis* μ Light	*P. malhamensis* μ Dark	*P. malhamensis* p	*P. malhamensis* Pooled μ	*Spumella* sp. μ
No food	0	−0.31 ± 0.09	−0.47 ± 0.02	0.86	−0.39 ± 0.16	−1.4 ± 0.02
Ultramicrobacteria	0.04	0.17 ± 0.083	0.12 ± 0.13	1.0	0.14 ± 0.07	−0.7 ± 0.18
	0.36	0.21 ± 0.17	0.31 ± 0.17	0.99	0.27 ± 0.11	0.3 ± 0.17
Large bacteria	0.18	1.46 ± 0.153	1.1 ± 0.06	0.47	1.3 ± 0.1	1.1 ± 0.4
	2.2	1.6 ± 0.13	1.7 ± 0.04	1.0	1.7 ± 0.06	2.8 ± 0.06

fore pooled. Growth of *P. malhamensis* feeding on the small bacteria was not significantly different for the 2 food concentrations tested (1-way ANOVA, Tukey test, p = 0.550; Fig. 1). In contrast, when feeding on the large bacteria growth rate was significantly higher for the high food concentration tested (ANOVA, Tukey test, p = 0.020). When no food was provided, the mortality rate of *Poterioochromonas* was low.

In contrast, *Spumella* sp. showed high mortality rates when no food was provided. Growth of *Spumella* sp. was significantly higher at high food concentrations for both types of bacteria (1-way ANOVA, Tukey test, p = 0.038 and 0.002 for the small and large bacteria, respectively; Fig 1).

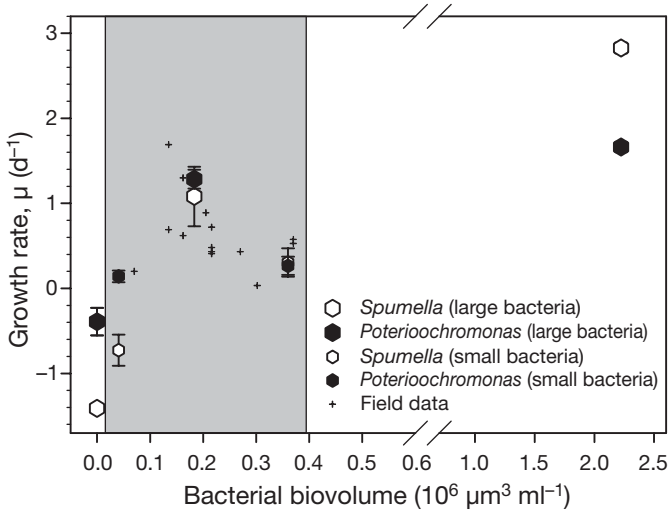

Fig. 1. *Poterioochromonas malhamensis* and *Spumella* sp. Growth rate of flagellates feeding on different biovolumes of large and small bacteria corresponding to roughly 0.8 and 8×10^6 bacteria ml^{-1}, respectively. Growth rates for flagellate communities in the field at temperatures between 13 and 20°C are also indicated. The corresponding bacterial biovolumes of the latter are calculated from abundances and cell volumes given in Sherr et al. (1984), Nagata (1988) and Weisse (1991) or from literature data from the same habitat at the same season. Grey area: bacterial biovolumes typical of oligotrophic/mesotrophic environments. Data are means ± 1 SE (n = 6 and 3 for *P. malhamensis* and *Spumella* sp., respectively)

In sum, without bacterial food *Poterioochromonas malhamensis* showed lower mortality rates (0.4 ± 0.1 d^{-1}) than *Spumella* sp. (1.4 ± 0.02 d^{-1}; Student's *t*-test, p < 0.001), but displayed lower growth rates at food concentrations exceeding typical field concentrations (Student's *t*-test, p < 0.001). Under food conditions comparable to those in the field both strategies were equally efficient (Fig. 1).

Expt 2: Numerical response to a broad range of prey concentrations

In the second set of trials we studied the effect of a wide range of bacterial concentrations (biovolumes) on the growth rate of *Poterioochromonas malhamensis* and *Spumella* sp. For *P. malhamensis*, the maximum growth rate in the dark was 1.0 and 1.7 d^{-1} when fed the small and the large bacteria, respectively (Table 2, Fig. 2A). The threshold food concentration for positive growth was 2.6 and 0.13×10^6 bacteria ml^{-1} for the small and the large bacteria, respectively (but see subsection on starvation below). Satiating food conditions corresponded to $>50 \times 10^6$ ultramicrobacteria ml^{-1} or to ca. 20×10^6 large bacteria ml^{-1}. In terms of bacterial biovolume, however, the differences are not so large and satiating food conditions corresponded to ca. 4.5×10^6 and ca. 3.5×10^6 μm^3 ml^{-1} for the small and the large bacteria, respectively.

Maximum growth rate was around 2 to 2.4 d^{-1} for our *Spumella* sp. strain on both bacterial prey strains, as estimated by non-linear regression (Table 2, Fig. 2A). For the ultramicrobacteria, i.e. bacteria representing the majority of natural aquatic bacteria, the threshold food concentration for positive growth seemed surprisingly high (ca. 6.4×10^6 bacteria ml^{-1}). For the large bacteria, the threshold food concentration for positive growth was 0.34×10^6. Satiating food conditions corresponded to $>50 \times 10^6$ ultramicrobacteria ml^{-1} or 5 to

Table 2. *Poterioochromonas malhamensis* and *Spumella* sp. Expt 2: Parameters indicating numerical response for flagellates feeding on either ultramicrobacteria or large bacteria. Parameters are for bacterial biovolumes ($\times 10^6$ µm^3 ml^{-1}) (in parentheses, recalculated for bacterial abundance, $\times 10^6$ cells ml^{-1}). μ_{max}: maximum growth rate d^{-1}; x' threshold concentration for growth; K_m: half-saturation constant for growth

Parameter	*P. malhamensis*	*Spumella* sp.
Large bacteria		
μ_{max}	1.7	2.4
x'	0.05 (0.13)	0.13 (0.34)
K_m	0.55 (1.45)	0.45 (1.18)
Ultramicrobacteria		
μ_{max}	1.0	2.0
x'	0.115 (2.6)	0.28 (6.4)
K_m	0.8 (18.2)	0.9 (20.5)

10×10^6 large bacteria ml^{-1}. Recalculated for bacterial biovolume, however, the differences are not so large and satiating food conditions correspond to ca. 3.5×10^6 and ca. 1×10^6 µm^3 ml^{-1} for the small and the large bacteria, respectively. For *Spumella* sp. feeding on the ultramicrobacteria, positive growth was realised only at biomasses higher than those realised in the field, suggesting that these bacteria may not suffice as the sole food source. For the large bacterium, positive growth was achieved at bacterial biomass typical for field conditions.

The numerical response curve fits with literature data for *Poterioochromonas malhamensis* (Fig. 2C,D). For *Spumella* sp., the general response also fits literature data, but higher maximum growth rates have been reported, even when temperature is taken into account (i.e. standardised to 16°C; Fig. 2C,D).

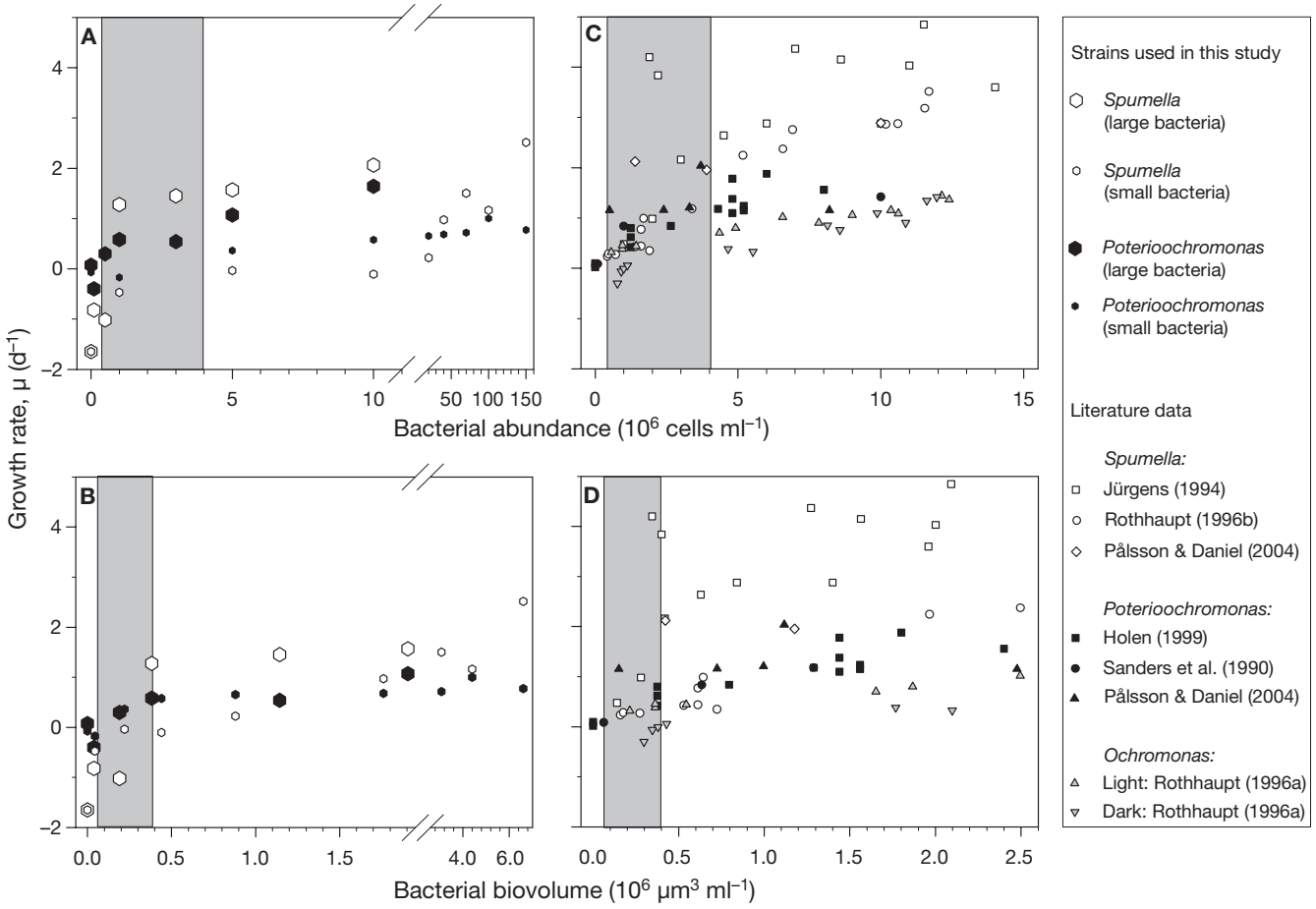

Fig. 2. *Poterioochromonas* spp./*Ochromonas* spp. and *Spumella* spp. Growth rate as a function of (A,C) bacterial concentration and (B,D) bacterial biovolumes. Grey areas: bacterial abundances and biovolumes typical of oligotrophic/mesotrophic aquatic habitats. (A, B) Numerical response of our isolates in present study; (C,D) literature data on the numerical responses of *Poterioochromonas* spp., *Ochromonas* spp. and *Spumella* spp. As some of the latter experiments were performed at 20°C, all data have been back-calculated for 16°C assuming a Q_{10} of 2. For calculating bacterial biovolumes we used bacterial cell size provided by relevant authors. For data of Holen (1999) we assumed mean bacterial cell volume of 0.3 µm^3

Our results indicate that the 2 flagellates display different characteristics with regard to starvation tolerance and maximum growth rates as indicated from the non linear regression derived from our data set (Fig. 3). *Poterioochromonas malhamensis* is slower growing than *Spumella* sp. at high prey concentrations, and thus *Spumella* sp. has the potential to outcompete *P. malhamensis* at the highest bacterial concentrations realised in oligotrophic/mesotrophic lakes. However, at the extreme low end of prey concentrations in natural waters *P. malhamensis* competes better, mainly due to the high mortality rate of *Spumella* sp. This relation, however, is modified by the bacterial size distribution, with *P. malhamensis* performing better when high percentages of large bacteria are present (Fig. 3).

Interspecific grazing of *Poterioochromonas malhamensis* on *Spumella* sp.

Besides bacterivory, our results show that *Poterioochromonas malhamensis* grazes on *Spumella* sp. In contrast to our expectations this was only significant for treatments using the large bacterium (Student's *t*-test, $p < 0.001$) even though the abundance of *Spumella* sp. was similar in both treatments. However, feeding on *Spumella* sp. made no significant contribution to the growth of *P. malhamensis* (*t*-test, $p = 0.174$). This was not unexpected, taking into account the ingestion rate of 0.47 flagellates d^{-1} per *P. malhamensis* (yielding an ingested biovolume of 47 μm^3 d^{-1}). Assuming a growth efficiency of 30%, this would comprise only to 5 to 6% of the volume necessary for cell division.

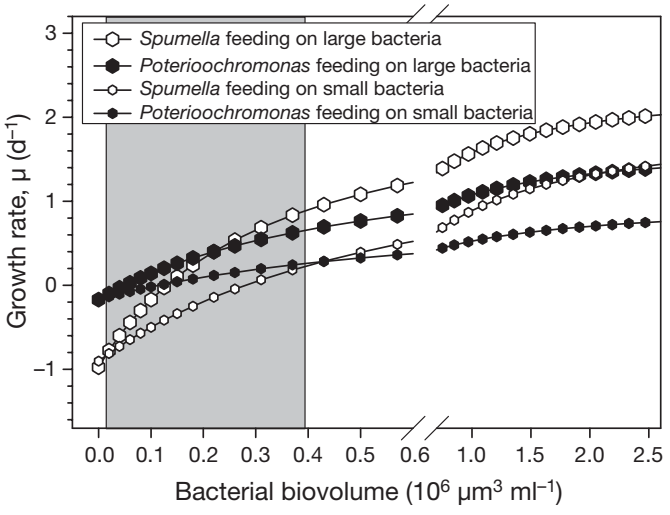

Fig. 3. *Poterioochromonas malhamensis* and *Spumella* sp. Growth rate as a function of prey biovolume (*P*) based on non-linear regression models (for explanation see section on non-linear regression in 'Materials and methods'). $\mu = 1 \times (P - 0.115)/(0.8 + P - 0.115)$ and $\mu = 1.7 \times (P - 0.05)/(0.55 + P - 0.05)$ for *P. malhamensis* feeding on small and large bacteria, respectively; $\mu = 2 \times (P - 0.28)/(0.9 + P - 0.28)$ and $\mu = 2.4 \times (P - 0.13)/(0.45 + P - 0.13)$ for *Spumella* sp. feeding on small and large bacteria, respectively

Starvation, residual division and mortality: effects of light, pH, and DOM

Poterioochromonas malhamensis underwent 2 cell divisions during the first 3 to 4 wk. Thereafter, cell concentration declined. However, even after 50 d starva-

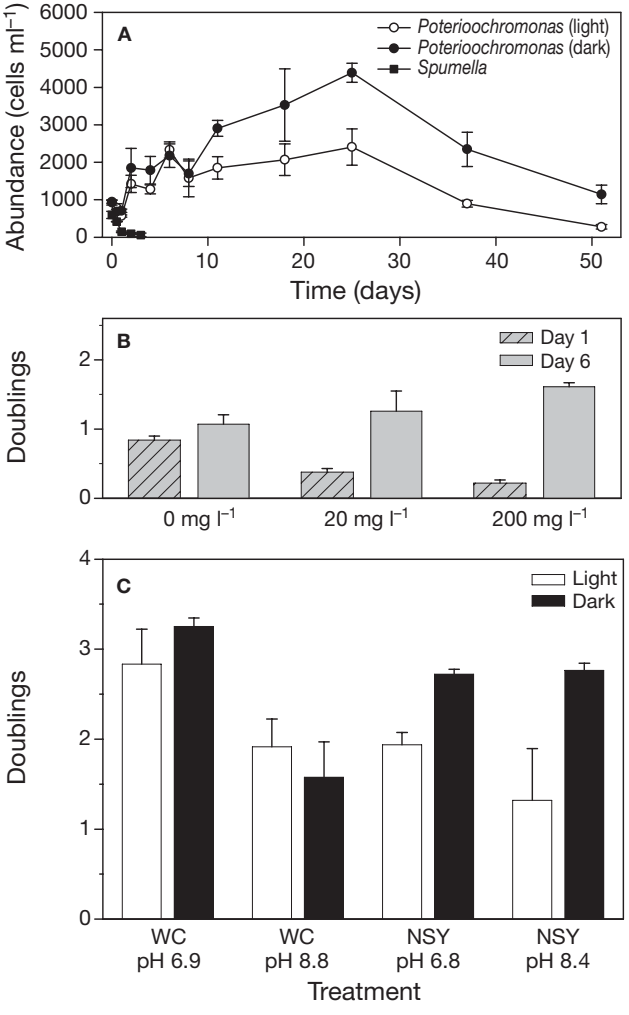

Fig. 4. *Poterioochromonas malhamensis* and *Spumella* sp. (A) Development of starved flagellate cultures as a function of time. Abundance of *P. malhamensis* and *Spumella* sp. in the dark and in light in sterile lake water with no food supply. (B) Doublings of *P. malhamensis* at 1 and 6 d in dark treatments without food supply and with different amounts of organic matter. (C) Maximum number of cell divisions during a 2 mo period without food supply in media containing different amounts of organic buffers and of different pH. Data are means ± 1 SE; n = 3

tion, abundance was barely below initial abundance (Fig. 4A) and cell size did not differ significantly between the light and dark treatments (Student's *t*-test, $p > 0.05$). The number of divisions was significantly higher for the dark than for the light treatment (Student's *t*-test, $p = 0.007$).

A possible explanation for the strong survival response of *Poterioochromonas malhamensis* could be that it consumed dissolved organic matter. To test this we further tested media (NSY medium) containing different amounts of organic matter. In experiments with no organic matter added, *P. malhamensis* underwent about 1 cell division during the first day of incubation. In incubations with added organic matter, the number of cell divisions was lower (Spearman rank correlation, $p < 0.001$). After 6 d of incubation, however, the number of cell divisions did not differ between the treatments (Spearman rank correlation, $p = 0.087$; Fig. 4B).

We also tested the significance of organic buffers in combination with different pH levels for the survival response of *Poterioochromonas malhamensis* (NSY medium: 4.4 mg l^{-1} Na_2EDTA, pH 6.8 and 8.4; modified WC medium: 115 mg l^{-1} TES buffer and 4.36 mg l^{-1} Na_2EDTA, pH 6.9 and 8.8). The overall survival response was similar to that using lake water (cf. Fig. 4A), i.e. an initial increase in cell number up to 30 d followed by a decrease. After 50 to 60 d, cell concentrations had almost reached their initial level (data not shown).

After allowing for effects of medium and pH, our results for the various test media show that in terms of cell division *Poterioochromonas malhamensis* performed better in the dark (3-way ANOVA, $p = 0.011$, Fig. 4C). This is in accordance with our findings for the lake water. This effect was significant depending on the medium ($p = 0.015$), i.e. enhanced dark growth was only observed in the medium with low organic matter concentration (NSY; $p < 0.001$) whereas it was not significant in the medium with high organic content (WC; $p = 0.901$).

In lake water, *Spumella* sp. died within 4 d without food and the mortality rate was 0.97 d^{-1} (regression, adjusted r^2: 0.86; $p = 0.047$; Fig. 4A). This fits with data from the numerical response data where experiments were also performed without additional food.

DISCUSSION

Basic survival: *r*-strategy or *K*-strategy?

Our data demonstrate that flagellates are severely food-limited at food concentrations present in the field. Different taxa have different strategies for coping with this food limitation (i.e. with living in a wet desert) that are, however, equally effective. *Poterioochromonas malhamensis* has relatively high growth rates, but is much more a *K*-strategist than *Spumella* sp. Growth rates at high food concentration were low, but during starvation mortality rates were also low. In contrast, *Spumella* sp. is a typical *r*-strategist, displaying high maximum growth rates under suitable food conditions and high mortality rates in the absence of food. The general strategy of *Spumella* sp. therefore is opportunistic: fast growth when food conditions allow, but little starvation tolerance. Based on our results we suspect that this flagellate most probably survives adverse conditions by re-colonisation from surrounding habitats or excystment. Encystment, in fact, is a basic part of survival strategies in many chrysophyte taxa (e.g. Sandgren 1988 and references therein, Duff et al. 1995).

Apart from a few studies (e.g. Fenchel 1982, Eccleston-Parry & Leadbeater 1994b), starvation tolerance is usually merely a side-issue in protist ecology. Laboratory studies so far have indicated that the bacterial concentrations of 2 to 4×10^6 bacteria ml^{-1} typical for oligotrophic/mesotrophic conditions allow fairly high flagellate growth (Eccleston-Parry & Leadbeater 1994a, Rothhaupt 1996a) with half-saturation constants (K_m) well below the field abundances of bacteria (e.g. Eccleston-Parry & Leadbeater 1994b, Jürgens 1994). Thus, based on laboratory findings starvation only becomes an issue at bacterial abundances below 0.1 to 0.5×10^6 bacteria ml^{-1}. With respect to natural bacterial cell volumes, however, starvation is highly relevant even at bacterial abundances exceeding 4×10^6 bacteria ml^{-1}.

Contribution of phototrophy to the nutrition of *Poterioochromonas malhamensis*

Although *Poterioochromonas malhamensis* and *Spumella* sp. are closely related and the latter is considered to be the colourless counterpart of *Ochromonas* sp., both taxa coexist in a variety of ecosystems (Preisig et al. 1991). In accordance with the niche concept, one would therefore expect a different utilisation of resources (Hutchinson 1957). For pigmented algae it is usually assumed that phototrophy contributes significantly to growth. Specifically, in a group of organisms such as chrysophytes which comprise both purely heterotrophic and mixotrophic taxa, phototrophy is thought to be the key issue in the differentiation of survival strategies (Sandgren 1988, Holen & Boraas 1995, Rothhaupt 1996b). The ability to use light is the obvious difference between taxa in this group — but the obvious need not be the essential issue.

In *Poterioochromonas malhamensis* we did not observe increased growth on an inorganic medium in the light compared to the dark. We did see an increased number of cells during the incubation period in the light. However, a similar increase was also observed in the dark. Thus the increase in cell number must be interpreted as residual division, i.e. no increase in biomass. In fact, sometimes the culture underwent more cell divisions in the dark. In addition, we found no differences in growth rate at low bacterial biomass in the light compared to the dark, indicating that residual cell division may be a starvation strategy to increase dispersal when food becomes scarce. A higher number of residual divisions may then occur in the absence of both light and dissolved organic carbon.

Considering its ability for residual cell divisions, the published phototrophic 'growth' rates of axenic cultures of *Poterioochromonas malhamensis* in inorganic media (i.e. in the range of <0.01 to 0.3 d^{-1}; Table 3) must be reconsidered. The great majority of published studies provide no data on the growth of *P. malhamensis* in inorganic media in the dark. In only 1 other case was a dark growth rate of 0.1 d^{-1} in axenic culture reported (Caron et al. 1990). Thus, reported growth rates may therefore largely represent residual cell division, simply because no dark treatments were performed that would have revealed this. In fact, a low contribution of photosynthesis in the nutrition of *P. malhamensis* has already been pointed out by Pringsheim (1952) and Myers & Graham (1956).

This does, however, not imply that *Poterioochromonas malhamensis* is not photosynthetically active. Taking the available inorganic carbon uptake rates from Sanders et al. (1990) at an irradiance of between 40 and 350 µE m^{-2} s^{-1} and assuming a light:dark period of 14:10 h and a growth efficiency of photosynthesis of 50% (i.e. 50% of the inorganic carbon taken is used for growth), a rough calculation suggests that *P. malhamensis* will take up 1.4 to 6.16 pg C $cell^{-1}$ d^{-1}, equivalent to ~7.5% of its cellular carbon content d^{-1}. Assuming a growth efficiency of 50% for photosynthesis, this implies that the contribution of photosynthesis to its growth will only be ~3.8% d^{-1}. The low contribution of photosynthesis to growth of *P. malhamensis* may indicate other benefits of the chloroplast. For instance, oxygen evolution under anaerobic conditions has been suggested by Myers & Graham (1956). However, as experimental proof is lacking or poor, these suggestions are highly speculative. The role of photosynthesis in the growth of *P. malhamensis* seems clearly different from its role in the closely related chrysophytes *Ochromonas* spp., for which published data clearly suggest increased growth rates in the light compared to the dark at low bacterial concentrations (e.g. Rothhaupt 1996a,b). A word of caution: As distinction between *Poterioochromonas* and *Ochromonas* species is difficult (and sometimes impossible in the field), care must be taken in interpreting literature data. For laboratory studies, however, it is in most cases possible to track the affiliation of the strains used, thus allowing some generalisation.

Contribution of different organic carbon sources to nutrition of *Poterioochromonas malhamensis* and *Spumella* sp.

The contribution of different sources of organic matter and of photosynthesis to the nutrition of both flagellates can be calculated for the range of particle concentrations realised in the field (Tables 4 & 5). In some cases, contribution to uptake was calculated from observed growth rates based on simple assumptions (see Tables 4 & 5). Our results indicate that DOM may contribute to the nutrition of *Poterioochromonas malhamensis* in the laboratory at levels present in media containing a high concentration of organic buffers (Fig. 4B, Table 3). However, the concentrations of DOM in natural ecosystems are probably too low to be of significant importance.

Table 3. *Poterioochromonas malhamensis.* Strains described in literature, their growth rates in light without particulate food, medium pH, medium type and contents of organic buffers

Strain	µ (d^{-1})	pH	Medium	Organic buffers (mg l^{-1})	Source
CCAP 933/1a = Starr Collection No. L1297	0.3/0.1	6.6/7.8	Modified *f*/2 medium	<1	Sanders et al. (1990)
	(<0.1 in the dark)	8.6	Modified *f*/2 medium	<1	Caron et al. (1990)
	0.32	≤8.0	Defined *Ochromonas* medium modification G	500	Lewitus & Caron (1991)
Zhang	0.32	6.6	AF-6 medium	4	Zhang & Watanabe (2001)
Holen	0.36	6.8	DY-IV medium	200	Holen (1999)

Table 4. *Spumella* sp. Typical range of biovolumes of different prey organisms in the field and mean (range) estimated gross carbon uptake from the different carbon sources (mean; range in parentheses). Estimates of gross field uptake based on laboratory experiments with natural range of particle concentrations, growth rate (μ) and estimated gross carbon uptake rate; biovolumes of bacteria in the field based on a literature survey and unpublished data (Schauer pers. comm.). For *Spumella* sp. without food a mortality rate of 0.97 d^{-1} is assumed. Growth yield for uptake rate of dissolved and particulate organic matter = 30% (Middleboe et al. 1992, Hansen et al. 1997); cellular carbon content *Spumella* sp.: 20 pg C $cell^{-1}$

Prey	Field		Laboratory		
	Biovolume ($\times 10^6$ μm^3 ml^{-1})	Gross C uptake (pg C $cell^{-1}$ d^{-1})	Biovolume ($\times 10^6$ μm^3 ml^{-1})	μ (d^{-1})	Gross C uptake (pg C $cell^{-1}$ d^{-1})
Ultramicrobacteria	0.02–0.1	20.4 (10.5–31.3)	0.04–0.36	–0.72–0.3	19.33–114.7
Large bacteria	0.1–0.4	88.9 (53.2–124.6)	0.18–2.22	1.1–2.8	140–246.7
Total		110 (63.7–160)			

Table 5. *Poterioochromonas malhamensis.* Typical range of irradiance and biovolumes of various prey organism in the field, and mean (range) estimated gross carbon uptake from the different carbon sources. Estimates of gross field uptake based on laboratory experiments carried out in natural range of light and carbon concentrations, growth rates and gross carbon uptake rates and (in parentheses) on gross carbon uptake rates estimated from growth rates. Biovolumes of bacteria and nanoplankton in the field are based on a literature survey and unpublished data (Schauer pers. comm.). Growth yield for uptake rate of particulate organic matter = 30% (Middleboe et al. 1992, Hansen et al. 1997); light:dark period for photosynthesis = 14:10 h; cellular carbon content of *P. malhamensis* = 50.4 pg C; mortality rate of *P. malhamensis* = 0.15 d^{-1}; data on photosynthesis from Caron et al. (1990)

	Field		Laboratory		
	Irradiance (μE m^{-2} s^{-1})/ Biovolume ($\times 10^6$ μm^3 ml^{-1})	Gross C uptake (pg C $cell^{-1}$ d^{-1})	Irradiance (μE m^{-2} s^{-1})/ Biovolume ($\times 10^6$ μm^3 ml^{-1})	μ (d^{-1})	Gross C uptake (pg C $cell^{-1}$ d^{-1})
Light	40–350	3.8 (1.4–6.16)	40–350		1.4–6.16
Ultramicrobacteria	0.02–0.1	12.4 (2.6–22.2)	0.04–0.36	0.14–0.26	(23.7–44)
Large bacteria	0.1–0.4	93.4 (49.4–137.3)	0.18–2.22	1.2–1.6	(203–270)
Piconanoplankton	0.2–1.0	8 (2.6–13.4)	0.7		9.4
Total		118 (56–179)			

The most important food source for *Poterioochromonas malhamensis* is large bacteria. At the natural levels of bacteria found in oligotrophic/mesotrophic habitats we estimate that the large bacteria contributed ~79% of the total carbon uptake, while the ultramicrobacteria contributed ~11%. *P. malhamensis* has been shown to feed on other protists of up to 2–3 times their own cell size (Zhang et al. 1996, Zhang & Watanabe 2001). Based on our grazing measurements of *P. malhamensis* on *Spumella* sp., we estimate that ~7% of its carbon uptake may come from the ingestion of pico/nanoplankton. Thus, we estimate that 97% of its carbon uptake comes from particulate sources. The remaining 3% of carbon uptake is met by photosynthesis.

For Spumella sp. *the* situation is much less complex since, unlike *Poterioochromonas malhamensis*, this flagellate seems to use a narrower spectrum of carbon sources. The data suggest that large bacteria will make up the bulk (~81%) of the food uptake by *Spumella* sp. in natural oligotrophic/mesotrophic lakes. However, ultramicrobacteria contributed substantially (~19%) to the overall bacterial carbon uptake of *Spumella* sp. in our study. Despite this, the mortality rates of *Spumella* sp. indicate that this flagellate cannot completely meet its basic maintenance requirements from feeding on ultramicrobacteria, probably because of its very high maintenance requirements (see Figs. 1 & 2, Table 4).

The contributions by different carbon sources correspond to the mean abundances of prey organisms. In different habitats and different seasons the contribution of these various sources may vary considerably due to fluctuations in abundance and size of prey. The degree to which this may influence the competition between the 2 flagellates can be judged from Fig. 3 and Tables 4 & 5.

In conclusion, *Poterioochromonas malhamensis* can utilise a wide selection of different organic food sources, while *Spumella* sp. relies on bacteria for food. Thus, *P. malhamensis* can be considered a food source generalist, and *Spumella* sp. more a food specialist. *P. malhamensis* will have no problem in existing in a fluctuating food environment: first, its maximum

growth and ingestion rates are fairly low; second, it can utilise very different food sources; and third it has a very high starvation tolerance.

The fact that *Spumella* sp. has a very low starvation tolerance could mean that this flagellate requires a very stable environment in terms of food availability in time and/or space. Alternatively, it may be patchily distributed or produce cysts, although we found no indications of cyst formation for our strain in this study. Our results clearly demonstrate that even closely related flagellates may have different life strategies.

Growth and survival strategies among bacterivorous flagellates

In this paper we have only dealt with 2 species of bacterivorous chrysophytes and only 1 strain of each species. Very little is known about the survival responses of bacterivorous flagellates in general, and thus strong generalisations concerning their growth/survival strategies cannot be made. Even strains with identical 18S rRNA may differ considerably in their ecophysiological responses. For the strain of *Spumella* sp. used in this study such variation has already been demonstrated (Boenigk & Stadler 2004, Boenigk et al. 2004) and we have further indications for strain-specific variation between *Poterioochromonas malhamensis* Strain DS and *P. malhamensis* Strain 933/1a even though both strains are identical in 18S rRNA sequence (Boenigk & Stadler 2004, authors' unpubl. data). We suggest, however, that the main difference (i.e. *K*-strategy in *P. malhamensis* vs. *r*-strategy in *Spumella* sp.) can be generalised for the genera *Poterioochromonas* and *Spumella* despite differences in grazing rates, food size spectrum, and optimum growth conditions between different strains of the 2 flagellates. Experimental data as well as extensive cultivation experience has given further evidence that, despite a high molecular and ecophyiological variation between different strains of the respective morphospecies, a general mode of nutrition and the basic nutritional strategy can be recognised for the morphotypes (cf. Boenigk 2005, Boenigk et al. 2005, K. Pfandl & J. Boenigk unpubl. data). The strength of adaptation to aquatic food-limited environments may, however, vary between different phylogentic clades of *Spumella* sp. (all phylogenetic clades seem, however, to be far more *r*-strategists than *P. malhamensis*).

Although not many data are available on growth strategies of bacterivorous flagellates, some indications can be gathered from the literature. In general, data so far indicate that with increasing importance of phototrophic nutrition (*Spumella*/*Paraphysomonas* → *Poterioochromonas* → *Ochromonas* → *Dinobryon*) there is a parallel decrease in maximum growth rate. This is expected, since development of a photosynthetic apparatus (chloroplasts, enzymes) is considered to be much more energetically expensive than heterotrophic degradation of food (e.g. Raven 1995). However, our data also suggest that fast growth rates are associated with decreased starvation tolerance. This topic needs to be studied in more detail to verify whether this observation is true for other groups of protists.

Current limitations in laboratory studies of bacterivorous flagellates

Although laboratory experiments and field studies should ideally complement each other, conditions in laboratory studies are frequently so far removed from *in situ* conditions that the relevance of their conclusions to the field situation is doubtful. Several recent key developments (axenic cultivation of heterotrophic flagellate strains, cultivation of natural-sized laboratory bacteria) and a deeper comprehension of factors significant for setting up laboratory experiments now allow experiments that are much closer to natural conditions than in the past. The use of axenic cultures to mimic natural conditions may seem strange, but it is the only way to avoid the occurrence of large laboratory bacteria that develop within hours or days even in freshly sampled bacteria from the field.

The physiological responses of axenic strains and laboratory strains that have been kept in culture for a long period may deviate from those of strains *in situ*. Such 'cultivation artefacts' have been used as an ultimate argument to explain data discrepancies; this, however, lacks a scientific basis. For instance, in the case of *Poterioochromonas malhamensis* this argument was used in regard to Type Strain 933/1a (e.g. Holen 1999, Pålsson & Daniel 2004), but careful re-evaluation of the data showed that the findings on the different strains used in the laboratory studies were not contradictory at all (see subsection on the contribution of phototrophy; see also Pringsheim 1952) and that only the interpretation of the results differed. In effect, even a 60 yr old laboratory strain still responds in the same way as recently isolated strains.

Recent major advances now allow us to set up experiments under more realistic conditions. Setting up such experiments, however, can be extremely time-consuming: cultivation, the need for multiple control treatments, and often many more than merely 3 replicate treatments is laborious. However, near-natural conditions and multiple control treatments are absolutely indispensable in order to understand the ecology of organisms instead of just getting significantly different

(but possibly meaningless) results. Instead of more natural experimental conditions extremely artificial setups are sometimes used with the excuse that the former are not essential to the question addressed or for the specific model system. However, ultimately such experiments often tend to be more misleading than helpful, and are a key reason for the current problems in applying laboratory results to the field situation.

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