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Potential toxicity of chrysophytes affiliated with *Poterioochromonas* and related '*Spumella*-like' flagellates

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The chrysophyte genera Poterioochromonas and Ochromonas and their heterotrophic analogons, i.e. the 'Spumella-like' flagellates, account for a significant and often dominating fraction of the pelagic nanoplankton. Even though several osmotrophically and autotrophically grown strains of Ochromonas and Poterioochromonas are assumed to produce toxins, the potential toxicity has been investigated neither for its association with bacterivorous nutrition nor within the related exclusively heterotrophic 'Spumella-like' flagellates. We investigated the toxic potential of several flagellate strains using cultures of flagellates, cell extracts and filtrate of flagellate cultures. The effect on potential predators was exemplarily tested for the cladoceran Daphnia magna and the rotifer Platyias sp. All tested heterotrophic and mixotrophic flagellate strains were toxic to zooplankton at abundances exceeding 10^4 *flagellates* mL^{-1}*. For the rotifers, survival on any of the flagellate strains was significantly lower than that in the control treatment (*$P < 0.001$*) already after 24 h. We conclude that (i) 'Spumella-like' flagellates can be toxic to zooplankton, (ii) all tested flagellates, i.e. heterotrophic and mixotrophic flagellates, feeding phagotrophically can be toxic to zooplankton and (iii) sublethal effects may be observed at typical field abundances, even though acute toxicity seems to be restricted to flagellate abundances observed only at peak events.*

INTRODUCTION

Heterotrophic and mixotrophic chrysomonads are abundant members of the freshwater planktonic communities (Carrick and Fahnenstiel, 1989; Carrias *et al.*, 1998; Auer and Arndt, 2001; Boenigk and Arndt, 2002; Weitere and Arndt, 2003). By grazing on bacteria, these organisms function as a link between microbial secondary production and higher trophic levels, since flagellates are an important food source for macrozooplankton (Havens and DeCosta, 1985; Knisley and Geller, 1986; Hessen *et al.*, 1989). Factors influencing zooplankton feeding, such as toxicity, as well as behavioral responses to prey characteristics, e.g. selective avoidance, could weaken this trophic link, and microbial toxins have been demonstrated to adversely affect aquatic organisms (Haney, 1987; Lampert, 1987). For instance, the effects of toxic cyanobacteria on metazooplankton, e.g. on rotifers (Fulton and Paerl, 1987; Rothhaupt, 1991; Gilbert, 1994, 1996a,b; Smith and Gilbert, 1995), on cladocerans (Infante and Abella, 1985; Hanazato and Yasuno, 1987; Fulton, 1988) and on copepods (DeMott and Moxter, 1991) have been extensively studied.

Even though mixotrophic and heterotrophic Ochromonadaceae are an often-dominating component of aquatic pelagic food webs (Happey-Wood, 1976; Bird and Kalff, 1989; Carrick and Fahnenstiel, 1989; Arndt *et al.*, 2000; Auer and Arndt, 2001; Boenigk and Arndt, 2002; Weitere and Arndt, 2003), little attention has been paid to the potential toxicity of these flagellates. Since the 1960s, chrysomonads in the genera *Poterioochromonas* and *Ochromonas* have been known or suspected to produce toxins (Reich and Spiegelstein, 1964; Spiegelstein *et al.*, 1967; Halevy and Avivi, 1968). Toxicity has been demonstrated for several species and strains, i.e. *Ochromonas danica*, *Ochromonas minuta*, *Ochromonas sociabilis* and other *Ochromonas* spp. and *Poterioochromonas malhamensis* strains L933/1A–C (Cambridge collection) (Spiegelstein *et al.*, 1967, 1969; Halevy and Avivi, 1968; Halevy *et al.*, 1971; Hansen, 1973; Magazanik and Halevy, 1973; Leeper and Porter, 1995; Boxhorn *et al.*, 1998). The toxins have been reported to be water-soluble, polar substances (Halevy *et al.*, 1971; Hansen, 1973) but have not been fully characterized. Extracted toxins have been demonstrated to inhibit bacterial growth (Hansen,

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1973), to lyse mammalian erythrocytes (Spiegelstein *et al.*, 1969; Halevy *et al.*, 1971; Magazanik and Halevy, 1973) and to possess ichthyotoxic activity (Reich and Spiegelstein, 1964; Spiegelstein *et al.*, 1969; Halevy *et al.*, 1971; Magazanik and Halevy, 1973). These toxins can be excreted by the cells and have been speculated to negatively affect aquatic organisms even if the cells are not ingested (Halevy *et al.*, 1971; Boxhorn *et al.*, 1998). Only few studies, however, have examined the effects of these toxins on potential grazers, i.e. to zooplankton (Leeper and Porter, 1995; Boxhorn *et al.*, 1998). Furthermore, these investigations focused only on mixotrophic strains. The closely related heterotrophic Ochromonadaceae, i.e. the '*Spumella*-like' flagellates, have not been investigated for toxic effects on zooplankton grazers, even though these flagellates are an important component of the flagellate community in freshwaters and reach abundances of several thousand individuals mL^{-1}. The mixotrophic genus *Ochromonas* and the heterotrophic genus *Spumella* have been separated on the basis of the presence or absence of a chloroplast (Preisig, 1995). Investigations into the 18S rRNA gene sequence similarity provide evidence that this separation is artificial and that both *Ochromonas* and *Spumella* have been considered to be polyphyletic (Andersen *et al.*, 1999). As the heterotrophic '*Spumella*-like' flagellates cannot be separated from these mixotrophic genera (Andersen *et al.*, 1999; J. Boenigk *et al.*, submitted for publication), but toxicity has been demonstrated for different strains of *Ochromonas* and *Poterioochromonas*, it can be suspected that toxins are present also in heterotrophic strains. Furthermore, the toxicity of *Ochromonas* and *Poterioochromonas* seems to be enhanced on heterotrophic (osmotrophic) nutrition (Leeper and Porter, 1995), also indicating that these toxins may be present in closely related heterotrophic flagellates, i.e. the '*Spumella*-like' flagellates.

We tested the hypothesis that (i) '*Spumella*-like' flagellates have a toxic potential comparable to that of the mixotrophic strains affiliated with *Ochromonas* and *Poterioochromonas* and (ii) toxicity can be demonstrated not only for osmotrophically growing flagellates but also for phagotrophically growing flagellates. Laboratory studies were conducted to evaluate the toxicity of bacterivorous '*Spumella*-like' flagellates. Antibiotic activity was tested using the freshwater bacterial strain *Listonella pelagia* CB5, and toxicity for zooplankton was tested using clonal cultures of the cladoceran *Daphnia magna* and the rotifer *Platyias* sp.

METHOD

Cultures and culturing techniques

The type strains of *P. malhamensis* (Pringsheim) Peterfi (strain 933-1a) as well as *P. malhamensis* strain 933-1c were obtained from the SAG culture collection (Göttingen, Germany). The strain '*Ochromonas* DS' (closely related to *P. malhamensis*, sequence similarity >99%; J. Boenigk *et al.*, submitted for publication) was isolated from Lake Constance. The heterotrophic '*Spumella*-like' flagellate strains JBC07, JBM10 and JBNZ41 were small bacterivorous chrysomonads isolated from lakes and ponds in different climatic zones (J. Boenigk *et al.*, submitted for publication): strain JBC07 originated from Lake Taihu, China; strain JBM10 originated from an artificial pond in Mondsee, Austria; strain JBNZ41 originated from a natural pond near Aviemore, New Zealand. The strains JBC07, JBM10 and JBNZ41 were closely related to each other (18S rRNA gene sequence similarity 100%) and distantly related to *P. malhamensis* (18S rRNA gene sequence similarity 92%; J. Boenigk *et al.*, submitted for publication). All strains were grown at 16°C under permanent illumination in 50 mL of inorganic basal medium (Hahn *et al.*, 2003) in 100-mL Erlenmeyer flasks. All strains were grown axenically and fed with heat-killed bacteria [Gamaproteobacterium *L. pelagia* CB5, obtained from M. Hahn (Hahn and Höfle, 1998)]. The Gamaproteobacterium *L. pelagia* CB5 was grown in inorganic basal medium (Hahn *et al.*, 2003) supplemented with 1 g L^{-1} of each nutrient broth, soyotone peptone and yeast extract (NSY medium).

Platyias sp. (0.35–0.5 mm) was purchased from Sciento (Manchester, UK) and was originally isolated from Kingfisher Pond near Manchester. *Platyias* sp. was grown in 50 mL cell-culture flasks at 20°C in inorganic basal medium (Hahn *et al.*, 2003) and fed with an algal food strain (*Cryptomonas* sp.). *Daphnia magna* was isolated from an artificial pond at the Institute for Limnology in Mondsee. Stock cultures of *D. magna* were grown in 1.0 L beakers at 20°C, filled with an artificial inorganic basal medium (Hahn *et al.*, 2003), and fed with *Cryptomonas* sp. Cladocerans were thinned out to a density of 5–30 individuals L^{-1} and transferred to fresh medium every week. The culture used for the experiments was established from one individual.

Preliminary tests for bioactive substances produced by osmotrophically and phagotrophically grown flagellates

Test for inhibition of bacterial growth

This test was performed following the protocol of Hansen (Hansen, 1973). Axenic flagellate cultures were grown phagotrophically on heat-killed bacteria (*L. pelagia* CB5) as well as osmotrophically in NSY medium (see above). Five-day-old cultures of JBC07, JBM10 and JBNZ41 and '*Ochromonas* DS', *P. malhamensis* 933-1a and *P. malhamensis* 933-1c with a flagellate density of 3–5 × 10^4 flagellates mL^{-1} were separated from the culture medium by 0.2 μm filtration, and the original pH was restored by adding a base or acid. The filtrate was added to a culture of the Gamaproteobacterium *L. pelagia* CB5 at a ratio of 1:1. As a control, NSY medium was added to *L. pelagia*. The experiments were run in triplicate at 20°C in the light. Similarly, 5-day-old cultures of phagotrophically grown flagellates (grown on heat-killed *L. pelagia* in the inorganic basal medium) were processed (flagellate density 3–5 $\times$ 10^4 flagellates mL^{-1}). Filtrate was added to a culture of *L. pelagia*, and inorganic basal medium served as a control. Growth of the cultures was surveyed by following changes in the optical density of the cultures at a wavelength of 575 nm during an 8 h period using a spectrophotometer (type UV-1202, Shimadzu). Growth rates were calculated from the linear part of the log-transformed densities. Growth rates of bacteria exposed to culture medium of the different phagotrophically and osmotrophically grown flagellates were tested against growth in the various control treatments using one-way ANOVA and subsequent Dunnet's test.

Daphnia magna toxicity tests using cell extracts as well as high densities of flagellates

Cell cultures of the different flagellate strains were grown axenically to densities of $10^5 - 10^6$ cells mL^{-1}. At the end of the growth period, the cells were separated by centrifugation (20 min at 7000 *g*) and extracted with 1 mL of acetone in a shaker for 15 min. The suspension was centrifuged, and the solvent was collected. Extraction was repeated until the solvent remained free of the green–brown pigment. In the case of the heterotrophic flagellates, extraction was repeated three times. The remaining pellets were extracted with methanol twice. The supernatants were then stored at -70°C until further use; the frozen extract can be stored for more than a month without any loss of activity (Hansen, 1973). Directly prior to the experiments, the solvent was removed in an evacuated excicator at 4°C in the dark, and the residue was shaken with inorganic NSY medium, corresponding to a concentration of cell extract of 5.5×10^6 cells mL^{-1}. These extract concentrates were added to test tubes, yielding a final concentration corresponding to 5×10^6 cells mL^{-1}. In addition, dense cultures of phagotrophically grown flagellate strains ($1–1.2 \times 10^5$ flagellates mL^{-1}) were used for the experiments. Clonal neonates (<24 h) of the second breed of mothers of *D. magna* were used for the toxicity test. Neonates were transferred to test vessels containing either cell extract or flagellate culture (one neonate per vessel). As a control, inorganic basal medium was used. Potential side effects of the heat-killed food bacteria were checked in separate control treatments. All experiments, i.e. all cell extracts and all flagellate cultures as well as the control treatment, were performed in 10 replicates. Experiments were run at 22.5°C under a light:dark cycle of 16:8 h. Vessels were inspected daily for survival of daphnids.

Survivorship experiments

Platyias sp.

Stock cultures were derived from a single individual. Experiments were conducted in 24 well tissue-culture plates. Into each well, 0.5 mL of culture medium was pipetted, and five adult rotifers were transferred to each well. Suspensions of phagotrophically growing flagellates feeding on heat-killed bacteria were prepared and adjusted to an abundance of 2×10^4 flagellates mL^{-1}. One milliliter of flagellate suspension was pipetted into each well. A treatment containing only inorganic medium served as control. Background concentrations of the food algae (*Cryptomonas*) was 5000 cells mL^{-1}, and final concentration of flagellates at the start of the experiment was 13 000 cells mL^{-1}. The experiment was run with all six flagellate strains, and experiments were conducted in 12 replicates each at 22.5°C and 14:10 h light:dark cycle. Periodically, the number of live individuals in each well was counted at ×25 to ×50 magnification using a binocular (Wild).

Daphnia magna

Prior to the experiments, all organisms were cultured at the experimental conditions, i.e. 22.5°C and 14:10 h light:dark cycle. To start an experiment, 5- to 6-day-old daphnids of the third breed from mothers from stock cultures were pipetted into 50 mL cell-culture flasks (five individuals in 40 mL). Experimental treatments consisted of seven different treatments, i.e. 1.5×10^4 cells mL^{-1} of *Cryptomonas* sp., *P. malhamensis* strains 933-1a and 933-1c, '*Ochromonas* DS', JBC07, JBM10 and JBNZ41 and a no-food control containing inorganic medium only. Experiments were run in three replicates. Survivorship and offspring were noted during daily transfer of daphnids into clean flasks containing fresh nanoflagellate suspensions, and daphnids were monitored until death.

RESULTS

Bacterial growth inhibition test and preliminary toxicity test

Growth of the bacteria was significantly different depending on the filtrate (ANOVA, $P < 0.001$ and $P = 0.006$ for the organic and the inorganic treatment respectively). In the inorganic treatment, growth rate of the bacteria was significantly higher on flagellate filtrate compared to that in the control treatment for the strains JBC07 and JBM10 (ANOVA, Dunnet's post-hoc test, $P < 0.05$; Table I). In the organic treatment, growth was significantly higher for the flagellate strains JBC07, JBM10, JBNZ41 and *P. malhamensis* 1a (ANOVA, Dunnet's post-hoc test, $P < 0.05$; Table I).

Table I: Growth rates of the bacterial strain Listonella pelagia CB5 on filtrates of phagotrophically and osmotrophically grown flagellates affiliated to Poterioochromonas malhamensis and 'Spumella-like' flagellates

Strain	Filtrate of phagotrophically grown flagellates (day^{-1})	Filtrate of osmotrophically grown flagellates (day^{-1})
JBC07	3.07 ± 0.08*	2.42 ± 0.03*
JBM10	2.86 ± 0.12*	2.09 ± 0.07*
JBNZ41	2.49 ± 0.06	2.01 ± 0.03*
P. malhamensis 933-1a	2.05 ± 0.26	2.05 ± 0.10*
P. malhamensis 933-1c	2.45 ± 0.02	1.39 ± 0.10*
'*Ochromonas* DS'	2.25 ± 0.02	1.79 ± 0.04
Control	2.21 ± 0.01	1.81 ± 0.02

An asterisk indicates growth rate to be significantly different from growth in the control treatments ($P < 0.05$).

Only for bacteria growing on filtrate of osmotrophically cultured *P. malhamensis* 1c was growth significantly lower compared to that in the control ($P < 0.05$).

Survival of neonates of *D. magna* was tested using dense cultures of flagellates (1–1.2 × 10^5 flagellates mL^{-1}). Survival was significantly lower in the presence of all flagellates (except strain JBM10) compared to that in the control (ANOVA on ranks, $P < 0.001$; Dunnet's post-hoc test, $P < 0.05$; Fig. 1). Accordingly, in the acetone extract, survival of the neonates was significantly shorter as in the control treatment for all flagellate strains except JBM10 (ANOVA on ranks, $P < 0.001$; Dunnet's post-hoc test, $P < 0.05$; Fig. 1). In contrast, survival was not significantly different from that in the control treatment for the methanol extracts (ANOVA on ranks, $P = 0.416$; Fig. 1).

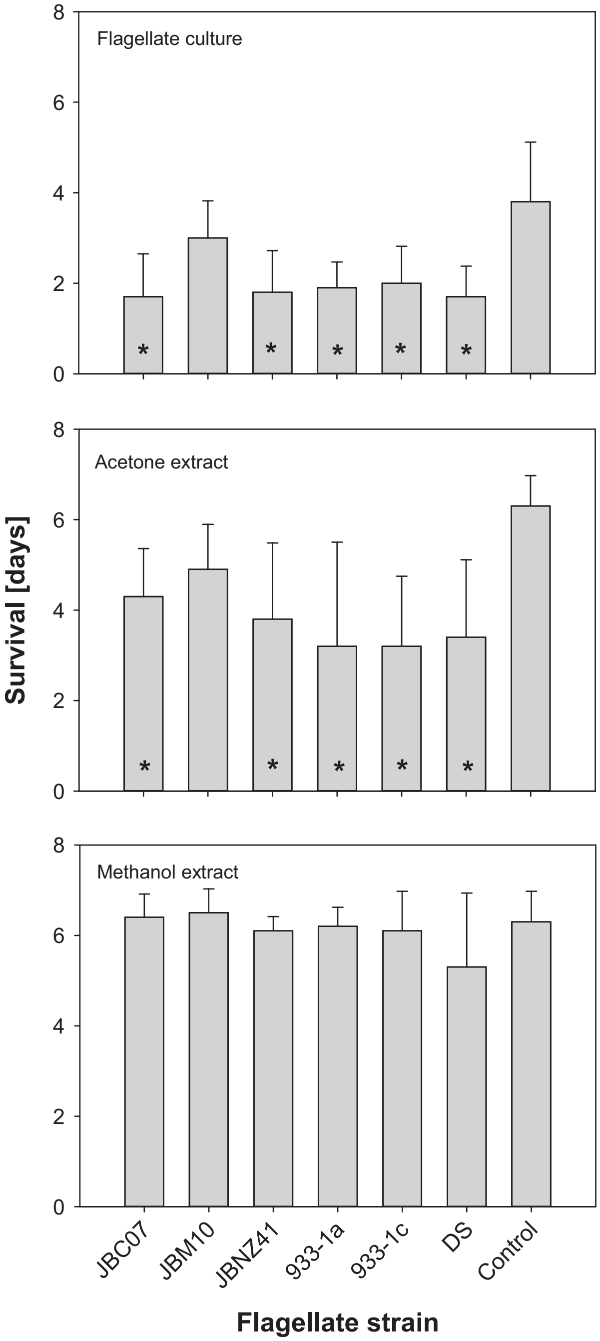

Fig. 1. Survival of neonates in the *D. magna* toxicity test using dense flagellate cultures and cell extracts. Flagellate cultures were tested at a density of 5–6 × 10^4 flagellates mL^{-1}. Cell extracts were tested at a concentration corresponding to 5 × 10^5 flagellates mL^{-1}. Significant differences of survival compared to the various control treatments are indicated by an asterisk.

Survivorship

Daphnia magna

Median survival of *D. magna* ranged from 5 to 17 days (Table II; Fig. 2), and survival was significantly different depending on the diet (Kruskal–Wallis one-way ANOVA, $P < 0.001$). *Daphnia magna* survived on all flagellate food strains longer than in the control treatment, but this was only significant for the green strains, i.e. *Poterioochromonas* strains 933-1a and 933-1c, the strain '*Ochromonas* DS' and *Cryptomonas* (Dunn's post-hoc test, $P < 0.05$; Table II). Survival of *D. magna* was significantly shorter on the heterotrophic strains compared to that in the green strains 933-1a, '*Ochromonas* DS' and *Cryptomonas* (except strain JBNZ41 versus *Cryptomonas*; Dunn's post-hoc test, $P < 0.05$; Table II). In all treatments except the treatments using the heterotrophic strains JBC07 and

Table II: *Survival and offspring of Daphnia magna fed with different flagellate food strains at food densities of* 1.5×10^4 *cells* mL^{-1}

	Survival (days)					Pairwise comparison of survival							
	n	25% quartile	Median	75% quartile	Offspring	JBC07	JBM10	JBNZ41	*Poterioochromonas malhamensis* 933-1a	*P. malhamensis* 933-1c	'*Ochromonas* DS'	*Cryptomonas*	Control
JBC07	15	6.25	8	9	0	—	0.109	0.111	3.283*	2.295	2.324*	3.152*	2.7
JBM10	15	7.25	8	8	0		—	0.221	3.174*	2.186	2.215*	3.035	2.817
JBNZ41	15	7	8	8.75	7			—	3.395*	2.406	2.435*	3.271*	2.581
P. malhamensis 933-1a	15	8	17	17.75	64				—	0.989	0.960	0.358	6.210*
P. malhamensis 933-1c	15	10	12	14	73					—	0.029	0.699	5.153*
'*Ochromonas* DS'	15	11	12	12	25						—	0.668	5.184*
Cryptomonas	20	10	13.5	17	33							—	6.321*
No food control	20	4	5	5	0								—

Significant differences of survival are given as *Q*-values of Dunn's pairwise comparison following a Kruskal–Wallis one way ANOVA. Asterisk indicates significant differences between treatments ($P < 0.05$).

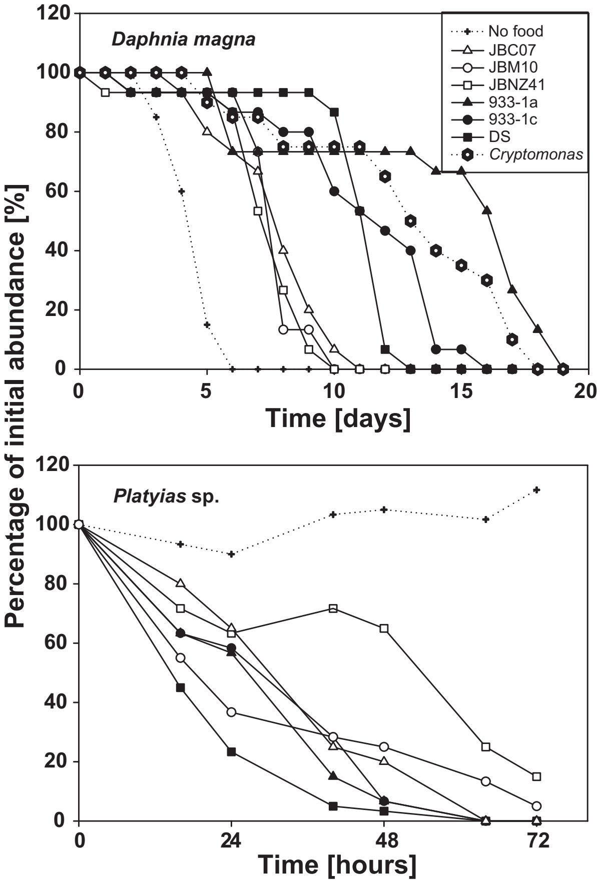

Fig. 2. Response of *D. magna* and *Platyias* sp. grown on flagellate cultures at a flagellate density of 1.5 and 1.3 × 10^4 flagellates mL^{-1} respectively.

JBM10, offspring were observed (Table II). Again, numbers of offspring produced were highest for the green strains. For the heterotrophic strains, only in the JBNZ41 treatment seven neonates were observed. In contrast, in the treatments using green strains, 25–73 neonates were observed (Table II). Both observations are suggestive of a lower food value of the heterotrophic flagellates compared to that of the green strains. Acute toxicity in terms of higher mortality compared to the no-food control could not be identified for the tested food concentrations in the range of 1–2 × 10^4 flagellates mL^{-1}.

Platyias sp.

The rotifers died back in all treatments with chrysomonad flagellates (Fig. 2). In contrast, in the control treatment, rotifer numbers remained high. After 24 h, survival was already significantly lower in all flagellate treatments compared to that in the control (ANOVA, $P < 0001$; Dunnet's post-hoc test, $P < 0.05$ for all flagellates). Consequently, mortality rate was significantly higher in all flagellate treatments compared to that in the control treatment (t-test on regression coefficients of log-transformed data; $P < 0.001$ in all cases). Except for strain JBNZ41, which showed a relatively high survival during the first 48 h, we observed no significant difference in rotifer survival between the tested chrysomonad strains (ANOVA, Dunnet's post-hoc test: $P > 0.05$).

DISCUSSION

Food particles must be considered toxic when, in lifespan experiments, the consumer feeding on them has a shorter lifespan than starving animals (Lampert, 1981). Toxicity has been demonstrated for osmotrophically grown strains of the mixotrophic genera *Ochromonas* and *Poterioochromonas*, even though the chemical composition of the toxin(s) is still uncertain: tests confirmed that the toxin(s) cause(s) bacterial growth inhibition (Hansen, 1973), fish mortality (Reich and Spiegelstein, 1964; Spiegelstein *et al.*, 1967, 1969; Halevy *et al.*, 1971; Magazanik and Halevy, 1973), zooplankton mortality (Leeper and Porter, 1995; Boxhorn *et al.*, 1998) and lysis of mammalian erythrocytes (Spiegelstein *et al.*, 1969; Halevy *et al.*, 1971; Magazanik and Halevy, 1973). We found that all tested '*Spumella*-like' flagellate strains as well as the related mixotrophic strains affiliated with *P. malhamensis* were toxic to metazoan plankton. The poor survivorship of the tested rotifers was indicative of cell toxicity. Furthermore, the *Daphnia* toxicity test proved that high densities of flagellates as well as of the acetone extracts were toxic to *D. magna*.

Our results demonstrate that phagotrophically grown flagellates, i.e. feeding on heat-killed bacteria, are toxic as well and further indicate that toxicity is not restricted to a few mixotrophic strains but present in different mixotrophic and heterotrophic strains, possibly a characteristic for the whole group. This finding matched with our expectations as both *Ochromonas* and *Spumella* are suspected to be polyphyletic, i.e. some *Ochromonas* and *Poterioochromonas* strains are more closely related to strains of *Spumella* than to other strains of *Ochromonas* (J. Boenigk *et al.*, submitted for publication), and toxicity has been shown to be stronger in heterotrophically growing flagellates compared to that in phototrophically growing flagellates (Leeper and Porter, 1995).

Our results indicate that acute toxicity is observed only at high flagellate abundances. We found no indication of toxicity for the filtered flagellate culture medium (3–5 × 10^4 flagellates mL^{-1}) on the basis of bacterial growth inhibition tests, except for the filtrate derived from the osmotrophically grown strain of *P. malhamensis* 1c. All other filtrates seemed to support bacterial growth. Toxicity (bacterial growth inhibition as well as toxicity to the fish *Danio malabricus*) has been reported for spent medium from *Ochromonas* and *Poterioochromonas* cultures (Halevy *et al.*, 1971; Hansen, 1973), a finding that is in contrast to our findings. These contrasting findings may be due to different flagellate densities in the growth media tested. The basic culture medium used in our study contained lower concentrations of organics (3 g of organics L^{-1}), and consequently, the flagellate abundance probably was much lower in our experiments than in the former studies. Even though we cannot exclude species-specific differences in toxin sensitivity, we assume that the different effects observed are related to the flagellate density in the growth medium and that adverse effects of extracellular substances on bacteria may only be observed at flagellate abundances exceeding 3–5 × 10^4 flagellate cells mL^{-1}. In contrast to the suggestion of Halevy *et al.* (Halevy *et al.*, 1971) and Boxhorn *et al.* (Boxhorn *et al.*, 1998), i.e. excreted toxins may negatively affect aquatic organisms even if the cells are not ingested, our results suggest that biological effects of excreted or dissolved toxins cannot be expected for natural cell densities. Low toxicity of the dissolved toxins is further supported by the low toxicity of the acetone and methanol extracts. The methanol extracts were less toxic compared to the acetone extracts, a finding that is in agreement with the findings of Hansen (Hansen, 1973).

Regarding the toxicity of ingested chrysomonads for zooplankton, our results indicate that abundances of 1–1.5 × 10^4 flagellates mL^{-1} are already toxic to the rotifer *Platyias* sp., which is in agreement with the study of Boxhorn *et al.* (Boxhorn *et al.*, 1998) who reported toxicity of *P. malhamensis* to *Brachionus angularis*. In that study, the lowest concentration tested was 2 × 10^4 flagellates mL^{-1}, which already showed adverse effects of the flagellates on rotifer survival. Our data further show that the heterotrophic '*Spumella*-like' flagellates affect *Platyias* sp. as strong as the *Poterioochromonas* strains. The cladoceran *D. magna* was also negatively affected at concentrations of 1.5–2 × 10^4 flagellates mL^{-1}, at least by the heterotrophic strains, but survivorship was still longer than that in the no-food control. The survivorship experiment using *D. magna* was therefore not indicative of cell toxicity, as the observed effects may partly be due to a low nutritional value of the different flagellate strains. However, the toxicity test proved the presence of substances toxic to *D. magna* in all flagellate strains, and the adverse effects in the survivorship experiment can therefore be assumed to be partly due to the presence of toxins. We assume that *D. magna* is negatively affected by the tested flagellates, but lethal effects are only observed for flagellate abundances exceeding 2 × 10^4 flagellates mL^{-1}. Toxicity of *P. malhamensis* for daphnids has been already demonstrated by Leeper and Porter (Leeper and Porter, 1995) using *Daphnia ambigua*. *Daphnia ambigua* seems to be more sensitive than *D. magna*, and this study indicates that toxicity is observed for flagellate abundances exceeding 1 × 10^4 flagellates mL^{-1}. As for the rotifers, our data indicate that the '*Spumella*-like' flagellates ar175*agellatesareastoxicastheirgreenrelativestocladocerans*.

Chrysomonads are an often-dominating component of the microbial food web: on annual average, 20–50% of pelagic heterotrophic nanoflagellate (HNF) biomass in freshwaters is formed by small heterokont taxa, mainly chrysomonads and bicosoecids (Salbrechter and Arndt, 1994; Carrias *et al.*, 1998; Arndt *et al.*, 2000), and '*Spumella*-like' flagellates have been reported to be common in freshwaters (Carrick and Fahnenstiel, 1989) and can reach abundances of several thousand flagellates mL^{-1} (Auer and Arndt, 2001; Weitere and Arndt, 2003). Similarly, *Ochromonas* and *Poterioochromonas* have been reported to be among the dominant mixotrophs (Bennet *et al.*, 1990), and abundances of the mixotrophic chrysomonads affiliated with *Ochromonas* and *Poterioochromonas* exceeding 1–3 × 10^3 cells mL^{-1} have been reported (Happey-Wood, 1976; Bird and Kalff, 1989). The potentially toxic chrysomonads may therefore reach abundances at which they appear to be toxic to zooplankton (Leeper and Porter, 1995; Boxhorn *et al.*, 1998), and adverse effects on the vertical carbon flow must be expected. Furthermore, toxicity may depend on environmental variables, and thus, even small flagellate populations in the field may appear toxic under certain conditions. We assume, however, that at normal field abundances of these organisms, i.e. $<10^3$ cells mL^{-1}, toxic effects are hardly detectable in the field, but this may change when abundance increases. For instance, a negative correlation between nanoflagellate abundance and rotifer birthrate has been reported by Baker (Baker, 1979). As far as we know, there are, however, no specific investigations focusing on the nutritional value of this flagellate group for zooplankton.

In conclusion, we have demonstrated that not only the mixotrophic chrysomonads affiliated with *Ochromonas* and *Poterioochromonas* but also related heterotrophic flagellates, i.e. '*Spumella*-like' nanoflagellates, can be toxic to zooplankton species. We found lethal effects on zooplankton species for mixotrophic as well as heterotrophic flagellate abundances exceeding 1–2 × 10^4 flagellates mL^{-1}, a finding that is in agreement with results of studies on zooplankton mortality due to ingestion of the mixotrophic *Poterioochromonas* (Leeper and Porter, 1995; Boxhorn *et al.*, 1998). We conclude that (i) the toxic potential of '*Spumella*-like' flagellates is similar to that of the mixotrophic *Poterioochromonas* spp. and (ii) acute toxicity is observed for flagellate abundances $>10^4$ flagellates mL^{-1}. The significance of toxicity of chrysomonads in the field has still to be investigated, but as both *Ochromonas* spp. and *Spumella* spp. often reach high abundances in the field and sublethal effects must already be expected at lower flagellate abundances, it can be assumed that these flagellates may achieve toxic densities in freshwaters.

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