

SHORT COMMUNICATION

Changes in the competitive abilities of two rotifers feeding on mixotrophic flagellates

MELANIE HARTWICH*, ALEXANDER WACKER AND GUNTRAM WEITHOFF

INSTITUTE FOR BIOCHEMISTRY AND BIOLOGY, UNIVERSITY OF POTSDAM, POTSDAM, GERMANY

*CORRESPONDING AUTHOR: melanie.hartwich@uni-potsdam.de

Received October 6, 2009; accepted in principle June 8, 2010; accepted for publication June 11, 2010

Corresponding editor: Roger Harris

The competitive abilities of two rotifer species (*Elosa worallii*, *Cephalodella* sp.) were influenced by the mode of carbon acquisition of the osmo-mixotrophic flagellate *Chlamydomonas acidophila* due to changes in cell biochemistry.

KEYWORDS: competition; mixotrophy; *Chlamydomonas*; *Elosa*; *Cephalodella*

Resource competition is a main driving force for the abundance and distribution of species. Exploitative competition is an indirect interaction between consumers by the exploitation of a shared resource (Lynch, 1978; Smith and Cooper, 1982; Rothhaupt, 1990) and is an important factor in structuring zooplankton communities (DeMott, 1989). In many water bodies, rotifers compete with crustaceans, especially with efficient filter-feeders such as large *Daphnia* (Vanni, 1986; Gilbert, 1988). Exploitative competition between rotifers is particularly expected when cladocerans are absent. This is the case, for example, in acidic mining lakes where the pH is too low for cladocerans. In lakes of the mining district of Lusatia, Germany, with pH <3, rotifers are the only planktonic metazoa, typically *Elosa worallii* and *Cephalodella* sp. (Deneke, 2000; Wollmann *et al.*, 2000). Many of these lakes also contain very few species of primary producers, often only two species: *Chlamydomonas acidophila* and *Ochromonas* sp. Both flagellates are mixotrophic, a nutritional strategy in protists

that is of growing interest (Spijkerman, 2007; Flynn and Mitra, 2009; Spijkerman, in press). They combine photosynthesis and the uptake of organic carbon as pathways for carbon acquisition. Laboratory experiments revealed that only *C. acidophila* is a suitable food source for the dominating rotifer species *E. worallii* and *Cephalodella* sp. (Weithoff, 2004; Weithoff, 2005). In Lake 111 (Lusatia, Germany), *C. acidophila* regularly builds up a deep chlorophyll maximum (DCM) at a water depth at which the light availability is reduced to <1% of surface irradiation (Tittel *et al.*, 2003; Kamjunke *et al.*, 2004). Therefore, the mode of carbon acquisition shifts from autotrophy through photosynthesis in the upper water layers to heterotrophy through the uptake of dissolved organic carbon in the deeper water layers. The mode of carbon acquisition in turn has a strong effect on the biochemical composition of the flagellates, especially on the polyunsaturated fatty acids (PUFAs) (Poerschmann *et al.*, 2004; Wacker and Weithoff, 2009). Recently, Weithoff and Wacker (Weithoff and Wacker,

2007) showed that the mode of carbon acquisition differentially determined the food quality for the two rotifers; in particular, *Cephalodella* sp. was little affected by the mode of carbon acquisition and grew on all types of *C. acidophila*, whereas *E. worallii* exhibited positive population growth only with autotrophically grown *C. acidophila*. Because of the different effect of the mode of carbon acquisition of *C. acidophila* on the population growth rates of the two rotifers, we investigated the direct competitive abilities of the two rotifer species under various environmental conditions: purely autotrophic, mixotrophic at two light intensities and purely heterotrophic in the dark.

All organisms originated from acidic mining lakes in the Lusatian area (*C. acidophila* and *E. worallii* from Lake 111, *Cephalodella* sp. from Lake 129). The stock cultures were semi-continuously cultured in a medium at pH 3, reflecting the chemical composition of lakes in the Lusatian area [i.e., it is characterized by high concentrations of sulphate, iron and aluminium (Bissinger *et al.*, 2000)]. *C. acidophila* was grown autotrophically at 20°C at a high light intensity of 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (HL), and at a low light intensity of 25 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (LL) under a light:dark cycle of 16:8 h. We chose this photoperiod to simulate the light conditions during summer in Lake 111, when the DCM builds up. For mixotrophic growth, *C. acidophila* was grown under the same light and temperature conditions in a medium that additionally contained glucose at a concentration of 20 mg C L^{-1} . Tittel *et al.* (Tittel *et al.*, 2005) have shown that *C. acidophila* uses both pathways, photosynthesis and osmotrophy, simultaneously when possible. Heterotrophically grown *C. acidophila* were cultured with glucose in the dark. Rotifer stock cultures were fed autotrophic *C. acidophila*.

Two experimental series were run. In the first series, the growth of the two rotifer species was investigated in single-species experiments; in the second series, competition in the same culture was investigated. Rotifer growth experiments were conducted in triplicate in 300-mL Erlenmeyer flasks with 10 rotifers mL^{-1} in 100 mL of the target algal suspension. The initial food concentration was above the limiting conditions for rotifer growth: 2.5 mg C L^{-1} for auto- and mixotrophic algae and 1.9 mg C L^{-1} for heterotrophic algae (Weithoff, 2005, 2007). Light and temperature conditions were the same as for the cultivation of algae. Every second day a subsample of 15 mL was taken and replaced by the fresh medium. From these subsamples, rotifer and algal densities were determined using an inverted light microscope (Thalheim, Germany). When algal concentration fell below the initial conditions, fresh algal suspension instead of pure medium was added,

resulting in a mean food concentration $>1.1 \text{ mg C L}^{-1}$. The experiments lasted 16 days, until rotifer densities remain constant. The growth rate r of the rotifers was calculated for the period of exponential growth, considering the dilution rate, from day 6 to 12 (exceptions: *Cephalodella* with autotrophic HL food day 8–14; *Cephalodella* with mixotrophic LL food day 0–8), according to $r = \ln(n_{t1}/n_{t0})/(t_1 - t_0)$, where n is the number of animals per millilitre and t_1 and t_0 is the time at the end and beginning of the time interval, respectively.

We determined carbon content, cell length and fatty acid composition of differently cultured *C. acidophila* to investigate the effects of the predominant nutrition mode of the algae on their physiology and biochemistry. For determination of the carbon content of the algae, algal suspensions were filtered on precombusted Whatman filters (GF/F; Whatman International Ltd, Maidstone, UK) and carbon was measured with a HighTOC (Elementar Analyse System GmbH, Hanau, Germany). From those data, cell numbers of *C. acidophila* were converted into carbon units. Cell length of differently grown *C. acidophila* was determined using a digital camera (JVC TK-C 1380E) and a computer-aided image analysis system (TSO-VID-MESS-HY 2.9). Aliquots of differently grown algal cultures were filtered onto Whatman GF/F filters, then were extracted with 7 mL dichloromethane/methanol (2:1; v:v) and stored under nitrogen at -20°C until analysis. Fatty acid composition was analyzed by gas chromatography as described previously (Wacker and Martin-Creuzburg, 2007; Sperfeld and Wacker, 2009). Data were analysed by one-way ANOVA and t -test with SPSS 15.0 (details in Tables I and II).

To investigate the competitive abilities of the two species, several approaches are possible. In one approach, the food level is kept constant by addition of resources according to the community consumption rate. Then, the species with the higher intrinsic growth rate will dominate, but competitive exclusion will not occur as long as both populations achieve positive intrinsic growth rates. An alternative approach is to allow the animals to reduce resource density. Then, the species that has the lower resource threshold survives and the other species goes extinct. This reflects the pure competitive exclusion principle. A disadvantage of this scenario is that populations might exhibit a highly dynamic behaviour and that random extinction might occur (Rosenzweig, 1971; Lande, 1993). We have chosen an intermediate approach by supplementing algae back to initial conditions every second day. This procedure has the advantage that strong population fluctuations are avoided and that severe resource depletion is prevented. The latter would have led to a food quantity effect, and

Table I: Maximum growth rates ($\text{day}^{-1} \pm \text{standard error}$) of *Cephalodella* and *Elosa* with differently cultivated *Chlamydomonas acidophila* as food during single-species and competition experiments

	Single species experiment		Competition experiment	
	<i>Cephalodella</i>	<i>Elosa</i>	<i>Cephalodella</i>	<i>Elosa</i>
auto HL	0.682 ± 0.020	0.459 ± 0.011	0.245 ± 0.068	$0.556 \pm 0.011^*$
mixo HL	0.663 ± 0.026	0.464 ± 0.017	0.582 ± 0.020	$0.354 \pm 0.015^*$
auto LL	0.662 ± 0.062	0.010 ± 0.029	0.346 ± 0.027	0.423 ± 0.044
mixo LL	0.565 ± 0.011	0.451 ± 0.004	0.483 ± 0.026	$0.307 \pm 0.027^*$
het	0.459 ± 0.012	-0.128 ± 0.033	0.095 ± 0.010	0.088 ± 0.014

HL, high light; LL, low light; auto, autotrophic; mixo, mixotrophic; het, heterotrophic.

*Significant differences between rotifer growth rates during the competition experiment (t -test: $P < 0.001$).

Table II: Physiological and biochemical parameters of *Chlamydomonas acidophila* cultivated under different nutrient and light conditions

	Growth rate (day^{-1})	Cell length (μm)	Total fatty acids ($\mu\text{g L}^{-1}$)	α -Linolenic acid ($\mu\text{g L}^{-1}$)
auto HL	0.754 ± 0.020^c	8.60 ± 0.78^b	147.77 ± 5.57^b	55.14 ± 2.08^a
mixo HL	1.177 ± 0.009^a	9.25 ± 0.90^a	174.56 ± 3.41^{ab}	53.83 ± 3.35^a
auto LL	0.156 ± 0.011^d	8.62 ± 0.70^b	180.63 ± 3.07^a	64.17 ± 1.04^a
mixo LL	0.936 ± 0.019^b	7.85 ± 0.69^c	176.87 ± 8.74^a	56.03 ± 2.87^a
het	0.053 ± 0.004^e	7.18 ± 0.66^d	54.54 ± 7.43^c	2.70 ± 0.40^b

Parameters were significantly different (ANOVA, growth rate: $F = 1236.76$, d.f. = 4/14, $P < 0.001$, $n = 3$; cell length: $F = 35.197$, d.f. = 4/149, $P < 0.001$, $n = 30$; total fatty acids: $F = 77.08$, d.f. = 4/14, $P < 0.001$, $n = 3$; α -linolenic acid: $F = 122.48$, d.f. = 4/14, $P < 0.001$, $n = 3$). Different letters show significant differences among growth conditions (Tukey-HSD, $P < 0.05$).

HL, high light; LL, low light; auto, autotrophic; mixo, mixotrophic; het, heterotrophic.

the food quality effect, the aim of our study, might have been masked (Sternier, 1997). We have used two different, though not independent, parameters, to assess the competitive abilities accounting for both growth and dominance pattern: (a) the maximum growth rate and (b) the final abundance when population densities remained constant. No competitive exclusion occurred.

We found a strong effect of the mode of carbon acquisition of *C. acidophila* on the competitive abilities of the rotifers alone and also during competition (Table I, Fig. 1). The results of the competition experiments were: (a) under high light autotrophic growth conditions, *E. worallii* was the superior competitor and (b) under high light mixotrophic growth conditions, *Cephalodella* sp. was the dominant species. Under low light conditions (c), both, autotrophic and mixotrophic, *Cephalodella* tended to dominate over *Elosa*, and this pattern was more pronounced under heterotrophic conditions (d). In detail:

- (a) High light, autotrophic conditions favoured the growth of *Elosa* and allowed for high positive

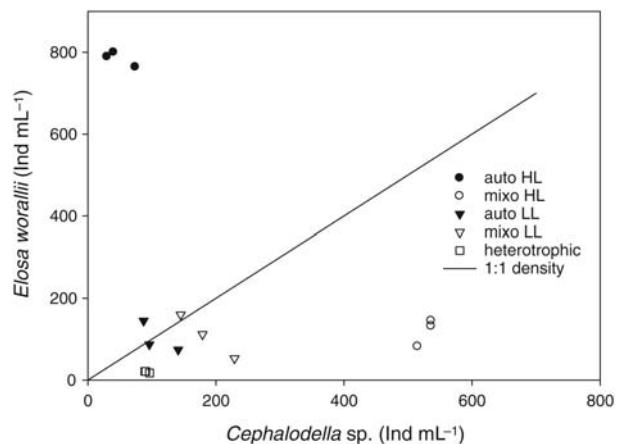


Fig. 1. Individual densities of *Elosa* and *Cephalodella* at the end of exponential growth with differently cultivated *Chlamydomonas acidophila* (HL, high light; LL, low light; auto, autotrophic; mixo, mixotrophic). The 1:1 line shows the equal share of both species.

growth rates and abundances (Table I, Fig. 1). Despite positive growth of *Cephalodella* in this treatment, growth rate and final population density of *Cephalodella* were suppressed by *Elosa* compared with growth in the single-species experiment due to resource competition. This can be explained by the lower food threshold concentration of *Elosa* compared with that of *Cephalodella* (Weithoff, 2007).

- (b) Under mixotrophic high light conditions, *Cephalodella* exhibited high growth rates and abundances and was able to dominate over *Elosa* whose growth rate was reduced. This resulted in an opposite pattern compared with autotrophic (HL) conditions and was in line with previous results demonstrating that mixotrophic food is of lesser quality for *Elosa* (Weithoff and Wacker, 2007); although, in that study, *Elosa* was more strongly affected by mixotrophic *C. acidophila*.
- (c) Under low light conditions, total rotifer abundances were lower than under high light. However, a similar pattern was found. Mixotrophic (LL) food

enabled *Cephalodella* to dominate over *Elosa*, whereas autotrophic conditions shifted the abundances towards an almost equal proportion of both rotifers. This is consistent with the inverse growth rates of the two species under mixotrophic and autotrophic conditions. The final abundances and the measured growth rates do not match perfectly because growth rates beyond that time interval was slightly variable. Nevertheless, the overall results were consistent.

- (d) Under heterotrophic conditions, *Cephalodella* was the superior competitor with significantly higher growth rates and density (Table I, Fig. 1). Contrary to the negative growth rates of *Elosa* on heterotrophic *C. acidophila* in our single-species results (Table I) and in Weithoff and Wacker (Weithoff and Wacker, 2007), *Elosa* exhibited slightly positive growth rates in the competition experiment with heterotrophic food, but only high enough to compensate for the dilution rate. This confirms that *Elosa* has low competitive abilities under heterotrophic food conditions.

Combining all results, an overall picture emerged: along a gradient from autotrophy to heterotrophy, the dominance pattern shifted from *Elosa* to *Cephalodella*.

The suitability of the differently cultured flagellates as diets for the rotifers may have varied due to differences in their biochemical composition, cell size and growth response under the different growth conditions (Table II). We found strong reductions in growth rate, cell length, total fatty acid content and especially α -linolenic acid (18:3n-3; ALA) content in heterotrophically grown *C. acidophila*. This difference in algal biochemistry might explain the low growth rates of *Elosa* with heterotrophic *C. acidophila* (Table I). Since *Elosa* has a lower food threshold than *Cephalodella* (Weithoff, 2007), the observed low competitive ability is not due to low food quantity and can only be explained by food quality. Thus, the low growth rate of *Elosa* seems to be the effect of the distinctly altered biochemistry of the heterotrophic algae. In particular, the low ALA and total fatty acid contents might have reduced the growth rate of *Elosa* (Ahlgren *et al.*, 1990). In general, *C. acidophila* does not have measurable amounts of eicosatetraenoic acid (20:4n-3; ETA) and eicosapentaenoic acid (20:5n-3; EPA) (Wacker and Weithoff, 2009), whereas *Elosa* has a high and constant content of ETA and also significant amounts of EPA. The production of these two fatty acids is presumably dependent on the presence of ALA as a potential precursor (Wacker and

Weithoff, 2009). Thus, limited concentrations of ALA in the food may limit the growth of *Elosa*.

In contrast, *Cephalodella* grew on the heterotrophically grown *C. acidophila* which is consistent with the results from Weithoff and Wacker (Weithoff and Wacker, 2007) although different strains were used in the two studies. This suggests that the ability to grow on heterotrophic food is not strain-specific, but common in acidophilic *Cephalodella*. Wacker and Weithoff (Wacker and Weithoff, 2009) found the fatty acid composition of *Cephalodella* to vary, depending on the mode of carbon acquisition of *C. acidophila*. Hence, the success of *Cephalodella* feeding on heterotrophic *C. acidophila* lies most likely in the better adaptability to the fatty acid composition of its food.

Our results have important implications for the abundance of the two rotifer species in their natural habitat, although the *Cephalodella* strain we used in this study originates from Lake 129, while a strain from Lake 111 was used for other studies (Weithoff and Wacker, 2007; Wacker and Weithoff, 2009). In subsequent studies, it was shown that both *Cephalodella* strains (or species) differ in some morphological and ecological characters, e.g. pH tolerance (G. Weithoff, unpublished data), but responded similar to the differently grown *C. acidophila* (this study; Weithoff and Wacker, 2007). A detailed taxonomic identification still needs to be carried out; however, both strains responded in the same way to their food source, demonstrating that the strain we used in the present study was reasonable for this investigation and allows to transfer our results to the field situation in Lake 111.

The dominance of *Elosa* over *Cephalodella* in the epilimnion in Lake 111 can be explained by the higher efficiency of *Elosa* in using autotrophic *C. acidophila* as food. In the hypolimnion, where *C. acidophila* occurs below the compensation point for purely autotrophic growth, the mixotrophic mode of nutrition prevails. This allows *Cephalodella* to dominate over *Elosa* because of its higher competitive potential under low light and mixotrophic conditions.

Although our results were derived from an extreme habitat, they might be transferable to circum-neutral lakes as well. A vertical segregation of rotifers is probably more common than detected, because most studies use depth-integrated sampling protocols. In addition, other factors, for example temperature, predation or ultraviolet radiation (Miracle and Armengol-Díaz, 1995; Obertegger *et al.*, 2008), food quality in deeper water layers might contribute to pronounced vertical distribution patterns. Deep chlorophyll maxima are a common characteristic of meso-eutrophic lakes, and they develop typically at a depth around or below the compensation point for autotrophic growth (Adler *et al.*, 2000; Gervais *et al.*, 2003). Thus, the exploitation by

zooplankton of this quantitatively rich resource might be hampered by the resource quality. Since at least some rotifers are able to persist with the described reduction in food quality, such species may also have a competitive advantage over cladocera that have a higher demand for long-chained PUFAs (Von Elert, 2002; Wacker and Martin-Creuzburg, 2007; Martin-Creuzburg *et al.*, 2009). Unfortunately, in studies on the DCM, the vertical distribution of micro-zooplankton is often not recorded.

ACKNOWLEDGEMENTS

We wish to thank S. Heim and Ch. Schirmer for assistance in the laboratory.

REFERENCES

- Adler, M., Gervais, F. and Siedel, U. (2000) Phytoplankton species composition in the chemocline of mesotrophic lakes. *Arch. Hydrobiol. Spec. Issues Adv. Limnol.*, **55**, 513–530.
- Ahlgren, G., Lundstedt, L., Brett, M. *et al.* (1990) Lipid composition and food quality for some freshwater phytoplankton for cladoceran zooplankters. *J. Plankton Res.*, **12**, 809–818.
- Bissinger, V., Jander, J. and Tittel, J. (2000) A new medium free of organic carbon to cultivate organisms from extremely acidic mining lakes (pH 2.7). *Acta Hydrochim. Hydrobiol.*, **28**, 310–312.
- DeMott, W. R. (1989) The role of competition in zooplankton succession. In Sommer, U. (ed.), *Plankton Ecology: Succession in Plankton Communities*. Springer, Berlin, pp. 195–252.
- Deneke, R. (2000) Review of rotifers and crustaceans in highly acidic environments of pH values ≤ 3 . *Hydrobiologia*, **433**, 167–172.
- Flynn, K. J. and Mitra, A. (2009) Building the “perfect beast”: modelling mixotrophic plankton. *J. Plankton Res.*, **31**, 965–992.
- Gervais, F., Siedel, U., Heilmann, B. *et al.* (2003) Small-scale vertical distribution of phytoplankton, nutrients and sulphide below the oxycline of a mesotrophic lake. *J. Plankton Res.*, **25**, 273–278.
- Gilbert, J. J. (1988) Suppression of rotifer populations by *Daphnia*: a review of the evidence, the mechanisms, and the effects on zooplankton community structure. *Limnol. Oceanogr.*, **33**, 1286–1303.
- Kamjunke, N., Gaedke, U., Tittel, J. *et al.* (2004) Strong vertical differences in the plankton composition of an extremely acidic lake. *Arch. Hydrobiol.*, **161**, 289–306.
- Lande, R. (1993) Risks of population extinction from demographic and environmental stochasticity and random catastrophes. *Am. Nat.*, **142**, 911–927.
- Lynch, M. (1978) Complex interactions between natural coexploiters—*Daphnia* and *Ceriodaphnia*. *Ecology*, **59**, 552–564.
- Martin-Creuzburg, D., Sperfeld, E. and Wacker, A. (2009) Colimitation of a freshwater herbivore by sterols and polyunsaturated fatty acids. *Proc. R. Soc. London B*, **276**, 1805–1814.
- Miracle, M. R. and Armengol-Diaz, X. (1995) Population dynamics of oxycinal species in lake Arcas-2 (Spain). *Hydrobiologia*, **313/314**, 291–301.
- Obertegger, U., Flaim, G. and Sommaruga, R. (2008) Multifactorial nature of rotifer water layer preferences in an oligotrophic lake. *J. Plankton Res.*, **30**, 633–643.
- Poerschmann, J., Spijkerman, E. and Langer, U. (2004) Fatty acid patterns in *Chlamydomonas* sp. as a marker for nutritional regimes and temperature under extremely acidic conditions. *Microb. Ecol.*, **48**, 78–89.
- Rosenzweig, M. L. (1971) Paradox of enrichment: destabilization of exploitation ecosystems in ecological time. *Science*, **171**, 385–387.
- Rothhaupt, K. O. (1990) Resource competition of herbivorous zooplankton: a review of approaches and perspectives. *Arch. Hydrobiol.*, **118**, 1–29.
- Smith, D. W. and Cooper, S. D. (1982) Competition among Cladocera. *Ecology*, **63**, 1004–1015.
- Sperfeld, E. and Wacker, A. (2009) Effects of temperature and dietary sterol availability on growth and cholesterol allocation of the aquatic keystone species *Daphnia magna*. *J. Exp. Biol.*, **212**, 3051–3059.
- Spijkerman, E. (2007) Phosphorus acquisition by *Chlamydomonas acidophila* under autotrophic and osmo-mixotrophic growth conditions. *Exp. Bot.*, **58**, 4195–4202.
- Spijkerman, E. (2010) High photosynthetic rates under a co-limitation for P_i and CO_2 . *J. Phycol.*, **46**, DOI: 10.1111/j.1529-8817.2010.00859.x.
- Sterner, R. W. (1997) Modelling interactions of food quality and quantity in homeostatic consumers. *Freshwat. Biol.*, **38**, 473–481.
- Tittel, J., Bissinger, V., Zippel, B. *et al.* (2003) Mixotrophs combine resource use to out-compete specialists: implications for aquatic food webs. *Proc. Natl. Acad. Sci. USA*, **100**, 12776–12781.
- Tittel, J., Bissinger, V., Gaedke, U. *et al.* (2005) Inorganic carbon limitation and mixotrophic growth in *Chlamydomonas* from an acidic mining lake. *Protist*, **156**, 63–75.
- Vanni, M. J. (1986) Competition in zooplankton communities: suppression of small species by *Daphnia pulex*. *Limnol. Oceanogr.*, **31**, 1039–1056.
- Von Elert, E. (2002) Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. *Limnol. Oceanogr.*, **47**, 1764–1773.
- Wacker, A. and Martin-Creuzburg, D. (2007) Allocation of essential lipids in *Daphnia magna* during exposure to poor food quality. *Funct. Ecol.*, **21**, 738–747.
- Wacker, A. and Weithoff, G. (2009) Carbon assimilation mode in mixotrophs and the fatty acid composition of their rotifer consumers. *Freshwater Biol.*, **54**, 2189–2199.
- Weithoff, G. (2004) Vertical niche separation of two consumers (Rotatoria) in an extreme habitat. *Oecologia*, **139**, 594–603.
- Weithoff, G. (2005) On the ecology of the rotifer *Cephalodella hoodi* from an extremely acidic lake. *Freshwater Biol.*, **50**, 1464–1473.
- Weithoff, G. (2007) Dietary restriction in two rotifer species: the effect of the length of food deprivation on life span and reproduction. *Oecologia*, **153**, 303–308.
- Weithoff, G. and Wacker, A. (2007) The mode of nutrition of mixotrophic flagellates determines the food quality for their consumers. *Funct. Ecol.*, **21**, 1092–1098.
- Wollmann, K., Deneke, R., Nixdorf, B. *et al.* (2000) Dynamics of planktonic food webs in three mining lakes across a pH gradient (pH 4–2). *Hydrobiologia*, **433**, 3–14.