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Influence of food-type on the population growth rate of the rotifer *Brachionus calyciflorus* in short-chronic assays *

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Abstract The type of food given during short chronic assays with the rotifer *Brachionus calyciflorus* might be one of the sources of variation in the reproductive rate of the rotifers. Ten green algal species were supplied as monospecific diets to examine variability in rotifer growth rate. In addition，rotifers were fed cyanobacteria as monospecific food and in mixtures with the green alga *Scenedesmus obliquus* to test the hypothesis that cyanobacteria are a valuable supplement in combination with food items such as *Scenedesmus*. The population growth rate of *B. calyciflorus* was influenced significantly by the different green algal food species. *S. obliquus* promoted the highest growth in *B. calyciflorus*（1.6 per day），while growth on *Desmodesmus abundans* was the lowest（0.3 per day）. As percentage of maximal growth on *S. obliquus*，the sequence of green algal food species is: *Desmodesmus subspicatus*（88%），*Chlorella vulgaris*（83%），*Monoraphidium minutum*（77%），*D. quadricauda*（74%），*S. falcatus*（71%），*S. acuminatus*（69%），*S. pectinatus*（64%），*Chlamydomonas reinhardtii*（57%）and *D. abundans*（19%）. The growth differences were not explained from size differences of the algae. The cyanobacteria *Microcystis aeruginosa* and *Synechococcus elongatus* depressed growth of *B. calyciflorus* not only as monospecific food but also in mixed diets with the good food source *S. obliquus*. The detrimental effect appeared not related to microcystins. The results do not support the hypothesis that nontoxic cyanobacteria are a valuable supplement in combination with other green algal food species. The variation in growth rates observed in current study underlines the influence food species might have on the growth of *B. calyciflorus* and potentially on the outcome of toxicity tests [*Acta Zoologica Sinica* 52（1）: 70－78，2006].
Key words Rotifer，*Brachionus calyciflorus*，Food，Population growth

食物种类对萼花臂尾轮虫种群增长率的影响 *

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摘 要 在短期慢性观测过程中，食物类型可能是造成萼花臂尾轮虫（*Brachionus calyciflorus*）种群繁殖率变化的一种原因。共观测了分别单独投喂 10 种不同绿藻对轮虫种群增长率的影响。为验证藻青菌是藻类饵料（如绿藻 *Scenedesmus*）有价值的佐剂这一假说，还用蓝细菌单独投喂或与斜生栅藻（*Scenedesmus obliquus*）混合投喂轮虫进行实验观测。结果发现食物种类对萼花臂尾轮虫种群增长率影响显著。斜生栅藻组获得最大种群增长（1.6/d），而 *Desmodesmus* 组增长率最低（0.3/d）。以占斜生栅藻组最大增长的百分率来表示，其它几种绿藻组种群增长由高到低依次为：*Desmodesmus subspicatus* 88%，小球藻（*Chlorella vulgaris*）83%，单壳缝藻（*Monoraphidium minutum*）77%，*D. quadricauda* 74%，*S. falcatus* 71%，*S. acuminatus* 69%，*S. pectinatus* 64%，莱茵衣藻（*Chlamydomonas reinhardtii*）57%，*D. abundans* 19%。轮虫增长率的差异不能用藻类饵料的大小差异来解释。蓝细菌（*Microcystis aeruginosa*）和（*Synechococcus elongates*）不论是单独投喂还是与优良藻类饵料（斜生栅藻）混合投喂都对萼花臂尾轮虫种群增长有抑制作用。这种副作用似乎与微囊藻素无关。该结果不支持无毒蓝细菌可作为与其他绿藻饵料配合使用的优良佐剂这一假说。本研究所观察到的生长变化显示了饵料种类对

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萼花臂尾轮虫种群增长的影响，也预示了对毒性实验结果的影响［动物学报 52（1）：70－78，2006］。
关键词 萼花臂尾轮虫 食物 种群增长

Planktonic rotifers are known to play an important role in the energy flow in lakes and ponds, and numerous studies have indicated the importance of rotifers as primary consumers (e.g., Dumont, 1977; Pilarska, 1977; Pourriot, 1977; Walz, 1995). Because of their importance in zooplankton communities and aquatic food chains rotifers have been used as model organisms in feeding and food chain studies, in aquaculture research and ecotoxicological research (e.g., Bogdan et al., 1980; Schlüter et al., 1987; Walz, 1995; Chial and Persoone, 2003).

In ecotoxicological research, two-day life cycle tests with the rotifer *Brachionus calyciflorus* in which multiple broods are produced and the F_1 generation also reproduces are used extensively (e.g., Snell et al., 1991; Snell and Moffat, 1992; Radix et al., 1999; Chial and Persoone, 2003; Maršálek and Bláha, 2004). In contrast to acute assays with mortality as endpoint, the two-day life cycle test has growth or reproduction as endpoint, which requires feeding of the test organisms. However, considerable variability in test results between studies might occur due to use of different food species and growth conditions (Chial and Persoone, 2003). In general, green algal species such as members of the common genera *Chlamydomonas*, *Chlorella*, *Desmodesmus* and *Scenedesmus* are fed to *B. calyciflorus*, but there is still no consensus on the nutritional adequacy of the food species. The results of some studies, for example, suggest *Chlorella* sp. to be a better food than *Scenedesmus* species (e.g., Xi et al., 2001; 2002; Flores-Burgos et al., 2003), whereas in others *B. calyciflorus* grew better on *Scenedesmus* than on *Chlorella* (e.g., Rothhaupt, 1990a). Comparison of the results from these studies, however, is difficult as different methods for long-term assays were used.

In several studies, *B. calyciflorus* in the 2-d growth assays were offered a monospecific diet of only live green algae (e.g., Cecchine and Snell, 1999; Radix et al., 2000, 2002). However, in other studies, the rotifers were fed a combined food of live green algae and dried cyanobacteria, *Spirulina* (Chial and Persoone, 2003, 2005). Cyanobacterial species might be a valuable supplement in combination with other green algal food species as, for example, a stimulatory effect has been reported for *B. calyciflorus* fed a 3∶1 mixture of the green alga *Monoraphidium* and the cyanobacterium *Planktothrix* (Weithof and Walz, 1995). In contrast, mixed feeds of the cyanobacterium *Anabaena* and the cryptophyte *Cryptomonas* hampered growth of *B. calyciflorus* (Gilbert, 1994, 1996). These studies were performed with filamentous cyanobacteria, but to our knowledge effects of mixed diets of green algae with non-filamentous cyanobacteria are virtually absent in literature.

The aim of the current study was to examine the effect of type of food given during short chronic assays with the rotifer *Brachionus calyciflorus* on the reproductive rate of the rotifers. According to Snell and Moffat (1992) growth rates should not be less than the 0.65 acceptability threshold for short chronic tests in order not to influence the outcome of the toxicity assays. Consequently, feed organisms should be of good nutritional value to avoid unsatisfactory reproduction. Inasmuch as the type of green algal food might seriously affect the growth of *B. calyciflorus*, in the current study, 10 green algal species as a variable in the growth rate of the rotifers were compared simultaneously in a 2-d growth assay. In addition to the effect of the type of green algal food also the effect of the non-filamentous cyanobacteria *Microcystis aeruginosa* and *Synechococcus elongatus* as monospecific food and in mixtures with the green alga *Scenedesmus obliquus* is studied. This was done to test the hypothesis that cyanobacteria are a valuable supplement in combination with food items such as *Scenedesmus*.

1 Materials and methods

1.1 Organisms

The green algae and cyanobacteria used in the current study are listed in Table 1. Semi-continuous stock cultures were maintained in 300 ml cellulose plug closed Erlenmeyer flasks containing 150 ml of modified WC (Woods Hole Chu)-medium (Lürling and Beekman, 1999) with vitamins added (Biotin B_1 and B_{12} at 50 ng/L, Thiamnine HCL at 100 ng/L). Flasks with green algae were kept in climate controlled room at 20℃ in continuous light of 60-μmol quanta/m^2/s and every three weeks for at least three months prior to the experiments, aliquots were transferred separately into fresh sterile medium. Flasks with cyanobacteria were kept at 20℃ and in 45 μmol quanta m/m^2/s light in a 16∶8 h light∶dark rhythm.

The rotifer *Brachionus calyciflorus* Pallas was obtained from Microbiotests Inc. (Nazareth, Belgium) and was supplied as cysts. Cysts were kept refrigerated until use.

Table 1 Green algae (Exp. Ⅰ) and cyanobacteria (Exp. Ⅱ) used as food for the rotifer *Brachionus calyciflorus* in short-chronic assays

Species	Exp. Ⅰ: Green algae	
	Strain	Culture collection
Chlamydomonas reinhardtii	NIVA-CHL13	NIVA[1], Oslo, Norway
Chlorella vulgaris	NIVA-CHL 19	NIVA, Oslo, Norway
Desmodesmus abundans	UTEX 1358	University of Texas, Austin, USA
Desmodesmus quadricauda	UTEX 614	University of Texas, Austin, USA
Desmodesmus subspicatus	NIVA-CHL 55	NIVA, Oslo, Norway
Monoraphidium minutum	SAG 243/1	University of Göttingen, Germany
Scenedesmus acuminatus	V411	University of Plovdiv, Bulgaria
Scenedesmus falcatus	SAG 2.81	University of Göttingen, Germany
Scenedesmus obliquus	SAG 276/3a	University of Göttingen, Germany
Scenedesmus pectinatus	v99	University of Plovdiv, Bulgaria
Species	Exp. Ⅱ: Cyanobacteria	
	Strain	Culture collection
Microcystis aeruginosa	SAG 17.85	University of Göttingen, Germany
Microcystis aeruginosa	SAG 18.85	University of Göttingen, Germany
Synechococcus elongatus	SAG 89.79	University of Göttingen, Germany

Food types used in Exp Ⅱ:
100% cyanobacterium − 0% *S. obliquus*; 75% − 25%; 50% − 50%; 25% − 75%;
100%; 75%; 50%; 25%; 0% *S. obliquus*

1: Norwegian Institute for Water Research

1.2 Exp. Ⅰ: effect of green algae

The first experiment evaluated the effect of 10 different green algal species (Table 1) on rotifer growth rate. To obtain exponentially growing algae with good nutritional value, aliquots of green algae from the semi-continuous stock cultures were transferred prior to the experiment into 100-ml Erlenmeyer flasks; containing 50 ml WC medium with vitamins added and that were closed with a cellulose plug. Algal densities and particle size distributions were determined in the size range 2.5 − 35 μm equivalent spherical diameter using a Coulter® Multisizer Ⅱ (capillary 100 μm orifice width, Coulter Electronics Limited, Luton, England). Each flask received identical initial concentration of algae, i. e. 5×10^6 μm^3/ml. These flasks were placed on a rotating shaking table (60 r/m) at 24℃ in continuous light of 100-μmol quanta/m^2/s. After three days the algae were transferred again at identical concentrations of $5 \times 10^6 \mu m^3$/ml into 100 ml Erlenmeyer's that contained 50-ml WC-medium. After three days the algae were used in the experiment. Meanwhile *B. calyciflorus* cysts were hatched in 100-ml suspensions of each of the corresponding green algae in the above mentioned WC-medium. The hatching flasks were placed on a rotating shaking device at 24℃ in continuous light of 100-μmol quanta/m^2/s. The cysts were hatched in the desired food inasmuch as a short-prefeeding period (2 h after hatching) has been found to have a pronounced positive effect on growth rates (Chial and Persoone, 2005).

The experiment was run in 6-welled covered culture plates positioned for two days on a rotating shaking table (60 r/m) in darkness in a climate-controlled room at 24℃. Each well was filled with 10-ml green algal food suspensions at identical concentrations of 10^7 μm^3/ml (≈5 mg C/L). For each food type (Table 1) six wells were used of which three received six freshly hatched (2 − 3 h old) *Brachionus*, while three other replicates received no rotifers. The newborn rotifers were collected in a dish with 15 ml fresh WC-medium and were counted and transferred from the dish to the culture plates under a dissecting microscope at 20× magnification using a prepared glass fine tip pipette. The experiment was run for 48 hours after which chlorophyll-a concentrations were determined in 1.5-ml samples from each well using a PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany). This is a non-destructive method in which chlorophyll concentrations are used to estimate algal disappearance rates and to estimate rotifer clearance rates (Lürling and Verschoor, 2003). Clearance rates (*CR*, ml/ind./h) were calculated using the equation:

$$CR = \left(\frac{\ln(A_{control}) - \ln(A_{rotifer})}{\Delta t}\right) \times \frac{V}{N}$$

where $A_{control}$ is the algal concentration (as chlorophyll-*a* (Chl_{F_0}) in $\mu g/L^{-1}$) after 2 days in controls, $A_{rotifer}$ is the algal concentration in the rotifer treatments, Δt is the time (h), V is the culture volume (ml) and N is the number of animals. For N mean values were estimated using the population growth rate and the exponential growth model. Algal disappearance rates (Dr, per day) were estimated from: $Dr = \frac{[\ln(Chla_{rotifer}) - \ln(Chla_{control})]}{2}$, where Dr is the algal disappearance rate (per day), $Chla_{control}$ is the chlorophyll-a concentration (in $\mu g/L$) after 2 days in controls and $Chla_{rotifer}$ is the chlorophyll-*a* concentration (in $\mu g/L$) after 2 days in presence of rotifers.

Then adding 0.5 ml of Lugol's fixative to each well terminated the experiment. The numbers of rotifers were counted under a dissecting microscope and population growth rates were calculated from: $r = \frac{[\ln(N_2) - \ln(N_1)]}{2}$, where r is the population growth rate (per day), N_2 is the number of animals after 2 days and N_1 the initial number of rotifers (=6).

1.3 Exp. Ⅱ: effect of cyanobacteria

A second experiment was run to study the effect on rotifer growth rate of mixtures of cyanobacteria with *S. obliquus* that included 0%, 25%, 50%, 75% and 100% cyanobacteria (Table 1). The cyanobacteria were harvested from one-week old cultures maintained at identical conditions as the cyanobacterial stock cultures (20℃ and in 45 μmol quanta/m/s light in a 16∶8 h light∶dark rhythm), *S. obliquus* from a culture as outlined above. Each food-type was run in triplicate at 10^7 μm^3/ml. The experiment was run in six-welled culture plates in darkness in a climate-controlled room at 20 ± 1℃. After 2 days, the numbers of rotifers were counted under a dissecting microscope. Growth rates were calculated as outlined above (Exp. Ⅰ).

1.4 Statistics

Population growth rates and algal disappearance rates in Exp. Ⅰ were analyzed by one-way ANOVA in the statistical toolpack SPSS 10.1.0 (SPSS, 2000). In Exp. Ⅱ, mean population growth rates were compared running two-way ANOVA with cyanobacterial species and food composition as the fixed factors. For comparisons among treatments and species also one-way ANOVA were run. Differences between means were distinguished by Tukey's *post hoc* comparison test ($P<0.05$). In Exp. Ⅰ, relationships between green algal particle volume, algal disappearance rates, rotifer clearance rates and population growth were estimated by non-linear regression using a sigmoid model (four-parameter Gompert function) in the tool-pack SigmaPlot 2000, version 6.00.

2 Results

2.1 Exp. Ⅰ: effect of green algae

The 10 different green algal food species exerted distinct effects on the population growth rate of *B. calyciflorus* (Fig.1). The one-way ANOVA indicated that growth rates were significantly different between food types ($F_{9,20} = 155.3$, $P < 0.001$). Tukey's *post-hoc* comparison revealed 8 homogenous groups that were significantly different (Fig.1). With the exception of *D. abundans* all green algal species supported good to excellent growth in *B. calyciflorus*. The highest growth rates were observed when *S. obliquus* was offered as food. Growth as a percentage of the maximum (= on *S. obliquus*) growth rate for other green algae varied between 19% (*D. abundans* as food) and 88% (*D. subspicatus* as food). For most species the variance coefficients were low (<7%), however, for *D. abundans* it was 47% (Fig.1). Green algal disappearance rates were also significantly different ($F_{9,20} = 41.6$, $P < 0.001$) between food-types (Fig.2). Tukey's *post-hoc* comparison revealed 6 homogenous groups with the highest disappearance rate for *S. obliquus* and the lowest for *D. abundans*, *C. reinhardtii* and *S. pectinatus* (Fig.2). Estimated clearance rates differed significantly ($F_{9,20} = 3.05$, $P = 0.018$), but Tukey's test revealed that only the clearance rates of rotifers fed *C. reinhardtii* were significantly lower than those feeding on *S. acuminatus* (Fig.3). Mean rotifer clearance rates varied between 1.5 and 7 μl microliter/rotifer/h (Fig.3). The mean particle volumes of the green algae showed considerable variation (Table 2). Because the data did not satisfy assumptions for ANOVA, separate *t*-tests were run. These tests showed that particle volumes of *D. quadricauda* and *S. pectinatus* were significantly larger than of all the other green algae, while those of *M. minutum* and *Chlorella vulgaris* were the smallest (Table 2). Non-linear regression analyses yielded no significant relationship between the particle volume and the algal disappearance rate ($r^2_{adj} = 0.00$, $F = 0.82$, $P = 0.528$), clearance rates ($r^2_{adj} = 0.00$, $F = 0.02$, $P = 0.994$) or rotifer growth rates ($r^2_{adj} = 0.00$, $F = 0.93$, $P = 0.481$). Algal disappearance rates and rotifer growth rates ($r^2_{adj} = 0.597$, $F = 5.45$, $P = 0.038$) as well as algal disappearance rates and clearance rates ($r^2_{adj} = 0.578$, $F = 5.12$, $P = 0.043$) were significantly correlated, but clearance rates and population growth rates showed no correlation ($r^2_{adj} = 0.190$, $F = 1.70$, $P = 0.265$).

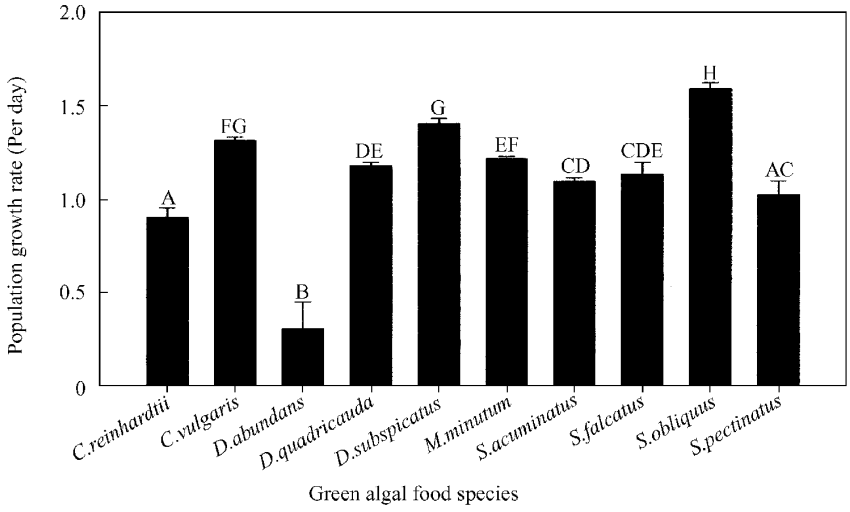

Fig.1 Population growth rate (per day) of the rotifer *Brachionus calyciflorus* in short-chronic assays (2-d) fed with ten different green algal species

Error bars indicate one standard deviation ($n=3$). Similar symbols above bars indicate homogeneous groups that are not significantly different (Tukey's *post hoc* comparison, $P<0.05$).

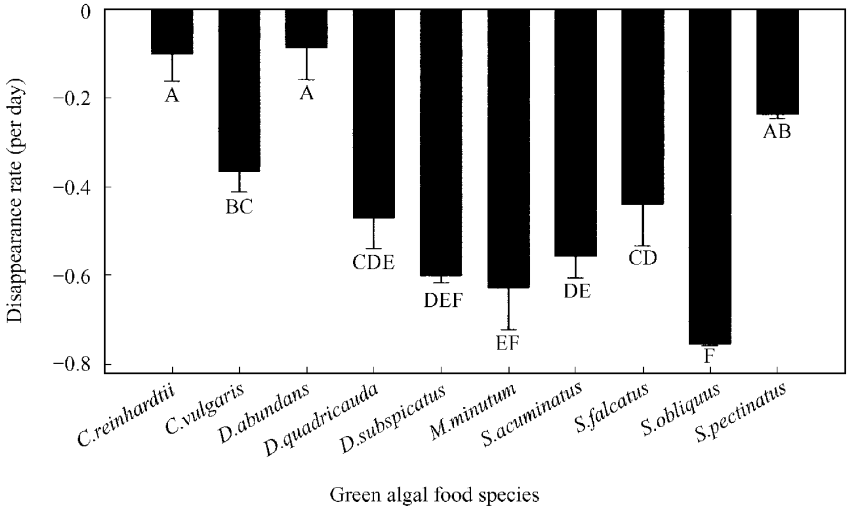

Fig.2 Disappearance rate (per day) of ten different green algal species used as food for the rotifer *Brachionus calyciflorus* in short-chronic assays (2-d)

Error bars indicate one standard deviation ($n=3$). Similar symbols below bars indicate homogeneous groups that are not significantly different (Tukey's *post hoc* comparison, $P<0.05$).

2.2 Exp. Ⅱ: effect of cyanobacteria

Unicells and bicells dominated the cyanobacteria used in this experiment. *Synechococcus* was comprised of 83% unicells and 17% two-celled colonies, making on average 1.17 cells per colony with a cell diameter of 2.7 (±0.4) μm. Strain SAG 17.85 was comprised of 88% unicells (diameter of 3.9 ± 0.4 μm) and 12% bicells (1.11 cells per colony), while SAG 18.85 consisted of 57% unicells (diameter of 3.2 ± 0.4 μm) and 17% bicells (2.18 cells per colony).

A two-way ANOVA indicated a significant cyanobacteria species effect on growth rates of *B. calyciflorus* ($F_{3,40}=21.5$, $P<0.001$), a significant mixture effect ($F_{4,40}=81.4$, $P<0.001$), and a significant species×mixture interaction ($F_{12,40}=4.60$, $P<0.001$). The latter is visualized in the different effects on growth rates of comparable mixtures (Fig.4). For example, population growth rates on monospecific cyanobacterial diets were slightly positive on *Microcystis* SAG17.85, slightly negative on *Synechococcus*, but a strong decline was found for animals fed *Microcystis* SAG18.85, which was comparable to unfed animals (0% *Scenedesmus*; Fig.4). The three replicate control series in which the rotifers were fed 100% *S. obliquus* were similar

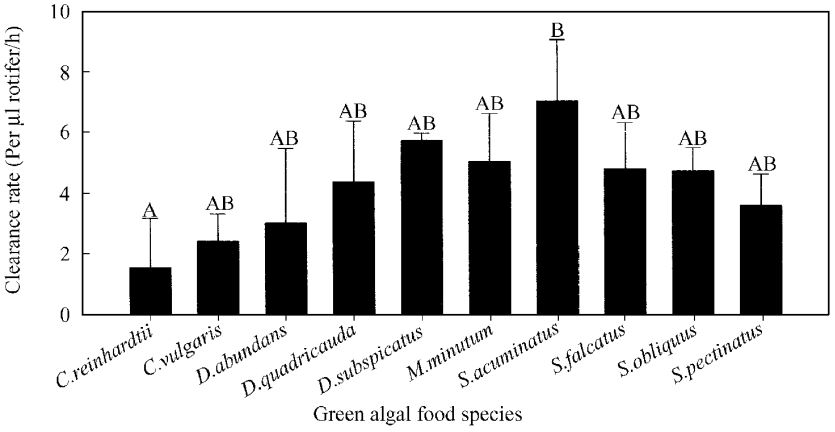

Fig.3 Estimated clearance rates (/µl rotifer/h) for the rotifer *Brachionus calyciflorus* in short-chronic assays (2-d) on ten different green algal species

Error bars indicate one standard deviation ($n=3$). Similar symbols above bars indicate homogeneous groups that are not significantly different (Tukey's *post hoc* comparison, $P<0.05$).

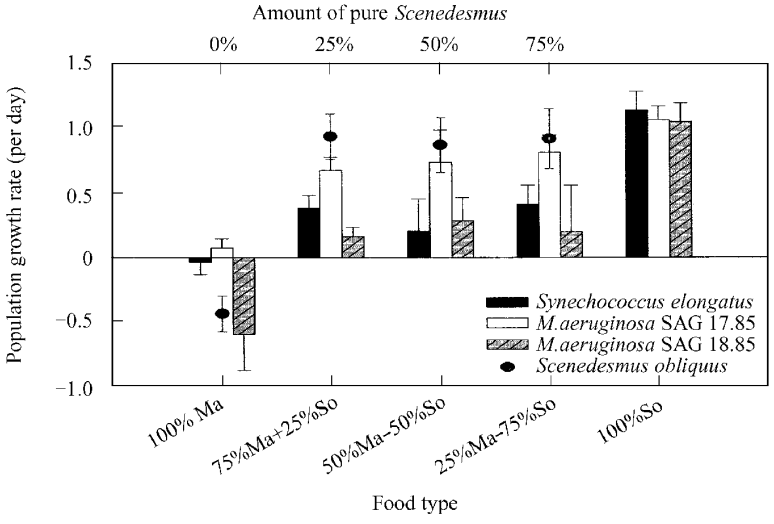

Fig.4 Population growth rate (per day) of the rotifer *Brachionus calyciflorus* in short-chronic assays (2-d) fed solely *Scenedesmus* or the cyanobacteria *Microcystis aeruginosa* or *Synechococcus elongatus* and each strain in different mixtures with *Scenedesmus* (bars) as well as proportional quantities of only *Scenedesmus* (filled symbols)

Error bars indicate one standard deviation ($n=3$).

($F_{2,6}=0.37$, $P=0.707$), whereas for rotifers fed different proportion *Scenedesmus* only the unfed treatment was significantly different from the other treatments (Tukey's test $P<0.05$, $F_{4,10}=34.6$, $P<0.001$). This means that the amount of *Scenedesmus* in the mixtures was sufficient to support good growth of the rotifers. Comparison of the different mixtures revealed that in the 75% *Scenedesmus* + 25% cyanobacterium mixtures the mixtures with strain SAG 18.85 were significantly ($F_{3,8}=6.18$, $P=0.017$) different from the pure *Scenedesmus* treatment (Fig.4). In the 50% *Scenedesmus* + 50% cyanobacterium mixtures only the treatment with *Synechococcus* differed from the *Scenedesmus* treatment ($F_{3,8}=6.48$, $P=0.016$). In the 25% *Scenedesmus* + 75% cyanobacterium mixtures rotifer growth rates in the mixtures with strain SAG 18.85 or *Synechococcus* were significantly ($F_{3,8}=24.8$, $P<0.001$) lower than those of animals fed with only 25% *Scenedesmus* (Fig.4).

3 Discussion

One of the major variables that might influence growth of rotifers is the size of the food algae, which acts through changes in feeding efficiencies over the range of ingestible food sizes (Rothhaupt, 1990b, c). Ingestion in *B. calyciflorus* follows an approximately bell shaped feeding efficiency curve (Rothhaupt, 1990b, c). Decreasing feeding efficiencies of *Brachionus* on algae smaller or larger than the optimal size of around 10 µm is reflected directly in lower rotifer population growth rates (Rothhaupt,

1990a). However, in the current study rotifer growth rates did not follow the predictions that might be derived from the bell-shaped feeding efficiency curve for *B. calyciflorus*. For example, when *B. calyciflorus* was fed the similarly sized *S. obliquus* and *C. vulgaris* significantly different growth rates resulted. Also growth rates on *D. abundans* as food were significantly lower than those of animals fed with *S. falcatus* despite the particle volumes were similar (see Table 2) and the estimated clearance rates were similar (*t*-test: $t = 1.08$, $P = 0.343$) for *Brachionus* feeding on either *D. abundans* or *S. falcatus*. The latter observation suggests that also potential digestion resistance of *D. abundans* can be excluded as an important factor. Green algae, such as Scenedesmaceae, have been found to pass through the gut of rotifers (Pourriot, 1977), but it should be noted that *Brachionus* is able to crush algae with a mastax that is specialised for grinding food items. It can therefore be expected that if viable gut passage of *D. abundans* had occurred, it would have been expressed in lower clearance rates. Disappearance rate for *D. abundans* is significantly lower than that of *S. falcatus*, which can be explained from fewer rotifers grazing down *D. abundans*. Hence, *D. abundans* is a poor food for *B. calyciflorus*, which can not be explained from morphology, i.e. ingestion or digestion resistance.

Table 2 Particle volumes (± one standard deviation, $n = 3$, in μm^3) of ten different green algal species used as food for the rotifer *Brachionus calyciflorus* in short-chronic assays (2-d)

Species	Particle volume (μm^3)
Chlamydomonas reinhardtii	79.0 (1.1)^{AB}
Chlorella vulgaris	31.8 (4.6)^{D}
Desmodesmus abundans	236.3 (16.3)^{C}
Desmodesmus quadricauda	454.1 (25.1)^{F}
Desmodesmus subspicatus	113.7 (27.4)^{AB}
Monoraphidium minutum	28.0 (1.0)^{D}
Scenedesmus acuminatus	71.4 (12.2)^{B}
Scenedesmus falcatus	204.9 (63.0)^{AC}
Scenedesmus obliquus	46.0 (4.6)^{E}
Scenedesmus pectinatus	514.9 (82.3)^{F}

Different superscripts indicate significant differences (*t*-tests: $P < 0.05$)

The results of the current study clearly show that although green algae may be closely related and cultured under similar conditions their suitability as feed to *B. calyciflorus* might differ considerably. Apart from food size-effects, *B. calyciflorus* feeds unselectively on algae that differ in nutritional quality (Rothhaupt, 1995). Inasmuch as the growth differences in the current study can not be explained from size differences, a difference in biochemical make-up of the algae seems the most plausible explanation. In that view, the poor growth of *B. calyciflorus* on *D. abundans* might be the result of an essential compound lacking in this alga.

Lack of essential compounds is considered one of the factors that determines the quality of cyanobacteria as food to zooplankton (e.g., DeMott and Müller-Navarra, 1997; Müller-Navarra et al., 2000; Von Elert and Wolffrom, 2001). Those studies do not support the finding that certain cyanobacteria might be a valuable supplement in combination with other green algal food species (Weithof and Walz, 1995). The results of the current study confirm that the cyanobacteria tested as monospecific food or as mixed diets might depress growth of generalist filter feeding zooplankton, such as *B. calyciflorus*. Although cyanobacteria such as *Microcystis* in the food might depress food intake, the reduction would probably not have been such that the rotifers became starved (Lürling and Verschoor, 2003). In all mixed diets growth rates in treatments with *Synechococcus* or *Microcystis* SAG18.85 were significantly lower than on the corresponding amounts of solely *Scenedesmus* and not different in the three mixtures. Because in nature suspension-feeding zooplankton generally consume a mixed diet of cyanobacteria and good food items, such as green algae, it has been proposed that the most suited measure of determining the effect of cyanobacteria on zooplankton is by using mixed diets (Ferrão-Filho et al., 2000). Using mixtures could overcome nutritional insufficiency (DeMott and Müller-Navarra, 1997; Ferrão-Filho et al., 2000). The results of the current study are, however, not in favour of the nutritional insufficiency hypothesis, because then no decrease in growth would have occurred in comparison to *Scenedesmus*-fed treatments as the amount of *Scenedesmus* alone appeared already sufficient to promote excellent growth.

In addition to nutritional inadequacy due to lack of essential compounds, determinants of cyanobacterial food quality are potential toxicity, digestive resistance and ingestion resistance. The size and shape of the cells or colonies mainly determine ingestion resistance, while digestion resistance is determined mostly by the presence of a thick mucous sheath. Inasmuch as all strains were almost completely comprised of unicell and bicells and not imbedded in a thick mucous envelope, and the cyanobacteria are eaten by *B. calyciflorus* (Fulton and Paerl, 1987), morphological characteristics seem not to have played a major causal role. This leaves potentially bioactive compounds the most probably candidates for the reduced growth of *B. calyciflorus*.

Many freshwater cyanobacteria are toxigenic and

the most frequently encountered cyanobacterial toxins are microcystins (Sivonen and Jones, 1999). However, microcystins have not been detected in *Synechococcus* and strain SAG 18.85 (unpubl. data) and thus these endotoxins did not contribute to the inadequacy of cyanobacteria as food. In addition to microcystins, cyanobacteria may contain a diverse group of other bioactive compounds, such as (cyclic) depsipeptides, which might be very potent inhibitors of proteases (Martin et al., 1993; Jakobi et al., 1996; Weckesser et al., 1996; Rohrlack et al., 2003; Bister et al., 2004).

Different cyanobacteria species or strains might exert detrimental (e.g., current study) or stimulating effects (e.g., Weithof and Walz, 1995). One plausible explanation for the contrasting impact of cyanobacteria on *B. calyciflorus* is the presence or absence of various bioactive compounds. In that view, as alternative to the potential lack of an essential compound in the green alga *D. abundans*, the possible production of a bioactive compound by this organism needs to be tested. Despite the vast majority of studies have reported green algae to be a suitable food source for zooplankton, occasionally green algal toxicity has been suggested (e.g., Halbach, 1971; Boersma and Vijverberg, 1995).

In summary, the results of the current study show that most of the green algae tested promoted good growth in *B. calyciflorus*, but that variation between the food species was considerable. The lowest growth rate on *D. abudans* as food was only 19% of the growth rate found for animals feeding on *S. obliquus*, which is an excellent food source for *B. calyciflorus* in 2-d assays. The rotifers grew better on this alga than on *Chlorella vulgaris* although some studies found *Chlorella* sp. to be a better food than *Scenedesmus* (e.g., Xi et al., 2002; Flores-Burgos et al., 2003). These studies of Xi et al. (2002) and Flores-Burgos et al. (2003) included the entire life span of *B. calyciflorus*, but over a two-day period the outcome could have been different. When in the study of Flores-Burgos et al. (2003) growth analysis is restricted to two days the numerical response of *B. calyciflorus* feeding on *Scenedesmus* was higher than of animals feeding on *Chlorella* (see Fig.1 in Flores-Burgos et al., 2003). Hence, based on the current study and literature data (Rothhaupt, 1990a; Wang et al., 1998; Xi et al., 2001; Flores-Burgos et al., 2003) *S. obliquus* can be considered a suitable live food. Because growth rates on mixed diets of *S. obliquus* and *Synechococcus* or *Microcystis* were below the 0.65 acceptability threshold for short chronic assays such diets should be avoided in order not to influence the outcome of the toxicity assays (Snell and Moffat, 1992). We did not observe mixis in any of our treatments, which means that our results apply only to asexual reproduction.

The variation in growth rates observed in current study underlines the influence food species might have on the growth of *B. calyciflorus* and potentially on the outcome of toxicity tests. More research with different algal strains might elucidate within-species sources of variation in *B. calyciflorus* growth and might contribute to further understanding of the impact of algal food items on the reliability and performance of short-chronic assays.

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