

Investigation of a rotifer (*Brachionus calyciflorus*) - green alga (*Scenedesmus pectinatus*) interaction under non- and nutrient-limited conditions

M. Lürling

Aquatic Ecology & Water Quality Management, Department of Environmental Sciences, Wageningen University, PO Box 8080, NL-6700DD Wageningen, The Netherlands

Two-day life cycle tests with the rotifer *Brachionus calyciflorus* were run to study the nutritional quality effects to rotifers of *Scenedesmus pectinatus* grown under non-limiting, nitrogen limiting and phosphorus limiting conditions and the feedback of the rotifers on the food algae. Under nutrient-limited conditions of its algal food *Brachionus* production was depressed, animals produced fewer eggs and were smaller sized. Clearance rates of *Brachionus* offered non-nutrient-limited and nutrient-limited food were similar. The number of cells per colony was similar for *S. pectinatus* under nitrogen limited and phosphorus limited conditions both in the absence and presence of *Brachionus*. Cell volumes of phosphorus limited *S. pectinatus* were larger than those of nitrogen limited cells. The most dramatic response of the food alga *S. pectinatus* was observed in non-nutrient-limited conditions: a strong size enlargement occurred only in the presence of *Brachionus*. This was caused by a higher share of eight-celled colonies and larger individual cell volumes in the presence of rotifers than in their absence. *S. pectinatus* might gain an advantage of becoming larger in moving out of the feeding window of its enemy, but nutrient limited conditions might undermine the effectiveness of such reaction.

Keywords : clearance rate, food quality, grazing, induced defence, morphology, plankton interactions

Introduction

Predator-prey interactions are crucial processes for the functioning of ecosystems as they represent the flow of energy along the food chains. At the base of typical aquatic food chains the alga-grazer interaction is of major importance because it represents the first step at which material and energy flows up the trophic levels (Lindeman 1942). Understanding the mechanisms that regulate these interactions is a key issue in aquatic ecology, and although quantitative and qualitative effects of algae on zooplankton growth have been studied intensively, the controlling factors are still poorly understood (Brett & Müller-Navarra 1997). Zooplankton may experience not only energy or food quantity

constraints on growth, but also food quality constraints. Energetic limitation depends on algal abundance, while food quality constraints involve algal characteristics such as the biochemical make-up of cells and those that affect ingestion and digestion. The rate at which algal material is ingested and digested by zooplankton depends on algal cell and colony shape and size, wall architecture, absence or presence of spines, bristles or secondary metabolites (Porter 1973, Lampert 1987).

Numerous studies have focused on deciphering what controls zooplankton growth limitation and determines food quality in which zooplankton is represented mostly by *Daphnia*. Food quality determinants were algal stoichiometry, mainly phosphorus limitation (e.g. Sterner 1993, Urabe et al. 1997, Boersma 2000), biochemical constraints or polyunsaturated fatty-acid limitation (e.g. Müller-Navarra et al. 2000, Park et al. 2002,

* Corresponding author :
E-mail: miquel.lurling@wur.nl

von Elert 2002), and morphology, such as cell wall thickening (Van Donk & Hessen 1993) and colony formation (Lürling & Van Donk 1996). In the vast majority of these studies the alga used was *Scenedesmus*, which is known for its extreme morphological variability (Trainor 1998). In the (chemical or physical) presence of zooplankton, *Scenedesmus* may increase its size to resist mortality from grazers (Lürling 2003). However, most studies in the wealth of research on effects of algal nutritional quality on the energy flow to zooplankton has been unidirectional, with no feedback between grazers and algae and no focusing on possible algal defences. Studies on divisive colony formation in *Scenedesmus* also considered one side of the coin as experiments on induced defences in *Scenedesmus* were run under nutrient replete conditions (Hessen & Van Donk 1993, Lampert et al. 1994, von Elert & Franck 1999, Lürling 2003, van Holthoorn et al. 2003).

A two-day life cycle test with the rotifer *Brachionus calyciflorus*, in which multiple broods are produced and the F₁ generation also reproduces (Snell & Moffat 1992), may provide an opportunity to study both sides of the grazer-algae interaction: the nutritional quality aspect on the grazers and their feedback on the algae. Rothhaupt (1995) showed that *Brachionus rubens* ingested nutrient (N- and P) limited *Scenedesmus* equally to non-limited cells, but that *Brachionus* growth was reduced. Also growth of *B. calyciflorus* was reduced when they were fed with nutrient-limited *Scenedesmus* (Jensen & Verschoor 2004). Therefore, it is expected that also in the current study nutrient limited food will result in lower rotifer abundance because of nutritional constraints of *Brachionus* growth. Nutrient limitation in itself probably will not have an effect on the number of cells per colony in *Scenedesmus* (Sternier & Smith 1993). However nutrient limited conditions may restrict the ability of *Scenedesmus* to respond optimally to grazing as active growth appeared to be a precondition for the activation of the morphological defence in *Scenedesmus* (Lampert et al. 1994). In that respect, it is expected that *Scenedesmus* will be able to activate its morphological defence only under nutrient replete conditions in the presence of grazers, but not in their absence or under nutrient limited conditions. Hence, the following hypotheses were tested:

1) Nutrient limited *Scenedesmus pectinatus* will result in lower *Brachionus* growth.

2) *Scenedesmus pectinatus* reacts to the presence of *Brachionus* by enlarged cell size and/or increasing colony size, but this reaction is impaired under nutrient limitation.

Materials and methods

Organisms

The green alga *Scenedesmus pectinatus* v99 was obtained from the culture collection of the University Plovdiv (Bulgaria). The green alga *Scenedesmus obliquus* SAG 276/3a originates from the culture collection of the University of Göttingen (Germany). Semi-continuous stock cultures of these algae were maintained in 300 ml Erlenmeyer flasks, closed with a cellulose plug, containing 150 ml of modified WC (Woods Hole Chu)-medium (Lürling & Beekman 1999) with vitamins added (Biotin B1 and B12 at 50 ng l⁻¹, Thiamine HCL at 100 ng l⁻¹). The flasks were placed in climate controlled room at 20°C in continuous light of 60-μmol quanta m⁻² s⁻¹ provided from above by fluorescent cool-white tubes (Osram L 36W/21-840). For at least a year prior to the experiments, every three weeks aliquots were transferred separately into fresh sterile medium.

The grazer *Brachionus calyciflorus* Pallas was obtained from Microbiotests Inc. (Nazareth, Belgium) and was supplied as cysts. Cysts were hatched in 100-ml suspensions of the green alga in the above mentioned WC-medium. The hatching flasks were placed on a rotating shaking device at 24°C in continuous light of 100-μmol quanta m⁻² s⁻¹.

Experimental procedure

An experiment was conducted to study the effect of *S. pectinatus* nutrient limitation on rotifer growth and vice versa of rotifers on algal growth and morphology. Non-limited *S. pectinatus* from the semi-continuous stock cultures were transferred prior to the experiment into 100 ml Erlenmeyer flasks, containing 50 ml of full WC medium with vitamins added and that were closed with a cellulose plug. These flasks were placed on a rotating shaking table (60 rpm) at 24°C in continuous light of 100-μmol quanta m⁻² s⁻¹. After three days the algae were transferred again into 100 ml Erlenmeyers that contained either 50-ml full WC-medium (non-limited) or 50-ml nutrient-limited medium. In the N-limited medium the N-concentration was reduced to 50 μM NaNO₃ (i.e.5% of the full medium) and in P-limited medium the concentration was reduced to 0.5 μM K₂HPO₄ (i.e.1% of the full medium). To compensate for decreasing potassium concentrations in the reduced P-medium KCl from a 100-μM stock solution was added to obtain the desired K balance. After three days incubation, algal carbon (C), nitrogen and phosphorus (N- and P) -contents were determined as described in Lürling & Van Donk (1997) and the algae

were used in a rotifer-growth experiment and a short-term grazing experiment. This experiment was run in 6-welled covered tissue plates positioned for two days on a rotating shaking table (60 rpm) at 24°C in continuous light of 100- $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Each well received a 10 ml suspension of non-limited, N-limited or P-limited *S. pectinatus* all at identical initial concentration of $5 \times 10^6 \mu\text{m}^3 \text{ml}^{-1}$. Full WC-medium was used for non-limited algae, medium lacking any N or P for treatments with N- or P-limited *S. pectinatus*, respectively. Three replicate wells for each algal type received 6 freshly hatched (2-3 hours old) rotifers (treatments), while three other replicates received no rotifers (controls). This yielded 3 algal food types (Non-, N- and P-limited *S. pectinatus*) \times 2 treatment (without/with rotifers) \times 3 replicates = 18 experimental units.

After two days of incubation the number of rotifers and the number of attached eggs were counted under a dissecting microscope at 25x magnification. Population growth rates were calculated from the increase in total numbers following $r = \{\ln(N_t) - \ln(N_{t-2})\} \times t^{-1}$ where r is the intrinsic growth rate (d^{-1}), N is the number of animals and t is the time (d). Lorica length and width of twenty *Brachionus* were measured with a Leica Quantimet 500 MC image analyser.

In each well the 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). The numbers of cells per colony were determined microscopically by counting at least 100 aggregates (i.e. unicells and colonies), using a Nikon light microscope at 600 x magnification.

Clearance rates (CR) of *B. calyciflorus* feeding on non-limited, N-limited or P-limited *S. pectinatus* were determined using a 24-welled culture plate. Each vial contained 1.1 ml of *S. pectinatus* in WC-medium ($10^7 \mu\text{m}^3 \text{ml}^{-1}$, $\sim 5 \text{ mg C l}^{-1}$); 8 vials were used per algal food type. Four vials of each food type received 20 non-egg bearing female *Brachionus*, while four others served as animal-free controls. The culture plates were incubated at 25°C in the dark for 4 h. Once every hour, settled material was resuspended by gently using a pipette and blow some water with it. After 4 h, 1 ml of water was pipetted from each vial and analysed on the numbers of particles and biovolume using the Coulter® Multisizer II. Hereto, each sample 1.0-ml was diluted with 9.0-ml electrolyte solution (ISOTON) and algal size distribution and concentrations were determined in the range from 2.5 to 35 μm (ESD; 100 μm -orifice tube). Rotifer clearance rates (CR, μl

rotifer $^{-1} \text{h}^{-1}$) were calculated using the following equation (Peters, 1984) :

$$\text{CR} = (b - a) \times \frac{V}{N}$$

$$\text{with } b = \left(\frac{\ln(A_{C,t=1}) - \ln(A_{C,t=0})}{\Delta t} \right) \text{ and } a = \left(\frac{\ln(A_{T,t=1}) - \ln(A_{T,t=0})}{\Delta t} \right), \text{ where } A_{C,t=0}$$

is the initial algal concentration (biovolume in $\mu\text{m}^3 \text{ml}^{-1}$) in controls, $A_{C,t=1}$ is the final algal concentration in controls, $A_{T,t=0}$ is the initial algal concentration in the treatments, $A_{T,t=1}$ is the final algal concentration in treatments, Δt is the time (4 h), V is the culture volume (1.1 ml) and N is the number of animals (20).

Data analysis

Population growth rates, egg-ratios and clearance rates of *B. calyciflorus* on non-limited, N-limited or P-limited *S. pectinatus* were analysed running a one-way ANOVA preceded by Levene's equality of variances test and followed by Tukey's HSD post-hoc multiple comparison test. *S. pectinatus* characteristics like the mean particle volume (MPV) and number of cells per colony were analysed by two-way ANOVA with nutrient conditions and the absence/presence of rotifers as fixed factors. Running t -tests tested other differences. All statistical analyses were run in the statistical tool pack SPSS® version 10.1.0.

Results

Brachionus

Population growth of *Brachionus* was the highest on non-limited *S. pectinatus*, intermediate on N-limited and lowest on P-limited *S. pectinatus* (Fig. 1). A one-way ANOVA indicated that those differences in growth were statistically significant ($F_{2,6} = 75.9$; $P < 0.001$) with *Brachionus* growth on each food type significantly differing from the others (Tukey's post hoc comparison, $P < 0.05$). The mean number of eggs per rotifer was significantly higher ($F_{2,6} = 44.1$; $P < 0.001$) in animals fed non-limited *S. pectinatus* than those reared on N- or P-limited food (Fig. 1). *Brachionus* reared on non-limited or N-limited *S. pectinatus* had a significantly longer lorica ($F_{2,57} = 4.99$; $P = 0.010$) than those grown on P-limited food (Fig. 2). The width of non-limited *Brachionus* was significantly larger ($F_{2,57} = 3.5$; $P = 0.034$) than animals fed P-limited *S. pectinatus* (Fig. 2). The short-term grazing experiment showed identical ($F_{2,9} = 0.52$; $P = 0.610$) *Brachionus* clearance rates (CR) on non-limited and nutrient limited *S.*

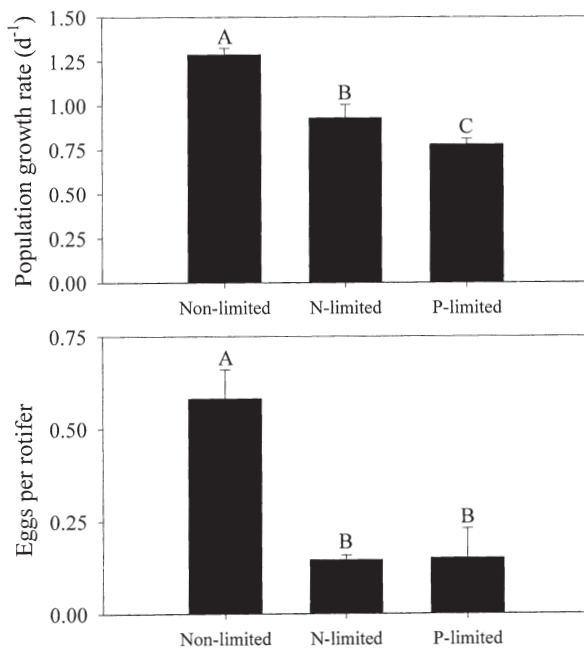


Fig. 1. Population growth rates (upper panel, in d⁻¹) and number of eggs per female (lower panel) of *Brachionus calyciflorus* grown for two days on non-nutrient-limited, nitrogen (N)-limited and phosphorus (P)-limited *Scenedesmus pectinatus*. Error bars indicate one standard deviation (N = 3). Different symbols above bars (A,B,C) indicate statistically significant differences (P < 0.05).

pectinatus (Fig. 3). However, CR estimated from the two-day growth experiment were significantly different ($F_{2,6} = 5.25$; $P = 0.048$). The CR of *Brachionus* feeding on N-limited food was significantly higher ($9.8 \pm 1.9 \mu\text{l rotifer}^{-1}\text{h}^{-1}$) than those of animals feeding on either non-limited ($5.2 \pm 0.6 \mu\text{l rotifer}^{-1}\text{h}^{-1}$) or P-limited food ($5.7 \pm 0.8 \mu\text{l rotifer}^{-1}\text{h}^{-1}$).

Scenedesmus

The C:P ratio of *S. pectinatus* that was used as food in the rotifer growth experiment as well as the short-term grazing experiment was the highest in P-limited algae, while the C:N ratio was the highest in the N-limited algae (Table I). The mean particle volume (MPV) of N-limited *S. pectinatus* was significantly smaller than P-limited *S. pectinatus* ($F_{2,6} = 5.11$; $P = 0.05$) while the numbers of cells per colony were similar (Table I). Moreover, N-limited algal cultures were yellow coloured, whereas non-limited and P-limited cultures were green; N-limited *S. pectinatus* contained significantly less ($F_{2,6} = 56.$; $P < 0.01$) chlorophyll-*a* per unit biovolume than non- and P-limited *S. pectinatus* did (Table I).

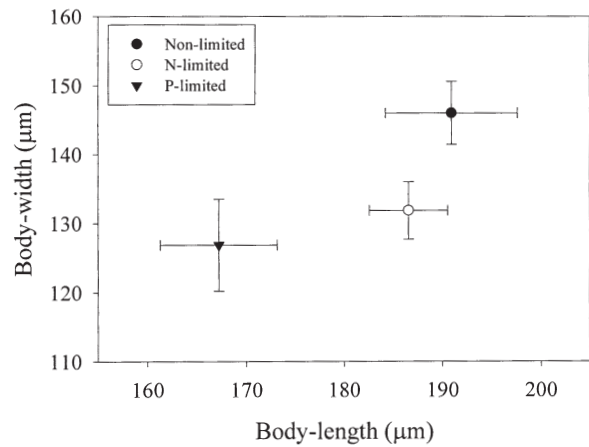


Fig. 2. Body-length and width dimensions (μm) of *Brachionus calyciflorus* grown for two days on non-nutrient-limited, nitrogen (N)-limited and phosphorus (P)-limited *Scenedesmus pectinatus*. Error bars indicate one standard deviation (N = 20).

After two days in the rotifer growth experiment, the mean particle volumes (MPV) that had been log transformed prior to statistical analysis to obtain homogeneity of variances were significantly different among *S. pectinatus* populations reared in the absence or presence of *Brachionus* ($F_{1,12} = 116.9$; $P < 0.001$) and under non- or nutrient-limiting conditions ($F_{2,12} = 109.8$; $P < 0.001$). The MPVs of N- or P-limited *S. pectinatus* were similar in the absence or presence of *Brachionus* (Fig. 4). However, in non-limited *S. pectinatus* the MPV was significantly higher in the presence of rotifers than in their absence (Fig. 4), which is reflected in a significant food types (Non-, N- and P-limited *Scenedesmus*) x treatment (without/with rotifers) interac-

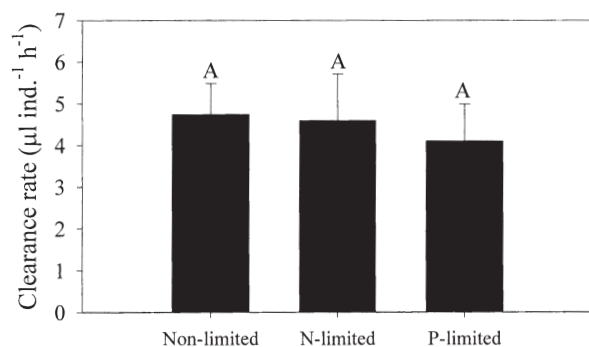


Fig. 3. Clearance rates (μl rotifer⁻¹ h⁻¹) of *Brachionus calyciflorus* feeding on non-nutrient-limited, nitrogen (N)-limited and phosphorus (P)-limited *Scenedesmus pectinatus*. Error bars indicate one standard deviation (N = 4).

Table 1. Characteristics of *Scenedesmus pectinatus* from cultures grown under non-limited, nitrogen (N)-limited and phosphorus (P)-limited conditions that were used as food for the rotifer *Brachionus calyciflorus*. Molar carbon to phosphorus (C:P) and carbon to nitrogen (C:N) ratios, mean particle volumes (MPV in μm^3), mean number of cells per colony and amount of chlorophyll-a ($\mu\text{g } \mu\text{m}^{-3}$) are given with standard deviations in parentheses ($N=3$); similar lettering (A, B) represent homogeneous groups that are not statistically different (Tukey's HSD; $P < 0.05$).

<i>S. pectinatus</i>	C:P	C:N	MPV	Cells colony ⁻¹	Chlorophyll-a
Non-limited	128 (29)	3 (1)	328.8 (16.9) ^{AB}	2.96	1.15 (0.03) 10^{-8} ^A
N-limited	105 (2)	20 (8)	296.9 (35.0) ^B	2.93	0.73 (0.08) 10^{-8} ^B
P-limited	811 (340)	9 (5)	375.7 (36.0) ^A	2.79	1.23 (0.07) 10^{-8} ^A

tion ($F_{2,12} = 81.6$; $P < 0.001$). The two-way ANOVA and post-hoc comparison on the number of cells per colony indicated that they were significantly higher in

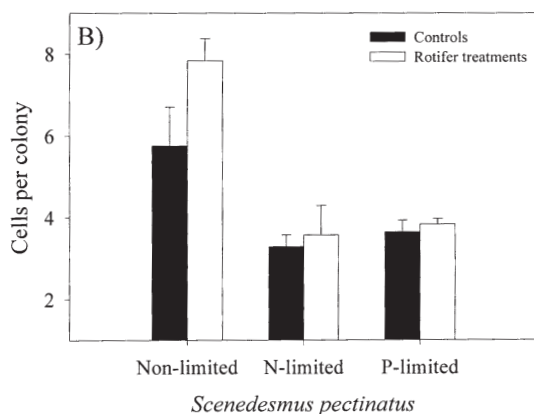
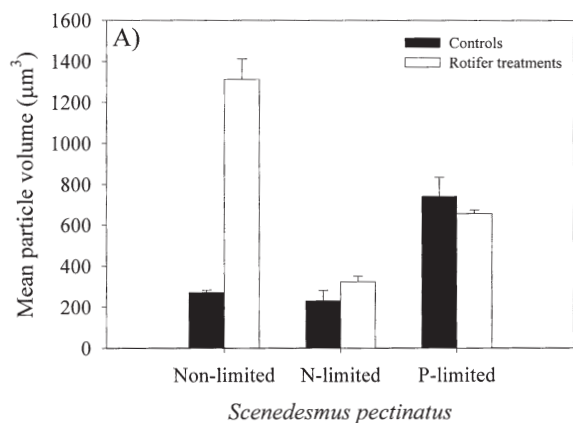


Fig. 4. Mean particle volumes (upper panel A, in μm^3) and number of cells per colony (lower panel B) of *Scenedesmus pectinatus* grown for two days under non-nutrient-limited, nitrogen (N)-limited and phosphorus (P)-limited conditions in the absence (filled bars) and presence (open bars) of the rotifer *Brachionus calyciflorus*. Error bars indicate one standard deviation ($N = 3$).

S. pectinatus under non-limiting than under nutrient-limiting conditions ($F_{2,12} = 65.2$; $P < 0.001$). Moreover, in the presence of *Brachionus* the number of cells per colony was significantly higher ($F_{1,12} = 10.3$; $P = 0.007$) than in their absence, but this was only observed for non-limited *S. pectinatus* (Fig. 4), also indicated by a significant interaction term ($F_{2,12} = 5.33$; $P = 0.022$).

Individual cell sizes, expressed as cell volume were significantly influenced by the nutrient conditions ($F_{2,12} = 64.7$; $P < 0.001$) and differed in the absence or presence of rotifers ($F_{1,12} = 21.4$; $P = 0.001$), but only for the non-limited *S. pectinatus*, which caused a significant interaction term ($F_{2,12} = 31.7$; $P < 0.001$). Tukey's HSD post-hoc comparison revealed two homogeneous groups: P-limited cell volumes in absence and presence of rotifers and of non-limited *S. pectinatus* in the presence of rotifers were significantly larger than

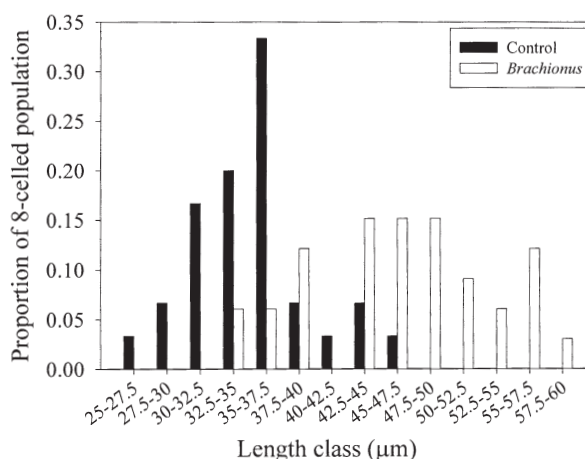


Fig. 5. Frequency distribution of different size classes of eight-celled *Scenedesmus pectinatus* grown for two days under non-nutrient-limited conditions in the absence (filled bars) and presence (open bars) of the rotifer *Brachionus calyciflorus*.

those of N-limited *S. pectinatus* cells in absence and presence of rotifers and non-limited in the absence of rotifers. Frequency distribution of different size classes of eight-celled *S. pectinatus* under non-limiting conditions, revealed that 45% of the colonies in the presence of rotifers exceeded the maximum sizes found in the absence of rotifers (Fig. 5). In the absence of rotifers eight-celled colonies of maximally 47.5 µm in length were found, while in the presence of rotifers they reached up to 60 µm in length (Fig. 5).

Inasmuch as the nutrient composition of the media differed and incubations were placed in continuous light in the two-day experiment, the resulting algal growth rates in the controls were significantly different (one-way ANOVA $F_{2,6} = 499.8$; $P < 0.001$). As expected non-limited medium permitted the highest volume-based growth of *S. pectinatus* ($1.49 \pm 0.04 \text{ d}^{-1}$), P-free medium a significantly reduced growth ($0.30 \pm 0.08 \text{ d}^{-1}$) and N-free medium supported no growth at all ($-0.01 \pm 0.08 \text{ d}^{-1}$). Also particle-based growth was significantly (one-way ANOVA: $F_{2,6} = 228.6$; $P < 0.001$ and Tukey's HSD; $P < 0.05$) lower in N-free ($0.15 \pm 0.16 \text{ d}^{-1}$) and P-free medium ($-0.01 \pm 0.03 \text{ d}^{-1}$) than in non-limited medium ($1.50 \pm 0.02 \text{ d}^{-1}$). In non-limited medium, particle-based and volume-based growth rates of *S. pectinatus* were similar ($t = 0.60$; $P = 0.579$) and also in N-free medium no statistical differences were detected ($t = 1.72$; $P = 0.161$). In contrast, for *S. pectinatus* in P-free medium particle-based and volume-based growth were significantly different ($t = 6.01$; $P = 0.004$). The positive volume-based growth for these P-limited *S. pectinatus* can be explained by cells becoming larger without dividing: the number of particles at the start and after two days of incubation was similar, i.e. 12500 particles ml^{-1} ($t = 0.10$; $P = 0.922$).

Discussion

Brachionus

The quality of *S. pectinatus* as food for *Brachionus* was influenced by the nature of the nutrient-limitation: the highest growth rate was found on non-limited *S. pectinatus*, N-limited food permitted intermediate growth and the lowest *Brachionus* growth occurred when P-limited *S. pectinatus* was the food source. These results are in concordance with the hypothesis that nutrient-limited algal food reduces growth of *Brachionus* and are comparable to those found by Rothhaupt (1995) and Jensen & Verschoor (2004) who offered *S. obliquus* that differed in nutritional status to *Brachionus rubens* and *B. calyciflorus*, respectively.

The nutritional constraints were not only reflected in lowered population growth rates, but also in the mean size of *B. calyciflorus* that was significantly reduced under nutrient limitation, especially when feeding on P-limited cells. Similar observations have been made for *Daphnia* feeding on non-, N- or P-limited *Scenedesmus* where animals reached the largest body-size on non-limited food (Lürling & Van Donk 1997, Urabe & Sterner 2001). Moreover, the reduced length and width dimensions of *B. calyciflorus* under nutrient limitation find support in the lower body volume at first reproduction and first day somatic growth found by Jensen & Verschoor (2004).

Brachionus also produced a significantly smaller number of eggs on nutrient limited food than on non-limited food, which has been observed in *Daphnia* too (Urabe & Sterner 2001). Nutrient limited food results in a limited amount of resources to be allocated to the three basic functions: maintenance, growth and reproduction and allocation to one of these functions implies less availability to the others. Feeding on N- or P-limited algae did not hamper the rotifers to produce offspring but probably at the price of reduced somatic growth and a lower reproductive rate. The latter is corroborated by the study of Jensen & Verschoor (2004) in which lower lifetime fertility was found in *B. calyciflorus* that was reared on nutrient limited *S. obliquus*.

As a consequence of the experimental design with different medium composition in the non-limited and nutrient-limited treatments, growth (and morphology) of the *S. pectinatus* was affected in the two-day experiment. In relation to the rotifers especially the volume-based growth is of interest, because positive values for growth reflect an increase in the actual biomass. Growth was the highest in the non-limited algae in non-limited medium and thus differences in food quantity could have influenced the population growth. However, food concentrations in all treatments were high and in the range that is expected to permit maximal growth (Rothhaupt 1990b, 1995). The lowest food concentration was found in the treatments with N-limited *S. pectinatus* (geometric mean of 1.5 mg C l^{-1}), which caused in the study of Rothhaupt (1995) a growth reduction of 25% compared to animals fed non-limited food at the same concentration. The 28% reduction found in the current study is in close match with the result of Rothhaupt (1995). Although food quantity may limit *Brachionus* production, the concentrations in the current study were such that the different population growth of *Brachionus* is most probably caused by the distinct food qualities of non-limited and

nutrient limited *Scenedesmus*. Inasmuch as the reduced population growth, egg production and size of *B. calyciflorus* under N- and P-limitation is also found for other food types and zooplankton these responses appear to be a more general pattern in herbivorous zooplankton.

The short-term feeding experiment showed no differences in *B. calyciflorus* clearance rates. This result is in good agreement with that of *B. rubens*, which ingested nutrient (N- and P) limited *S. obliquus* equally to non-limited cells (Rothhaupt 1995). These findings do not support the digestion resistance hypothesis, which postulates that under nutrient-limitation chlorophytes like *Scenedesmus* gain increased resistance against digestion through a thickening of the wall (Van Donk & Hessen 1993, 1995, Van Donk et al. 1997). The duration of the short-term experiment was such that it exceeded several times the maximum gut passage times of *B. calyciflorus* (Starkweather & Gilbert 1977) and it can therefore be expected that if viable gut passage would have occurred, it would have been expressed in lower clearance rates. Although *Scenedesmus* has been found to pass through the gut of cladocerans (Van Donk & Hessen 1993) and rotifers (Pourriot 1977), *Brachionus* is able to crush algae with a mastax that is specialised for grinding food items, which might explain the absence of differences in clearance rates in the short-term grazing experiment. In the two-day growth experiment, however, rotifer clearance rates differed. The highest clearance rates were found for *B. calyciflorus* feeding on N-limited food and significantly lower clearance rates for animals feeding on P-limited and non-limited *S. pectinatus*. The difference in clearance rates for *Brachionus* feeding on N-limited and P-limited *S. pectinatus* can be explained from the differences in food concentrations (Rothhaupt 1990a). The clearance rates that were estimated of *Brachionus* feeding on non-limited *S. pectinatus* were similar to those that preyed on P-limited food. Because clearance rates drop continuously with food concentration above a critical concentration (Rothhaupt 1990a), the similar clearance rates in this case could be the result of the larger animals in non-limited conditions that through higher feeding activity might have outbalanced expected lower clearance rates. On the other hand, it remains possible that in both non-limited and P-limited conditions lower clearance rates were obtained, but that these were not detected in the experimental set-up employed in the current study. In the short-term grazing experiment the algae were all in the same size range (see Table I), however after the two days growth experiment distinct differences occurred (see Fig. 4). P-li-

mitted *S. pectinatus* had gained in size, N-limited dropped in size and the most dramatic increase was observed in the non-limited *S. pectinatus*, but only in the presence of rotifers. The large eight-celled *S. pectinatus* colonies probably reached sizes beyond the ingestion capacity of *B. calyciflorus* (Rothhaupt 1990a). Further research should incorporate estimates of feeding efficiencies of *Brachionus* supplied with identical amounts of such distinct morphologies of the food.

Scenedesmus

Under non-limiting conditions *S. pectinatus* became much larger through an increase in the number of cells per colony as well as larger individual cell size. The increased number of cells per colony can be explained from selective feeding on colonies with fewer than eight cells and from induction of the eight-celled morph (Lürling 2003). Eight-celled *S. pectinatus* colonies were also observed in the absence of rotifers, but more than 45% of the eight-celled colonies in the presence of rotifers exceeded the maximum size (length and width) found in populations without natural enemies. A larger cell size and consequently larger colonies appears to be linked to the presence of grazers and not the result of selective feeding. This reaction most probably will have moved the majority of the population that were eight-celled colonies with dimensions of 46 x 37 µm out of the feeding window of *B. calyciflorus*. These rotifers feed most efficiently on particles with an equivalent spherical diameter around 10 µm (Rothhaupt 1990a). The feeding efficiency curve of *B. calyciflorus* is approximately bell shaped and lower ingestion rates are reflected in lower *Brachionus* growth rates (Rothhaupt 1990b). The underlying mechanism is most probably interference due to the handling time needed for processing large algae. Hence, the result is in favour of the hypothesis that an increment of cell/colony dimensions in the presence of natural enemies could be an efficient response to minimise the risk of being consumed (Lehman 1988). In fact, enlargement of prey to reduce or prevent attack success of natural enemies is a widespread phenomenon among aquatic taxa in the animal kingdom ranging from multicellular organisms such as fish (Brönmark & Miner 1992), cladocerans (Dodson 1989), and rotifers (Halbach 1971), to unicellular ciliates (Kusch 1993). However, although divisive colony formation is a well-known example of an induced defence in phytoplankton and has been found across different algal taxa in both the freshwater and marine environment (e.g. Hessen & Van Donk 1993, Tang 2003), the concomitant enlargement of cells has gone unnoticed.

In contrast to the strong size enlargement of *S. pectinatus* in reaction to the presence of *B. calyciflorus* under non-nutrient-limited conditions, no reaction of *S. pectinatus* to the rotifers was observed in N- or P-limited cultures. Thus, nutrient-limitation could undermine the effectiveness of a defence against grazers through lowering the algal growth rate and concomitantly the ability to form protective colonies or at least by reducing the speed in which the defence can be activated. This is corroborated by the observation that also for colony formation in another *Scenedesmus* species active growth appeared to be a precondition for the activation of a morphological defence (Lampert et al. 1994). However, especially under P-limitation when growth rates drop other morphological properties like cell wall structure and covering might determine largely the resistance of algae to grazing (Van Donk & Hessen 1993). To fully understand the impact of such morphological changes in the alga-grazer interaction assessing the population consequences of both grazers and algae in prolonged exposure studies is needed. It is conceivable that through nutrient recycling (Moegenburg & Vanni 1991, Urabe 1993) some growth of nutrient-limited *S. pectinatus* might take place, which could make the formation of colonies possible in more than the 48 hours that were used in the current experimental set-up. The formation of colonies and enlargement of cells will require time to be activated and fully expressed, because the algae need time to accumulate a certain amount of protoplasm, which is formed during photosynthesis, before the cells undergo division (Setlík et al. 1972, Siver & Feeda 1982). Hence, population consequences to algal prey and rotifers may be more pronounced in longer exposures.

When P-limited *S. pectinatus* were placed for two days in P-free medium, still a positive volume-based growth rate was found, but the number of cells per ml were similar. This means that P-limited cells were no longer capable of division and probably accumulated photosynthesis products in their cells. In contrast, N-limited cells did not increase in size, but rather decreased in size and some cell division still occurred. An increase in cell size in P-limited chlorophytes has been observed in several other studies but for N-limited cells patterns are less clear and both increased and decreased cell sizes have been found (Van Donk & Hessen 1993, 1995, Kilham et al. 1997, Lürling & Van Donk 1997).

The results of the current study showed that under nutrient-limited conditions *Brachionus* production is strongly depressed, animals produced fewer eggs and were smaller sized. The food alga *S. pectinatus* showed

a strong size enlargement in the presence of *Brachionus*, but only under non-limited conditions. Hence, to activate this response growth appears to be essential and nutrient limited conditions seem to undermine it. *S. pectinatus* might gain an advantage of becoming larger in moving out of the feeding window of its enemy, but nutrient limited conditions might undermine the effectiveness of such reaction.

Acknowledgements

Drs Detlina Belkinova and Ruben Mladenov (University of Plovdiv, Bulgaria) are cordially thanked for the delivery of *Scenedesmus pectinatus*. This study was supported by a fellowship of the Royal Netherlands Academy of Arts and Sciences (KNAW).

References

- Boersma M. 2000. - The nutritional quality of P-limited algae for *Daphnia*. *Limnol. Oceanogr.*, 45, 1157-1161.
- Brett M.T. & Müller-Navarra D.C. 1997. - The role of highly unsaturated fatty acids in aquatic food web processes. *Freshwater Biol.*, 38, 483-499.
- Brönmark C. & Miner J.G. 1992. - Predator-induced phenotypical change in body morphology in crucian carp. *Science*, 258, 1348-1350.
- Dodson S.I. 1989. - The ecological role of chemical stimuli for zooplankton : predator-induced morphology in *Daphnia*. *Oecologia*, 78, 361-367.
- Halbach U. 1971. - Zum Adaptivwert der zyklomorphen Dornenbildung von *Brachionus calyciflorus* Pallas (Rotatoria). *Oecologia*, 6, 267-288.
- Hessen D.O. & Van Donk E. 1993. - Morphological changes in *Scenedesmus* induced by substances released from *Daphnia*. *Arch. Hydrobiol.*, 127, 129-140.
- Jensen T.C. & Verschoor A.M. 2004. - Effects of food quality on life history of the rotifer *Brachionus calyciflorus* Pallas. *Freshwater Biol.*, 49, 1138-1151.
- Kilham S.S., Kreeger D.A., Goulden C.E. & Lynn S.G. 1997. - Effects of nutrient limitation on biochemical constituents of *Ankistrodesmus falcatus*. *Freshwater Biol.*, 38, 591-596.
- Kusch J. 1993. - Induction of defensive morphological changes in ciliates. *Oecologia*, 94, 571-575.
- Lampert W. 1987. - Feeding and nutrition in *Daphnia*. Pages 143-192 in Peters, R.H. & De Bernardi, R. (eds) *Daphnia*. Memorie dell'Istituto Italiano di Idrobiologia, 45.
- Lampert W., Rothhaupt K.O. & von Elert E. 1994. - Chemical induction of colony formation in a green alga (*Scenedesmus acutus*) by grazers (*Daphnia*). *Limnol. Oceanogr.*, 39, 1543-1550.
- Lehman J.T. 1988. - Selective herbivory and its role in the evolution of phytoplankton growth strategies. Pages 369-387 in Sandgren, C.D. (ed) *Growth and reproductive strategies of freshwater phytoplankton*. Cambridge Univ. Press.
- Lindeman R.L. 1942. - The trophic-dynamic aspect of ecology. *Ecology*, 23, 399-418.
- Lürling M. 2003. - Phenotypic plasticity in the green algae *Desmodesmus* and *Scenedesmus* with special reference to the induction of defensive morphology. *Ann. Limnol. - Int. J. Limnol.*, 39, 85-101.
- Lürling M. & Beekman W. 1999. - Grazer-induced defenses in *Scenedesmus* (Chlorococcales; Chlorophyceae); coenobium and spine formation. *Phycologia*, 38, 368-376.

- Lürling M. & Van Donk E. 1996. - Zooplankton-induced unicell-colony transformation in *Scenedesmus acutus* and its effect on growth of herbivore *Daphnia*. *Oecologia*, 108, 432-437.
- Lürling M. & Van Donk E. 1997. - Life history consequences for *Daphnia pulex* feeding on nutrient limited phytoplankton. *Freshwater Biol.*, 38, 693-709.
- Moegenburg S.M. & Vanni M.J. 1991. - Nutrient regeneration by zooplankton: effects of nutrient limitation of phytoplankton in a eutrophic lake. *J. Plankton Res.*, 13, 573-588.
- Müller-Navarra D.C., Brett M.T., Liston A.M. & Goldman C.R. 2000. - A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature*, 403, 74-77.
- Park S., Brett M.T., Müller-Navarra D.C. & Goldman C.R. 2002. - Essential fatty acid content and the phosphorus to carbon ratio in cultured algae as indicators of food quality for *Daphnia*. *Freshwater Biol.*, 47, 1377-1390.
- Peters R.H. 1984. - Methods for the study of feeding, grazing and assimilation by zooplankton. Pages 336-412 Downing, J.A. & Rigler, F.H. (eds) A manual on methods for the assessment of secondary production in fresh waters. IBP Handbook 17, 2nd edition, Blackwell, Oxford.
- Porter K.G. 1973. - Selective grazing and differential digestion of algae by zooplankton. *Nature*, 244, 179-180.
- Pourriot R. 1977. - Food and feeding habits of rotifera. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, 8, 243-260.
- Rothhaupt K.O. 1990a. - Changes of the functional responses of the rotifers *Brachionus rubens* and *Brachionus calyciflorus* with particle sizes. *Limnol. Oceanogr.*, 35, 24-32.
- Rothhaupt K.O. 1990b. - Population growth rates of two closely related rotifer species: effects of food quantity, particle size and nutritional quality. *Freshwater Biol.*, 23, 561-570.
- Rothhaupt K.O. 1995. - Algal nutrient limitation affects rotifer growth rate but not ingestion rate. *Limnol. Oceanogr.*, 40, 1201-1208.
- Šetlík I., Berková E., Doucha J., Kubín Š., Vendlová J., & Zachleder V. 1972. - The coupling of synthetic and reproduction processes in *Scenedesmus quadricauda*. *Algol. Stud.*, 7, 172-213.
- Siver P.A. & Freeda S.J. 1982. - The interaction of growth rate and cell cycle on the number of cells in a *Scenedesmus* coenobium. *Proc. Penn. Acad. Sci.*, 56, 133-137.
- Snell T.W. & Moffat B.D. 1992. - A 2-d life cycle test with the rotifer *Brachionus calyciflorus*. *Environ. Toxicol. Chem.*, 11, 1249-1257.
- Starkweather P.L. & Gilbert J.J. 1977. - Radiotracer determination of feeding in *Brachionus calyciflorus*: the importance of gut passage times. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, 8, 261-263.
- Sterner R.W. 1993. - *Daphnia* growth on varying quality of *Scenedesmus*: mineral limitation of zooplankton. *Ecology*, 74, 2351-2360.
- Sterner R.W. & Smith R.F. 1993. - Clearance, ingestion and release of N and P by *Daphnia obtusa* feeding on *Scenedesmus acutus* of varying quality. *Bull. Mar. Sci.*, 53, 228-239.
- Tang K.W. 2003. - Grazing and colony size development in *Phaeocystis globosa* (Prymnesiophyceae): the role of a chemical signal. *J. Plankton Res.*, 25, 831-842.
- Trainor F.R. 1998. - Biological aspects of *Scenedesmus* (Chlorophyceae)-phenotypic plasticity. *Nova Hedwigia, Beiheft*, 117, 1-367.
- Urabe J. 1993. - Coupling N to P cycling through grazers' activities: food quality and nutrient release by zooplankton. *Ecology*, 74, 2337-2350.
- Urabe J., Clasen J. & Sterner R.W. 1997. - Phosphorus limitation of *Daphnia* growth: is it real? *Limnol. Oceanogr.*, 42, 1436-1443.
- Urabe J. & Sterner R.W. 2001. - Contrasting effects of different types of resource depletion on life history traits in *Daphnia*. *Funct. Ecol.*, 15, 165-174.
- Van Donk E. & Hessen D.O. 1993. - Grazing resistance in nutrient-stressed phytoplankton. *Oecologia*, 93, 508-511.
- Van Donk E. & Hessen D.O. 1995. - Reduced digestibility of UV-B stressed and nutrient-limited algae by *Daphnia magna*. *Hydrobiologia*, 307, 147-151.
- Van Donk E., Lürling M., Hessen D.O. & Lokhorst B. 1997. - Changed cell wall morphology in nutrient deficient phytoplankton and the significance for grazers. *Limnol. Oceanogr.*, 42, 357-364.
- van Holthoorn F.L., van Beek T.A., Lürling M., Van Donk E. & de Groot Æ. 2003. - Colony formation in *Scenedesmus*: a literature overview and further steps towards the chemical characterisation of the *Daphnia* kairomone. *Hydrobiologia*, 491, 241-254.
- von Elert E. & Franck A. 1999. - Colony formation in *Scenedesmus*: grazer-mediated release and chemical feature of the infochemical. *J. Plankton Res.*, 21, 789-804.
- 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.