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Effects of food quality on life history of the rotifer *Brachionus calyciflorus* Pallas

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SUMMARY

1. Herbivorous zooplankton face considerable temporal and spatial variation in food quality, to which they respond by adapting their life histories. Zooplankton may even take up mineral nutrients directly, and use these to counter the effects of algal nutrient limitation (mineral compensation). This study examined the life history of the rotifer *Brachionus calyciflorus* fed phosphorus-, and nitrogen-limited *Scenedesmus obliquus* (Chlorophyta), and investigated whether *B. calyciflorus* was capable of mineral compensation.
2. Both phosphorus- and nitrogen-limited algae gave similar life history responses: somatic growth and reproduction were reduced, whereas lifespan remained unaffected.
3. No evidence was found for mineral compensation in *B. calyciflorus* in relation to detrimental life history effects, so mineral compensation does not seem to be relevant for this species under field conditions.
4. The similarity in life history responses of *B. calyciflorus* and the low levels of ω-3 PUFAs in both phosphorus- and nitrogen-depleted algae suggest that ω-3 PUFAs were limiting to *B. calyciflorus*, although other (bio)chemicals or mineral nutrients may also have been important.
5. No trade-off was observed between life span and reproduction during algal nutrient limitation. Reduced population growth rates of *B. calyciflorus* were caused by shorter reproductive periods.

Keywords: food quality, growth, reproduction, *Scenedesmus obliquus*, stoichiometry

Introduction

Suspension-feeding zooplankters encounter a variety of food items of different quality in space and time. Whereas 'edible algae' were once classified largely according to morphological aspects such as size and shape (Burns, 1968; Gliwicz, 1977), ecologists now recognise that the chemical composition of the food cannot be neglected (Gulati, Siewertsen & Van Liere, 1991; Müller-Navarra, 1995; Gulati & DeMott, 1997), and that nutrient-limited algae can be a poor food source for zooplankton (Hessen, 1992; Urabe, Clasen & Sterner, 1997; DeMott, Gulati & Siewertsen, 1998). Furthermore, nutrient-limited algae accumulate carbon-rich compounds such as carbohydrates and saturated fatty acids, whereas the synthesis of proteins and polyunsaturated fatty acids (PUFAs) usually declines under nutrient limitation in algae (Müller-Navarra, 1995; Weers & Gulati, 1997). Some compounds for zooplankton cannot be replaced by chemically related compounds; PUFAs in particular have been shown to be important for zooplankton growth and reproduction (e.g. Müller-Navarra, 1995, 2000; Urabe *et al.*, 2002). There has been some debate on the importance of carbon : nutrient ratios (ecological stoichiometry: Sterner & Elser, 2002)

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versus PUFAs as determinants of algal food quality, but both seem to be important and should be studied together (e.g. Boersma, 2000; Becker & Boersma, 2003).

Little is known about the physiological strategies that herbivorous zooplankters use to maximise their fitness in periods of low algal food quality. Current theories on how consumers react to changing food quality are at the population level, and do not take into account the physiological trade-offs that animals face (e.g. Huxel, 1999; Grover, 2003). This is probably because of the complex biochemical and physiological mechanisms that are involved in animal nutrition. For example, the stoichiometric requirements for body growth and reproduction may differ, depending on the materials required for synthesis of body structure and eggs. Different life history strategies have contrasting requirements, with body growth perhaps requiring P-rich materials such as phospholipids and ribosomes, development of reproductive structure requiring N-rich materials such as amino acids, and maintenance and egg production needing C-rich materials like lipids and carbohydrates. Therefore, differing life histories may emerge under different types of algal nutrient limitation. Furthermore, recent studies have shown that the stoichiometric requirements of crustacean zooplankton may vary depending on the developmental stage of the individuals (Urabe & Sterner, 2001; Villar-Argaiz & Sterner, 2002; Færøvig & Hessen, 2003). However, for non-crustacean zooplankton (i.e. rotifers), virtually nothing is known about the importance of food quality during ontogeny. Research on different species should shed more light on whether herbivorous zooplankton in general have similar nutritional constraints in their life histories. Only when we have more insight into these strategies, will we be able to assess how zooplankton maximise fitness during periods of poor food quality. This should allow us to predict the outcome of competitive interactions in zooplankton communities under changing nutrient regimes.

Besides life history changes, zooplankton may overcome algal nutrient limitation by direct uptake of the mineral nutrients (mineral compensation). Heterotrophic ciliates and flagellates can take up phosphate directly from the medium (Hadas, Pinkas & Wynne, 1992); Hadas and co-workers suggested that this uptake of phosphate could compensate for high C/P ratios of the food of these protozoans. Urabe *et al*. (1997) showed that growth reduction of *Daphnia* on P-limited algae was relieved when high phosphate concentrations (4 mmol L^{-1}) were supplied to the medium. Parker & Olson (1966) found that *Daphnia* also take up phosphate at lower concentrations. Furthermore, Sterner & Schwalbach (2001) suggested that phosphorus from the food can be stored by *Daphnia* for later use (luxury uptake). Cladocera may therefore (partly) compensate for low phosphorus content in the algae by either using previously stored phosphorus reserves (Sterner & Schwalbach, 2001) or by direct uptake of phosphate (Urabe *et al*., 1997). Rothhaupt (1995) observed a growth enhancement of *Brachionus rubens* after short-term phosphate supplementation of their P-limited algal food. The phosphorus content of phosphorus limited algae increased rapidly after addition of phosphate (Rothhaupt, 1995), whereas biochemical synthesis usually occurs over longer time spans. Thus, the observed growth enhancement upon supplying *B. rubens* with phosphorus limited, phosphate pulsed algae suggests that rotifers may be able to take up and use mineral P. Anisotropic crystals in the guts of rotifers have been hypothesised to have a reserve function (Wallace, 1993), and may provide a form of mineral storage. Larger zooplankton are probably incapable of direct uptake of inorganic nitrogen (Brett, 1993) but uptake of inorganic nitrogen is more widespread in smaller heterotrophic plankton, such as bacteria (Kirchman & Wheeler, 1998; Allen *et al*., 2002) and flagellates and ciliates (Finlay, Span & Harman, 1983; Hadas *et al*., 1992). Zooplankton have a much lower uptake efficiency of mineral nutrients than autotrophs (Urabe & Watanabe, 1993), but their mobility and larger reserve storage capacity might still enable them to take up, store and use mineral nutrients in a temporally or spatially heterogeneous environment. If mineral compensation can occur under (semi-)natural conditions, this has important implications for our current view of nutrient cycling in food webs.

The suspension-feeding rotifer *Brachionus calyciflorus* Pallas is a cosmopolitan zooplankton species that reproduces parthenogenetically. Rotifers are typical 'r-strategists' and may dominate the zooplankton in lakes during short periods of the year. Within such fast-growing species, it is not inconceivable that nutrients become limiting for particular life history traits (Elser *et al*., 2000). In the present experiments, individual *B. calyciflorus* were allowed to feed on algae

(nutrient sufficient, N- or P-limited) for most of the day. Both elemental stoichiometry (C : N : P) and fatty acid profiles were determined for these algae and compared with elemental stoichiometry and fatty acid profiles of well-fed *B. calyciflorus*. To test for mineral compensation, the animals were placed in media either lacking inorganic nutrients or with high concentrations of inorganic nutrients (N and P) for a brief period of the day. We tested the following hypotheses:

1 Algal nitrogen and phosphorus limitation both affect tissue production-related life history parameters of *B. calyciflorus*, i.e. somatic growth and reproduction.

2 *Brachionus calyciflorus* is capable of mineral compensation for nutrient limitation in the food at mineral concentrations that could occur in the field.

Methods

Cultures

The green alga *Scenedesmus obliquus* (Turpin) Kützing was obtained from the Max Planck Institute of Limnology (MPI), Plön, Germany. The algae were cultured semi-continuously in 10 L vessels, kept in suspension using magnetic stirrers. At least 100 μmol quanta m^{-2} s^{-1} light (PAR) was supplied by circular fluorescent lights. Per culture, 1 L of algal suspension was harvested every 24 h and replaced by fresh medium. Before the start of the experiment, the algae had been in stable culture for 1 month. The algae were cultured on three types of modified COMBO medium (Kilham *et al*., 1998): Full nutrient sufficient medium (F) with a phosphate concentration of 50 μmol L^{-1} and a nitrate concentration of 1000 μmol L^{-1}, N-depleted medium (N) with nitrate concentration reduced to 40 μmol L^{-1} and P-depleted medium (P) with a phosphate concentration of 2 μmol L^{-1}. Vitamins were omitted from all three algal media. Daily measurements were carried out for: (i) cell concentrations, estimated by measuring the optical density at 750 nm on a Unicam Helios δ photospectrometer (Unicam, Dublin, Ireland); (ii) the phytoplankton size distribution, measured on a CASY© TTC1 Cell Counter (Schärfe Systems, Reutlingen, Germany); (iii) Species composition and colony formation were examined using an inverted microscope.

Samples for dry weight (DW) and stoichiometry of the algae were taken twice a week. The harvested algae were centrifuged and used for food suspensions in experiments and the surplus of the algal pellet was resuspended in a small volume of basal COMBO medium (Dobberfuhl & Elser, 1999) and dried for 24 h at 70 °C in 15 cm siliconised Petri dishes. Dried algae were scraped off the dishes, put into preweighed Eppendorf vials, weighed, and stored in a desiccator for stoichiometric analysis.

Brachionus calyciflorus were hatched from commercially available dormant eggs (cysts: MicrioBioTest inc., Nazareth, Belgium), in order to have identical cyst quality (size, food history). This is important as embryonic development depends strongly on egg size. Another reason for using *B. calyciflorus* hatched from cysts was to get animals born during a short time interval and thus of similar size. In order to hatch individuals for the life history experiments cysts were placed in shallow dishes in an incubator at 20 °C, moving at 60 rpm and under dim light (40 μmol quanta m^{-2} s^{-1}).

Zooplankton life history experiments

B. calyciflorus were raised on each of the three types of *S. obliquus* (F, P or N). For a brief period of the day animals fed any of the three types of algae were placed in one of three different zooplankton mineral media containing different combinations of inorganic phosphorus and nitrogen. We supplied animals fed P-depleted algae with mineral phosphorus, and animals fed N-depleted algae with either nitrate or ammonium. The compositions of the three zooplankton mineral media were: (i) without mineral nitrogen or phosphorus (–), (ii) containing phosphate plus nitrate in concentrations of 50 μmol L^{-1} and 1000 μmol L^{-1} respectively (+) and (iii) containing phosphate plus ammonium in concentrations of 50 μmol L^{-1} and 1000 μmol L^{-1} (#), respectively. This allowed for a completely cross-classified experimental design: three types of algal suspension × three types of zooplankton mineral media resulting in nine treatments: F+, F–, F#, P+, P–, P#, N+, N– and N#. Trace metals were omitted from all three zooplankton mineral media. Ionic strength of the P-depleted algal medium and nutrient free zooplankton mineral medium was maintained by addition of KCl in a concentration of 100 μmol L^{-1}.

After cysts had been incubated for 30 h, newly born *B. calyciflorus*, up to 2-h old, were transferred into clear

polystyrene 96-wells microtiterplates, with one individual per well in 250 μl food suspension. Each plate contained one of the three food suspensions; F, N and P algae. Food suspensions were prepared from the harvested, centrifuged algae by resuspending algae in P- and N-free medium (–), to obtain an algal concentration of 40 mg DW L^{-1}. This was computed from previously made regressions for each type of algae culture between absorbance and dry weight yield. During experiments rotifers were also incubated at 20 °C and 60 rpm in dim light. Eighteen hours of incubation in high concentrations (40 mg DW L^{-1}) of either type of food algae was followed by 6 h of incubation in either type of the three zooplankton mineral media described before. These mineral media contained a very low concentration of dried, sonified F algae (0.5 mg DW L^{-1}) in order to stimulate maximum filtering activity of the animals. The algal food concentration chosen was near the threshold for *B. calyciflorus* to maintain zero growth (R_0: Stemberger & Gilbert, 1987; Walz, 1995) and below the incipient limiting level of 2 mg C L^{-1} (Walz, 1995) to keep a maximum filtering rate. Dried algae were used because they hardly exchange inorganic nutrients with their environment (Dobberfuhl & Elser, 1999), whereas live algae may take up inorganic nutrients very rapidly (Rothhaupt, 1995). After 6 h of incubation in the zooplankton mineral media, the rotifers were washed twice using N- and P-free medium. The washing prevented uptake by algae of inorganic nutrient medium brought in with the rotifers. The animals were then transferred to freshly prepared food suspensions. This 18 : 6 h cycle (food suspension of 40 mg DW L^{-1} : zooplankton mineral medium with low concentration of dried algae) was repeated daily.

We performed three different life history experiments: one life-table (LT) experiment, one to measure size at first reproduction (SFR) and one to measure somatic growth (SG). In the two first experiments (LT and SFR), 12 individuals per treatment were used. The numbers of surviving individuals, eggs and offspring were counted every 12 h (during the first 2 days every 6 h), and dead individuals and offspring were removed. Additional counting was carried out when the animals were transferred from the food suspension to the inorganic medium. All neonates removed from the plates were preserved in an isotonic salt solution, which quickly killed the animals (Beckman Coulter Isoton II, Fullerton, CA, U.S.A.), and stored in a refrigerator (5 °C). SFR animals were followed until they reached maturity (the time they released their first offspring), and together with their offspring these individuals were killed, preserved and stored. In SG, somatic growth was measured during the first 24 h. This experiment had 48 animals per treatment, which were sacrificed and preserved immediately after the first 24 h cycle (18 h food suspension: 6 h zooplankton mineral medium).

Length of preserved individuals was measured to the nearest 10 μm using an inverted microscope. Body volume was calculated following Ruttner-Kolisko (1977) and was increased by 10% to account for volume of the foot. Egg volume was calculated from measurements of length (L) and width (W) using the general formula for an ellipsoid: $V = \frac{\pi}{6} LW^2$. Volume based body growth rate (somatic and overall) for the first 24 h were calculated as $r_b = (lnV_1 - lnV_0)$, where r_b = the body growth rate, V_1 = the body volume after 24 h and V_0 = the body volume at the start. Note that 'somatic' growth in zooplankton excludes eggs but includes gonad volume, which may be considerable in small rotifers prior to first reproduction.

Population growth rates (intrinsic rates of natural increase) were calculated by iterative solution of Euler's stable age equation for each treatment (Stearns, 1992):

$$1 = \sum_{x=0}^{n} (e^{-rx} l_x m_x),$$

where e = the base of the natural logarithm, x = age (day), l_x = proportion of individuals surviving to the end of age x, and m_x = average number of offspring produced by females surviving to the end of age x. By solving this equation for jackknifed pseudovalues of each individual, we were able to calculate the associated standard errors and to statistically test the differences between calculated population growth rates.

Analytical procedures

Algal carbon and nitrogen were determined in samples of dried algae placed in precombusted, preweighed 7.5 × 6 mm pressed silver cups (Elemental Microanalysis Ltd., Okehampton, U.K.) and weighed to the nearest μg on an Ohaus AS120 microbalance. For carbon and nitrogen determinations in *B. calyciflorus* the animals were separated from their food

suspension and washed in demineralised water. For each determination 40–100 individuals were transferred to a precombusted, preweighed silver cup and dried at 70 °C and weighed again as for algae. Carbon and nitrogen content were measured on a Unicarb Universal Carbon and Nitrogen Analyser (Salonen, 1979).

Phosphorus in algae was determined using dried algae in precombusted and preweighed silver cups, which were combusted for 2 h at 550 °C. For the rotifers, the samples used for carbon and nitrogen analysis were also used for phosphorus determination after combustion. All combusted samples were analysed for phosphorus content, largely following Murphy & Riley's (1962) colorimetric determination with molybdene blue. Before measurements, the samples were centrifuged (10 min, 840 **g**) and transferred to a 5 cm quartz cuvette to measure extinction at 890 nm (Perkin Elmer UV/VIS photospectrometer, Boston, MA, U.S.A.). Three replicate measurements per sample were averaged.

Fatty acids (FAs) were analysed after Folch, Lees & Sloane Stanley (1957). For the algae, a known volume of each culture which was filtered on a prerinsed, precombusted 25 mm GF/F glass fibre filter, and frozen to destroy the algal cells. Algal FAs were extracted with chloroform/methanol 2 : 1 with 0.003% butylated hydroxytoluene (BHT, Sigma, St Louis, MO, U.S.A.) in order to prevent autoxidation of PUFAs. *Brachionus calyciflorus* were directly homogenised and extracted in chloroform/methanol. After addition of an internal standard (heneicosanoic acid: C21:0, Sigma), several homogenisation, centrifugation and purification steps followed. The purified extract in chloroform was evaporated and re-dissolved in hexane, free FAs were methylated (3% H_2SO_4 in dry methanol under N_2 atmosphere, 4 h at 80 °C, H_2O added after cooling down), and the FA methyl esters were extracted in hexane. FA methyl esters were analysed on a gas chromatograph. For further details see Weers & Gulati (1997).

Statistics

All statistical analyses were carried out with the STATISTICA analytical package (StatSoft, Inc., 2001, Tulsa, OK, U.S.A.). Data were first tested for homoscedasticity (Levene's test for ANOVA) and normality (Kolmogorov–Smirnoff test) and, if necessary, data were subjected to an appropriate transformation. Interactions between food quality and inorganic nutrient medium were tested by two-way analysis of variance (ANOVA), followed by *post hoc* comparison [Tukey HSD or Tukey HSD for unequal N (Spjotvoll and Stoline test)]. Data that were non-normal or heteroscedastic or both, even after transformation, were analysed using the Scheirer-Ray-Hare extension of the Kruskal–Wallis test (Sokal & Rohlf, 2000). Survival analysis was carried out by comparing the distribution of the cumulative proportions of surviving animals (Kaplan–Meier test), allowing for censored data. Individual data were censored if mortality was caused by a non-natural cause, such as experimental handling or floating.

Results

Algal food quality

Physiological differences between nutrient-sufficient and nutrient-depleted algae were visible detectable by the paler colour of the nutrient-limited algae and their somewhat larger size. The size increase was apparently caused by a slight increase in the proportion of multicellular colonies, and, more importantly, by aggregates of mucus-forming algal particles. Algae growing on nutrient-depleted media were clearly nutrient limited, as indicated by their dry weight yields, which were four to 10 times lower than algae grown on full medium. Nutrient limitation of these algae was also visible in both nutrient contents (% of dry weight) and C : P and C : N ratios (Table 1). The FA content of the nitrogen limited algae was twice as high as that of the other two types of algae. In nitrogen limited algae, a threefold increase in saturated palmitic acid (C16:0) and a tenfold increase in mono-unsaturated *cis*-oleic acid (18 : 1ω9c) were observed, compared with full medium algae (Table 2). In addition, phosphorus and nitrogen limited algae showed qualitatively similar changes in FA composition compared with full medium algae: a doubling of the ratio of saturated fatty acids to unsaturated fatty acids (SAFA/UFA, from 0.23 to 0.5), and a concomitant decrease in both total PUFAs [from 65 to 21% (N) respectively 34% (P) of total FA's] and omega-3 FAs [ω3: from 57% to 14% (N) respectively 24% (P)]. The latter is largely attributable to a threefold reduction in the relative contents of α-linolenic acid (C18 : 3ω3).

Table 1 Stoichiometric composition of the three qualities of *Scenedesmus obliquus* used, and of *Brachionus calyciflorus* fed *ad libitum* on full medium algae (dry weight of well-fed individual *B. calyciflorus* was 0.15 ± 0.03 μg). In the first three rows, carbon, nitrogen and phosphorus values are given as percentage of dry weight (DW). In the bottom three rows, atomic ratios between the different elements are given. Averages (±1 SD) for algal stoichiometry were calculated over triplicate samples from start ($t = 0$ day), middle ($t = 7$ day) and end ($t = 14$ day) of the life history experiment ($n = 9$). For *B. calyciflorus*, averages (±1 SD) were calculated from 14 samples containing 40–100 individuals per sample. The letters in the table refer to algal culture conditions: algae cultured with full nutrients (F) or N-deficient (N) or P-deficient (P) media

	S. obliquus			*B. calyciflorus*
	F	N	P	F
C [DW (%)]	45.60 (2.79)	34.37 (2.73)	48.78 (2.43)	40.08 (4.92)
N [DW (%)]	5.80 (0.63)	1.83 (0.06)	4.62 (0.26)	11.59 (2.11)
P [DW (%)]	1.292 (0.074)	1.088 (0.285)	0.202 (0.002)	1.32 (0.64)
C : N	9.35 (0.10)	21.85 (1.06)	12.38 (0.08)	4.09 (0.38)
C : P	91.5 (10.8)	85.9 (28.5)	626.0 (23.7)	92.45 (36.1)
N : P	9.78 (1.44)	3.89 (1.11)	50.79 (2.24)	22.7 (8.9)

Somatic growth

Somatic growth rates of *B. calyciflorus* differed significantly after 24 h (Fig. 1; Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P \leq 0.0001$, $H = 97.54$, d.f. $= 2$, $n = 270$): individuals fed nutrient replete algae had significantly higher growth rates than those fed both N- or P-limited algae. Some F individuals had already produced their first egg within 24 h. Including an average egg volume of 3.469×10^5 μm^3 when calculating SG rates of the egg-bearing individuals yielded even greater differences (Fig. 1; Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P \leq 0.0001$, $H = 138.73$, d.f. $= 2$, $n = 270$). A significant food effect was also found for size (body volume) at first reproduction (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P \leq 0.0001$, $H = 19.30$, d.f. $= 2$, $n = 84$): individuals grown on nutrient replete algae were larger than those grown on N- or P-limited algae. The medium did not have significant effects on somatic growth during 24 h (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.51$, $H = 1.36$, d.f. $= 2$, $n = 270$), nor on overall growth during 24 h (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.58$, $H = 1.09$, d.f. $= 2$, $n = 270$), nor on the SFR (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.54$, $H = 1.21$, d.f. $= 2$, $n = 84$). The interaction between food and medium was significant for somatic growth rates after 24 h, because of the treatment with phosphate and ammonium in the zooplankton medium (Fig. 1; Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.015$, $H = 12.37$, d.f. $= 4$, $n = 270$). Such an interaction effect was not found for SFR (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.054$, $H = 9.29$, d.f. $= 4$, $n = 84$).

Reproduction

Reproduction was observed in all treatments. Some individuals fed nutrient sufficient algae produced their first egg within 24 h (data not shown). The age at which the first eggs appeared differed significantly between food types (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P \leq 0.0001$, $H = 129.05$, d.f. $= 2$, $n = 197$), but not between medium types ($P = 0.65$, $H = 0.87$, d.f. $= 2$, $n = 197$). The same significant differences in food type were seen for age at first reproduction (Fig. 2; Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P \leq 0.0001$, $H = 125.15$, d.f. $= 2$, $n = 197$), but again with no significant differences for the medium effect (Fig. 2; $P = 0.29$, $H = 2.49$, d.f. $= 2$, $n = 197$). The interaction between food and medium types did not differ significantly, neither for age at first egg appearance nor for age at first reproduction (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.68$ and 0.98 respectively, $H = 2.30$ and 0.43, respectively, d.f. $= 4$, $n = 197$). The percentage of matured eggs that hatched (hatching success: data not shown) was 87 ± 18% (SD), irrespective of food and medium type.

In addition to earlier maturation, animals fed nutrient sufficient algae reproduced for a longer period than those fed P-limited algae, which in turn had a longer reproductive period than animals fed N-limited algae (Fig. 2; Scheirer-Ray-Hare extension

Table 2 Fatty acid contents of *Scenedesmus obliquus* used, and of *Brachionus calyciflorus* fed *ad libitum* on full medium algae. Algal fatty acid content is expressed as microgram per milligram dry weight (±1 SD) and was calculated from three replicate samples measured for the different foods at the end of the experiment ($n = 3$). For the rotifers, only a single determination was carried out; here, percentages of total fatty acids are given

	S. obliquus			*B. calyciflorus*
Fatty acid	F	N	P	F (%)
C14:0	0.1 (0)	–	–	–
C16:0	11.02 (1.17)	32.08 (1.19)	21.98 (2.11)	38.80
C16:1ω7t	–	–	–	–
C16:1ω7c	0.38 (0.02)	1.19 (0.09)	0.85 (0.09)	1.60
C17:0	0.15 (0.09)	1.98 (0.11)	0.66 (0.56)	–
C16:2ω4	–	–	–	–
C16:3ω4	–	–	0.18 (0.18)	–
C16:4ω3	10.38 (1.84)	2.75 (1.74)	4.94 (2.23)	–
C18:0	–	2.64 (0.09)	1.22 (0.41)	4.80
C18:1ω9t	–	–	–	1.60
C18:1ω9c	4.83 (0.54)	50.09 (1.33)	17.65 (1.01)	5.60
C18:1ω7c	3.75 (0.12)	1.21 (0.08)	1.83 (0.07)	2.00
C18:2ω6	4.91 (0.45)	7.31 (0.72)	6.78 (1.21)	9.60
C18:3ω3	20.53 (2.52)	11.98 (1.95)	9.15 (3.73)	22.40
C18:4ω3	3.72 (0.61)	1.86 (0.5)	3.07 (1.24)	–
C20:1ω9	1.08 (0.01)	1.01 (0.06)	0.59 (0.25)	2.80
C20:4ω6	–	–	–	–
C20:4ω3	–	–	–	3.20
C20:5ω3	–	–	–	2.00
C24:0	0.22 (0)	0.3 (0.05)	0.07 (0.07)	3.60
C22:5ω3	–	–	–	2.00
C22:6ω3	–	–	–	–
Total	60.98 (7.19)	114.4 (6.91)	68.97 (11.49)	100.00
SAFAs (%)	18.72 (0.43)	32.39 (1.45)	35.06 (3.23)	47.20
MUFAs (%)	16.51 (0.81)	46.83 (1.60)	30.80 (4.12)	13.60
PUFAs (%)	64.77 (1.24)	20.78 (2.98)	34.14 (7.33)	39.20
SAFA/UFA	0.23 (0.01)	0.48 (0.03)	0.54 (0.08)	0.89
ω3 (%)	56.71 (1.45)	14.40 (2.71)	24.09 (7.00)	30
ω6 (%)	8.07 (0.21)	6.38 (0.28)	9.81 (0.13)	10
ω3/ω6	7.03 (0.37)	2.25 (0.34)	2.45 (0.68)	3.08

SAFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; UFA, unsaturated fatty acids.

of the Kruskall–Wallis test, $P \leq 0.0001$, H = 28.91, d.f. = 2, $n = 108$). Medium type had a weak effect on the duration of the reproductive period (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.044$, H = 6.26, d.f. = 2, $n = 108$). There was no significant effect of the interaction of food and medium on reproductive period (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.77$, H = 1.81, d.f. = 4, $n = 108$). Although the length of the postreproductive (senile) period was highly variable for all treatments (Fig. 2; *c.v.* = 55–102%) it was shortest in individuals grown on nutrient replete algae (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.0002$, H = 16.93, d.f. = 2, $n = 108$), irrespective of medium type (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.065$, H = 5.48, d.f. = 2, $n = 108$). Neither for the postreproductive period did the interaction between food and medium have a significant effect (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.29$, H = 4.99, d.f. = 4, $n = 108$).

Besides length of the reproductive period, the reproductive rate (rate of egg- and offspring production during the reproductive period) was also affected by algal nutrient limitation. When reproductive period was plotted against number of eggs or offspring (data not shown), slopes of regressions (the reproductive rate) showed that animals fed nutrient replete algae produced 1.88 eggs day^{-1} and 1.71 offspring day^{-1} during their reproductive period, animals fed P-limited algae produced 1.31 eggs day^{-1} and 1.14 offspring day^{-1} and animals fed N-limited 1.05 eggs day^{-1} and 0.96 offspring day^{-1}.

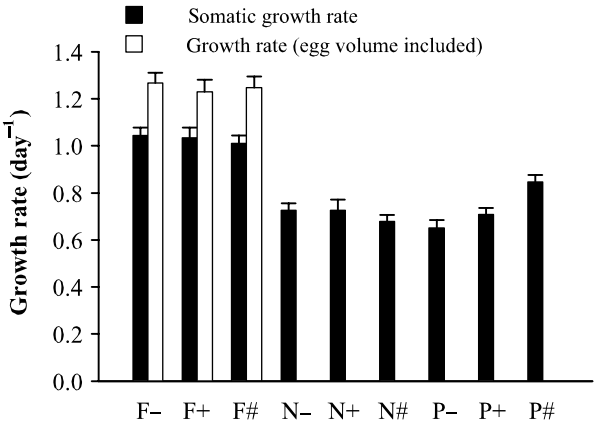

Fig. 1 Body volume-based somatic growth rates of *Brachionus calyciflorus* in the different treatments during the first 24 h. Also shown are the growth rates with average egg volumes of egg-bearing individuals included ('egg volume included'). Error bars are ±1 SE. The letters in the figure refer to algal culture conditions. Animals were kept for 18 h day^{-1} in food suspensions (40 mg dry weight L^{-1}) of algae cultured with full nutrients (F) or N-deficient (N) or P-deficient (P) media. The remaining 6 h of the day animals were kept in either of three different zooplankton mineral media: medium lacking inorganic N or P(−), medium with phosphate and nitrate (+) or medium with phosphate and ammonium (#).

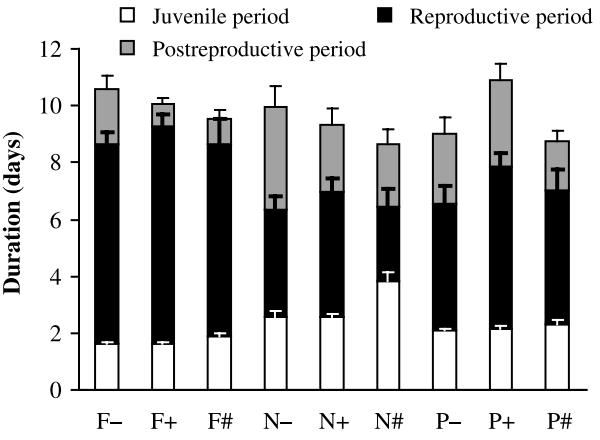

Fig. 2 Duration of juvenile, reproductive and postreproductive periods of *Brachionus calyciflorus*. Error bars are ±1 SE. Note that total bar height represents total lifespan. Treatments coded as in Fig. 1.

Lifespan

In contrast to growth and reproduction, lifespan was not affected by food type (Fig. 3; Kaplan–Meier test, $P = 0.29$, $\chi^2 = 2.45$, d.f. = 3) or medium type (Kaplan–Meier test, $P = 0.12$, $\chi^2 = 4.22$, d.f. = 3).

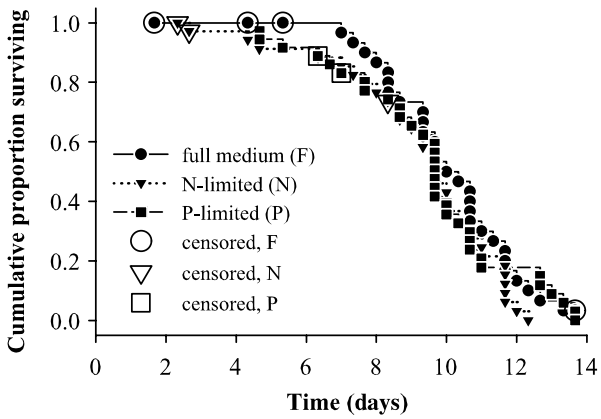

Fig. 3 Cumulative proportion surviving *Brachionus calyciflorus* (including censored animals). Data for the three different food treatments are pooled over the three different medium treatments. Algae culture conditions coded as in Fig. 1.

Fitness

To assess overall fitness, we compared three different fitness parameters: lifetime fecundity (number of eggs produced per female), lifetime fertility (number of offspring produced per female) and population growth rate (intrinsic rate of increase). Lifetime fecundity and fertility differed significantly for the different food types (Fig. 4a; ANOVA, $P = 0.0021$ and 0.0018 respectively, $F_8 = 6.64$ and 6.83, respectively). Animals fed nutrient sufficient algae had higher lifetime fecundity and fertility than those fed P-depleted algae, which again had higher fecundity and fertility than those fed N-depleted algae. Medium type had no significant effect on lifetime fecundity and fertility (Fig. 4a; ANOVA, both $P = 0.19$, $F_8 = 1.69$). The interaction between food and medium types did not differ significantly, neither for fecundity nor fertility (ANOVA, $P = 0.87$ and 0.84 respectively, $F_8 = 0.31$ and 0.36, respectively). Population growth rates differed significantly between the different food qualities (Fig. 4b; Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P < 0.0001$, H = 95.12, d.f. = 2, $n = 108$). Individuals fed nutrient sufficient algae had higher population growth rates than those fed P-limited algae, which again had higher growth rates than individuals fed N-limited algae. The different nutrient media also had a significant effect on population growth rates (Fig. 4b; $P = 0.029$, H = 7.09, d.f. = 2, $n = 108$): animals from medium with phosphate and ammonium (#) had significantly lower population growth rates than animals from the two

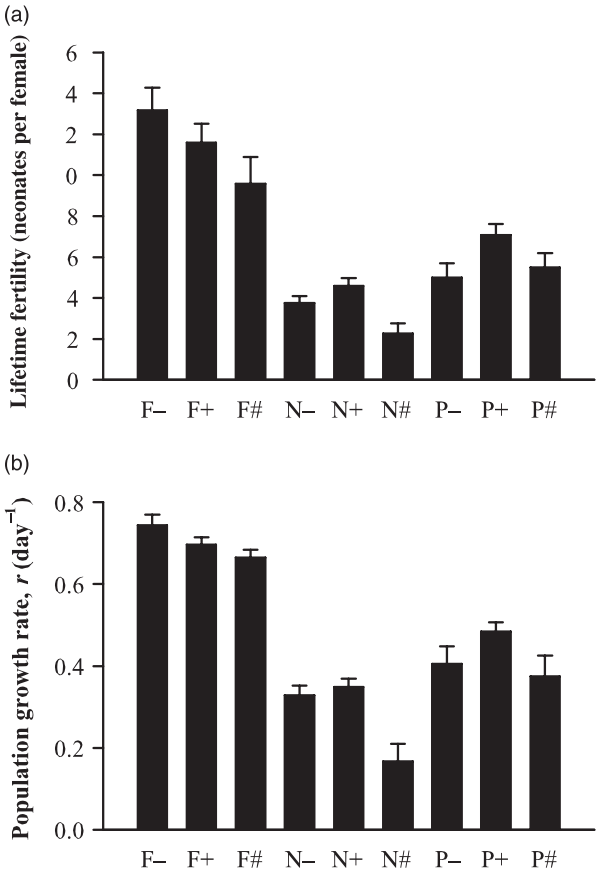

Fig. 4 Lifetime fertility of *Brachionus calyciflorus* for the different treatments (a). Population growth rates of *Brachionus calyciflorus* for the different treatments (b). Error bars are ±1 SE. Treatments coded as in Fig. 1.

other zooplankton medium types (− and +). The interaction between food and medium had no significant effect on the population growth rates (Scheirer-Ray-Hare extension of the Kruskall–Wallis test, $P = 0.61$, $H = 2.69$, d.f. $= 4$, $n = 108$).

Discussion

Algal food quality

We observed clear and significant effects of algal food quality on most investigated life history traits of *B. calyciflorus* (Table 3). This effect can either be direct, if it is caused by the nutritional content of the food, or indirect, if it is caused by reduced ingestion and/or assimilation rates. Rothhaupt (1995), using *B. rubens* feeding on the same strain of *S. obliquus* used here and cultured on similar media (full, N-depleted and P-depleted), found no differences in functional responses (ingestion rates) among the three types of algae. In our study, nutrient-depleted algal cells were slightly larger (5–6 μm equivalent spherical diameter, ESD) than nutrient sufficient algae (4–5 μm ESD). However, as long as the algae are below 10 μm ESD, the ingestion rate of *B. calyciflorus* is expected to be maximum at high food concentrations (Rothhaupt, 1990). Thus, differences in life history parameters are probably not because of different ingestion rates. However, nutrient-limited algae may divert their excess carbon either in thicker cell walls or via excretion as an extracellular mucus layer (Van Donk & Hessen, 1993; Van Donk *et al.*, 1997), thus reducing digestion (assimilation efficiency) of such algal cells. Nevertheless, in contrast to cladocerans, rotifers are able to crush ingested algae with a stomach (mastax) that is specialised for grinding the food. This may explain why Rothhaupt (1995) did not find differences in growth rates of rotifers among nutrient-limited algae with carbon-based (green algae) or silica-based (diatoms) cell walls. This, in combination with the high food concentrations applied, also makes reduced assimilation rates unlikely. The observed effects of food quality on life history traits were probably caused by the nutritional contents of the algae.

Phosphorus deficiency

We found a high P-content in *B. calyciflorus* (C : P 92: Table 1). Rothhaupt (1995) observed for the closely related *B. rubens* under similar feeding conditions a slightly lower P-content (C : P 111). In our study, *B. calyciflorus* still grew and reproduced when fed P-depleted *S. obliquus* (C : P 626). In contrast, however, *B. rubens* showed no positive population growth, irrespective of food concentration if fed either P-limited *S. obliquus* (C : P 874) or P-limited *Cyclotella meneghiniana* (C : P 684; Rothhaupt, 1995). This is a striking difference, because of the higher C : P ratios of *B. rubens* and the comparable P contents of the algae. This is even more surprising given the higher dilution rate in Rothhaupt's (1995) algal culture (0.4 compared with 0.1 d^{-1} in our study), which should improve the biochemical food quality. However, Rothhaupt (1995) used a food concentration of 1 mg C L^{-1}, which is much lower than the food concentration used in our study (20 mg C L^{-1}). This suggests that in the genus *Brachionus*, R_0 increases with

Table 3 Summary of food, inorganic nutrient medium effects, and their interaction, on life history of *Brachionus calyciflorus*

Variable	Food effect	Medium effect	Interaction	*n*	Test
Body growth					
Somatic growth rate	***	ns	*	270	SRH
Body volume at first reproduction	***	ns	ns	84	SRH
Reproductive lifespan					
Age at first egg appearance	***	ns	ns	197	SRH
Age at first offspring (=juvenile period)	***	ns	ns	197	SRH
Reproductive period	***	*	ns	108	SRH
Postreproductive (senile) period	***	ns	ns	108	SRH
Lifespan					
Survival	ns	ns	ND	108(9)†	KM
Overall fitness					
Lifetime fecundity	**	ns	ns	108	AV
Lifetime fertility	**	ns	ns	108	AV
Population growth rate	***	*	ns	108	SRH

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
†Within brackets, the number of censored individuals is given.
ND, not determined; ns, not significant; SRH = Scheirer-Ray-Hare extension of the Kruskall–Wallis test; KM, Kaplan–Meier test; AV, analysis of variance (ANOVA).

decreasing food quality, similar to what has been found for *Daphnia* (Boersma & Kreutzer, 2002).

In our experiments, supplementation with inorganic P to *B. calyciflorus* fed P-deficient algae did not have any significant effect on life history parameters. We did, however, find a significant effect of the interaction between type of food algae and zooplankton medium for SG rates. We have no evident explanation for this interaction effect, but it may be worth further investigation. Our experiments suggest that *B. calyciflorus* is not capable of mineral P compensation, at least not at the mineral concentrations we used. We know of only one study where mineral compensation for zooplankton has been proven (Urabe *et al*., 1997); this was for *Daphnia* under extremely high mineral concentrations and never resulted in complete compensation of the deleterious effects of P-limited algae. Rothhaupt (1995) also prepared food concentrations of P-limited algae with phosphate-sufficient medium. This resulted in an algal P-content similar to P-sufficient algae, but in only slightly higher population growth rates of *B. rubens*. This effect was most likely a result of uptake of phosphate from the ingested algae, as algae are a highly concentrated P source (Urabe *et al*., 1997). In our study, phosphate was only supplied to *B. calyciflorus* through the medium. Uptake of mineral P from the medium by *Daphnia* only takes place at unnaturally high concentrations (Parker & Olson, 1966; Urabe *et al*., 1997). Thus the lack of mineral compensation could be because *B. calyciflorus* is not able to take up inorganic P from the medium directly at the concentrations used (50 μmol P L^{-1}).

Nitrogen deficiency

In our study, the animals fed N-depleted algae showed similar life history responses as P-depleted algae: decreased fecundity and somatic growth, but no reduction in lifespan. Both N per DW and C : N ratio indicate a very high nitrogen content in *B. calyciflorus*, which has also been found in other studies of *B. calyciflorus* (Awaïss & Kestemont, 1997) and related *Brachionus* species (Scott, 1980; Yúfera & Pascual, 1989; Rothhaupt, 1995). We did not observe any significant mineral compensation in *B. calyciflorus* supplied with ammonium or nitrate. Probably *B. calyciflorus* is incapable of inorganic nitrogen uptake, like larger zooplankton (Brett, 1993). Therefore, Urabe & Watanabe (1993) suggested that protein limitation is a more appropriate term than N-limitation. *B. calyciflorus* is particularly rich in

proteins (53.3–54.1% of DW: Awaïss & Kestemont, 1997) which could explain the high nitrogen content of *Brachionus*. Growth rate of the related *B. plicatilis* is positively correlated with individual protein content and protein/lipid ratios (Øie & Olsen, 1997). This can be explained by the fact that high growth rates require high protein synthesis rates (=high amino acid demands). Algal N-limitation directly limits amino acid/protein synthesis. Both types of algal nutrient limitation could have limited protein synthesis in *B. calyciflorus*: N-limitation directly reduces the (essential) amino acid and protein content in the algae, whereas P-limitation may affect rotifer protein synthesis through decreased contents of ribosomal RNA (Elser *et al.*, 2000).

Fatty acid deficiency

Although the total FA content of the algae did not change or even increased under nutrient limitation, the FA composition of the algae showed similar changes in both P- and N-limited algae. The ω3 FAs as a proportion of total FAs decreased in nutrient limited algae, which is probably a general phenomenon in nutrient-limited green algae (cf. Weers & Gulati, 1997). FA composition of the food algae is important for *Brachionus* growth (Olsen, 1999), and rotifer growth rates depend in particular on the PUFA content of their food (Lubzens, Tandler & Minkoff, 1989). *Brachionus sp*. appear to be particularly rich in ω3 PUFAs, and have a limited ability to elongate and desaturate these from precursors (Lubzens, Marko & Tietz, 1985). As the fraction of ω3 FAs in the algae decreased under nutrient limitation, this may have limited the synthesis of other ω3 PUFAs such as C20: 4ω3, C20: 5ω3 and C22: 5ω3 in *B. calyciflorus*. In particular, the predominant and essential FA α-linolenic acid (α-LA: C18: 3ω3, 22% of the FAs in well-fed *B. calyciflorus*, Table 2) decreased under both types of nutrient limitation. Furthermore, both types of algal nutrient limitation resulted in similar changes in *B. calyciflorus* life history. This suggests that ω3 FAs may have been limiting growth and reproduction of *B. calyciflorus* under both N and P-limitation, although this does not exclude the importance of other compounds, such as essential amino acids or direct mineral limitation.

Rothhaupt (1995) already suggested that mineral P limitation may not be the only factor causing limitation in *Brachionus*, but that biochemicals such as FAs or amino acids may also be important in P-limited algae. When considering the relationship between P- and PUFA-limitation in *Daphnia*, both elemental composition and PUFAs seem to be important and even substitutable (Boersma, Schops & McCauley, 2001). Generally P is most important for *Daphnia* under severe P-limitation of algae whereas PUFAs become more important under more moderate algal P-limitations (Boersma, 2000; Becker & Boersma, 2003). However, one study found P- and FA-limitation effects on *Daphnia* to be independent (Park *et al.*, 2002). Considering the observed effects on N-limited algae in the present study, a similar relation may exist between N and PUFAs in N-limited systems, i.e. the N-/protein-/amino acid content of N-limited algae is most important under severe N-limitations and PUFAs become more important under more moderate N-limitation.

Somatic growth

Because the cysts in our experiment came from well-fed mothers, they were expected to contain comparable levels of reserves. However, somatic growth rates of *B. calyciflorus* even differed after the first 24 h, and these growth differences became more pronounced by the time of first reproduction because of the cumulative effect of food limitation. Both N and P algal limitation reduced somatic growth rates. This observation is in accordance with experiments where cladocerans fed nitrogen or phosphorus-limited algae also showed reduced somatic growth rates (Sterner, 1993; Urabe & Sterner, 2001). Obviously, both algal N- and P-limitation inhibit the formation of somatic (and reproductive) structures in herbivorous zooplankton.

Reproduction effects

The lower quality of nutrient-deficient algae strongly limited reproduction-related parameters, but did not inhibit reproduction completely. The total number of offspring produced during the lifespan decreased, not only because the animals had shorter reproductive periods but also because of a lower daily reproduction. Nutrient-limited *B. calyciflorus* did not abort eggs, as has been observed for *Daphnia* under algal nutrient

limitation (Weers & Gulati, 1997; Urabe & Sterner, 2001).

Lifespan

Lifespan, in contrast to all other life history parameters, was not affected by algal nutrient limitation (Table 3). This may be due to the fact that high food concentrations could still support maintenance metabolism, which requires mainly C-rich compounds (e.g. Sterner & Robinson, 1994). However, at low food quality, zooplankton survival may already be affected at food (carbon) concentrations that would normally support positive population growth (Rothhaupt, 1995; Boersma & Kreutzer, 2002), which suggests that also nutrient intake may be important for maintenance. Apparently, the high food concentrations of low quality food used in our experiment were sufficient to support the nutrient requirements of maintenance and hence did not affect lifespan.

Fitness

Only one previous study has investigated the effects of algal nutrient limitation on rotifer life history (Ramos-Rodríguez & Conde-Porcuna, 2003). However, as the C : N : P ratios in this study did not indicate severe limitation of the 'nutrient-limited' algae, we cannot directly compare our results with this study. A trade-off between lifespan and reproduction has been observed for rotifers under food quantity limitation (Kirk, 1997). In Kirks study some rotifer species had higher survival and reduced reproduction during food quantity limitation, whereas others had a lower survival and higher reproduction. Such a trade-off between lifespan and reproduction was not found in our study under food quality limitation. However, as lifespan was similar for all food qualities in our study, we observed a shift from a highly reproductive lifespan towards a low-reproductive lifespan (Fig. 2), which has also been found for other rotifers (Enesco, 1993). Guisande *et al.* (1993) noted the juvenile period in *B. calyciflorus* to be a crucial selecting factor for population growth rate. In addition to this, our study shows that under food quality limitation, both juvenile period and total non-reproductive period influence population growth rate and lifetime reproductive success. The apparent time constraint on fitness can be explained by the fact that malnutrition, through a slower build-up of certain (essential) chemical reserves, prolongs both the maturation time (juvenile period) and the production time between consecutive eggs. Other studies have also shown that algal N and P-limitation cause reduced somatic growth and reproduction in *Daphnia* leading to decreased population growth rates (Sterner, 1993; Urabe & Sterner, 2001). Thus, reduction of somatic growth and reproduction appears to be a general pattern found in herbivorous zooplankton under both N and P limitation. Although this strategy reduces individual fitness, it does not seem to reduce individual survival in *B. calyciflorus*, and may thus increase the chance of individuals to overcome periods of inferior food quality.

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