

Does excess carbon affect respiration of the rotifer *Brachionus calyciflorus* Pallas?

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SUMMARY

1. Herbivorous zooplankton maintain a rather constant elemental composition in their body mass as compared with the variability commonly encountered in their food. Furthermore, their high phosphorus (P) and nitrogen (N) content means that they often face an excess of carbon (C) in their diet. Regulation of this surplus of energy may occur via modulation of assimilation efficiency, or postassimilation by increased respiration (CO₂) and/or excretion dissolved organic carbon, DOC. Whereas several studies have examined the effect of elemental imbalance in the genus *Daphnia*, few have examined other zooplankton taxa.

2. We investigated whether the rotifer *Brachionus calyciflorus* uses increased respiration as a means of stoichiometrically regulating excess dietary C. Growth rate and respiration were measured under different food qualities (C : N and C : P ratios).

3. Both C : N and C : P ratios in food had strong effects on growth rate, demonstrating strong nutrient limitation of rotifer growth when nutrient elements were depleted in the diet and indicating the need for stoichiometric regulation of excess ingested C.

4. Respiration measurements, supported by a stoichiometric model, indicated that excess C was not released as CO₂ in *B. calyciflorus* and that nutrient balance must therefore be maintained by other means such as excretion of DOC or egestion in faecal material.

Keywords: *Brachionus*, food quality, growth, homeostatic regulation, stoichiometry

Introduction

The flux of carbon (C) through lake ecosystems depends on the availability of key nutrient elements such as phosphorus (P) and nitrogen (N) (Sterner & Elser, 2002; Hessen *et al.*, 2004). Herbivorous zooplankton are of particular importance, being the link between the primary producers and higher trophic levels. These consumers maintain relatively high and rather constant N and P contents compared with their food (Andersen & Hessen, 1991; DeMott, Gulati & Siewertsen, 1998), so they often face diets with excess C relative to nutrient elements. This

imbalance leads to decreased C growth efficiencies and ultimately to lower food chain production (Hessen, 1992; Sterner & Hessen, 1994; Sterner *et al.*, 1998). Reduced growth efficiencies implies that the grazers have cope with the excess C, which may be released as faeces, dissolved organic carbon (DOC) or CO₂. These different fates have important consequences for C cycling and sequestration in ecosystems. An understanding of homeostatic regulation in zooplankton is therefore important when trying to link organism to ecological processes.

Zooplankton may use different strategies in homeostatic regulation. The acquisition of resources can be regulated by adjusting the uptake of carbon relative to nutrients through changed food selectivity, ingestion rates or assimilation efficiencies (DeMott, 1995; DeMott *et al.*, 1998; Plath & Boersma, 2001). Zooplankton

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may also release assimilated excess C postabsorption by the gut by excretion of carbon rich organic compounds (Darchambeau, Færøvig & Hessen, 2003), or by respiring the excess (Anderson *et al.*, 2005). This potential role of respiration as a regulatory mechanism for homeostasis in zooplankton is not well understood. The phenomenon was emphasised by Sterner (1997), who advocated the need for estimates of specific dynamic action (SDA; the increase in respiration associated with feeding) and how it relates to food quality. Recent stoichiometric modelling approaches have accordingly distinguished between basal metabolism and the costs of assimilation and growth (Anderson *et al.*, 2005; Anderson & Hessen, 2005). As well as measuring SDA, obtaining estimates of basal metabolic rates (BMR) at different food qualities is also important (Anderson & Hessen, 2005). Basal metabolism depends not only on food quantity, food quality may also be important. Elevated BMRs may play a role in the disposal of excess C in some animals (Curcio *et al.*, 1999; Even *et al.*, 2003; Fu & Xie, 2004). The time of acclimation to a given food quality could also be important for which mechanism zooplankton use to obtain nutrient balance. Increased respiration could be just a transient phenomenon in response to excess dietary C, but it could also be important after long-term acclimation to food of high C, although regulating food uptake might also be important in this situation.

A large number of stoichiometric studies have focused on *Daphnia*, a particularly important genus in the ecology of freshwater systems. However, the response of *Daphnia* to elemental imbalance in its food may not be representative of those of other taxa. Rotifers may also play an important ecological role at some periods of the year (Walz, 1995) and in particular lakes when environmental factors, such as fish predation pressure, becomes unfavourable for larger crustacean zooplankton (Jeppesen *et al.*, 1990). Although daphnids and rotifers belong to the same functional group, they are distant taxonomic relatives, and there appear to be important physiological differences between the two groups. Recent modelling work by Anderson *et al.* (2005) suggested that *Daphnia* regulates excess C in the diet primarily postabsorption by the gut, either by respiration of CO₂ or by excretion of DOC. In common with *Daphnia*, suspension feeding rotifers of the genus *Brachionus* show reduced growth on P-deficient diets (Rothhaupt, 1995; Jensen &

Verschoor, 2004). However, in contrast to *Daphnia*, N-limitation may also be prevalent in this rotifer (Sterner, 1993; Rothhaupt, 1995; Jensen & Verschoor, 2004). Rotifers may therefore experience different challenges when dealing with excess carbon in the many lakes where food reaches high C : nutrient ratios (Elser *et al.*, 2000) and may use different mechanisms than *Daphnia* in order to regulate their C balance.

We studied stoichiometric regulation in a cosmopolitan, commonly occurring zooplankton, the rotifer *Brachionus calyciflorus* and in particular examined the role of respiration in the disposal of excess C in the diet. Respiration of *B. calyciflorus* was measured when feeding on different food qualities (C : N and C : P ratios) at high food concentrations. The food treatments included nutrient sufficient and phosphorus- and nitrogen-depleted algae. Respiration measurements were conducted on animals after both short- and long-term acclimation to the respective food treatments. Finally, we used the stoichiometric model of Anderson *et al.* (2005), parameterised for *B. calyciflorus*, in order to study the experimental results in greater detail.

Methods

Cultures

The green alga *Selenastrum capricornutum* was grown in continuous cultures (dilution rate 0.2 day⁻¹) in 2 L Nalgene polycarbonate vessels (Nalge Company, Rochester, NY, U.S.A.) with a magnetic stirring device receiving a nominal light of 70 µmol quanta m⁻² s⁻¹ from 25 W blue-white fluorescent tubes. Algal cultures were allowed to run for 2 weeks before the start of the experiments in order to obtain stable cultures. The algae were cultured on the same three types of COMBO medium (Kilham *et al.*, 1998), as described by Jensen & Verschoor (2004): full nutrient sufficient medium (F) with a phosphate concentration of 50 µmol L⁻¹ and a nitrate concentration of 1000 µmol L⁻¹; P-depleted medium (-P) with phosphate concentration reduced to 2 µmol L⁻¹; and N-depleted medium (-N) with a nitrate concentration of 40 µmol L⁻¹.

As a proxy of cell density, daily measurements of optical density at 663 nm were performed using a spectrophotometer (UV-210A, Shimadzu Seisakusho

LTD, Kyoto, Japan). Samples were taken twice a week for phytoplankton size distribution and for samples for particulate C, N and P. Those for size distribution of the algae were fixed with Lugol's iodine (1% final concentration), inspected visually using a microscope and analysed for cell number and cell volume on a CASY TTC1 Cell Counter (Schärfe Systems, Reutlingen, Germany). Subsamples from the cultures were filtered on precombusted, acid-washed GF/F filters for analysis of particulate C, N, and P. C and N were analysed on a Flash EATM 1112 automatic elemental analyser (Thermo Finnigan, Milan, Italy). Particulate P was analysed following the persulphate method according to Hessen, Færøvig & Andersen (2002). Algae from the different cultures were used as food in the zooplankton experiments. Harvested algae were diluted with nutrient free COMBO medium to a standardised food concentration of 6 mg C L⁻¹. This concentration was calculated from pre-established regressions for each food type relating absorbance and the carbon content of the cultures.

Brachionus calyciflorus were hatched from dormant eggs (MicroBioTest inc., Nazareth, Belgium). This ensured identical egg quality and synchronised age of the population. Rotifers were cultured in COMBO medium without phosphate and nitrate in order to avoid uptake of N and P from the medium by the food algae. The ionic strength of the nutrient-free zooplankton medium was maintained by the addition of KCl at a concentration of 100 µmol L⁻¹. All experiments were carried out at constant temperature (19 °C) in dim light.

Zooplankton experiments

Brachionus calyciflorus for the experiments were hatched from cysts placed in nutrient-free COMBO medium in shallow dishes. Hatching started after 36 h of incubation at 19 °C. Because of the small individual size of *B. calyciflorus*, large numbers were needed for the respiration experiments. To obtain sufficient numbers, newborns hatched from the same batch of cysts within 36–48 h were collected, divided into three groups and transferred to large glass beakers and fed F, -P or -N algae at a food concentration of 6 mg C L⁻¹ for 48 h before the respiration experiment. The food was refreshed daily.

A previous study by Olsen, Reinertsen & Vadstein (2002) indicates that *Brachionus* species can have a

relatively flexible stoichiometry. Thus, the stoichiometric composition of *B. calyciflorus* fed different algal qualities in terms of C : N : P was measured in order to determine the extent to which body C : N : P was affected by food quality. Rotifers from cultures grown under the same conditions as for the respiration experiment were rinsed in nutrient-free COMBO medium in several steps. Three hundred individuals were then transferred to small acid-washed glass vials in 10 mL of distilled water and the samples with animals were filtered on precombusted acid-washed GF/F filters, before being analysed for C, N and P as described above. Samples for the determination of dry masses were also taken from the same rotifer cultures. For this purpose 70–100 rotifers were transferred to preweighed silver cups, dried for 24 h at 60 °C, cooled in a desiccator and weighed to the nearest microgram on a Mettler Toledo MX5 microbalance (Mettler Toledo, Greifensee, Switzerland).

We measured somatic growth rates of the juvenile *B. calyciflorus* that were fed different food qualities of algae. Newborn individuals (up to 2 h old) were transferred into clear polystyrene 96-well microtiter plates, with one individual per well in 250 µL food suspension (6 mg C L⁻¹). Animals were transferred from the food suspension after 24 h and preserved in an isotonic salt solution (CASYTON, Schärfe Systems, Reutlingen, Germany) that killed them quickly. The animals were measured in an inverted microscope and body volumes calculated following Ruttner-Kolisko (1977). Measured values were increased by 10% in order to account for the volume of the foot. Volume-based somatic growth rates were calculated as $r = (\ln b_1 - b_0)/t$, where r = somatic growth rate, b_1 = body volume at the end of the experiment, b_0 = body volume at the beginning and t = duration of experiment in days.

The respiration rate of *B. calyciflorus* was determined by measuring oxygen consumption by the 'closed bottle method' (Lampert, 1984). Respiration in animals is affected by their feeding activity (Fig. 1). When an animal starts feeding the respiration rate increases from the BMR (here considered synonymous with fasting or standard metabolic rate) to a maximal level and then decreases asymptotically towards BMR again when feeding stops.

In order to determine if and how food quality affects it, the respiration of *B. calyciflorus* was measured under four different treatments (see also Fig. 1;

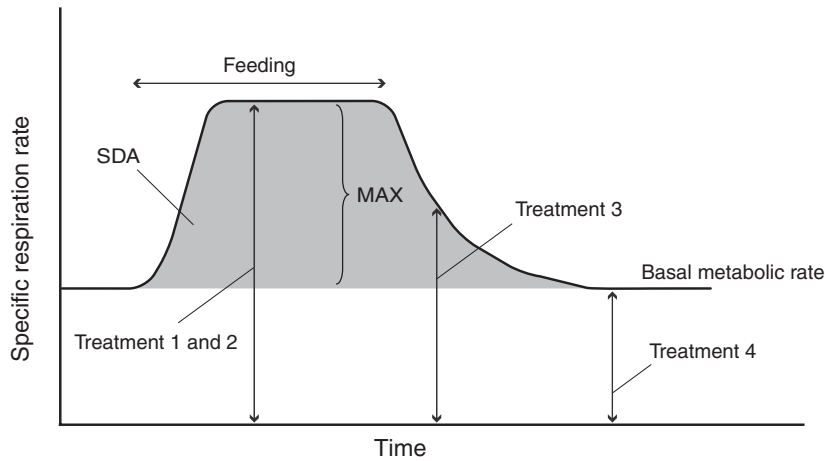


Fig. 1 Schematic representation of how feeding of an animal affects its respiration. The respiration rate increase from the basal metabolic rate (BMR) to a maximal respiration rate when the animal starts feeding. When feeding stops the respiration rate will approach BMR again asymptotically. The feeding event is shown in the figure. The specific dynamic action (SDA; the area shown in grey below the curve above BMR) comprises an obligatory part related to digestion and growth, but it may also include a facultative part related to dissipation of excess energy (Krieger, 1978; Simonson & Defronzo, 1990). Respiration measured in our experiments under different conditions is also shown: Respiration of feeding animals (treatment 1 and 2), respiration of well fed animals without food (treatment 3) and BMR (treatment 4). MAX is maximal weight specific respiration rate above fasting metabolic rate. See text for further details. Modified from Sigsgaard *et al.* (2003).

Table 1). In treatment 1 (Table 1), respiration measurements were conducted on feeding animals after short-term acclimation to nutrient limited food. The animals were grown from birth to the start of the experiment (48 h) on nutrient sufficient algae (F). Animals were then transferred to food suspensions (6 mg C L⁻¹) with either nutrient sufficient or nutrient deficient algae and left to acclimate for a short period (30 min) before being transferred with food suspension to the respiration chambers. In treatment 2 (Table 1), respiration measurements were conducted on feeding animals after a long period of acclimation to the different food qualities. In this treatment animals were grown from birth to the start of the experiment (48 h) on either nutrient sufficient or

nutrient deficient algae (F, -P or -N). Animals were then transferred to the food suspensions (6 mg C L⁻¹) with algae of the same quality as with which they were raised, and left to acclimate for 30 min before being transferred with food suspension to the respiration chambers. In treatment 3 (Table 1) animals were also long acclimated to the different food qualities as in treatment 2 but respiration was measured in pure medium without food. Again animals were grown from birth and onwards for 48 h to the start of the experiment on either nutrient sufficient or nutrient deficient algae. Before measurement, the animals were transferred to pure medium and allowed to empty their gut for 30 min, before being rinsed with nutrient-free COMBO medium and transferred to the

Table 1 Treatment definitions and experimental conditions during the growth and respiration measurement phases. Food algae were offered at a concentration of 6 mg C L⁻¹ during growth phases in all treatments and when respiration measurements were taken in treatments 1 and 2.

Treatment	Acclimation period	Algae offered during growth	Conditions during respiration measurement	Acclimation time to algae quality
1	Short	F (48 h)	F, -N or -P	0.5 h
2	Long	F, -N or -P (48 h)	F, -N or -P	48 h
3	Long	F, -N or -P (48 h)	No food	48 h
4	Long	F, -N or -P (48 h) followed by 18 h fasting period	No food	48 h

Letters in the table refer to algal culture conditions: nutrient sufficient (F), P-deficient (-P) algae or N-deficient algae (-N).

respiration chamber in pure nutrient-free COMBO medium. In treatment 4 (Table 1) measurements of basal metabolism were performed on animals long acclimated to the different food qualities. In this treatment respiration measurements were conducted after a period of fasting. During fasting the respiration of previously fed *B. calyciflorus* decreases asymptotically to a constant lower level reached after approximately 18 h (Kirk, Ellis & Taylor, 1999). This lower level can be taken as a measure of the basal respiration rate of the animal (Sigsgaard, Petersen & Iversen, 2003). As in treatment 2 and 3 animals were grown for 48 h (from birth) on each of the three food qualities. Then animals were rinsed with nutrient-free COMBO medium, transferred to new medium and allowed to fast for 18 h. After fasting the animals were rinsed again with nutrient free COMBO medium and transferred to the respiration chamber in pure nutrient-free COMBO medium.

Respiration experiments were made using a MRCh System (Unisense A/S, Århus, Denmark). Animals were placed in a 0.370 µL glass respiration chamber in a thermostatically controlled waterbath (19 ± 0.1 °C). The chambers contained either food suspension or pure nutrient-free COMBO medium depending on the experiment. Dissolved oxygen content was measured with a calibrated Clark-type oxygen microsensor (model OX-MRCh; Unisense A/S). Animals were allowed to acclimate for 10 min, after which oxygen consumption rate was taken as the linear slope of the O₂ concentration plotted against time for the next 20 min (Fig. 2). The short incubation times minimised the problem of decreasing oxygen- and food-concentrations. O₂ concentration in the chamber never declined below 85% saturation. The number of animals in the chambers was between 80 and 150. These are high densities but animal densities only have a minimal effect on respiration rate in rotifers (Kirk *et al.*, 1999). In order to correct for the background oxygen consumption, controls were set up in a separate chamber containing only food suspension or pure nutrient-free COMBO medium (Fig. 2). Background respiration was below 10% of animal respiration. After measuring respiration, the animals were removed from the respiration chamber, preserved, measured and body volumes calculated as described above. These were transformed to dry masses from predetermined relations between body volume and dry mass of individuals fed nutrient sufficient algae

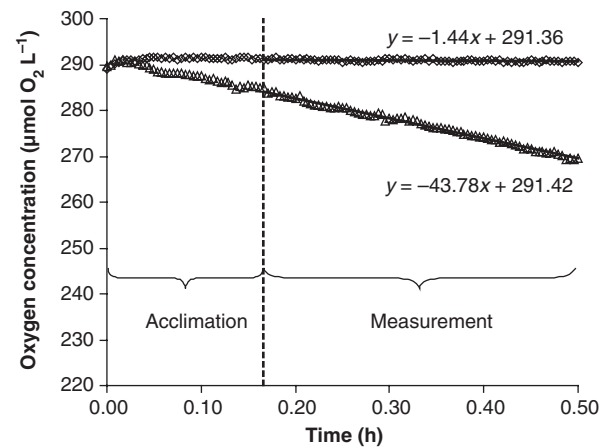


Fig. 2 Example of oxygen consumption curves from the respiration experiments with *Brachionus calyciflorus*. Upper curve (diamonds): background oxygen consumption in respiration chamber with food suspension. Lower curve (triangles): rotifer oxygen consumption in respiration chamber with food suspension. Incubations started with 10 min of acclimation. Then oxygen consumption rate was taken as the linear slope of the curves for the next 20 min (indicated by the lines in the figure).

[$W = (4.3 \times 10^{-7} \times b) - 0.296$ where W is dry mass ($\mu\text{g individual}^{-1}$) and b is calculated body volume (μm^3); $n = 26$, $R = 0.72$, $\text{MS}_{\text{residual}} = 0.00166$, $P < 0.0001$].

Respiration rates in *Brachionus* decrease at very low food concentrations. However, as long as concentrations are kept at saturating levels this effect of food concentration is minimal (Hirata & Yamasaki, 1987). Assuming maximum ingestion rates for *B. calyciflorus* (Rothhaupt, 1990a) of $18 \text{ ng C individual}^{-1} \text{ h}^{-1}$, food concentrations were kept above saturating levels during the incubation.

In the respiration experiments with *B. calyciflorus* only a few individuals were carrying eggs. In order to estimate 'somatic' respiration rates the egg respiration was subtracted from the total respiration assuming that specific respiration in eggs is 50% of that of the animals (Pilarska, 1977; Galkovskaya, 1995). To account for variation in respiration rates because of size, oxygen consumption rates can be adjusted using relations between respiration rates and size (Fu & Xie, 2004). We did this for *B. calyciflorus* using a relation between respiration and size for rotifers (Stemberger & Gilbert, 1987). Mass-specific respiration rates represent an animal size of $0.21 \mu\text{g dry mass}$.

Specific dynamic action of animals can be characterised indirectly by calculating the maximal specific

respiration rate above the BMR (MAX) (Sigsgaard *et al.*, 2003). In our experiments we calculated this for *B. calyciflorus* grown and fed on different food qualities as the difference between specific respiration rate of feeding animals (treatment 2) and specific respiration rate after fasting, i.e. BMR (treatment 4, see also Fig. 1).

Statistics

The effect of food quality treatment on growth rates and specific respiration rates was tested by the Kruskal–Wallis test. For pair-wise comparisons of means of growth rates and specific respiration rates, Tukey's HSD test was applied.

Effects of food quality treatment on elemental ratios and MAX values of *B. calyciflorus* were tested using bootstrap procedures. For each food quality treatment, elemental ratios (C : N, C : P or N : P) were first calculated for all combinations of samples for C, N and P. Similarly, MAX values were calculated for all combinations of measurements of respiration during treatment 2 and 4. Then, we used a between-class variance analysis to test for the overall effect of food quality treatment. Between-class variance was calculated either on three subsamples of the total possible number of combinations for each food quality treatment (1), or on three subsamples of the total possible number of combinations for all food quality treatments pooled (2). If there is a food quality treatment effect, (1) should be greater than (2). Food quality treatment effect was thus evaluated by calculating the proportion of (1) – (2) values greater than zero (one-tailed test). We also performed pairwise comparisons (pairwise tests) of the effects of food quality treatments on elemental ratios and MAX values of *B. calyciflorus*. Considering two food quality treatments (t1 and t2), this was assessed by calculating the difference between means of subsamples of the total possible number of combinations for t1 and t2. We checked that the difference between means was non-null using a two-tailed test. In addition, we used Bonferroni corrections (Sokal & Rohlf, 1997) to deal with the multiplicity of tests. For all tests, we used 10 000 randomisations and subsamples of four values for elemental ratios and five values for respiration (corresponding to the minimal numbers of measures realised in the different experiments). Statistical analyses were carried out with the JMP (SAS Institute

Inc., 2002, Cary, NC, U.S.A.) and Splus (Lucent Technologies, Inc., 2002, Seattle, WA, U.S.A.) analytical packages.

Results

Food quality

The degree of nutrient depletion of the algal cultures was visible from the C yields (data not shown). Algal cultures (–P and –N) grown on nutrient-depleted medium always had reduced yields as well as reduced specific N and P content, compared with their nutrient-sufficient (F) counterparts (Table 2). Both nutrient-deplete and nutrient-sufficient algae had a size of 4–5 µm equivalent spherical diameter (ESD).

Stoichiometry of *B. calyciflorus*

The elemental composition of *B. calyciflorus* was far less flexible than its food (Table 2). Food treatment had no overall effect on the body C : P ratios of *B. calyciflorus* (Table 2; between-class variance analysis, $P = 0.1637$). Pairwise comparisons showed that individuals receiving nitrogen limited algae had significantly higher C : P ratios than animals receiving nutrient sufficient food (Table 2; pairwise test, $P = 0.0000$). Although the change in C : P ratios of *B. calyciflorus* is not significant with the P-limited diet (Table 2; pairwise test, $P = 0.0990$), the ratio is still

Table 2 Elemental composition of the green alga *Selenastrum capricornutum* used as food and of the rotifer *Brachionus calyciflorus* (fed nutrient replete, phosphorus-deplete and nitrogen-deplete algae)

	C : P	C : N	N : P
<i>S. capricornutum</i>			
F	66 (11)	7.6 (0.4)	8.8 (1.8)
–P	484 (99)	12.9 (1.6)	38.1 (8.4)
–N	76 (5)	16.3 (1.2)	4.7 (0.4)
<i>B. calyciflorus</i>			
F	70 (4)	5.6 (0.3)	12.6 (0.8)
–P	105 (18)	7.0 (0.3)	15.1 (2.6)
–N	121 (18)	5.6 (0.2)	21.7 (3.3)

Values given are atomic ratios (mean ± SD). Algal stoichiometry were analysed from six separate samples. Rotifer stoichiometry were analysed from four samples containing 300 individuals per sample. Abbreviations as in Table 1. Mean values and standard deviations for *B. calyciflorus* were calculated using a bootstrap method (10 000 permutations).

about 50% higher compared with individuals fed the nutrient sufficient diet. This lack of significance could be due to large variation in the data. Concerning body C : N ratios of *B. calyciflorus*, food treatment tended to have an effect (Table 2; between-class variance analysis, $P = 0.0595$). The pairwise comparisons showed that individuals receiving P-limited algae had significantly higher C : N ratios than those receiving nutrient sufficient and N-limited food respectively (Table 2; pairwise test $P = 0.0162$ and $P = 0.0000$ respectively). As for the N : P ratios of *B. calyciflorus* food treatment had no overall effect (Table 2; between-class variance analysis, $P = 0.1722$), but animals receiving N-limited algae somewhat surprisingly had significantly higher N : P ratios than animals receiving nutrient sufficient food (Table 2; pairwise test, $P = 0.0000$).

Somatic growth

Nutrient-limited food caused reduced growth of *B. calyciflorus*. Somatic growth rates differed significantly after 24 h (Kruskal–Wallis test, $P < 0.0001$,

d.f. = 2, $n = 108$). Individuals fed nutrient sufficient algae had an average growth rate of $0.73 \pm 0.04 \text{ day}^{-1}$ (mean \pm 1 SE). This was significantly higher than those fed P-limited algae (Tukey's HSD test, $P < 0.05$), having a growth rate of $0.60 \pm 0.03 \text{ day}^{-1}$ (mean \pm 1 SE). This again was significantly higher than animals receiving N-limited algae (Tukey's HSD test, $P < 0.05$), they had the lowest rates of $0.43 \pm 0.03 \text{ day}^{-1}$ (mean \pm 1 SE).

Respiration

Specific respiration rates of feeding *B. calyciflorus* were not affected after a short period of acclimation to poor food algae (treatment 1) with high C : nutrient ratios (Fig. 3a; Kruskal–Wallis test, $P = 0.1057$, d.f. = 2, $n = 32$).

In treatment 2, 3, and 4 individuals were acclimated for a long period to the respective foods (i.e. they were fed F, –P or –N algae from birth, 48 h) and thus had a different size because of different growth rates. In treatment 2 the respiration of feeding animals was examined and specific respiration rates of *B. calyci-*

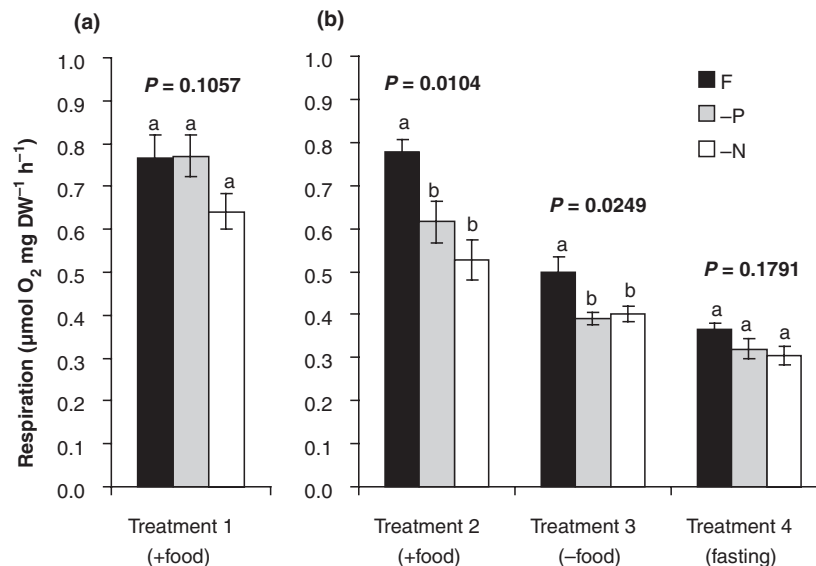


Fig. 3 Respiration rates (mean \pm 1 SE) of *Brachionus calyciflorus*. The letters in the figure refer to algal culture conditions: Nutrient sufficient (F, black), P-deficient (–P, grey) or N-deficient algae (–N, white). (a) In treatment 1 respiration measurements were carried out on feeding animals after a short period (30 min) of acclimation to the respective food algae. The animals in treatment 1 had been grown from birth on nutrient sufficient algae (48 h). (b) In treatment 2 respiration measurements were carried out on feeding animals after a long period (48 h, i.e. from birth) of acclimation to the respective food algae. Treatment 3: as in treatment 2 but respiration measurements were measured without food. Treatment 4: as in treatment 2 but respiration measurements were measured after 18 h of starvation. Results from the statistical analysis (P -values) are shown in the figure, significant values in bold. Different letters above columns indicate a significant difference between means (Tukey–Kramer HSD test, $P < 0.05$).

florus differed significantly between food qualities (Fig. 3b, Kruskal–Wallis test, $P = 0.0104$, d.f. = 2, $n = 15$): individuals fed and grown on nutrient-replete algae had higher respiration rates than individuals grown on P-limited and N-limited algae (Tukey's HSD test, $P < 0.05$). In treatment 3, animal respiration was measured in pure medium without food, i.e. prefed but fasting animals that would rely on energy storage for respiration. Under these conditions respiration rates were significantly affected by food quality (Fig. 3b; Kruskal–Wallis test, $P = 0.0249$, d.f. = 2, $n = 25$): individuals fed and grown on nutrient-replete algae had higher respiration rates than individuals grown on nutrient-limited algae (Tukey's HSD test, $P < 0.05$). Treatment 4 examined the effect of food quality on basal metabolism (i.e. respiration of fasting rotifers). Under these conditions respiration rates of *B. calyciflorus* did not differ for the different food qualities (Fig. 3b; Kruskal–Wallis test, $P = 0.1791$, d.f. = 2, $n = 30$).

The results from the respiration experiments clearly demonstrate the effect of feeding on the respiration rate. The respiration rates of feeding individuals were always considerably higher than for non-feeding individuals under all food qualities (Fig. 3b). Food quality had no overall significant effect on the maximum respiration above basal metabolic level (MAX) for *B. calyciflorus* (between-class variance analysis, $P = 0.1036$, $n = 10\ 000$). However, the pairwise comparisons showed that MAX was significantly higher for animals fed and grown on nutrient sufficient algae (F) as compared with animals receiving nitrogen limited (–N) food (pairwise test, $P = 0.0048$).

The group of rotifers with the highest growth rate (nutrient sufficient, F) also had significantly higher respiration rates (both when considering respiration in feeding animals and MAX as an indirect measure of SDA) than the group of animals with lowest growth rates (nitrogen limited, –N).

Modelling

We used a stoichiometric model (Anderson *et al.*, 2005) to aid in the interpretation of the experimental results for *B. calyciflorus* and in particular to examine whether or not excess C in the diet was respired or disposed of by some other mechanism by the animal. The model includes C, N and P as currencies, assimilation, maintenance and production as sequential steps for

substrate use, and subdivides maintenance into separate terms for protein turnover and the associated energetic costs and other basal costs such as osmoregulation. Anderson *et al.* (2005) used their model to study the fate of excess nutrients and carbon in *Daphnia*, the model indicating in that case that regulation of excess C seems to occur primarily postassimilatory by respiration and excretion of DOC.

Comparison of data with the model requires that respiration data be converted into biomass-specific units. This conversion was done using the measured C content of rotifers fed the three different food algae. Rates of oxygen consumption were converted to carbon units by assuming respiratory quotients (RQ) of 1.0 for feeding and non-feeding animals (treatments 2 and 3) and 0.7 for fasting animals (treatment 4) (Richman, 1958).

The model was originally parameterised by Anderson *et al.* (2005) for *Daphnia*. When applying it to *B. calyciflorus*, several adjustments were made: (i) assimilation efficiency (AE) of P, parameter β_P , was increased from its original setting of 0.8 to a new value of 0.95, which was necessary to reconcile the budget of the –P food treatment. Assimilation efficiencies for P as high as 0.95 are not unusual in zooplankton (DeMott *et al.*, 1998); (ii) AE for C was decreased by 10% so that predicted growth in the F food treatment better matched the observed rate of $0.73\ \text{day}^{-1}$. Carbon is divided between protein and non-protein fractions in the model, with new values for the associated assimilation efficiencies, β_N and β_M , being 0.62 and 0.56, respectively. The result is an average C AE of 0.59 for a food item with C : N of 7.6 (algae in the F food treatment; assumes protein C : N of 3.7), nearly equal to an efficiency of 0.6 estimated for *B. calyciflorus* by Verschoor, Boonstra & Meijer (2005) for algae of comparable quality; (iii) Respiration measured under starvation conditions (treatment 3) was 0.22, 0.17 and $0.17\ \text{day}^{-1}$ for the F, –P and –N food treatments respectively, averaging $0.18\ \text{day}^{-1}$. Parameter ζ_B , which represents 'other basal costs', was increased from its original setting of $0.052\ \text{day}^{-1}$ in Anderson *et al.* (2005) to a new value of $0.09\ \text{day}^{-1}$, thereby giving a total basal respiration rate (but which excludes any assimilation costs, which are absent in starving animals) matching that of the experimental treatments, i.e. $0.18\ \text{day}^{-1}$. The remaining parameters were set to their default settings as in Anderson *et al.* (2005), except that, because *Brachionus* does not moult,

Parameter	Definition	Value	Unit of measure
$\theta_{CN(H)}$	C : N ratios of consumer	See Table 2	mol C (mol N) ⁻¹
$\theta_{CP(H)}$	C : P ratios of consumer		mol C (mol P) ⁻¹
$\theta_{CN(F)}$	C : N ratios of food		mol C (mol N) ⁻¹
$\theta_{CP(F)}$	C : P ratios of food		mol C (mol P) ⁻¹
β_N	Assimilation efficiency: protein	0.62	Dimensionless
β_M	Assimilation efficiency: non-protein C	0.56	Dimensionless
β_P	Assimilation efficiency: P	0.95	Dimensionless
ζ_B	Other basal costs	0.09	day ⁻¹
θ_V	C : N of protein	3.7	mol C (mol N) ⁻¹
τ_{CN}	Biomass turnover: C, N	0.094	day ⁻¹
τ_P	Biomass turnover: P	0.094	day ⁻¹
η_{CN}	Fraction CN turnover reclaimed	0.38	Dimensionless
η_P	Fraction P turnover reclaimed	0.38	Dimensionless
ζ_I	Cost of assimilation	0.06	mol C (mol C) ⁻¹
φ_C	Cost of biosynthesis	0.75	mol C (mol C) ⁻¹

Table 3 Parameter values used in the model (see Anderson *et al.*, 2005)

the moult parameters were rendered redundant. A complete list of parameter values is provided in Table 3.

We use the model to study the factors underlying the total respiration of *B. calyciflorus* as measured in the animals that were fed a saturating ration and that were long acclimated to different food qualities (treatment 2). Algae were supplied at a concentration of 6 mg C L⁻¹. The first step when using the model is to specify intake, which was estimated from this food concentration as follows. Measured growth and respiration for the F treatment were 0.73 and 0.66 day⁻¹ respectively. Using a C AE of 0.59 (see above), intake can then be estimated as $(0.73 + 0.66)/0.59 = 2.36$ day⁻¹. By means of comparison, a functional response curve for *Brachionus rubens* ingesting *Scenedesmus* subjected to three treatments – nutrient sufficient, P-limited or N-limited – is provided by Rothhaupt (1995). Ingestion rate, which was insensitive to algal nutrient status, saturated at approximately 18 ng C individual⁻¹ h⁻¹, equivalent to 2.4 day⁻¹ (body weight 0.18 µg C), remarkably close to our estimate above. *Brachionus calyciflorus* has a similar saturated ingestion rate of 0.18 ng C individual⁻¹ h⁻¹ (Rothhaupt, 1990a) and ranged in length between 220 and 285 µm (Rothhaupt, 1990b). Calculated ingestion rates, using a conversion to biovolume according to Ruttner-Kolisko (1977), conversion to dry mass as in this study, and assuming C as 33.4% of dry mass (this study, *B. calyciflorus* fed F algae), are 1.3–4.2 day⁻¹, a range that encompasses the 2.36 day⁻¹ used here.

Model results are shown in Fig. 4. Animals in the F food treatment were predicted to be limited by

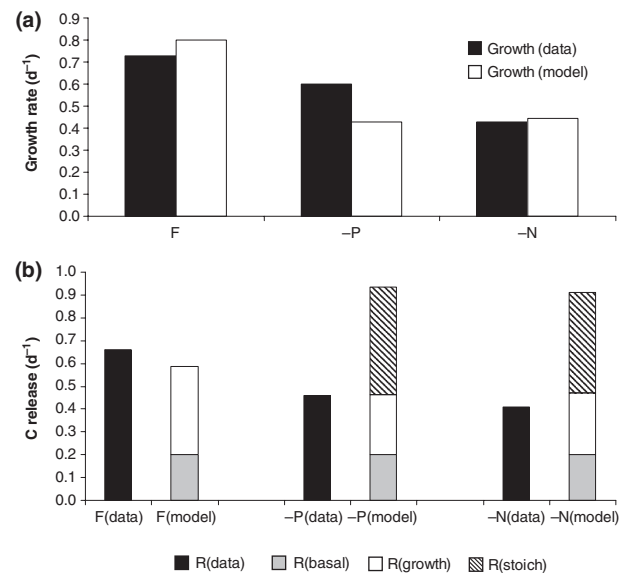


Fig. 4 Effects of food quality on growth and C release in *Brachionus calyciflorus* (a) Growth rates as predicted by the model of Anderson *et al.* (2005) (white bars), compared with data (black bars) from our experiments. (b) Modelled release of C in *Brachionus calyciflorus*; consisting of a standard part [respiration related to maintenance and production, R(basal) and R(growth)] and stoichiometric release of excess C [R(stoich)]. The model is compared with data from our experiments on respiration of feeding animals long term acclimated to different food qualities (treatment 2) at a saturating concentration of 6 mg C L⁻¹.

carbon, the modelled growth rate of 0.82 day⁻¹ being slightly higher than the experimentally observed rate of 0.73 day⁻¹ (Fig. 4a). As might be expected, predicted growth rates were significantly decreased in the -P and -N food treatments because of limitation of *B. calyciflorus* production by phosphorus and nitrogen

respectively. Calculated threshold elemental ratios (TERs) for C : P and C : N in the -P and -N treatments were 280.6 and 9.65 respectively, substantially lower than the food C : P and C : N of 484 and 16.3, thereby indicating strong limitation by nutrient elements rather than carbon in each case. Predicted growth rates of 0.80 and 0.44 day⁻¹ for the F and -N food treatments show a close match with the experimental rates. However, the predicted growth rate under P limitation of 0.43 day⁻¹ is somewhat lower than the experimental value of 0.60 day⁻¹. This discrepancy suggests that P was in fact used for growth by the animals more effectively than was predicted by the model. It is difficult to increase P use efficiency in the model by increasing assimilation given the already high AE of 0.95 and low usage of P in basal metabolism. Postabsorptive mechanisms that enable a more effective use of P under P-limitation could be hypothesised to explain this discrepancy. One possibility is a higher protein synthesis rate per ribosome under P-limiting conditions, acting to increase growth rate without increasing requirements for P-rich rRNA. Experiments in which *B. calyciflorus* were fed P-limited algae showed a higher production per unit RNA as compared with animals fed nutrient sufficient and N-limited algae (T.C. Jensen and M. Kyle, unpubl. data), indicating that such an increase in protein synthesis rate per ribosome actually does take place. Further evidence for such a mechanism comes from the study of Acharya, Kyle & Elser (2004) showing the same type of response in *Daphnia galeata* under P-limitation. Such a 'P-saving' mechanism is not incorporated in the model.

Limitation of growth in the F food treatment was predicted by the model to be by carbon, in which case one would expect there to be no stoichiometric excess of this element and modelled and observed respiration to show good agreement. The predicted respiration rate for the F food treatment of 0.58 day⁻¹ is indeed reasonable close to the observed rate of 0.66 day⁻¹ (Fig. 4b). The modelled rate is shown subdivided into its separate components, namely that associated with basal metabolism (31% of the total) and that associated with growth and assimilation over and above basal metabolism, i.e. SDA (69% of the total). One would expect the respiration associated with assimilation and growth to be lower in the -P and -N food treatments compared with the F food treatment because of the lower growth rates involved.

Total respiration rates [the sum of the R(basal) and R(growth) terms in Fig. 4b] are indeed lower in the modelled -P and -N food treatments. Agreement is good with data, but only if release of C in stoichiometric excess is not included as part of respiration. If this C was released as respired CO₂ by the animals, then the total respiration predicted would unacceptably exceed the observed respiration rates. Therefore respiration does not appear to be used by *B. calyciflorus* as a postabsorptive regulative mechanism for disposing of excess C in the diet, other mechanisms such as excretion of DOC being used instead to achieve this disposal.

Discussion

Somatic growth of *B. calyciflorus* was clearly reduced when consuming nutrient depleted food. Decreased intake is one potential cause. However, as noted previously, algal nutrient content did not affect ingestion rates in the closely related species *B. rubens* (Rothhaupt, 1995). Furthermore, nutrient-deficient and nutrient-sufficient algae in the present study were of similar size and therefore any influence of size on ingestion (Rothhaupt, 1990b) can be excluded. Nutrient-limited algae of high C : nutrient ratios can have thickened cell walls reducing digestion (Van Donk & Hessen, 1993; Van Donk *et al.*, 1997). However, food algae in our experiments were not so heavily nutrient limited as in these previous studies. The models requirement for very high P AE is also contrary to digestion resistance, which would reduce AE for all elements. Further, rotifers possess a specialised stomach, the mastax, with which they are able to crush ingested food which should thereby minimise problems associated with reduced digestibility. Our results indicate that the observed decreased growth of *B. calyciflorus* was caused by limitation by N and P when consuming nutrient-deplete algae, a result supported by the modelling analysis.

Biomass-specific respiration rates of non-feeding *B. calyciflorus* (receiving F algae) in the present study were similar to previous results reported in the literature (Table 4). The rates from our experiment with animals starved for 18 h were twice as high as those found by Kirk *et al.* (1999). We have no obvious explanation for this difference (Table 4), although the acclimation period in the respiration chamber before measurement was much shorter in our experiments. We found

Table 4 Specific respiration rates of *Brachionus calyciflorus* and *Brachionus plicatilis* at 20 °C from different studies under different conditions (feeding, previously fed non-feeding, starved 18 h). To account for variations in specific respiration rates (represent animal size of 0.21 µg dry mass) because of size, rates from previous studies were adjusted using relations between respiration rates and size (Stemberger & Gilbert, 1987).

Species	Specific respiration rates at 20 °C (µmol O ₂ mg DW ⁻¹ h ⁻¹)			Experimental temperature (°C)	References
	Feeding	No food	Basal/fasting (18 h starvation)		
<i>B. calyciflorus</i>	–	0.67	–	20	Doohan (1973)
	–	0.47	–	20	Galkovskaya (1995)
	–	0.52	0.20	20	Kirk <i>et al.</i> (1999)
	0.86	0.55	0.40	19	Present study*
<i>B. plicatilis</i>	0.67	0.55	0.28	20	Hirata & Yamasaki (1987) [†]

*Only results from the F food treatment included, results were temperature corrected from 19 to 20 °C assuming a Q_{10} for *B. calyciflorus* of 2.66 (Galkovskaya, 1995).

[†]Specific respiration rates not allometrically scaled, because animal size was not reported.

respiration rates of feeding *B. calyciflorus* to be considerably higher than those of individuals starved for 18 h. For the F algae this increase was approximately twofold (Table 4), comparable with the results of Hirata & Yamasaki (1987) for *Brachionus plicatilis*.

The results from treatment 1 (short acclimation to respective food quality) in the respiration experiments suggest that *B. calyciflorus* does not transiently increase respiration to dispose of excess C, as suggested for *D. magna* (Darchambeau *et al.*, 2003). However, respiration could still play a role when animals are long-term acclimated to a diet with excess C; i.e. feeding or fasting metabolic rate could increase after long-term exposure to a high C : P or C : N diet. The results from treatments 2, 3 and 4 (long acclimation to respective food quality) show that this is not the case. Instead, feeding metabolic rate or MAX (i.e. SDA) decrease with growth rates of animals fed nutrient-limited diets. SDA consists of an obligatory part related to digestion and protein synthesis (growth). It may also, however, comprise a facultative component related to the dissipation of excess C or energy (Krieger, 1978; Simonson & DeFronzo, 1990). Respiration should increase with growth and intake where SDA is primarily related to growth and digestion (increased metabolic activity). If, however, respiration is also involved in the stoichiometric regulation of homeostasis then it could remain high or even be inversely related to growth when animals feed on excess C diets. Thus our experimental results, in which respiration decreased sharply when animals were fed on nutrient deficient diets, indicate that SDA

in *B. calyciflorus* comprises components for digestion and growth only, with no stoichiometric component. The modelling work further emphasised this finding, with predicted and observed respiration rates showing good agreement only if excess dietary C was not respired as CO₂. Even altering the total predicted respiration by ±10% makes little difference to the main conclusion, namely that there is a large stoichiometric excess of C under nutrient limitation that is not respired. Similarly, possible errors in the respiration measurements, because of the applied RQ in converting oxygen consumption rates to C units, will not alter this conclusion.

The model is not dynamic in nature and therefore does not predict acclimation of animals to changing circumstances, such as occurred in treatment 3. In this instance we measured the metabolic rate of well-fed animals subjected to long-term acclimation to different food qualities and then deprived of food for a short period. Although growth in mass cease immediately when the food is removed, some growth related processes such as protein synthesis would probably continue for a while during a period of fasting. As respiration associated with growth is a significant fraction of the total, the metabolic rates from this treatment are probably intermediates between the feeding metabolic rate and the BMR. Thus the starvation treatment (treatment 4) appears to be the best indicator of basal metabolism, although down regulation (metabolic depression) under starvation is possible as observed for *Daphnia* (Glazier & Calow, 1992).

The respiration measurements together with the model predictions strongly suggest that excess C in *B. calyciflorus* is not respired. In the model, food intake and assimilation efficiencies were constant, and the release of excess assimilated C was postabsorptive. An alternative to respiration as a disposal mechanism is excretion of surplus C as DOC. Two previous investigations both demonstrated high release of DOC in *B. plicatilis* (Olsen *et al.*, 2002; Vadstein *et al.*, 2003). The origin of this grazer-derived DOC was not clear, but may have been excretion. The study of Darchambeau *et al.* (2003) suggested that *Daphnia* can release excess C by excretion of DOC as well as CO₂. Yet another alternative regulatory mechanism to achieve homeostatic balance that could be used by *B. calyciflorus* is reduced C AE for nutrient deficient food. This could be incorporated in the model, and thus excess C would be released as faecal material (POC and DOC). The study of DeMott *et al.* (1998) showed that *Daphnia* can use such a decrease in AE of C coupled to a high P AE to cope with P-deficient high C diets. Thus, further studies are clearly needed to reveal how *B. calyciflorus* cope with high dietary C.

In conclusion, P- and N-limited food had a strong effect on growth of *B. calyciflorus*, indicating the need for stoichiometric regulation of excess ingested C. Furthermore, our study shows that this rotifer does not use respiration as a physiological mechanism in coping with high dietary C, in which case excess C must be released in other ways, either as faecal material or excreted as DOC. Different zooplankton taxa may regulate the release of ingested excess C in different ways (defecation, excretion or respiration). This has potentially important implications for the sequestration of C in ecosystems. If regulation occurs by adjusting assimilation within the gut one would expect an increased vertical flux of POC of faecal origin, to the sediments of lakes. Excretion of excess C as DOC would lead to recycling of organic C, a potential source for bacterial production. The composition of the zooplankton community and the physiology of dominating species could therefore be important factors in determining the C-flux in nutrient limited systems.

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References

- Acharya K., Kyle M. & Elser J.J. (2004) Biological stoichiometry of *Daphnia* growth: An ecophysiological test of the growth rate hypothesis. *Limnology and Oceanography*, **49**, 656–665.
- Andersen T. & Hessen D.O. (1991) Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnology and Oceanography*, **36**, 807–814.
- Anderson T.R. & Hessen D.O. (2005) Threshold elemental ratios for C versus P limitation in *Daphnia*. *Freshwater Biology*, **50**, 2063–2075.
- Anderson T.R., Hessen D.O., Elser J.J. & Urabe J. (2005) Metabolic stoichiometry and the fate of excess carbon and nutrients in consumers. *American Naturalist*, **165**, 1–15.
- Curcio C., Lopes A.M., Ribeiro M.O., Francoso O.A., Carvalho S.D., Lima F.B., Bicudo J.E. & Bianco A.C. (1999) Development of compensatory thermogenesis in response to overfeeding in hypothyroid rats. *Endocrinology*, **140**, 3438–3443.
- Darchambeau F., Færøvig P.J. & Hessen D.O. (2003) How *Daphnia* copes with excess carbon in its food. *Oecologia*, **136**, 336–346.
- DeMott W.R. (1995) Food selection by calanoid copepods in response to between lake variation in food abundance. *Freshwater Biology*, **33**, 171–180.
- DeMott W.R., Gulati R.D. & Siewertsen K. (1998) Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. *Limnology and Oceanography*, **43**, 1147–1161.
- Doohan M. (1973) Energy budget for adult *Brachionus plicatilis* Muller (Rotatoria). *Oecologia*, **13**, 351–362.
- Elser J.J., Fagan W.F., Denno R.F. *et al.* (2000) Nutritional constraints in terrestrial and freshwater food webs. *Nature*, **408**, 578–580.
- Even P.C., Bertin E., Gangnerau M.N., Roseau S., Tomé D. & Portha B. (2003) Energy restriction with protein restriction increases basal metabolism and meal-induced thermogenesis in rats. *American Journal of Physiology. Regulatory, integrative and comparative physiology*, **284**, 751–759.
- Fu S.J. & Xie X.J. (2004) Nutritional homeostasis in carnivorous southern catfish (*Silurus meridionalis*): is there a mechanism for increased energy expenditure during carbohydrate overfeeding? *Comparative Bio-*

- chemistry and Physiology A. *Molecular & Integrative Physiology*, **139**, 359–363.
- Galkovskaya G.A. (1995) Oxygen consumption rate in rotifers. *Hydrobiologia*, **313**, 147–156.
- Glazier D.S. & Calow P. (1992) Energy allocation rules in *Daphnia Magna*. Clonal and age-differences in the effects of food limitation. *Oecologia*, **90**, 540–549.
- Hessen D.O. (1992) Nutrient element limitation of zooplankton production. *American Naturalist*, **140**, 799–814.
- Hessen D.O., Færøvig P.J. & Andersen T. (2002) Light, nutrients, and P:C ratios in algae: grazer performance related to food quality and quantity. *Ecology*, **83**, 1886–1898.
- Hessen D.O., Ågren G.I., Anderson T.R., Elser J.J. & De Ruiter P.C. (2004) Carbon, sequestration in ecosystems: The role of stoichiometry. *Ecology*, **85**, 1179–1192.
- Hirata H. & Yamasaki S. (1987) Effect of feeding on the respiration rate of the rotifer *Brachionus plicatilis*. *Hydrobiologia*, **147**, 283–288.
- Jensen T.C. & Verschoor A.M. (2004) Effects of food quality on life history of the rotifer *Brachionus calyciflorus* Pallas. *Freshwater Biology*, **49**, 1138–1151.
- Jeppesen E., Søndergaard M., Sortkjaer O., Mortensen E. & Kristensen P. (1990) Interactions between phytoplankton, zooplankton and fish in a shallow, hypertrophic lake: a study of phytoplankton collapses in Lake Søbygård, Denmark. *Hydrobiologia*, **191**, 149–164.
- Kilham S.S., Kreeger D.A., Lynn S.G., Goulden C.E. & Herrera L. (1998) COMBO: a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia*, **377**, 147–159.
- Kirk K.L., Ellis J. & Taylor J. (1999) Physiological responses to variable environments: storage and respiration in starving rotifers. *Freshwater Biology*, **42**, 637–644.
- Krieger I. (1978) Relation of specific dynamic action of food (SDA) to growth in rats. *American Journal of Clinical Nutrition*, **31**, 764–768.
- Lampert W. (1984) The measurement of respiration. In: *A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters* (Eds J.A. Downing & F.H. Rigler), pp. 413–468. Blackwell Scientific Publications, Oxford.
- Olsen L.M., Reinertsen H. & Vadstein O. (2002) Can phosphorus limitation inhibit dissolved organic carbon consumption in aquatic microbial food webs? A study of three food web structures in microcosms. *Microbial Ecology*, **43**, 353–366.
- Pilarska J. (1977) Eco-physiological studies on *Brachionus rubens* Ehrbg (Rotatoria). II. Production and respiration. *Polskie Archiwum Hydrobiologii*, **24**, 329–341.
- Plath K. & Boersma M. (2001) Mineral limitation of zooplankton: Stoichiometric constraints and optimal foraging. *Ecology*, **82**, 1260–1269.
- Richman S. (1958) The transformation of energy by *Daphnia pulex*. *Ecological Monographs*, **28**, 274–291.
- Rothhaupt K.O. (1990a) Changes of the functional responses of the rotifers *Brachionus rubens* and *Brachionus calyciflorus* with particle sizes. *Limnology and Oceanography*, **35**, 24–32.
- Rothhaupt K.O. (1990b) Differences in particle size-dependent feeding efficiencies of closely related rotifer species. *Limnology and Oceanography*, **35**, 16–23.
- Rothhaupt K.O. (1995) Algal nutrient limitation affects rotifer growth rate but not ingestion rate. *Limnology and Oceanography*, **40**, 1201–1208.
- Ruttner-Kolisko K.O. (1977) Suggestions for biomass calculations of planktonic rotifers. *Ergebnisse der Limnologie*, **8**, 71–76.
- Sigsgaard S.J., Petersen J.K. & Iversen J.J.L. (2003) Relationship between specific dynamic action and food quality in the solitary ascidian *Ciona intestinalis*. *Marine Biology*, **143**, 1143–1149.
- Simonson D.C. & Defronzo R.A. (1990) Indirect calorimetry: Methodological and interpretative problems. *American Journal of Physiology*, **258**, 399–412.
- Sokal R.R. & Rohlf F.J. (1997) *Biometry*. W. H. Freeman and Company, New York.
- Stemberger R.S. & Gilbert J.J. (1987) Rotifer threshold food concentrations and the size-efficiency hypothesis. *Ecology*, **68**, 181–187.
- Sterner R.W. (1993) *Daphnia* growth on varying quality of *Scenedesmus*: Mineral limitation of zooplankton. *Ecology*, **74**, 2351–2360.
- Sterner R.W. (1997) Modelling interactions of food quality and quantity in homeostatic consumers. *Freshwater Biology*, **38**, 473–481.
- Sterner R.W. & Elser J.J. (2002) *Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton.
- Sterner R.W. & Hessen D.O. (1994) Algal nutrient limitation and the nutrition of aquatic herbivores. *Annual Review of Ecology and Systematics*, **25**, 1–29.
- Sterner R.W., Clasen J., Lampert W. & Weisse T. (1998) Carbon : phosphorus stoichiometry and food chain production. *Ecology Letters*, **1**, 146–150.
- Vadstein O., Olsen L.M., Busch A., Andersen T. & Reinertsen H.R. (2003) Is phosphorus limitation of planktonic heterotrophic bacteria and accumulation of degradable DOC a normal phenomenon in phosphorus-limited systems? A microcosm study. *FEMS Microbiology Ecology*, **46**, 307–316.

- Van Donk E. & Hessen D.O. (1993) Grazing resistance in nutrient-stressed phytoplankton. *Oecologia*, **93**, 508–511.
- Van Donk E., Lürling M., Hessen D.O. & Lokhorst G.M. (1997) Altered cell wall morphology in nutrient-deficient phytoplankton and its impact on grazers. *Limnology and Oceanography*, **42**, 357–364.
- Verschoor A.M., Boonstra H. & Meijer T. (2005) Application of stable isotope tracers to studies of zooplankton feeding, using the rotifer *Brachionus calyciflorus* as an example. *Hydrobiologia*, **546**, 535–549.
- Walz N. (1995) Rotifer populations in plankton communities: Energetics and life history strategies. *Experientia*, **51**, 437–453.

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