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## LETTER

# Rapid adaptation of herbivore consumers to nutrient limitation: eco-evolutionary feedbacks to population demography and resource control

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### Abstract

Humans alter biogeochemical cycles of essential elements such as phosphorus (P). Prediction of ecosystem consequences of altered elemental cycles requires integration of ecology, evolutionary biology and the framework of ecological stoichiometry. We studied micro-evolutionary responses of a herbivorous rotifer to P-limited food and the potential consequences for its population demography and for ecosystem properties. We subjected field-derived, replicate rotifer populations to P-deficient and P-replete algal food, and studied adaptation in common garden transplant experiments after 103 and 209 days of selection. When fed P-limited food, populations with a P-limitation selection history suffered 37% lower mortality, reached twice the steady state biomass, and reduced algae by 40% compared to populations with a P-replete selection history. Adaptation involved no change in rotifer elemental composition but reduced investment in sex. This study demonstrates potentially strong eco-evolutionary feedbacks from shifting elemental balances to ecosystem properties, including grazing pressure and the ratio of grazer:producer biomass.

### Keywords

*Brachionus calyciflorus*, chemostat, contemporary evolution, continuous culture, experimental evolution, micro-evolution, microsatellites, phosphorus, selection, zooplankton.

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## INTRODUCTION

Global change involves drastic modifications to biogeochemical cycles of elements that are essential to life, such as carbon (C), nitrogen (N) and phosphorus (P). In recent decades, human activities have strongly altered the amounts and ratios of such key elements in natural systems through nutrient enrichment and more recently limitation (Stockner *et al.* 2000; Elser *et al.* 2009). These changes have far-reaching consequences for biota, because many organisms require essential elements in specific ratios (Sterner & Elser 2002; Hessen *et al.* 2013). Heterotrophs are more confined in their elemental composition than autotrophs. Mismatches between the elemental stoichiometry of consumers and their food therefore often result in reduced consumer growth rates, reproductive output and survival (Bukovinszky *et al.* 2012). Such mismatches may not only affect the abundance and persistence of single populations, but also the diversity, composition and functioning of entire communities (Elser *et al.* 1998; Hall 2009; Hillebrand *et al.* 2009).

Until recently, evolutionary change in populations was assumed to take place at time scales much longer than that of ecological dynamics (Schoener 2011). The potential of populations to show rapid evolutionary responses to changing selection conditions has only recently become appreciated (Hendry & Kinnison 1999; Cousyn *et al.* 2001). The notion that evolu-

tionary change may be realised at similar time scales as ecological interactions implies a potential for eco-evolutionary feedbacks (Hairston *et al.* 2005; Fussmann *et al.* 2007; Schoener 2011), where ecological processes are altered by evolutionary change and vice versa (Decaestecker *et al.* 2007; Becks *et al.* 2012; Hiltunen & Becks 2014). We are unlikely to fully understand and predict the responses of biota to anthropogenic environmental changes if we continue to ignore rapid evolutionary adaptations and their potential eco-evolutionary feedbacks (Matthews *et al.* 2011; Urban *et al.* 2012). Given the global alteration of biogeochemical cycles of essential elements, there is a clear need for an integration of ecology, evolutionary biology and the framework of ecological stoichiometry (Elser *et al.* 2000a; Kay *et al.* 2005; Jeyasingh *et al.* 2014). Micro-evolutionary responses of consumers to changes in the availability of essential elements are poorly documented, especially for metazoans (Frisch *et al.* 2014). Even less is known about ecological feedbacks of such evolutionary responses to fundamental ecosystem functions (Elser 2006; Matthews *et al.* 2011).

Across metazoan taxa there is an impressive diversity of strategies to maintain homeostasis when confronted with element limitation and imbalance (Hessen & Anderson 2008). One may therefore also expect a high diversity of micro-evolutionary adaptations to such conditions. The prediction of adaptive trajectories for populations under specific stoichiom-

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etric selection regimes is further complicated by its potential dependence on genetic architecture. Nevertheless, it is worthwhile to search for generalities (Jeyasingh *et al.* 2014). If elemental limitation reduces consumer fitness, one may expect natural selection to act on the elemental composition of the organism, as has been suggested by studies that related inter-specific stoichiometric variation in consumers to nutrient availability in their habitat (Kay *et al.* 2005). However, adaptation can also be achieved in other ways as selection may benefit genotypes with higher uptake, assimilation or retention efficiencies of limiting elements or reduced costs associated with the disposal of excess elements. Adaptations may also involve important life history changes. In particular, we expect that genetically determined resource allocation patterns, such as investment in sex, can affect the relative fitness of genotypes under limiting conditions. Evolution of such traits may have important consequences for the demographic features of populations (Kokko & López-Sepulcre 2007; Cameron *et al.* 2013).

Another key issue concerns the consequences of rapid evolutionary consumer adaptations for consumer-driven nutrient cycling, trophic dynamics, and ecosystem functioning (Elser *et al.* 2000a; Mizuno & Kawata 2009; Matthews *et al.* 2011). Insight into the ecosystem consequences of evolutionary responses to nutrient imbalance requires knowledge of the associated modifications in the consumer elemental phenotype (Jeyasingh *et al.* 2014). Ecological stoichiometry predicts that herbivore elemental composition determines the relative recycling rates of nutrients (Sterner 1990; Elser & Urabe 1999). A fixed herbivore stoichiometry results in enhanced excretion of excess elements when adaptations involve higher relative assimilation and/or retention of the limiting element. In such a scenario herbivore adaptation would further distort elemental ratios in its food (Hall 2009) and reinforce existing stoichiometric selection pressures on the herbivore, thus closing a positive eco-evolutionary feedback loop. For example, an organism will increase the N:P ratio in excretion products if adaptation to P-limitation involves increased P-assimilation or P-retention. This further distorts N:P ratios in the environment and, hence, the algal food, reinforcing the initial selection pressure. Conversely, adaptations that involve a reduction in the limiting element in the herbivore are likely to weaken such positive feedback but may instead have consequences for the interactions of herbivores with their predators. Specifically, adaptive adjustment of herbivore elemental composition towards its nutrient-limited food may alter the degree of elemental mismatch between predators and their herbivore prey, and as such affect the strength of predator top-down control, in analogy to a stoichiometry-driven bottom-up cascade (Boersma *et al.* 2008).

With this study, we aimed to investigate the potential of natural populations of a small metazoan cyclical parthenogenetic zooplankter, the rotifer *Brachionus calyciflorus*, to rapidly adapt to P-limited food. Using second stage chemostats, we performed a replicated selection experiment where we allowed multi-clonal rotifer populations to adapt to different types of stoichiometric food quality (P-limited vs. P-replete phytoplankton) under continuous culture conditions. This selection experiment was combined with two common garden

transplant experiments in semi-continuous cultures, where populations were exposed to the different food quality treatments after being under selection for 103 and 209 days. A main objective of these experiments was to identify and quantify population adaptation to the food quality treatments by comparing population-level traits associated with fitness. We also wanted to test whether adaptation involves modifications in body elemental composition. Furthermore, we wanted to investigate eco-evolutionary feedbacks by considering the consequences of adaptation for consumer demographic features and grazing pressure on producers and explore the potential for a positive eco-evolutionary feedback loop through altered nutrient cycling. We evaluated the generality of the results by including populations with different genetic backgrounds in our design.

## MATERIAL AND METHODS

We made use of the unique characteristic of rotifers to be the only known metazoans capable of reaching steady state population growth in (semi-)continuous cultures (Walz 1993). Using a second stage chemostat set-up, we subjected populations to different selection regimes, that is, low P vs. high P food. To compare features of evolved populations at steady state under each of the selection regimes, we performed two common garden transplant experiments using semi-continuous cultures. Semi-continuous cultures represent growth conditions very similar to those in second-stage chemostats (Walz 1993), while allowing for sufficient replication. Common garden experiments in semi-continuous cultures have the great advantage that they provide a test for local adaptation, while simultaneously allowing the study of the population level consequences of that adaptation and its potential impact on some important ecosystem functions, such as grazing pressure exerted on food resources.

### Selection experiment

We continuously supplied second stage rotifer chemostats harbouring *B. calyciflorus* populations with food produced in phytoplankton chemostats containing *Chlamydomonas reinhardtii* (See Appendix S1 in fig. S1). Four replicate rotifer chemostats (volume: 1.55 L) were supplied with P-deficient phytoplankton (mean molar C:N:P = 414:40:1; further referred to as 'LP'), whereas another four chemostats were supplied with P-replete food (mean C:N:P = 61:4:1; 'HP') (Appendix S2). These elemental ratios are within the natural range of freshwater habitats (Elser *et al.* 2000b). Rotifer chemostats received daily 300 mL of fresh medium (dilution rate:  $0.19 \text{ day}^{-1}$ ) containing  $1550 \mu\text{mol C L}^{-1}$  of either LP or HP algal food. Second stage chemostats were kept permanently in the dark at approximately 22 °C. Appendices S1 and S2 give more details on the chemostat set-up and food quality monitoring results, respectively.

The *Brachionus* populations were seeded with mixtures of parthenogenetic descendants of females extracted from the resting egg banks of two Dutch ponds (see Appendix S1 for more information on the ponds and their populations). The mixtures consisted of 14 ('Pond 7') and 30 clones ('Pond 22'),

respectively. Each clone mixture (Pond 7 vs. 22) was allocated to two replicates per food treatment (LP vs. HP) in a  $2 \times 2$  design. Given that *B. calyciflorus* consists of a cryptic species complex, we sequenced the nuclear internal transcribed spacer I genetic locus of each of these clones prior to the experiment, and ensured that all clones belonged to the same putative cryptic species.

### Transplant experiments

In the two common garden experiments rotifer populations with different prior selection history (LP vs. HP chemostats) were exposed to LP- and HP-food in reciprocal transplants in semi-continuous culture. Experiment 1, which was initiated on day 103 of the selection experiment, only involved populations with a Pond 22 origin and consisted of 24 experimental units: 4 chemostat populations (each selection history represented by two populations)  $\times$  2 food quality levels  $\times$  3 replicates. Experiment 2 started on day 209 of the selection experiment and involved populations with both Pond 22 and Pond 7 origins. This experiment consisted of 48 units: 8 chemostat populations  $\times$  2 food quality levels  $\times$  3 replicates.

Experimental units were started by transferring 50 haphazardly selected rotifer individuals from the second stage chemostats to 100 mL flasks. Every day, we replaced 20% of the culture volume (including rotifers) with a fresh *Chlamydomonas* suspension prepared from the phytoplankton chemostats and diluted with nutrient-free medium to an average food concentration of  $2080 \mu\text{mol L}^{-1}$  C in Experiment 1 and  $1660 \mu\text{mol L}^{-1}$  C in Experiment 2. Flasks were kept on a shaking tray (75 rpm) in continuous darkness at  $22^\circ\text{C}$ . The cultures were transferred into clean flasks every 3 days.

### Sampling and sample analysis

Common garden experiments were sampled at steady state as inferred from a stabilisation of residual food concentrations and rotifer densities after approximately 2 weeks. Samples were obtained from the culture volumes that were removed for daily dilution. The rotifer populations were sampled on days 23, 32 and 41 of Experiment 1 and on days 20 and 27 of Experiment 2. In addition, rotifers were collected on a daily basis throughout these periods to compose samples for rotifer elemental composition. Seston stoichiometry and dissolved nutrients were sampled once at the end of the experiment (day 41 and 27 respectively). We manually counted and characterised rotifers using photographic images collected by a FlowCam (Fluid Imaging Technologies, Inc., ME, USA) optimised for our purposes (Appendix S1). The images were used to estimate the densities of males, females (fecund and non-fecund) and sexual resting eggs, and the number of asexual eggs per fecund female (fecundity). Seston samples were obtained by filtering culture medium on glass fibre filters (GF/F) following the removal of rotifers with an  $80 \mu\text{m}$  screen. The filtrate was kept for the analysis of dissolved nutrients (ammonia, nitrites, nitrates and orthophosphates). Rotifer C, N and P content was determined from two samples of 150 haphazardly selected individuals per replicate. We measured the C and N content of samples using a FLASH 2000 organic elemental analyser

(Interscience B.V., Breda, The Netherlands). For P, samples were incinerated at  $550^\circ\text{C}$  for 30 min and autoclaved in a 2% potassium persulfate ( $\text{K}_2\text{S}_2\text{O}_8$ ) solution at  $121^\circ\text{C}$ . Subsequently, P-content was determined using a QuAAtro segmented flow autoanalyser (Beun de Ronde, Abcoude, The Netherlands).

We developed 12 microsatellite primers and assessed the multilocus genotype (MLG = genotype identified by a unique combination of alleles on the investigated microsatellite loci) of each of the clones used to start up the selection experiment (see Appendix S3 for primer sequences and other details). In addition, we monitored MLG composition of the chemostat populations by genotyping 20 rotifer individuals per chemostat on days 16, 30, 44, 75, 103, and 166 of the selection experiment.

### Data analysis

Rotifer population biomass was calculated as the product of population density times the carbon content of individual rotifers. For the common garden experiment, we calculated three measures of rotifer population performance at steady state: (1) population biomass B, (2) residual food concentration  $C^*$ , and (3) rotifer yield calculated as  $B/(C_{\text{in}} - C^*)$ , where  $C_{\text{in}}$  and  $C^*$  are the food carbon concentrations in the daily supply and in the experimental flasks at steady state, respectively.  $C^*$  reflects the ability of a population to suppress resources and is therefore a reliable indicator of competitive ability (Kreutzer & Lampert 1999). We estimated population birth rate  $b$  as  $\ln(E + 1)/D_e$ , where  $E$  is the per capita number of asexual eggs and  $D_e$  the egg development time at  $22^\circ\text{C}$  (0.64 days; Herzig 1983). At steady state, the realised specific population growth rate equals the dilution rate  $D$ , and specific death rate  $d$  can be calculated as  $b - D$ .

For the selection experiment we used linear mixed effects models to statistically analyse effects of stoichiometric food quality and time on rotifer female density, residual food concentration and the per capita number of sexual eggs. Using the program GenoDive v.2b27, we distinguished MLGs that had originally been introduced in the chemostats from MLGs generated *de novo* by sexual reproduction.

For the common garden experiments, we used linear mixed effects models to analyse the effects of stoichiometric food quality, selection history, and population origin on the following rotifer variables (averaged over steady state samples): population biomass and yield, body C and P content and C:P ratio, death rate, fecundity, and the per capita number of males, sexual eggs, and females with asexual eggs. We also analysed  $C^*$ , and the C:P and N:P ratios of residual seston.

Mixed effects analyses were performed with the lme4-package (Bates *et al.* 2014) in R (R Core Team 2014). Individual chemostats were always specified as random variable by design. We removed non-significant variables from the fixed model component via the backward elimination procedure of the 'step' function in the lmerTest package (Kuznetsova *et al.* 2014). Significance values for the remaining fixed effects were based on F-tests with Kenward-Roger approximation. *Post hoc* comparisons between multifactorial combinations of the fixed model part were performed with the 'diffmeans' func-



tion of the lmerTest package (Kuznetsova *et al.* 2014). Except for death rate  $d$ , variables were logit-transformed (per capita numbers) or log-transformed (all other variables) prior to analysis.

For response variables for which we detected a significant effect of both food quality and selection history (or a Food  $\times$  Selection interaction) in the common garden experiments, we quantified the relative contribution of ecology and evolution to observed treatment differences. Applying the Geber method (Hairston *et al.* 2005; Ellner *et al.* 2011, Eq. 12) we calculated the impact of evolution as  $\Delta V = 0.5 [(X_{LP,LP} - X_{HP,LP}) + (X_{LP,HP} - X_{HP,HP})]$  and of ecology as  $\Delta C = 0.5 [(X_{LP,LP} - X_{LP,HP}) + (X_{HP,LP} - X_{HP,HP})]$ , where  $X_{i,j}$  is a treatment mean observed in prior rotifer selection regime  $i$  and experimental food quality treatment  $j$ . From these two components we calculated the proportional effect of evolution on treatment means as  $|\Delta V|/(|\Delta V|+|\Delta C|)$ . We also calculated the impact of evolution in each of the food quality treatments separately as the proportional mean change in a response variable in the LP- relative to the HP-selection treatment.

## RESULTS

### Selection experiment

During most of the selection experiment, rotifer population densities were considerably lower and residual food concentrations consistently higher in LP- than in HP-food treatments (Fig. 1a and b, Appendix S4). In LP-chemostats, rotifer densities declined and residual food concentrations increased during the first 60 days; subsequently, rotifer densities rebounded to almost initial levels, whereas residual food concentration tended to decline again. In HP-chemostats, rotifer densities increased and residual food concentrations declined during the first 80 days and continued fluctuating around the same levels throughout the rest of the experiment.

Per capita numbers of sexual eggs were highest at the start of the experiment and steadily declined to very low levels in both food quality treatments (Fig. 1c, Appendix S4). The decline was slower under LP than under HP conditions but treatment differences disappeared after approximately 100 days.

New multilocus genotypes appeared early in the experiment as the result of sexual reproduction with recombination. Chemostats with Pond 22 populations became entirely dominated by new genotypes after 20 days (Fig. 1d). Relative abundances of new MLGs increased more gradually in Pond 7 populations but eventually reached 100%, with the exception of one population fed LP-food.

### Common garden experiments

Experiments 1 and 2 yielded very similar results. We therefore present results of the larger Experiment 2 (including populations of both pond origins) in detail here and refer to Appendix S6 for results of Experiment 1.

#### Population performance

The mixed effects models indicate significant Food  $\times$  Selection history interactions for steady state rotifer population

biomass, yield, and residual food concentration (Appendix S5). LP-food reduced rotifer population biomass (Fig. 2a) and yield (Fig. 2b) compared to HP-food but these reductions were less pronounced in populations with an LP- compared to an HP-selection history. When fed with LP-food, the yield of LP-adapted populations was on average 83% higher than that of HP-adapted populations (*post hoc* test:  $P = 0.003$ ). No such difference was found with HP-food. Populations originating from Pond 22 reached on average 28% higher steady state biomass than populations from Pond 7 (Appendix S5).

Residual food concentrations were substantially higher in LP- than in HP-food treatments. This indicates a reduced capacity of rotifers to exploit P-limited food (Fig. 2c). Yet, LP-selected populations were able to graze down LP-food to 40% lower levels than HP-selected populations (*post hoc* test:  $P = 0.006$ ). No such differences were observed in HP-food treatments.

#### Demographic features

Estimated birth and death rates were considerably higher in LP- than in HP-food treatments and there was a significant Food  $\times$  Selection history interaction (Fig. 3a, Appendix S5): LP-selected populations had lower birth and death rates than HP-selected populations when fed with LP-food (*post hoc* test:  $P < 0.001$ ). These differences were caused by differences in the per capita number of fecund asexual females (Fig. 3b, Appendix S5), while fecundity did not differ among treatments.

Food quality interacted strongly with selection history in affecting the per capita number of sexual eggs and males (Fig. 3c and d, Appendix S5). The production of both types of sexual progeny was systematically lower in LP-selected than in HP-selected populations in both food treatments (*post hoc* tests: sexual eggs: HP-food:  $P = 0.003$ , LP-food:  $P < 0.001$ ; males: HP-food:  $P < 0.066$ , LP-food:  $P = 0.007$ ). HP-selected populations produced considerably more males and resting eggs in LP- than HP-food conditions (*post hoc* tests:  $P < 0.001$ ). Such food quality effects were much weaker for LP-selected populations (sexual eggs:  $P = 0.390$ ; males:  $P = 0.018$ ).

#### Rotifer body elemental composition

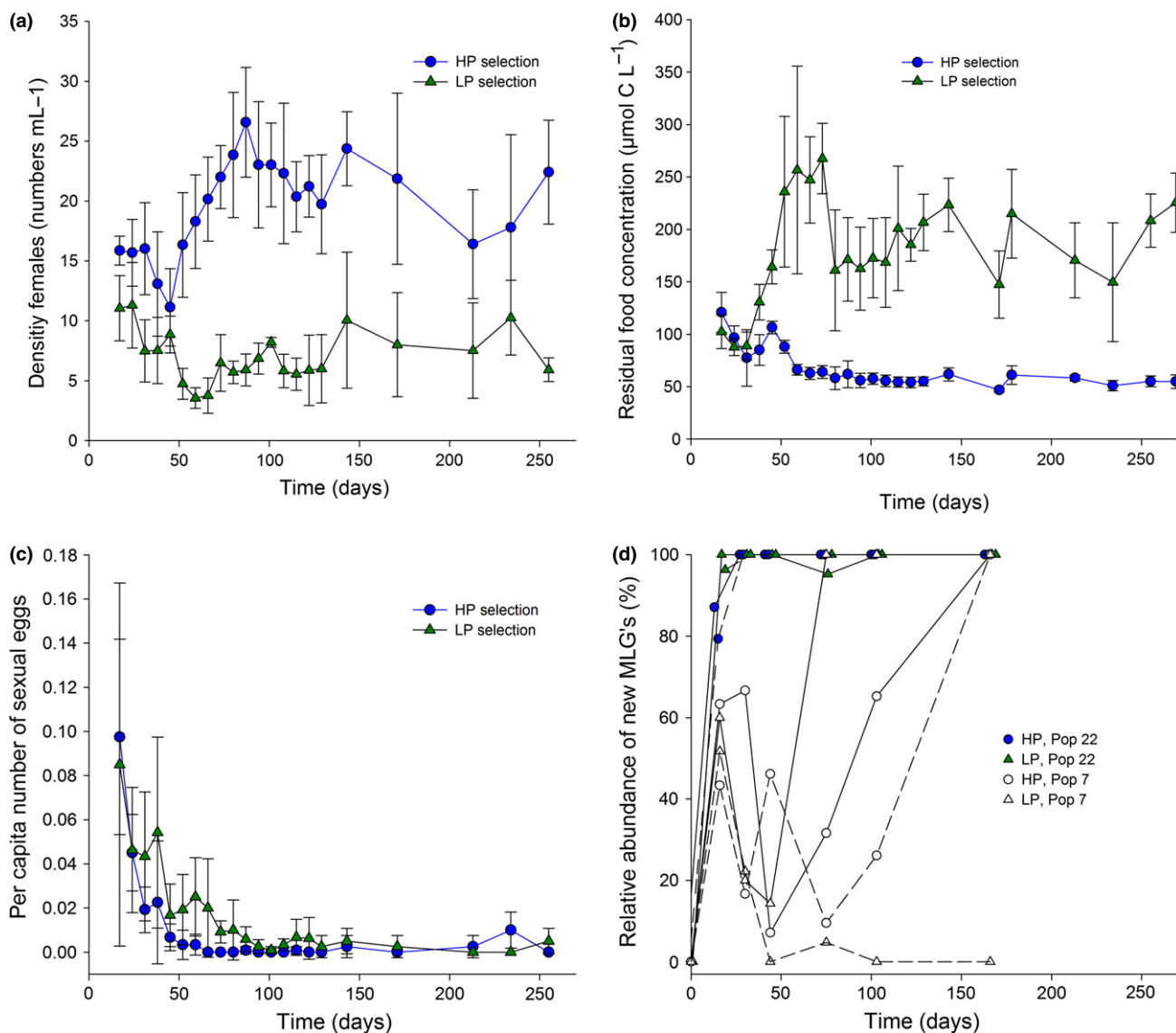
Individual rotifers contained slightly less P and considerably more C, yielding two-fold higher C:P body mass ratios, in LP- than in HP-food treatments. These effects were independent of prior selection regime or population of origin (Fig. 4a–c, Appendix S5). C:N ratios were only slightly lower in LP- than in HP-food treatments. Patterns of rotifer N- and N:P-content therefore followed closely the corresponding patterns of rotifer C- and C:P-content and are not shown.

#### Distribution of nutrients and seston stoichiometry

Dissolved P was below the detection limit in all LP-food treatments and prevented us from analysing the ratio of dissolved N to dissolved P. Seston elemental composition strongly reflected the food treatments (Fig. 4d and e). Seston C:P and N:P differed between populations of origin, but remained unaffected by prior rotifer selection history (Fig. 4d and e).

#### Contribution of evolution to food treatment responses

For response variables that were significantly affected by selection history, estimates of the relative contribution of evo-



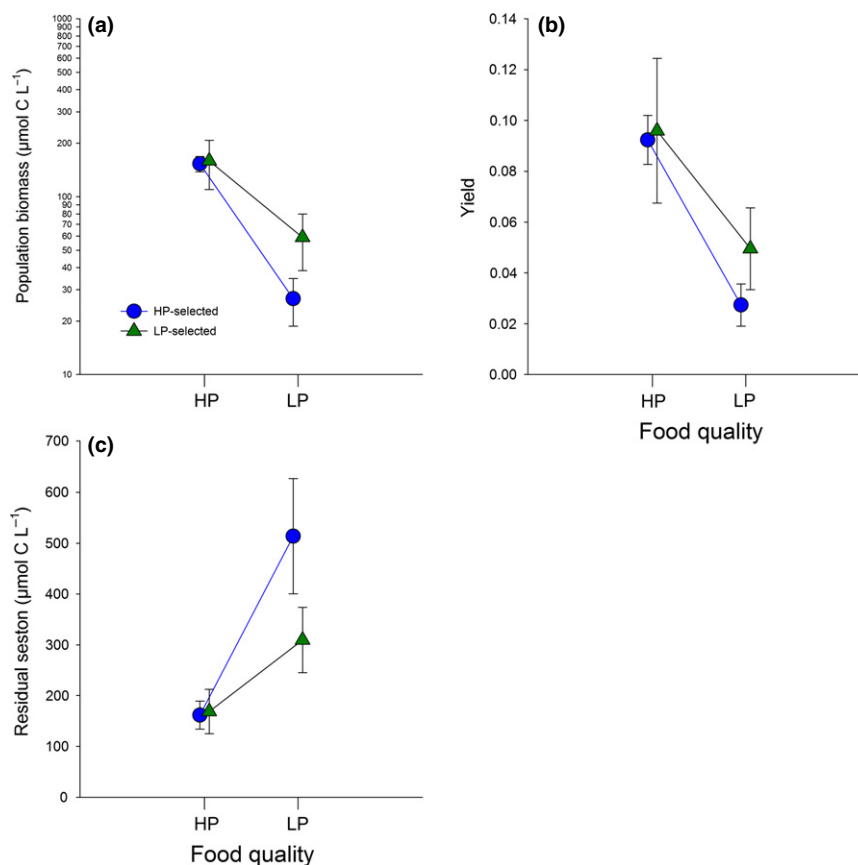
**Figure 1** (a–d). Temporal dynamics of rotifer female density (a), residual seston C-content (b), per capita number of sexual rotifer eggs (c) and the relative abundance of newly formed rotifer multilocus genotypes (d) in the rotifer reactors of the second stage chemostat system during the selection experiment. Circles are HP-selection treatments, triangles are LP-selection treatments. In panels (a–c) means  $\pm$  2 standard errors of the mean are shown ( $n = 4$ ). In (d) all eight chemostat populations are shown separately; symbols are slightly offset to avoid overlap. Open and filled symbols indicate Pond 7 and Pond 22 origins, respectively. Solid and broken lines distinguish the two replicates of each treatment.

lution to observed food treatment responses ( $100 \cdot |\Delta V| / (|\Delta V| + |\Delta C|)$ ) were as follows: rotifer steady state biomass: 14%, yield: 19%, residual food: 29%, death and birth rates: 35%; proportion of fecund asexual females: 34%; per capita number of males and resting eggs: 52 and 61%, respectively.

In the LP-food treatment, effect sizes of selection history (expressed as relative changes of treatment means in LP- compared to HP-selected populations) were: rotifer steady state biomass: 121%; yield: 81%; residual food:  $-40\%$ ; death and birth rates:  $-37\%$ ; proportion of fecund asexual females:  $-25\%$ ; per capita number of males and resting eggs:  $-78$  and  $-94\%$ , respectively. In the HP-food treatment differences were statistically insignificant for most variables (ranging from  $-10$  to  $4.5\%$ ), except for per capita number of males and resting eggs for which the change equaled  $-71\%$ .

## DISCUSSION

Our study clearly shows that natural rotifer populations can rapidly adapt to stoichiometrically imbalanced, P-limited food, and it simultaneously identifies potential feedbacks of such adaptation to population performance and resource levels. When fed P-limited food, LP-adapted populations suffered lower mortality rates, realised higher steady state biomasses, achieved higher yields, and grazed down food to lower levels than did populations with an HP-selection history. The ability to persist at a lower residual concentration of P-limited food strongly suggests competitive superiority of LP-selected populations under P-limited conditions. These micro-evolutionary responses proved highly reproducible both in time and across populations with different genetic backgrounds.

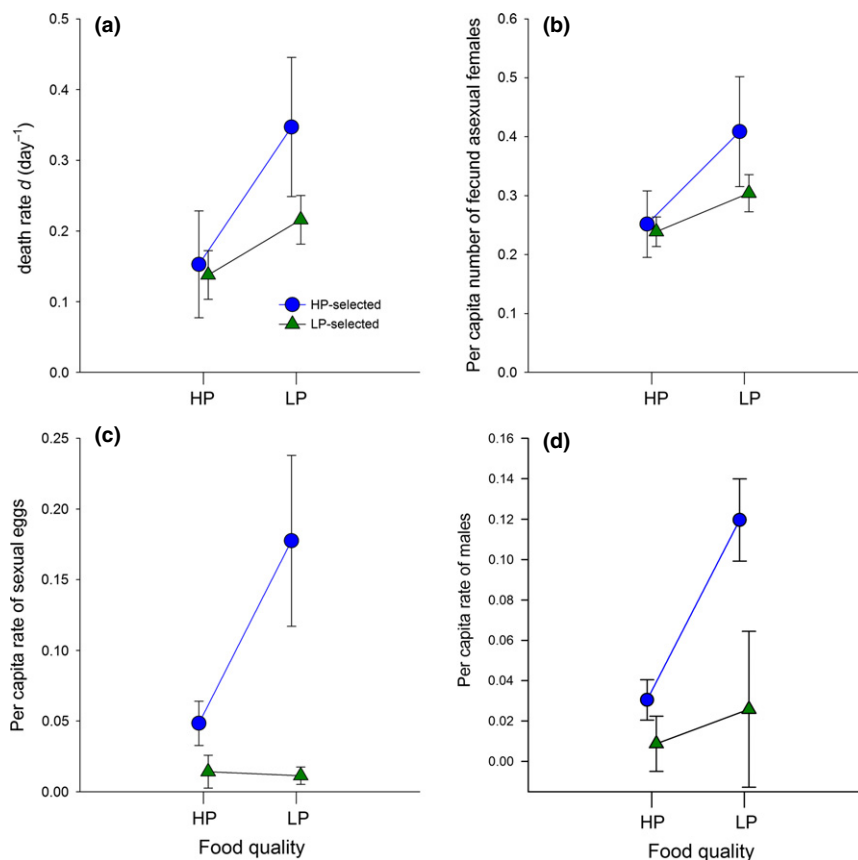


**Figure 2** (a–c). Responses of performance related population variables to ambient food quality and selection history in common garden transplant Experiment 2. Steady state rotifer population biomass (a) and yield (b), and residual seston concentration (c). Circles are treatments with a P-replete selection history, triangles are treatments with a P-limitation selection history. Ambient food quality treatments are: HP: food with high P-content; LP: food with low P-content. Shown are means across chemostat origins  $\pm 2$  standard errors ( $n = 4$ ). Note the log-scale in (a).

We found no evidence for adaptive changes in rotifer body elemental composition. Instead, adaptation was associated with strong changes in resource allocation patterns. HP-selected populations responded to P-limited food with an increased per capita production of sexual eggs and males, whereas no such response was observed for LP-selected populations. The lower investment in sex of the LP-selected populations seemed constitutive, as these populations also produced less sexual progeny than the HP-populations when fed P-replete food. Sex involves great costs that trade off with asexual reproduction (Snell 1987). Sex has been shown to be highly evolvable in rotifer populations (Fussmann *et al.* 2003; Smith & Snell 2012) and to be maladaptive in chemostats at dilution rates similar to ours (Becks & Agrawal 2010). This is also suggested by the steady decline of males and resting eggs in the chemostats of our selection experiment. Reduction in sexual investment may have been an important strategy to cope with P-limitation and likely contributed to the relatively high performance of LP-adapted populations under P-limiting conditions. It cannot be excluded, however, that adaptation also involved changes in other, physiological, traits such as altered ingestion rates (Suzuki-Ohno *et al.* 2012), possibly in combination with a more efficient P-assimilation and/or P-retention (Frisch *et al.* 2014). Adapted populations may also

have been better at reducing costs associated with the disposal of excess elements like C and N (Darchambeau *et al.* 2003) or at coping with other properties of P-limited algae, such as a reduced biochemical quality (Müller-Navarra 1995) or digestibility (Van Donk *et al.* 1997).

P-limited food resulted in important demographic changes, but these changes were less pronounced in LP-adapted populations. According to our estimates, all experimental populations experienced higher steady state birth and mortality rates in P-limited food. Compared to populations in P-replete food, higher death rates were associated with lower population biomass and were compensated by higher birth rates at higher residual food concentrations. However, LP-food may also have increased egg mortality and egg development time, and both may have contributed to an overestimation of birth and death rates. Higher birth rates were associated with a higher per capita number of egg bearing females and not with higher fecundity. Most likely, this shift in demographic structure under LP-food conditions reflects changed transition rates between life stages, for example as a consequence of increased mortality rates and/or decreased development rates of eggs or juveniles (De Roos & Persson 2013). Independent measurements of development and mortality rates of LP- and HP-selected clones in LP- and HP-



**Figure 3** (a–d). Responses of demographic rotifer variables to ambient food quality and selection history in common garden transplant Experiment 2. Death rate (a), per capita number of fecund asexual females (b), per capita number of sexual eggs (c) and males (d). Rotifer birth rates can be inferred from death rates by addition of the dilution rate ( $0.2 \text{ day}^{-1}$ ). Circles are treatments with a P-replete selection history, triangles are treatments with a P-limitation history. Ambient food quality treatments are: HP: food with high P-content; LP: food with low P-content. Symbols represent means across chemostat origins  $\pm 2$  standard errors ( $n = 4$ ).

food will be required to provide a better understanding of such demographic shift.

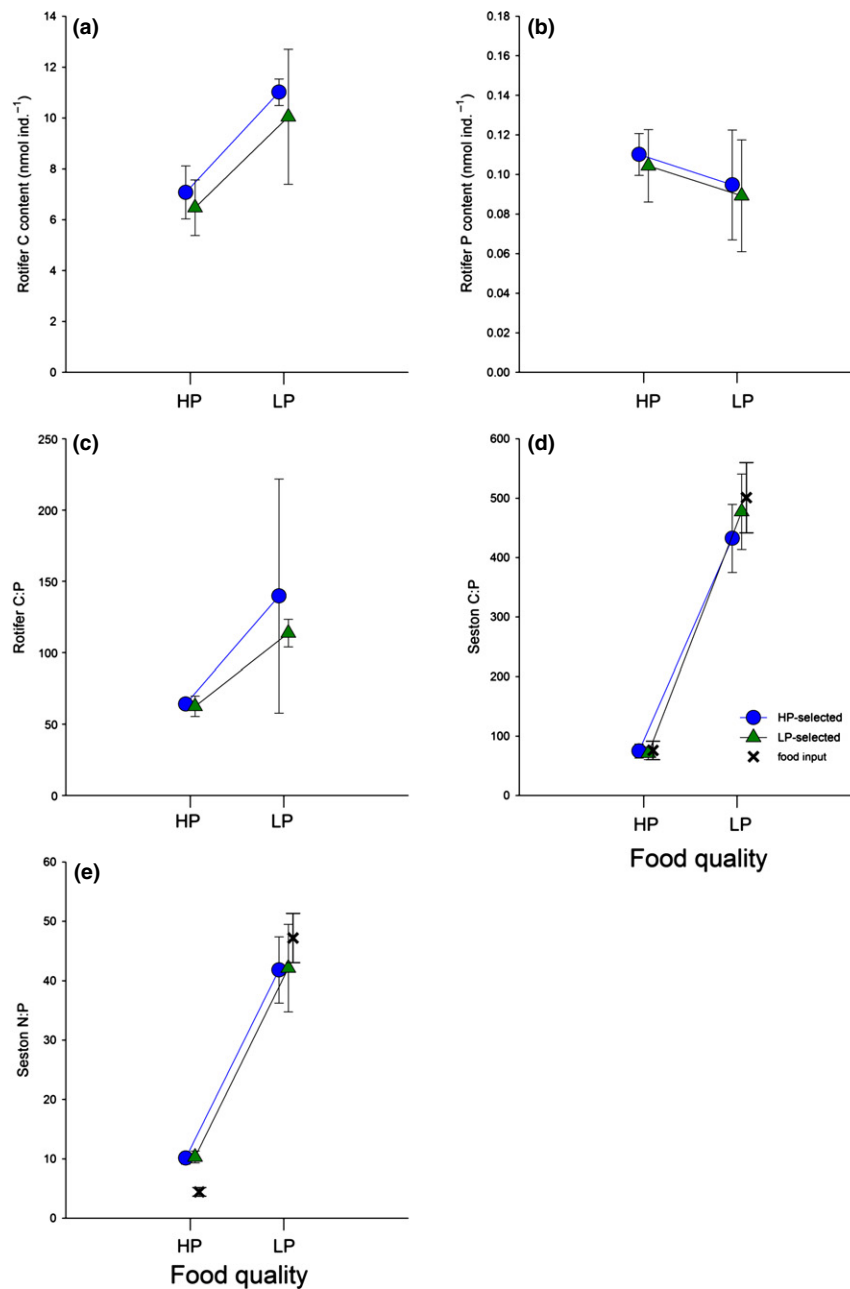
Most likely, the observed trait shifts resulted from selection on genetic diversity that was generated by sexual recombination of standing genetic variation (Barrick & Lenski 2013). Clonal diversity of the experimental populations was relatively low at the start of the selection experiment but increased substantially following an initial bout of sexual reproduction. With the exception of one population, we observed a complete replacement of the initial set of clones by new clones during the experiment. Although it cannot be entirely excluded, mutation-driven evolution was probably negligible, given the relatively small size of the experimental populations and the low numbers of generations involved. Our efforts to use clones of a single putative cryptic species rule out that trait shifts resulted from cryptic species sorting.

Remarkably, adaptation to P-limited conditions did not appear to trade-off with population performance under other conditions. Indeed, populations with an LP-selection background performed equally well under nutrient replete conditions as the HP-selected populations with respect to measures of immediate fitness such as steady state biomass, yield, and the ability to deplete food sources ( $C^*$ ). Note, however, that the constitutively lower resource allocation of LP-selected

populations to sex may involve fitness costs in the longer term, for example by reducing evolvability of the populations and recruitment success after major disturbances.

A central task in eco-evolutionary research is to identify how environmental change propagates through both ecological and evolutionary pathways and to assess the relative speed and strength of responses through each route (Ellner *et al.* 2011; Cameron *et al.* 2013). To be relevant, feedback of evolution to ecology should be rapid and its effects should be important (Hairston *et al.* 2005). Taking advantage of the ability of rotifer populations to approach steady state in (semi-)continuous culture, we could not only demonstrate adaptation of rotifers to different food quality selection regimes but simultaneously disentangle the relative impact of ecology (effect of ambient food quality) and of evolutionary history (effect of selection background) on observed responses. Compared to the ecological effects of ambient food quality, the relative effects of prior selection history ranged from insignificant (e.g. rotifer body stoichiometry) through substantial (e.g. steady state biomass, yield, residual food concentration, birth and death rates) to dominant (production of males and resting eggs). In addition, evolution had very strong absolute effects on some response variables. For example, in the LP-food treatment, prior adaptation to LP-food





**Figure 4** (a–e). Responses of rotifer elemental content and rotifer and seston elemental ratios to ambient food quality and selection history in common garden transplant Experiment 2. Rotifer C-content (a), rotifer P-content (b), and molar elemental ratios of rotifer C:P (c), seston C:P (d), and seston N:P (e). Circles are treatments with a P-replete selection history, triangles are treatments with a P-limitation history. Ambient food quality treatments are: HP: food with high P-content; LP: food with low P-content. Symbols represent means across chemostat origins  $\pm$  2 standard errors ( $n = 4$ ).

resulted in 80 and 120% increases of key variables such as rotifer biomass and yield, respectively. Clearly, population level consequences of food quality adaptation can be very substantial under certain conditions.

Evolutionary responses were also rapid. The potential for evolutionary change to feed back to ecology will largely depend on whether both dynamics operate on comparable time scales (Hairston *et al.* 2005). Depending on the water body and its landscape context, rates at which seston stoichiometry changes may vary strongly and range from short-term

fluctuations over seasonal variations to long-term interannual trends. The two transplant experiments revealed very similar adaptive responses of the rotifer populations in the second stage chemostats to food quality, suggesting that the strongest rotifer trait changes had been realised already during the first 3 months of selection. Rotifer dynamics in the selection experiment can be interpreted along these lines. Population size increased during the first 100 days in HP-chemostats and rebounded after an initial 60 days of decline in LP-chemostats. These observations may reflect improving performance

as a result of clonal selection and possibly indicate evolutionary rescue in the LP-chemostats. The results thus suggest that eco-evolutionary feedbacks may strongly alter ecological dynamics on the time scale of a growing season.

Notably, rapid adaptation to food quality altered herbivore grazing pressure on algae, a variable that is tightly related to important ecosystem functions. Specifically, adaptation to LP-food enabled rotifer populations to graze down food to on average 40% lower levels than non-adapted populations. The ability of herbivorous zooplankton to adapt to nutrient limitation in their food may therefore feedback on standing stocks of aquatic primary producers and, as a consequence, also on primary production (Miner *et al.* 2012). An improved ability to reduce food levels may furthermore affect the outcome of interspecific competition between consumer species (Kreutzer & Lampert 1999). While we studied just one single plankton species, we expect other species to have similar adaptive abilities. We therefore postulate that population persistence under conditions of intense interspecific exploitative competition may in part depend on the capacity to rapidly adapt to a changing environment.

We found no evidence for a positive eco-evolutionary feedback loop. Higher rotifer biomass of LP- compared to HP-adapted populations in the LP-food treatment was not a consequence of higher realised growth per unit of ingested P (body elemental composition did not differ between rotifers with different selection histories). Currently, we do not know whether adaptation to LP-food involved increased feeding activity (clearance rate), higher assimilation efficiency of P and/or higher retention efficiency of metabolised P. In the latter two cases one would expect higher excretion rates of N relative to P in adapted compared to non-adapted populations, potentially reinforcing the imbalance among nutrients available to algae and thus also in algal stoichiometry. Due to the limitations of our method, we were unable to assess imbalances among dissolved nutrients. Furthermore, we found no evidence for effects of rotifer adaptation on seston stoichiometry. This may indicate that adaptation was not realised through enhanced assimilation and/or retention efficiencies of P. Yet, the observed seston N:P ratio of ca. 40 is close to the optimal N:P ratio predicted for severely P limited algae (Klausmeier *et al.* 2004), suggesting that phytoplankton in LP-treatments may have been too saturated with N to be affected by grazer-driven nutrient cycling.

## CONCLUSIONS

Our study demonstrates a high capacity of a plankton consumer to adapt rapidly to changing environmental conditions, *in casu* resource P-limitation and associated stoichiometric imbalances. When fed P-limited food, populations with a P-limitation selection history reached higher steady state biomass, and grazed down food to lower levels compared to non-adapted populations. Adaptation involved no changes in elemental composition but was associated with reduced investment in sex. From our results we infer that rapid adaptations have important ecological implications. We observed eco-evolutionary feedbacks to population demographic features, such as birth and death rates and population structure, as well as

to grazing pressure on algae, a variable that is strongly related to important ecosystem functions. We found no evidence for the idea that adaptation may reinforce selection pressure on consumers by enhancing nutrient imbalance in the food source.

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## AUTHORSHIP

SAJD developed the core idea and designed the experiment. DW and SAJD developed the chemostat set-up. SP and KP developed the microsatellite markers and performed the molecular analyses. The clones were collected and maintained by SP and DW and genotyped and sequenced by SP and KP. DW supervised the chemostat experiments. ARM carried out the common garden experiments and the corresponding sample analyses. SAJD and SP performed the data analyses. SAJD wrote the article with important input from all authors, especially SP, KL and SD.

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