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Research

Maternal effects in zooplankton consumers are not only mediated by direct but also by indirect effects of phosphorus limitation

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Nutrient limitation of primary producers has repeatedly been shown to negatively affect consumers, directly through stoichiometric mismatch and indirectly via alterations in the producer's biochemical quality or palatability. In this study, we assessed whether direct and indirect impacts of phosphorus-limitation on a planktonic consumer are transferred to the next generation via maternal effects and whether these effects reflect an anticipatory adaptive strategy. For this, we subjected cultures of the algalivorous monogonont rotifer *Brachionus calyciflorus* to three food quality treatments, i.e. P-limited (LP), P-replete (HP) and P-enriched LP algae (i.e. algae with an LP-growth history but with molar C:P ratios equal to those of HP-algae). After two generations, we subjected offspring of these cultures to each of the three food quality treatments and monitored life history traits. In addition, we tested starvation resistance. Our results showed very strong negative maternal effects of low P food on offspring performance. These negative effects prevailed irrespective of contemporary diets, suggesting transmissive and selfish maternal effects rather than anticipatory adaptive effects. The relative strength of direct and indirect maternal P-limitation effects varied among different traits. Adult body size was predominantly determined by direct effects of P-shortage in maternal as well as contemporary food ($LP < LP + P$ and $LP + P = HP$). In contrast, whereas egg size was negatively affected by direct effects of P-limitation in the maternal diet, a contemporary diet of LP and LP + P algae resulted in larger eggs than HP algae. Animals born from such larger eggs showed no higher growth rates, but they were more resistant to starvation, likely as the result of higher maternal allocation of energy rich molecules to the eggs. The present study shows that maternal food conditions represent an important factor that should be taken into account in studies of stoichiometric mismatch between producers and consumers.

Keywords: ecological stoichiometry, elemental mismatch, life history, plasticity, rotifer, stoichiometric mismatch

Introduction

The phenotype of an organism is the product of the interaction between its genotype and its environment. In addition, organisms may also be profoundly affected by the environment that has been experienced by previous generations (Marshall and Uller



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2007, Yanagi and Tuda 2010, Pajk et al. 2012, Kuijper and Hoyle 2015, Harney et al. 2017). Maternal generations may indeed affect the quality of their offspring via non-genetic transgenerational mechanisms ('maternal effects', Mousseau and Fox 1998, Marshall and Uller 2007). When confronted with changed levels of a stressor or threat, they may increase their fitness by anticipating the environment of the future generation and by enhancing the success of their offspring in this environment ('anticipatory maternal effects', Marshall and Uller 2007, Sheriff and Love 2013). For example, upon reception of cues by predators, prey may induce the expression of traits in their offspring that reduce their vulnerability to predation (Yin et al. 2015, Yule and Burns 2017), while deteriorating feeding conditions may result in changed allocation patterns of energy resources among eggs (Yanagi and Tuda 2010, Kaneko et al. 2011, Stahlschmidt and Adamo 2015, Harney et al. 2017). In contrast, organisms may also respond to worsening environmental conditions by increasing their own fitness at the expense of their offspring ('selfish maternal effects', Marshall and Uller 2007), e.g. by allocating fewer resources to offspring under worsening feeding conditions (Frost et al. 2010, He et al. 2016). Alternatively, some maternal effects represent the legacy of maternal growing conditions but lack adaptive value ('transmissive maternal effects', Marshall and Uller 2007). For example, exposure of the mother generation to food limitation, toxins or parasites may translate in a reduced performance of the offspring (Guinnee et al. 2007, Beyer et al. 2017).

A good understanding of fitness variation in consumers requires knowledge of their response to environmental change and how the effects of these responses propagate across generations (West-Eberhard 2003, Kuijper and Hoyle 2015). One important factor that may strongly affect the performance of animals is resource quality. Resource quality is often determined by the relative ratios of essential biogenic elements, such as nitrogen (N), phosphorus (P) and carbon (C) (Hessen et al. 2013). Many studies have demonstrated that a mismatch between the elemental requirements of consumers and their prey (i.e. 'stoichiometric mismatch') results in altered phenotypes and reduced performance of consumers (Elser et al. 2001, Frost and Elser 2002, Zhou and Declerck 2019). However, the role of maternal effects in determining the phenotypic response to stoichiometric mismatch is still poorly understood. It is also unclear to what extent such maternal effects represent an adaptive strategy.

In addition to the negative effects of a stoichiometric mismatch, imbalanced nutrient supply rates to a system may also influence the performance of consumers by causing non-stoichiometric alterations in their food (Rothhaupt 1995, Ravet and Brett 2006, Zhou et al. 2018). Phosphorus is generally considered as one of the most important elements that limit the primary productivity of freshwater systems. P-limitation of primary producers often results in changes in their biochemical composition (Müller-Navarra 1995, Weers and Gulati 1997, Spijkerman and Wacker 2011, Challagulla et al. 2015) and morphology (van Donk and Hessen 1995, van Donk et al. 1997), which may affect consumer performance

independently of P-content. Although never studied, it is likely that such indirect effects may also be transferred to next generations through maternal effects. Variation in the biochemical composition of maternal diets has been shown to affect the performance of subsequent generations (Sperfeld and Wacker 2015), likely as the result of altered allocation of biochemical components to eggs (Sperfeld and Wacker 2009, 2012). Changes in the biochemical quality of food as result of P-limitation could be transferred to the next generation in a similar way and it remains an open question to what extent such indirect effects would be transmissive or adaptive.

Zhou et al. (2018) showed that individuals of the zooplanktonic rotifer *Brachionus calyciflorus* produced larger eggs when fed P-limited algae (LP) compared to animals that were fed with a diet of P-replete algae (HP). Given that egg size tends to be associated with higher offspring quality in many taxa (Dias and Marshall 2010, Krist 2011, Segers and Taborsky 2011, Moore et al. 2015), such increased egg size may reflect an anticipatory adaptive response. Using a P enrichment method (Rothhaupt 1995), Zhou et al. (2018) were able to disentangle direct from indirect effects of P-deficient food by creating a food quality treatment (LP + P) involving algae with an LP growth history but having molar C:P ratios equal to that of the HP treatment. They showed that rotifers underperformed in LP + P compared to HP diets, possibly as the result of indirect effects of P-limitation (e.g. disadvantageous alterations in algal biochemical composition or morphology). Notably, the eggs of animals grown in LP + P were also larger than in HP and equaled the size of eggs produced in LP. This also leads to the question whether the egg size response in the LP + P treatment has any adaptive value or, conversely, reflects a legacy of the indirect effects of P-limitation.

The objective of our study was to assess whether both direct and indirect impacts of P-limitation on the rotifer *B. calyciflorus* are transferred to the next generation via maternal effects and to evaluate to what extent these effects reflect anticipatory adaptive strategies. For this, we raised animals under LP, LP + P and HP conditions for multiple generations, confronted their offspring to either of the three food quality conditions according to a multifactorial design and monitored a number of key life history traits. We considered maternal effects to be anticipatory if offspring with an ancestral history of P-limitation (LP or LP + P) perform better under these respective conditions than offspring born from mothers grown under P-replete conditions. In addition, we tested if the increased egg size associated with exposure of the maternal generation to P-limitation or its indirect effects is associated with an increased starvation resistance of the offspring.

Methods

Rotifer and algae cultures

We cultured the green alga *Chlamydomonas reinhardtii* as food for the rotifers in ten 2-liter-chemostats with modified WC medium (Guillard and Lorenzen 1972) at $23 \pm 1^\circ\text{C}$ and

a dilution rate of 0.33 day^{-1} . Five of the chemostats received phosphorus rich medium ('HP', $65 \mu\text{mol l}^{-1} \text{ K}_2\text{HPO}_4$) and $40 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ of light and produced algae with a low molar C:P ratio (C:P=131), whereas the other five chemostats were provided with a phosphorus poor medium ('LP', $15 \mu\text{mol l}^{-1} \text{ K}_2\text{HPO}_4$) and $120 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, resulting in algal cultures with a high C:P ratio (C:P=582).

We used one *Brachionus calyciflorus* clone obtained from the resting egg bank of a Dutch lake ($52^\circ 5' 26.50'' \text{ N}$, $4^\circ 20' 18.40'' \text{ E}$). *Brachionus calyciflorus* is actually known to consist of a species complex, of which four species have recently been (re-)described based on phylogenetic and morphological analyses (Papakostas et al. 2016, Michaloudi et al. 2018). Based on its ITS1-sequence information, the clone used for this study was identified as *B. calyciflorus* (Michaloudi et al. 2018). Rotifer stock cultures were maintained at room temperature under continuous light conditions, and fed with HP food.

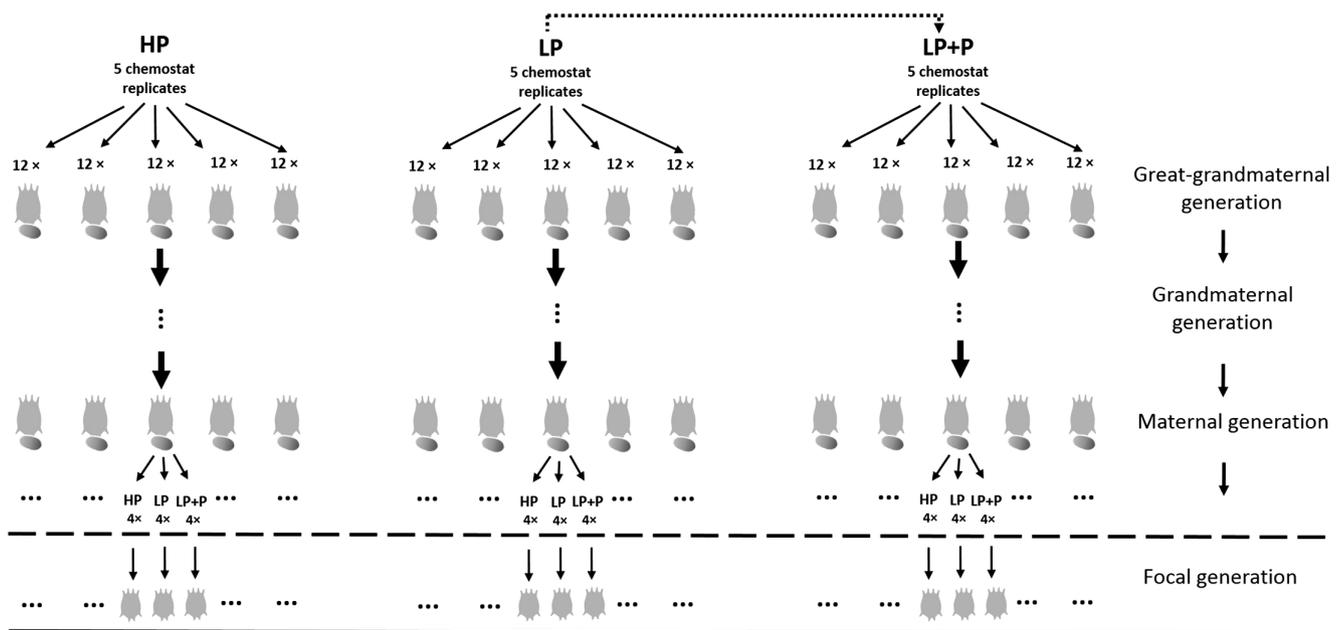
Creation of food quality treatments for the experiment

The rotifers in the experiment were fed every 24 h at a fixed time of the day. Algae were harvested from the LP and HP chemostats, centrifuged (2500 rpm for 10 min) and resuspended in nutrient free WC medium. Following Rothhaupt (1995) and Zhou et al. (2018), we created an LP+P food

quality treatment by adding inorganic phosphate (K_2HPO_4) to a suspension of LP algae just after harvesting and 90 min before feeding. The amount of added P was based on the algal C content estimated from cell counts. The algae of all three treatments were kept in the dark between the moments of harvesting and feeding to the rotifers.

Maternal effects experiment

The aim of this experiment was to study how the effects of food quality on the performance and life history traits in a focal rotifer generation (further referred to as 'contemporary diet') is mediated by the food quality conditions experienced by the grandmaternal and maternal generations ('maternal diet'). The design of this experiment is illustrated in Fig. 1. Starting from great-grandmaternal adults, we independently raised multiple clonally reproducing lines of single individuals under each of the three food quality types (i.e. HP, LP+P and LP) for two generations (the grandmaternal and maternal generations). We then randomly redistributed the offspring (further referred to as 'focal' generation) of the maternal generation across these same food quality treatments. Each food quality treatment was independently replicated by five chemostats. In the focal generation, each replicate in each cross-factorial food quality combination was represented by four animals from clonal cultures that had been independently raised since the grandmaternal generation. This thus resulted in an experiment with



Note: dashed line with arrow indicates that replicates of the LP+P food treatment were created by enriching LP chemostat replicates with inorganic P.

Figure 1. Design of the experiment. We created three food quality treatments (HP, LP + P and LP) that were each replicated by five chemostats. We used randomly selected individuals (great-grandmothers) from stock cultures to start 12 clonal lines per food quality replicate. Clonally producing lines were independently raised for two generations, i.e. the grandmaternal and maternal generations. Once individuals of the maternal generation produced their first parthenogenetic eggs, they were haphazardly assigned to one of the three food qualities. These mothers were checked hourly for neonates. When born, these neonates (i.e. the 'focal generation') were individually raised and monitored to record life history traits.

180 experimental units (i.e. three ancestral food quality treatments \times three contemporary food quality treatments \times five chemostat replicates \times four individuals).

To start the experiment, great-grandmothers with parthenogenetic eggs from stock cultures were randomly collected and used to start 180 individual cultures, i.e. 12 cultures per food quality replicate. The individuals were assigned to individual wells of tissue culture plates filled (volume: 1 ml) with a suspension of the respective food quality treatment ($1000 \mu\text{mol C l}^{-1}$). Plates were incubated at $23 \pm 1^\circ\text{C}$ in the dark at a random location in the incubator. Different food qualities cause differences in generation times (Zhou et al. 2018). To maximally synchronize the moment of birth of the focal generations in the three food quality treatments, we initiated the great-grandmaternal generation of the LP and LP+P cultures, respectively, 42 and 27 h earlier than of the HP cultures. Every day the cultures were checked twice for the presence of neonates under a stereomicroscope. Females were discarded once their first parthenogenetically produced offspring was born, and the neonate was further cultured as the next generation. Following the production of their first eggs, females of the mother generation were immediately transferred to a food suspension of the destined food quality treatment (see experimental design in Fig. 1) to ensure that experimental animals of the focal generation were born in the target food quality treatment. These gravid females were checked on an hourly basis to assess the time of birth of the neonate. From an age of 8 h on, animals from the focal generation were checked every two hours and the age at first egg production was recorded. Two hours after the observation of the first egg the individuals were preserved in 4% formaldehyde for later microscopic measurement of body and egg size.

Starvation experiment

This experiment was conducted to evaluate the effect of ancestral food quality treatments on starvation resistance of newborns. Starvation resistance of each rotifer individual was estimated as the time between its birth and death in the absence of food. Similar as with the maternal effects experiment, clonally reproducing culture lines of single individuals were established from stock cultures and independently grown under the three food quality treatments (i.e. LP, HP and LP+P) for two full generations. The experiment consisted of 45 experimental units, i.e. three food quality treatments \times five chemostat replicates \times three lines. Upon production of their first eggs, females from the maternal generation were transferred into nutrient-free WC medium and checked every hour. The hour of birth was recorded and the newly hatched neonates were transferred to an individual well with 1 ml nutrient-free WC medium. The neonates were monitored every two hours until the time of their death.

Algae stoichiometry

During the experiment, the molar C:P ratio of the phytoplankton in each replicate of each food quality treatment was

measured at two occasions. Algal samples were obtained by filtering culture medium on glass filters (GF/F) following by drying at 60°C for at least 24 h. C and N contents of algae were determined using an organic element analyzer. For P content, algal samples were incinerated at 550°C for 45 min and then autoclaved in 2.5% potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) at 121°C . These samples were subsequently determined using a segmented flow autoanalyzer.

Data analysis

Body volume was calculated as $Vb = \pi \times Lb \times (Wb/2)^2$, where Lb and Wb are rotifer body length and width, respectively. Egg volume was calculated with the geometric formula for an ellipsoid: $Ve = (4/3) \times \pi \times (Le/2) \times (We/2)^2$, where Le and We represent egg length and egg width (Zhou et al. 2018). Somatic growth rate was estimated as $g = (\ln Vb - \ln Ve) / t$, where t is the age at which the first egg were produced (Zhou et al. 2018). By estimating the initial egg size of the focal generation by the size of the eggs they produced themselves we assumed that egg size of maternal and focal generations remained the same within food quality treatments.

To evaluate the effects of maternal and contemporary diets and their interactions on life history traits, we applied general linear mixed effects models specifying contemporary and maternal diet treatments as fixed factors and chemostat replicates for contemporary and maternal diets as random factors. Size at first egg production and egg size were \log_2 -transformed prior to analysis. The effect of maternal diet on offspring starvation resistance was studied by taking maternal food quality as fixed factor and food chemostat replicate as random factor. Tukey post hoc comparisons were applied to evaluate the significance of differences among factor levels. All statistical analyses were performed in R software environment ver. 3.4.2 (<www.r-project.org>). Mixed effects analyses were performed with the lme4 package in R (Bates et al. 2015).

Results

Throughout the experiment, the algal molar C:P ratios of the HP and LP+P food quality treatments were very similar (HP: mean: 131, SD: 22, $n=5$; LP+P: mean: 99, SD: 13, $n=5$) and much lower than of the LP treatment (mean: 582, SD: 59, $n=5$; Supplementary material Appendix 1).

Mortality of focal animals during the life table experiment was low as only four out of 180 individuals died before maturation. Size at first egg production of the focal generation was determined by both maternal and contemporary diets, but there was no interaction between these factors (Fig. 2a, Table 1). The contribution of the maternal diet to the variation in size at first egg production was considerably higher than the contribution of the contemporary diet (Table 1). Under any of the contemporary diets, HP and LP+P maternal diets resulted in a similar size at first egg production and both were significantly larger than animals born to mothers cultured in LP food (Fig. 2a, Tukey post hoc comparisons:

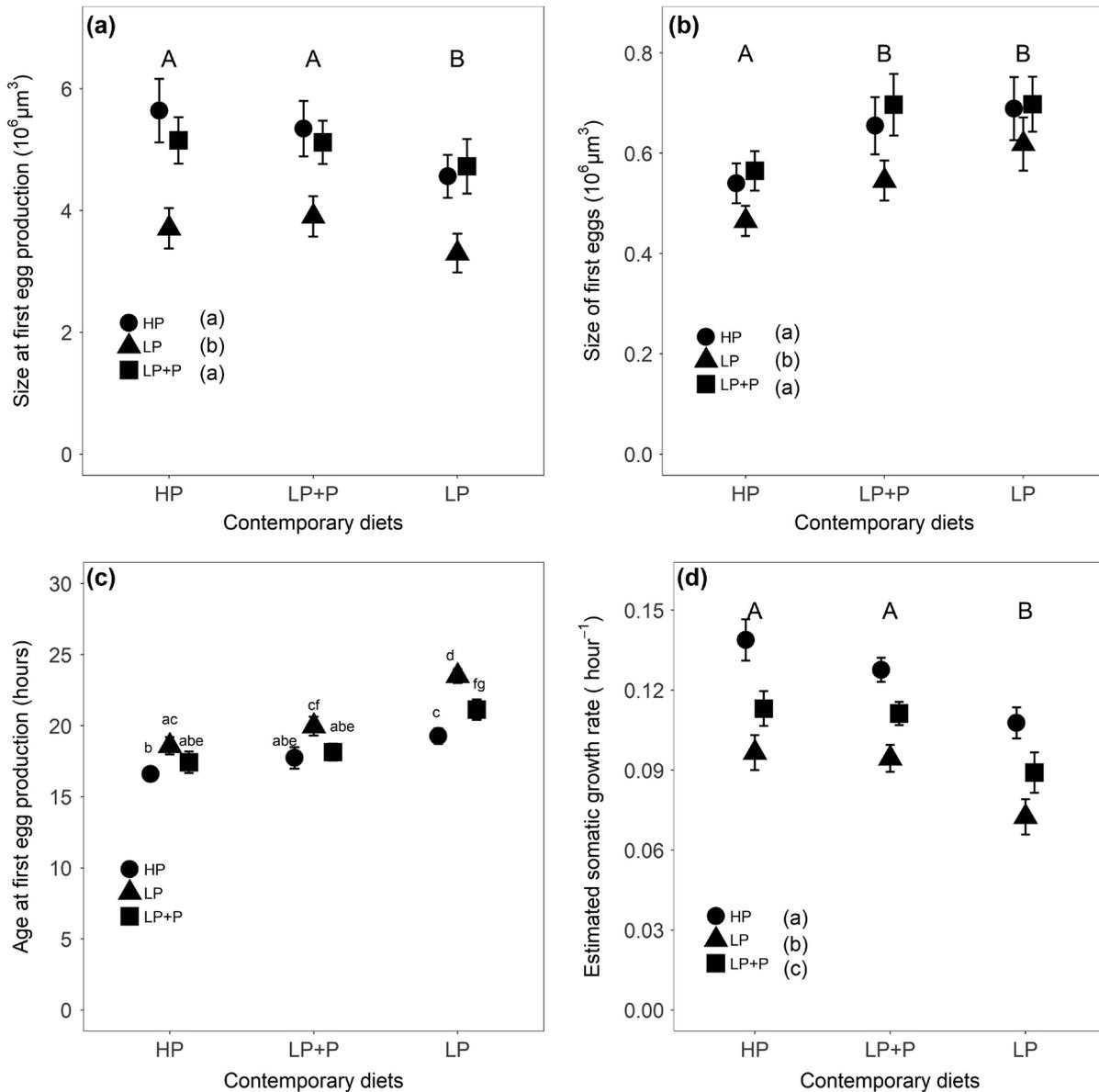


Figure 2. Response of rotifer life history traits in the focal generation to contemporary and maternal food quality treatments. (a) Size at first egg production, (b) size of first eggs, (c) age at first egg production, (d) somatic growth rate. HP: P-rich diet, LP: P-poor diet, LP+P: LP diet enriched with inorganic P. The circles, triangles and squares represent offspring for which the two preceding maternal generations were cultured in HP, LP and LP+P food, respectively. Different uppercase letters indicate a significant difference among contemporary diet treatments according to Tukey post hoc comparisons. Different lowercase letters indicate significant differences among maternal diet treatments, except for age at first egg production (c) for which a significant two-way interaction was found and different lower case letters indicate differences among any multifactorial combinations. Symbols and error bars represent the mean \pm 2 SE.

HP versus LP maternal diets: $p < 0.001$, LP+P versus LP maternal diets: $p = 0.001$). Similarly, for focal animals with a same maternal diet history, there was no difference in size at first egg production between HP and LP+P contemporary diets, but both were larger than in the LP contemporary diet (Fig. 2a, Tukey post hoc comparisons: HP versus LP contemporary diets: $p = 0.005$, LP+P versus LP contemporary diets: $p = 0.008$).

The size of the first eggs was also affected by both maternal and contemporary diets while no significant interaction

was found (Fig. 2b, Table 1). Under a given contemporary diet, the size of the first eggs of focal animals born to HP and LP+P mothers were similar, and both were larger than those produced by offspring born to LP mothers (Fig. 2b, Tukey post hoc comparisons: HP versus LP maternal diets: $p = 0.020$, LP+P versus LP maternal diets: $p = 0.006$). However, for animals with a same maternal diet, LP and LP+P food resulted in the production of larger eggs than HP food (Tukey post hoc comparisons: HP versus LP+P contemporary diets: $p = 0.027$, HP versus LP contemporary

Table 1. Summary of mixed model analysis results of the maternal effect experiment. Maternal and offspring diets and their interaction were specified as fixed factors in the models. SS: sum of squares, MS: mean square, df: degrees of freedom.

| Variables | SS | MS | df | F value | p |
|-------------------------------------|-------|-------|----|---------|--------|
| Size at first egg production | | | | | |
| Maternal diets | 1.34 | 0.67 | 2 | 22.6 | <0.001 |
| Contemporary diets | 0.69 | 0.34 | 2 | 11.7 | 0.004 |
| Maternal diets × Contemporary diets | 0.09 | 0.02 | 4 | 0.8 | 0.516 |
| Size of first eggs | | | | | |
| Maternal diets | 0.45 | 0.22 | 2 | 9.9 | 0.006 |
| Contemporary diets | 0.40 | 0.20 | 2 | 8.8 | 0.006 |
| Maternal diets × Contemporary diets | 0.07 | 0.02 | 4 | 0.8 | 0.545 |
| Age at first egg production | | | | | |
| Maternal diets | 93.2 | 46.6 | 2 | 29.8 | <0.001 |
| Contemporary diets | 282.2 | 141.1 | 2 | 90.1 | <0.001 |
| Maternal diets × Contemporary diets | 30.6 | 7.7 | 4 | 4.9 | 0.001 |
| Estimated somatic growth rate | | | | | |
| Maternal diets | 0.94 | 0.47 | 2 | 47.0 | <0.001 |
| Contemporary diets | 0.30 | 0.15 | 2 | 15.2 | 0.001 |
| Maternal diets × Contemporary diets | 0.067 | 0.017 | 4 | 1.66 | 0.163 |

diets: $p = 0.005$), whereas no significant difference was found between LP + P and LP treatments.

Age at first egg production of rotifers was determined by both maternal and contemporary diets and a relatively weak but significant interaction between these two factors (Fig. 2c, Table 1). Animals born to mothers with an LP history always needed a significantly longer time to produce their first eggs than those born to mothers with an HP history (Fig. 2c, Tukey post hoc comparisons: HP versus LP maternal diets: $p < 0.01$ in all levels of contemporary diets). Age at first reproduction of animals with a maternal LP + P diet was similar to those with an HP maternal diet and lower than in the LP maternal diet (Tukey post hoc comparisons: LP + P versus LP maternal diets: $p < 0.001$) except in an LP contemporary diet, where age at first reproduction of animals with a maternal LP + P diet was intermediate to the other maternal diets (Fig. 2c, Tukey post hoc comparisons: LP + P versus LP maternal diet: $p < 0.001$, HP versus LP + P maternal diet: $p = 0.009$). Contemporary diets also strongly affected the age at first reproduction, given that animals in LP food needed a longer time to produce their first eggs than those in HP and LP + P diets (Fig. 2c, Tukey post hoc comparisons: HP versus LP contemporary diets: $p < 0.001$, LP + P versus LP contemporary diets: $p < 0.001$).

Both maternal and contemporary diets significantly affected somatic growth rate (Fig. 2d, Table 1). Maternal diets contributed considerably more to variation in somatic growth rate than the contemporary diet (Table 1). Under a given contemporary diet, animals in the HP treatment tended to have a higher somatic growth rate than those in the LP + P treatment, and both were higher than in the LP treatment (Fig. 2d, Tukey post hoc comparisons: HP versus LP + P maternal diets: $p = 0.005$, HP versus LP maternal diets: $p < 0.001$, LP + P versus LP maternal diets: $p = 0.005$). The diet of the focal generation also strongly affected the somatic growth rate of animals. Animals in HP diets had a similar growth as those with an LP + P diet, whereas both were higher than animals in the LP treatment (Tukey post hoc

comparisons: HP versus LP contemporary diets: $p = 0.002$, LP + P versus LP contemporary diets: $p = 0.004$).

Neonate starvation resistance proved to be significantly affected by maternal diet. After being inoculated in a food free environment, neonates hatched from eggs produced by LP and LP + P mothers lived on average 63 and 59 h, respectively. In contrast, neonates from HP mothers only lived 51 h (Fig. 3, Tukey post hoc comparisons: HP versus LP + P: $p = 0.05$, HP versus LP: $p = 0.002$, LP versus LP + P: $p = 0.51$).

Discussion

Our study demonstrates strong effects of maternal food quality on key life history traits of rotifer consumers. Consistent with our previous study (Zhou et al. 2018), rotifers that had been cultured in LP food across multiple generations produced larger eggs than those cultured in HP food. However, we found no evidence for an anticipatory maternal effect. In contrast to the idea that larger egg size should result in a higher offspring quality, offspring born to P-limited mothers had a reduced fitness (e.g. smaller size at first egg production, lower somatic growth rates and a higher age at first reproduction) compared to offspring born to mothers reared with P-sufficient food. This pattern was found irrespective of the diet in which the focal generation was grown. More specifically, when fed LP food, animals with an ancestral history of P-limitation had no advantage to animals of which maternal generations had grown under P-sufficient conditions. The fact that LP raised mother animals produced larger eggs and neonates with higher starvation resistance than HP raised mother animals suggests that they increased allocation of carbon under the form of energy rich biomolecules (e.g. lipids) to their offspring. Unless food scarcity typically follows periods of algal P limitation in the habitat where the population of the investigated genotype evolved, the increased C-allocation is more likely a coincidental positive side-effect (Urabe and Sterner 2001) rather than an anticipatory adaptive response.

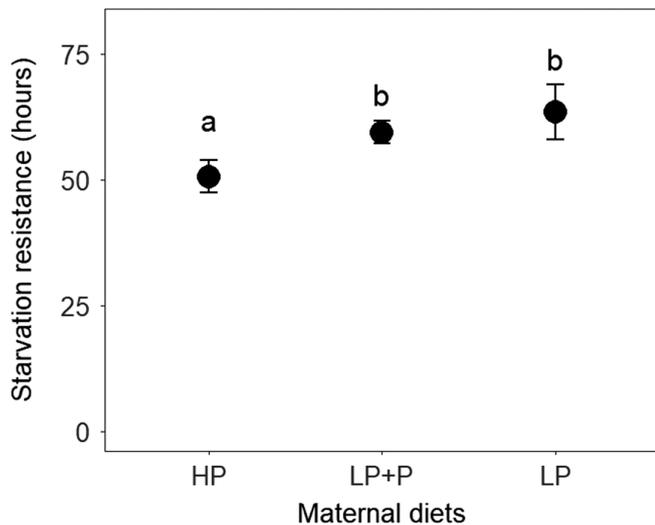


Figure 3. Starvation resistance of neonates in function of maternal diet. HP: P-rich maternal diet, LP: P-poor maternal diet, LP + P: LP maternal diet enriched with inorganic P. Different letters indicate significance differences according to Tukey post hoc comparison. Symbols and error bars represent the mean \pm 2 SE.

The strong negative maternal effects of P-limited food are likely the result from the transmission of poor growing conditions to the offspring (i.e. transmissive maternal effects), possibly in combination with a selfish maternal strategy where the mother generation increases its own fitness at the expense of that of the next generation (i.e. selfish maternal effect, Cunningham and Russell 2000, Kudo 2006), e.g. by allocating less P or valuable biochemical resources or by shunting excess C towards the eggs. Somatic growth rates were more strongly determined by maternal than contemporary diets. Animals with a maternal history of P limitation underperformed compared to those with an HP history. The magnitude in growth difference between both categories proved to be larger than those resulting from differences in contemporary food quality. These results show that animals have difficulties to recover from the negative effects of a P-deficient maternal diet even if their contemporary diet contains sufficient P. It is reasonable to question if such pattern can be well explained by transmissive effects alone given that it fits better to what would be expected from strong selfish maternal effects. Similar cases with relatively strong maternal effects have been reported for consumers exposed to food with imbalanced biochemical composition (Sperfeld and Wacker 2015), and toxic cyanobacteria (Beyer et al. 2017).

Only a few studies have provided evidence for negative maternal effects of stoichiometric mismatch. In *Daphnia*, P-limited mothers allocate less P to their eggs than P-sufficient mother animals (Boersma and Kreutzer 2002, Frost et al. 2010), which seems to result in reduced offspring performance (Frost et al. 2010). Similarly, He et al. (2016) demonstrated that the copepod *Pseudodiaptomus annandalei* reduced its investment of N and the fatty acid DHA into its offspring when feeding on N limited food, which resulted

in a longer naupliar development time and smaller body length at copepodite stage I compared to mothers fed with an N-rich diet. Interestingly, in contrast to our results, poor offspring performance due to nutrient limitation was associated with reductions in egg size in these studies.

P-limitation of algal resources may affect the performance of consumers not only via direct effects of P-shortage but also through indirect effects via other food quality aspects (Rothhaupt 1995, Zhou et al. 2018). Unique to our study is that its design allows decomposing maternal effects of P-limitation into these two components. As such it has the potential to reveal hidden anticipatory maternal responses to indirect effects of P-limitation that may be masked by non-adaptive transmitted effects of direct P-limitation or vice versa. If maternal generations allow their offspring to cope better with negative indirect effects of P-limitation, offspring with an LP + P ancestral feeding history should outperform offspring with an HP ancestral feeding history under LP + P feeding conditions. Alternatively, if maternal generations prepare their offspring only in coping with negative direct effects of P-limitation, offspring with an LP ancestral feeding history should outperform offspring with an LP + P ancestral feeding history under LP feeding conditions. However, our results provide no evidence for such anticipatory effects. Under LP + P feeding conditions, somatic growth rate was lower in animals with a LP + P than an HP ancestral feeding history while age at first reproduction was higher. Similarly, we found no evidence for a higher performance between animals with an LP compared to an LP + P ancestral feeding history under LP feeding conditions.

Our data also show that the relative strength of direct and indirect maternal effects of P limitation vary strongly among different life history traits. Maternal diets had large effects on the size at first egg production of the focal generations, which seemed entirely determined by the P content of the algae in the maternal diets and not by the culture history of these algae. In contrast, the indirect effect of P limitation was more pronounced in somatic growth rate and age at first egg production, for which values of offspring from LP + P mothers tended to be intermediate to those from HP and LP mothers. The most remarkable response, however, was found for the size of the eggs produced by the focal animals where effects of maternal diets were mainly generated by direct effects in contrast to the effects of contemporary diets which were largely determined by indirect mechanisms of food P limitation. This latter observation clearly indicates that the mechanisms through which maternal diets affect egg size of the focal animals differ from those of the contemporary diets. Generally, in a given environment, the initial size of animals is known to be positively correlated to the body size of their mothers (Boersma 1997, Boersma et al. 2000, Neuheimer et al. 2015, Rollinson and Rowe 2016). Animals that were continuously cultured with HP or LP + P food across generations were consistently larger than animals cultured with LP food. In line with the idea of a positive body size–egg size relationship, we found a very similar pattern of size differences between

eggs of different maternal feeding histories within each of the contemporary food quality treatments. However, this pattern was broken by the response pattern of egg size to contemporary food quality.

Our results were obtained from the study of one single clone. This limits our ability to generalize our findings. However, given that we worked with a haphazardly selected clone, it likely represents other genotypes of the same and possibly also other zooplankton species as well. In any case, our results can be considered as proof of concept for the idea that the direct and indirect consequences of P limitation may strongly impact zooplankton consumers through maternal effects. However, our results also indicate that the relative impact of these direct and indirect effects may largely be trait-dependent.

Conclusion

Our study demonstrates that phosphorus limitation of the maternal generation may strongly reduce the performance of zooplankton consumers through transmissive maternal effects, possibly in combination with selfish maternal effects. Importantly, depending on the traits under consideration, such maternal effects were not limited to the direct effects of P-limitation but were also caused by indirect effects of such limitation. We found no evidence for anticipatory maternal effects. Although P limitation resulted in the production of larger eggs and a higher starvation resistance, it did not contribute to a higher ability to cope with P-limitation. The relative strength of the maternal effects is remarkable, given that the growth rate of the focal generation was found to be more determined by the ancestral than by the contemporary diet. First, it demonstrates the need for taking into account maternal effects in the design of ecological stoichiometry studies by acclimatizing animals to experimental food quality treatments during at least one, but preferentially two or more maternal generations. Second, stoichiometric studies in which experimental manipulations of stoichiometric mismatch are created by artificially enriching P-limited algae to various degrees, may result in an underappreciation of the effects of such mismatch because they exclude indirect effects of P-limitation (see also Zhou et al. 2018). Third, strong maternal effects may also be important for the interpretation of consumer-producer dynamics under natural conditions, as they may produce time lags in the responses of consumer growth (Frost et al. 2010) and associated ecosystem functions (e.g. such as grazing and nutrient cycling; Frost et al. 2004, He and Wang 2008, Suzuki-Ohno et al. 2012, Urabe et al. 2018) to changes in the degree of P-limitation. The latter is expected to be mainly relevant in aquatic systems where the time scale of recurrent changes in stoichiometric seston quality approximates the generation time of consumers.

Data availability statement

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.f4qrfj6sg>> (Zhou and Declerck 2020).

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Statement of authorship – LZ conceived the ideas, LZ and SAJD designed methodology. LZ conducted the experiments, collected and analyzed the data. LZ and SAJD wrote the manuscript.

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Supplementary material (available online as Appendix oik-06898 at <www.oikosjournal.org/appendix/oik-06898>). Appendix 1.