FEAR AND FOOD: EFFECTS OF PREDATOR-DERIVED CHEMICAL CUES AND STOICHIOMETRIC FOOD QUALITY ON *DAPHNIA*

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Abstract

While resource quality and predator-derived chemical cues can each have profound effects on zooplankton populations and their function in ecosystems, the strength and direction of their interactive effects remain unclear. We conducted laboratory experiments to evaluate how stoichiometric food quality (i.e., algal carbon (C):phosphorus (P) ratios) affects responses of the zooplankter, *Daphnia pulicaria*, to predator-derived chemical cues. We compared growth rates, body P content, metabolic rates, life-history shifts, and survival of differentially P-nourished *Daphnia* in the presence and absence of chemical cues derived from fish predators. We found effects of predator cues and/or stoichiometric food quality on all measured traits of *Daphnia.* Exposure to fish cues led to reduced growth and increased metabolic rates but had little effect on the body %P content of *Daphnia*. Elevated algal C:P ratios reduced growth and body %P, and increased mass-specific respiration rates. While most of the effects of predator cues and algal C:P ratios of *Daphnia* were non-interactive, reduced survival and relatedly reduced population growth rates that resulted from P-poor food were amplified in the presence of predator-derived cues. Our results demonstrate that stoichiometric food quality interacts with anti-predator responses of *Daphnia*, but these effects are largely trait-dependent and appear connected to animal life-history evolution. Given the ubiquity of predators and P-poor food in lake ecosystems, our results highlight the importance of the interactive responses of animals to predator cues and poor nutrition.

Introduction

The mere presence of predators can change the behavior, morphology, and life-history traits of prey (Stibor 1992; Tollrian 1995; Clinchy et al. 2013). In aquatic ecosystems, the presence of predators can be signaled through a variety of chemicals present in the water column, including kairomones, which are released directly by the predator, and alarm cues, which are released from prey species that have been attacked or alerted to the presence of predators (Pijanowska 1997; Dicke and Grostal 2001; Pestana et al. 2013). Prey have evolved complex behavioral, physiological, and demographic responses to these chemicals to reduce the risk of mortality due to predation (Lima 1998; Eklöv et al. 2006). The type and strength of such predator-induced responses and their subsequent effects on prey population dynamics can be influenced by environmental conditions, including food availability. Predator cues and the availability of food interactively affect prey reproduction (Pauwels et al. 2010), growth (DeLong and Walsh 2015), and behavior (Heithaus and Dill 2002). Recent work has also found variable stoichiometric responses in vertebrate prey exposed to predators (Costello and Michel 2013), especially when confronted with poor food quality (Dalton and Flecker 2014) and different environmental temperatures (Schmitz et al. 2016). Here we examine whether and how food quality affects the physiological responses of zooplankton exposed to predator cues and consequences on their population growth rates.

Low nutrient content in food results in elemental imbalances between zooplankton consumers, such as *Daphnia*, and their food resources (Sterner and Elser 2002). Elemental imbalances alter a range of physiological processes in consumers, including feeding rates, carbon assimilation, metabolic rates, and growth (Sterner and Elser 2002; Hessen et al. 2013). Specifically, herbivores that consume food with high carbon (C):phosphorus (P) ratios exhibit

decreased body P content and reduced P excretion rates (DeMott et al. 1998), which is consistent with acute P-limitation. Similarly, animals consuming food having high C:P ratios alter C-based metabolic processes that appear to eliminate excess intake of C (Darchambeau et al. 2003; Frost et al. 2005). Due to constraints on animal energy and material budgets imposed by poor elemental nutrition, prey may alter their physiological responses when exposed to predator cues (Hawlena and Schmitz 2010; Dalton and Flecker 2014; Zhang et al. 2016). Poor stoichiometric food quality could also affect key life-history responses in prey to predator exposure (Jeyasingh and Weider 2005). *Daphnia* typically respond to chemical cues released from large bodied, visual predators by reproducing earlier and at a smaller size, which increases reproductive output, at least in younger population cohorts (Stibor 1992; Weider and Pijanowska 2003). These life-history traits are also sensitive to poor stoichiometric food quality, which generally limits reproductive rates by increasing age of first reproduction and reducing brood sizes (Weider et al. 2008; Prater et al. 2016). Because poor stoichiometric food quality constrains reproduction, poorly-nourished prey may have reduced abilities to respond to perceived increases in predation risk. When present together, the effects of predator cues and poor elemental food quality could thus amplify or nullify the effects of each other and result in significant interactive effects.

The responses of life-history traits of prey to environmental stress, both in terms of predator exposure and poor elemental food quality, could translate into altered population growth rates. Population growth rates are a function of prey survival and reproduction as these rates determine gains and losses of the prey population (Sibly and Hone 2002). As mortality rates increase with the presence of predators, exposed prey generally respond by shifting to earlier reproduction (Stearns 1992; Stibor 1992). Predator-induced shifts in life-history traits may require higher investments of resources at the time of first reproduction, which may be limited by

nutrient-poor food (Zhang et al. 2016). If so, low nutrient food may constrain the earlier initiation of population growth, which can result from exposure to predator-derived cues. The nature of such interactive effects of predator cues and elemental food quality, if present, would depend of the relative strength and timing of effects on reproduction and animal mortality.

In this study, we examined whether and how predator-induced responses of *Daphnia pulicaria* are mediated by stoichiometric food quality. We did so by raising *Daphnia* in the laboratory in the absence and presence of cues released from juvenile bluegill (*Lepomis macrochrius*), a common fish predator, and manipulating C:P ratios in their algal food. We then measured physiological and life-history responses of *Daphnia* including mass-specific growth rates, metabolic rates, reproduction, and survival. We further used reproduction and survival data to examine population growth responses. We expected both increased diet C:P ratios and exposure to predator cues to reduce growth rates, increase metabolic rates, and reduce animal survival. We also predicted that animals exposed to predator cues would optimize reproduction, in terms of number and timing of offspring produced, brood size, and neonate size, but that these responses to predator exposure would be limited by higher C:P ratios in the diet. Finally, we predicted exposure to predator cues would reduce population growth rates and that the size of this reduction would increase with food C:P ratios.

Methods

Experimental design and treatments. We examined responses of differentially P-nourished *Daphnia pulicaria* to fish-predator cues. The daphnid clone used in this study was originally collected from Big Cedar Lake in south-central Ontario (44.606638, -78.171669) and cultured in the laboratory for ~4 years prior to use in our experiments. We collected juvenile

bluegill (*Lepomis macrochirus*) from the Otonabee River near Peterborough, Ontario, Canada (44.371780, -78.286718) to produce predator cues.

Daphnia brood mothers were reared in COMBO media (Kilham et al. 1998) and fed green algae (*Scenedesmus obliquus*, Canada Physiological Culture Centre, Strain 10). Neonates (<24 hr old) were collected from the 2nd – 5th broods of these mothers and randomly assigned to different treatment combinations. For all experiments, *Daphnia* were reared individually in 50 ml plastic conical centrifuge tubes, which were maintained in a temperature-controlled growth chamber (20°C) and under low irradiance with a 14:10 light:dark photoperiod.

We measured several response variables during a series of full factorial 3×2 laboratory experiments where *D. pulicaria* were exposed to all six treatment combinations of food quality (C:P 100, 300, and 600) and predator cues (absent or present). These algal C:P ratios were selected to fit within the range previously reported for lake ecosystems (Hassett et al. 1997; Elser et al. 2000a). To prepare the experimental diets, we cultured *S. obliquus* with varying media P concentrations and dilution rates in multiple culture flasks. After harvesting, algal suspensions were centrifuged for 10 min at 5000 rpm and re-suspended in N- and P-free COMBO media. Algal P content was determined by persulfate digestion followed by molybdate-blue ascorbic acid colorimetry and spectrometry (APHA 1992), which allowed us to mix algae of different P contents to produce the three food P content levels. We determined the C and N content of algae with an elemental analyzer (Vario EL III, Elementar Incorporated, Mount Laurel, New Jersey). The algal C:N:P ratios were determined on post-mixed food, using the same methods, to ensure that the nominal food types were created.

Fish cues were collected from juvenile *L. macrochirus* (80-110mm) housed for 24 hrs in tanks with 4 L of N- and P-free COMBO media. The fish were fed live *Daphnia* during their

incubation, so the media contained both predator kairomones as well as conspecific alarm cues. The fish-conditioned media was then membrane filtered (0.40 µm) and frozen at -20° C. Fresh fish cues were harvested and frozen every four days, and before use in experiments the cue media was thawed and diluted to a concentration equivalent to that produced by 0.1 fish L^{-1} day^{-1}. We selected this concentration of the predator cue based on reported concentrations from past experiments that also examined *Daphnia* responses to fish-predators (Pauwels et al. 2010; Tollrian et al. 2015). While the natural relevance of this concentration is unclear due to differences between lakes and laboratory bottles (e.g., Schindler et al. 1997), the selected fish density (#/L) is moderately higher than that reported for lake littoral zones (Mittelbach 1988). Growth media was refreshed in all the experimental tubes every other day.

To account for the effects of additional P from the fish cue on food algal P content, we determined the concentration of soluble reactive phosphorus (SRP) in the cue media prior to food algae preparation. By using P concentration values from both the fish cue and algal food cultures, we mixed food concentrations to the desired dietary C:P ratio (100, 300, and 600) under the assumption that 100% of the fish derived-P would be acquired by the food algae.

Growth and body elemental content. We examined the effects of food C:P ratios and predator cues on growth rates of *D. pulicaria* with a 6-day growth experiment. Age-synchronized neonates were collected (<24 hr old), and subsets of these neonates were randomly assigned into replicate tubes to be later used to quantify growth rates (n=10) for each treatment combination. Initial neonate mass was determined by collecting three subsets of 20 neonates, which were transferred into pre-weighed aluminum tins, placed into a drying oven (at 60°C) for 24 hr, and reweighed with a microbalance. Experimental animals were reared in treatment conditions for 6 days and fed 4 mg C L^{-1} of either C:P 100, 300, or 600 food every other day. These food rations

were based on animal mass and feeding rates and provided food quantities in excess of those estimates of daily food requirements for fast-growing daphnids as previously verified in similar experiments (Wagner and Frost 2012). After 6 days of growth, all experimental animals were removed from tubes, rinsed with N and P free COMBO, and dried for 24 hr. Mass-specific growth rates (MSGR) were calculated as:

$$\text{MSGR} = \frac{ln(M2) - ln(M1)}{time}$$

where M_2 is the final mass per *Daphnia*, M_1 is the average initial neonate mass, and time is number of days of growth. Animals were saved for P analysis using the same methods as above on persulfate-digested animal bodies. The remaining animals were pooled and weighed in tin cups, which were then used to measure body C and N content; however, due to mortality issues and sample pooling that resulted in reduced sample size, the results of these analyses were not included here but can be found in our supplementary material (Supplementary material Appendix 1, Fig. A2).

Metabolic rates. To assess metabolic responses of *D. pulicaria*, we measured rates of O_2 consumption of 6-day old animals following the approach of McFeeters and Frost (2012). Animals were raised following the same procedure as outlined above. Small groups of *Daphnia* (3–6 individuals) were placed in respiration vessels (0.5 ml) containing COMBO and the assigned algal food C:P ratio-fish cue treatment. The vessels were placed in a water bath (21°C) and animals allowed to acclimatize for 10 min. Oxygen consumption was recorded using a micro-oxygen probe (MRCh system; Unisense A/S; Aarhus, Denmark) for 10 min under low light to limit photosynthetic activity. For every five samples, two blank vessels (without *Daphnia*) were measured to determine the effect of algae and fish cues on oxygen concentrations; these baseline values were then used to correct oxygen consumption slopes for

each treatment. These baseline changes in O_2 concentration were relatively small (~1%) relative to oxygen changes that occurred when *Daphnia* were in the vessels. After respiration measurements were made, animals were collected, dried for 24 hr, and weighed. Mass-specific respiration rates were calculated by dividing the average corrected oxygen consumption by both total *Daphnia* mass and time of measurement.

Life table experiment. A 30-day laboratory life table experiment was conducted to investigate differences in life history trade-offs between animals fed different food C:P ratios and in the presence or absence of fish predator cues. For each treatment, 25 *Daphnia* were individually reared in tubes containing 30 mL (age 0–6 day) and then 40 mL (age 7–30 day) of N-and P-free COMBO media. Animals were fed 4 mg C L^{-1} of algal food every other day for the first 6 d and then 8 mg C L^{-1} for the rest of the experiment. These food rations were based on animal mass and feeding rates and provided food quantities in excess of those estimates of daily food requirements for fast-growing daphnids. Each tube was checked daily for animal mortality and reproduction. We measured daily production of offspring, size at reproductive maturity, brood size, and survival. Mortality and survival data were then used to calculate intrinsic rate of increase (r) using Euler's equation,

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

where x is age (days), l_x is age-specific survivorship, and m_x is age-specific fecundity. Reproductive output (R_0) was calculated by summing the product of lx and mx from each treatment on each day of the experiment. To measure differences in body size, 10 additional animals of each treatment were reared simultaneously; photos were taken of each animal immediately after the first clutch of neonates was released and then every 7 d for the rest of the experiment. Body length measurements were made from the top of the eye to the base of the tail.

Neonate experiment. To assess how food C:P ratios and fish cues affected neonate size, neonates were collected from the 1st-3rd broods of animals raised in treatments for 15 days. Ten replicate mother *Daphnia* were raised in each treatment; three neonates from each the first five reproducing animals from each brood were selected randomly for measurement. Photos were taken of the neonates and of the mothers for body length measurements. Daphnid mass was estimated with a length-mass power function which was originally developed from a *D. pulicaria* clone from a nearby lake (Prater et al. 2017). Mass-specific reproductive investment was calculated as neonate mass/mother mass *100.

Statistical analyses. Effects of food C:P ratios and predator cues on MSGR, respiration, body %P, body size, and brood size were analyzed using 2-way ANOVA, with food C:P ratio and predator cue treatments used as fixed effects. Body size, size at first reproduction, neonate size, and reproductive investment were analyzed with a 2-way repeated-measures ANOVA using linear mixed-effect models with either individual or mother identity as random effects. The effect sizes from the 2-way ANOVA models were estimated using eta-squared (η^2), which is a measure of the proportion of variance accounted for by each main effect and interaction term. Tukey's HSD was used to assess treatment differences for all parametric tests. Differences in survival were assessed using Cox proportional hazards regression models (Cox 1972), using food C:P ratios and predator cue treatments as covariates. The food C:P failed to pass the assumption of proportional hazards, so the levels of each covariate were analyzed individually. To compare population parameters, 1000 values of r and R_o were generated for each treatment combination using a jackknife technique (Meyer et al. 1986). As the residuals violated parametric assumptions of normality, they were statistically compared using a Kruskal–Wallis H test with Scheirer–Ray–Hare extension (Ashforth and Yan 2008, Prater et al. 2016), which is a non-

parametric equivalent to a 2-way ANOVA. We used $\alpha = 0.05$ and all data were checked for normality by visually assessing residuals of each model. Homogeneity of variances was assessed by plotting residuals of each model against fitted values. We used R statistical software (version 3.3.3 with R studio) for all statistical analyses and image analysis software (IMT i-Solution) for all daphnid body measurements.

Results

Growth and respiration. We found a significant interactive effect of food C:P ratios and predator cues on the mass-specific growth rates of *D. pulicaria* (Table 1). While this indicates that the response of growth rates to predator cues varied with food C:P ratios, this interactive effect ($\eta^2 = 0.04$) was dwarfed by the main effects of slower growth produced by higher food C:P ratios ($\eta^2 = 0.36$) and the presence of cues ($\eta^2 = 0.42$, Fig. 1a). We also found effects of treatments on *Daphnia* body sizes over the span of the 30-day experiment; a food C:P and predator cue interaction was only found on the last day of the experiment, while relatively large main effects of both treatments (η^2=0.13-0.63) were found on body sizes on animals aged 14, 21, and 30 days (Table 1, Supplementary material Appendix 1 Fig. A1). No significant interactive effects were found on respiration rates of *Daphnia* (Table 1, Fig. 1b). Respiration rates were elevated at the highest food C:P ratios and by exposure to fish cues (Table 1; Fig. 1b). We also did not detect significant interactive effects between food C:P ratios and predator cues on the body %P content of *Daphnia* (Table 1)*;* increasing algal C:P ratios reduced %P in *Daphnia*, regardless of cue exposure (Fig. 1c).

Life history traits and survival. While there was no interactive effect of food C:P ratios and exposure to predator cues on the size at first reproduction of *Daphnia* (Table 1), this parameter was reduced in response to each treatment independently (Fig. 2a). In terms of neonate

size, there was no food C:P ratio × cue interaction (Fig. 2b) but increasing food C:P ratios consumed by *Daphnia* resulted in smaller offspring (repeated-measures: $F_{2,58}$=37.55, p<0.001). There was an interactive effect of food C:P ratios and predator cues on mass-specific reproductive investment, which was measured as neonate mass relative to maternal mass (repeated-measures interaction: $F_{2,297}$=4.62, p=0.011; Fig. 2c). While this parameter increased in response to cues, the size of this effect was larger for the food C:P ratio 100 and especially for the food C:P ratio 600 (Fig. 2c). In our brood size analysis, we found an interactive effect between our two treatments with strong effects of cues on animals consuming high food C:P ratios. In these animals, exposure to predator cues increased the number of neonates born in the first brood compared to that produced by unexposed animals (Table 1, Fig. 2d, Food C:P × Cue: η^2=0.12). In contrast, predator cues had minimal influence on brood sizes of animals eating food C:P ratios of 100 and 300 (Fig. 2d).

Cox regression showed food quality and cue exposure interacted to affect *Daphnia* survival (Supplementary material Appendix 1, Table A1). Survival in food C:P 100 and 300 treatments was high (92-96%) both in the presence and absence of predator cues (Fig. 3a,b). At food C:P ratio 600, survival of non-cue exposed animals was reduced to approximately 50% of animals at the end of the experiment. This effect in P-stressed animals was amplified by exposure to fish cues, as we observed near population extinction (~96% mortality) of predator-exposed animals fed P-poor algae over the 30-day experiment (Fig. 3c). Cox proportional hazard models showed significant treatment effects on survival, but only in the food C:P 600 treatment. In the absence of cues, the hazard ratio of animals fed food C:P 600 (HR=20.8, 95% CI=2.7-157.7) indicated that mortality risk increased by ~ 20 times relative to animals raised on high P. When animals were raised on food C:P 600 and additionally exposed to predator cues, the

estimated hazard ratio increased to 59.4 (95% CI=7.9-445.5), meaning that animals exposed to both cues and high food C:P ratios had ~ 60 times higher mortality risk relative to unexposed animals consuming high food quality.

Reproductive output and population growth rates. There was no interactive effect of treatments on reproductive output (R_0, Table 2). As food C:P ratios increased from 100 to 600, there was a reduction of R_0 of over 85% in both cue treatment levels (Fig. 4a). Additionally, exposure to predator cues led to reduced R_0 across all diet treatments (Table 2). Observed differences in reproduction and survival translated into differences in the population-level parameter of intrinsic rate of increase (r), which is a measure of population growth and organismal fitness. Food quality and cue treatments had significant interactive effects on r (Table 2, Fig. 4b). In high-P conditions, r was approximately 5% in higher in the fish cue treatment, but as food C:P ratios increased, there was a 27% reduction of r of *Daphnia* exposed to predator cues, relative to those in the absence of cues (Fig. 4b).

Discussion

Prey species face the difficult task of maximizing fitness in environments having variable predator and nutritional conditions. This challenge is not trivial from a prey's perspective because small changes in their reproduction and survival can yield substantial differences in population growth rates (Frost et al. 2010; Storm and Lima 2010). Following this, prey that can detect and respond to mortality risks should be at an advantage if this leads to altered life-history traits, including reproduction, that account for changes in expected longevity. Such life-history shifts, in terms of reproductive timing and effort, might only be observed in predator-exposed prey if these responses are otherwise costly in the absence of high predator mortality risk

(Tollrian 1995; Riessen 2012). Matching these expectations, prey modify their physiology, life-history traits, and morphology when exposed to cues that signal predator presence and a higher risk of mortality (Stibor 1992; Beckerman et al. 2007). It remains unclear, however, whether and how life-history responses to predator exposure would change with the nutritional state of prey species.

From a physiological perspective, strong interactive responses to the combined effects of predator cues and poor nutrition could emerge if these responses share a common energetic or material basis. Yet, our individual-based measurements failed to match this prediction, as interactive effects were weak or insignificant and animals exposed to fish cues grew slower, respired faster, and exhibited smaller body size across all ages, regardless of diet. These main effects of predator cues are consistent with previously documented patterns of prey life-history shifts in response to size-selective predation (Stibor 1992; Gliwicz and Maszczyk 2007). In this case, reduced growth rates and smaller body sizes may have resulted from energy and nutrients being redirected away from biomass accumulation and used for other purposes. For example, the maintenance of high body P in our study animals despite reduced growth rates produced by exposure to predator cues suggests that P-rich RNA was instead used for reproduction (e.g., oogenesis; Markow et al. 2001) or cellular maintenance functions (Bertram et al. 2006). It thus appears that elemental food quality may not strongly modify or constrain physiologically-linked prey responses to predator cues (Jeyasingh and Weider 2005).

Similar to life-history traits and body %P, we found no interactive effects of predator cues and food quality on metabolic rates. While predator cues increased *Daphnia* respiration rates in well-fed animals, no apparent effect of cues were observed in the most P-stressed animals, who also exhibited elevated respiration rates in the absence of predator cues. It is

unclear why cue exposure did not further increase mass-specific respiration rates beyond that produced by poor food quality. While there may be an upper ceiling to elevated *Daphnia* respiration rates that cannot be exceeded regardless of the combined presence of predator cues and poor food quality, these effects may be partly mediated by direct treatment effects on animal body mass. As our experiments were not designed to separate out the drivers (e.g., mass vs. metabolic reshaping) of elevated mass-specific respiration, future work is needed to better examine how nutrition and predator cues alter metabolic rates of *Daphnia.*

Predator cues are known to elicit changes in prey life-history traits related to reproduction (Macháček 1991; Weider and Pijanowska 1993; Reede 1997). To compensate for lower lifetime reproductive success resulting from reduced longevity, reproductive output can be increased in younger *Daphnia* exposed to predator cues (Macháček 1991; Stibor 1992). We found evidence of these changes, with interactive effects of predatory cues and food quality on daphnid mass-specific reproductive investment and brood size. *Daphnia* invested more strongly in reproduction when exposed to predator cues largely due to smaller maternal mass and invariant neonate production. We found that this mass-specific reproductive investment increased the most in animals consuming poor food quality, which indicates this predator cue induced response was not eliminated with acute nutrient stress in *Daphnia*. The number of neonates in the first brood was only increased in animals experiencing increased risk while consuming good food quality. Otherwise, cues either had no effect (for food C:P ratios 100 and 300) or possibly reduced brood size (food C:P ratio 600) on the first three broods. Consequently, despite changes in animal growth and metabolism, exposure to predator cues yielded little effect on total reproduction. The insensitivity of reproductive output (R_0) to predator cue-food quality interactions further indicates its importance even in animals experiencing nutritional and predator-induced stress.

Mortality rates of *Daphnia* were modified by the presence of predator cues and by food quality. Highest survival occurred in animals consuming high-P food, regardless of predator treatment. Thus, shifting growth and timing of reproduction to younger instars did not appear to affect short-term survival (~30 days) of well-nourished animals. While predator cues had minimal effects on survival of well-nourished prey, mortality in P-stressed animals increased dramatically with exposure to predator cues. Therefore, sustained P-stress appears to exacerbate trade-offs between sustaining reproduction and maintenance processes underlying survival (McNamara and Buchanan 2005).

While we found interactive effects of high food C:P ratios and predator cues on a number of physiological and life-history responses, it is unclear how these effects would change the ecological role of *Daphnia* in lake ecosystems. For example, in terms of respiration, predator exposure increased daphnid respiration only in well-nourished animals. These changes in respiration, if accompanied by increased feeding rates or altered feeding behavior, could alter *Daphnia*-phytoplankton dynamics at a whole-lake scale. Such changes may not appear in lakes with high food C:P ratios where increased respiration rates were found in *Daphnia* regardless of predator-cue exposure. In addition, it is unclear how interactive effects of food quality and predator cues on *Daphnia* population growth (r) observed in this lab setting would translate to natural conditions in lakes. This uncertainty rests, in part, on the extent that mortality rates of *Daphnia* are higher in predator-rich lakes due to direct losses to predators beyond that generated simply by exposure to predators. In other words, higher mortality rates observed in P-stressed, predator exposed animals may be lower than those generated by predation itself. Alternatively, direct predator effects on nutrient-limited *Daphnia* populations may be over-estimated if individuals escaping consumption nevertheless experience high mortality from exposure to

predator cues. To reduce this uncertainty, future work could focus on *in situ* zooplankton population responses to predators and their cues in lakes with variable food quality and predator densities.

For a parthenogenetic invertebrate such as *Daphnia*, the timing and quantity of early reproduction can strongly drive population growth over relatively short, but environmentally relevant (~30 days), time periods (McCauley et al. 1996; Pestana et al. 2013). We found population growth rates in well-nourished *Daphnia* increased modestly with exposure to predator cues, which was driven primarily by higher reproductive investment, larger first brood sizes, and high survival. This indicates that *Daphnia* feeding on high quality food while under threat of fish predation compensate by accelerating reproduction (Zhang et al. 2016). This pattern was opposite in P-deprived *Daphnia*, whose population growth rates were depressed by the presence of fish cues. This reduced population growth rate corresponded with both smaller brood sizes and remarkably higher mortality rates. It thus appears that stress generated by low P food limits the ability of *Daphnia* to maintain early and increased reproduction without sacrificing survival over subsequent time periods. Overall, our findings of strong interactive effects on survival and population growth, despite limited evidence from individual-based parameters, highlight the importance of using integrated parameters such as intrinsic growth rates for understanding the effects of predators and food quality on prey dynamics in natural populations.

We have shown how predator cues and food C:P ratios interact to affect survival and some aspects of reproduction, with significant consequences for *Daphnia* population growth rates. As large differences in predation pressure and food quality occur in lakes across summer growing seasons (Sommer et al. 2012), the interactive nature of these effects may combine to influence population dynamics and biomass production in nature. A fuller understanding of these

stoichiometric food quality effects on predator-induced responses will partly include deeper knowledge of molecular underpinning of prey physiological changes (Wagner et al. 2013; Mitchell et al. 2017). Such work could include studying how cue detection specifically translates into changes in growth, reproduction, and metabolism. In addition, information on the elemental requirements of predator cue responses at the biochemical level (Elser et al. 2000b) is necessary to understand how poor food quality may constrain anti-predator life-history traits. Our results also indicate that differences in reproduction and survival produced by predator cues may lead to significant effects on population growth rate or organismal fitness. These life-history responses should be incorporated into models of predator-prey interactions, including stoichiometrically-explicit models (Elser et al. 2012; Leroux and Schmitz 2015), to better understand potential impacts of these changes over multiple generations. Similar studies should be conducted on more diverse taxa with other forms of nutrient limitation to determine the nature and ubiquity of stoichiometric constraints on life-history responses to predation risk. Our results on an important zooplankton consumer thus provide insights into a relatively understudied connection between the bottom up effects of food resources and top down effects of predation and point to a need to better connect predator-prey dynamics to emerging issues in nutritional ecology.

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List of Tables

Table 1: Main and interactive effects of food C:P ratios and fish cues on *Daphnia* traits, using two-way ANOVAs. η^2 values estimate the proportion of error associated with main effects and interaction terms, out of total variation. Significant effects are in bold.

	Food C:P				Cue				Food C:P × Cue			
	df	η^2	F	p	df	η^2	F	p	df	η^2	F	p
MSGR	2,53	0.36	53.9	**<0.001**	1,53	0.42	126.0	**<0.001**	2,53	0.04	6.51	**0.003**
Body size												
Day 14	2,44	0.64	94.4	**<0.001**	1,44	0.12	61.4	**<0.001**	2,44	0.00	0.68	0.51
Day 21	2,42	0.75	202.9	**<0.001**	1,42	0.17	89.8	**<0.001**	2,42	0.00	0.21	0.81
Day 30	2,38	0.71	129.6	**<0.001**	1,38	0.17	61.3	**<0.001**	2,38	0.02	3.56	**0.038**
Respiration	2,24	0.76	20.1	**<0.001**	1,24	0.06	4.35	**0.048**	2,24	0.02	1.59	0.23
% P	2,21	0.89	120.9	**<0.001**	1,21	0.01	3.39	0.08	2,21	0.02	2.63	0.10
SFR	2,47	0.38	42.8	**<0.001**	1,47	0.38	83.0	**<0.001**	2,47	0.03	2.78	0.07
Brood size												
1st brood	2,135	0.18	17.6	**<0.001**	1,135	0.00	0.18	0.67	2,135	0.12	11.5	**<0.001**
2nd brood	2,128	0.65	125.9	**<0.001**	1,128	0.02	6.43	**0.012**	2,128	0.01	1.94	0.15
3rd brood	2,121	0.51	67.3	**<0.001**	1,121	0.01	2.89	0.09	2,121	0.01	1.82	0.17

Table 2: Main and interactive effects of food C:P ratios and fish cues on reproductive output (R_0) and population growth rate (r) using Kruskal–Wallis H test with Scheirer–Ray–Hare extension. Significant effects are in bold.

	Food C:P		Cue		Food C:P × Cue	
	H	p	H	p	H	p
R_0	5442.6	**<0.001**	485.1	**<0.001**	0.63	0.73
r	5379.9	**<0.001**	52.4	**<0.001**	444.9	**<0.001**

List of Figures

Figure 1. (a) Mass-specific growth rates, (b) respiration rates, and (c) phosphorus body content of *Daphnia* fed a gradient of food C:P ratios in the absence and presence of predator cues. Asterisks indicate means that are significantly different within each diet treatment ($p<0.05$, Tukey's HSD). Error bars show ±1 SE.

Figure 2. Effects of food C:P ratios and fish cues on (a) size of first reproduction, (b) neonate size, and (c) mass-specific reproductive investment. Neonates used for analysis were collected from the first 3 broods of mothers raised in each treatment combination. Mass-specific reproductive investment was calculated as neonate mass/ mother mass *100. Bottom panels show *Daphnia* brood size differences from the first 3 broods of animals fed algal food ratios of (d) C:P 100, (e) C:P 300, and (f) C:P 600 and in the absence (open triangles) or presence (filled triangles) of predator cues. Asterisks indicate means that are significantly different within each diet treatment ($p<0.05$, Tukey's HSD). Error bars show ±1 SE.

Figure 3. Effects of predator cues on survival of *Daphnia* fed algal food ratios of (a) C:P 100, (b) C:P 300, and (c) C:P 600. Arrows indicate the relative timing of the first broods and dotted lines show 95% confidence intervals.

Figure 4. Effects of food C:P ratios and fish cues on (a) reproductive output (R_0) and (b) population growth rate (r). Each point shows the mean and ± 1 SD of values generated for each treatment using a jackknife procedure.

Figure 1.

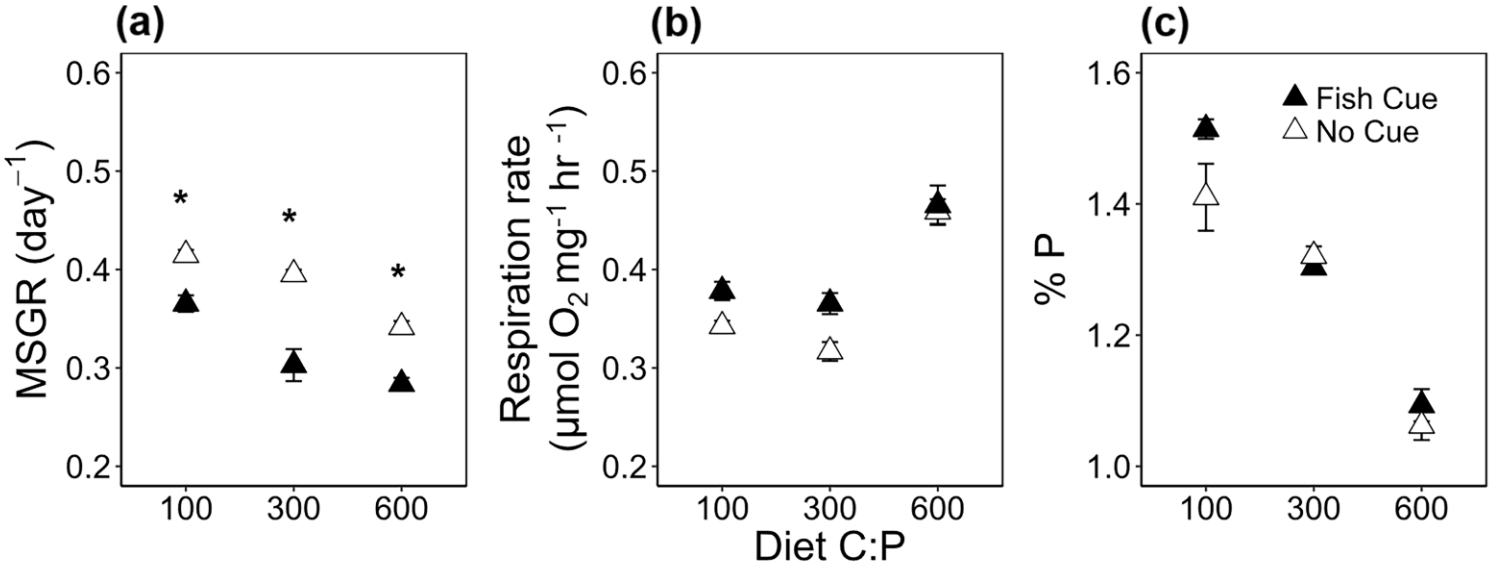

(a)
(b)
(c)
MSGR (day^{-1})
Respiration rate
(µmol O_2 mg^{-1} hr^{-1})
% P
Diet C:P
Fish Cue
No Cue
0.6
0.5
0.4
0.3
0.2
1.6
1.4
1.2
1.0
100
300
600
*

Figure 2.

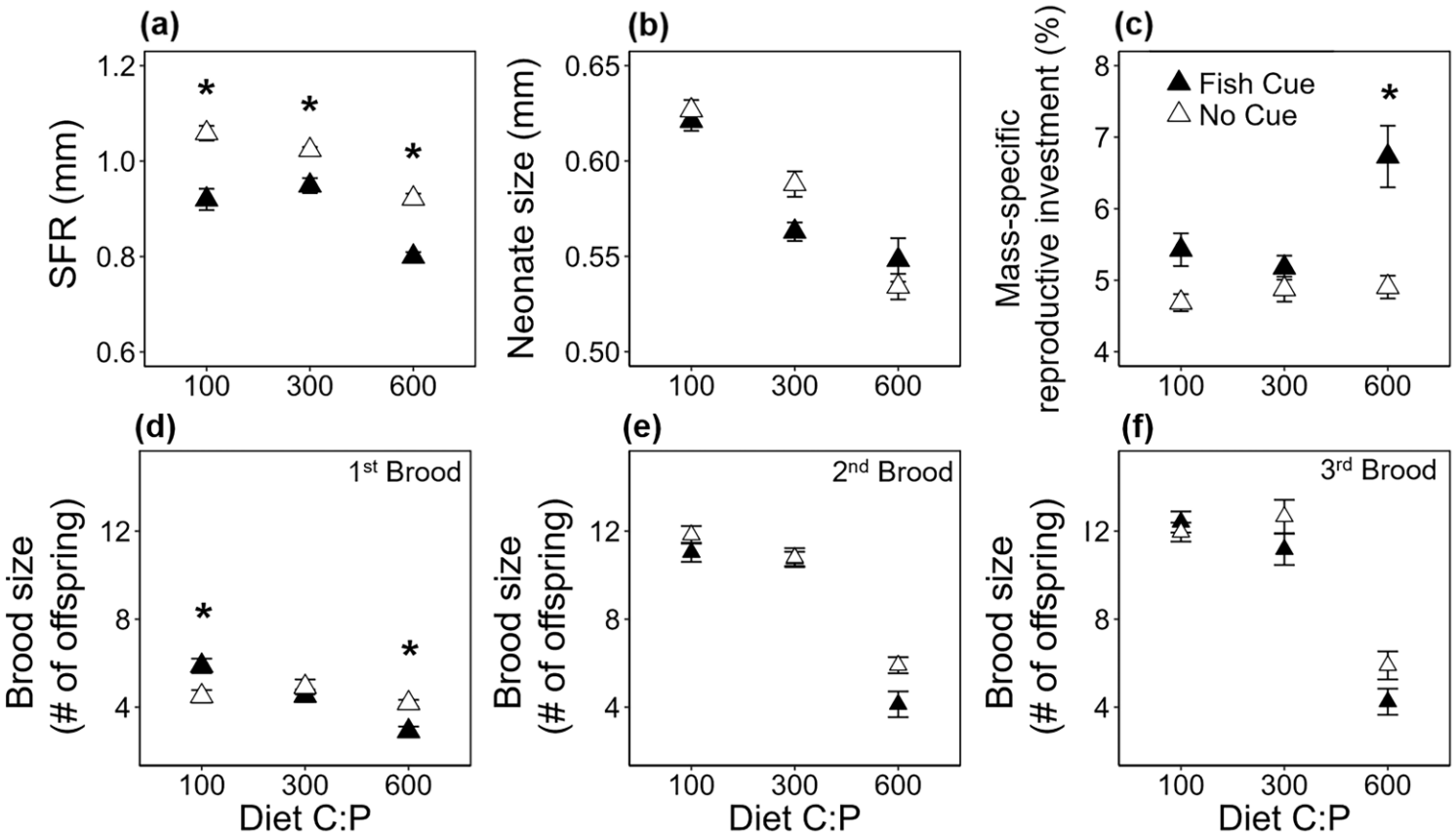

(a)
SFR (mm)
1.2
1.0
0.8
0.6
100
300
600
(b)
Neonate size (mm)
0.65
0.60
0.55
0.50
(c)
Mass-specific reproductive investment (%)
Fish Cue
No Cue
(d)
1st Brood
Brood size (# of offspring)
Diet C:P
(e)
2nd Brood
(f)
3rd Brood

Figure 3.

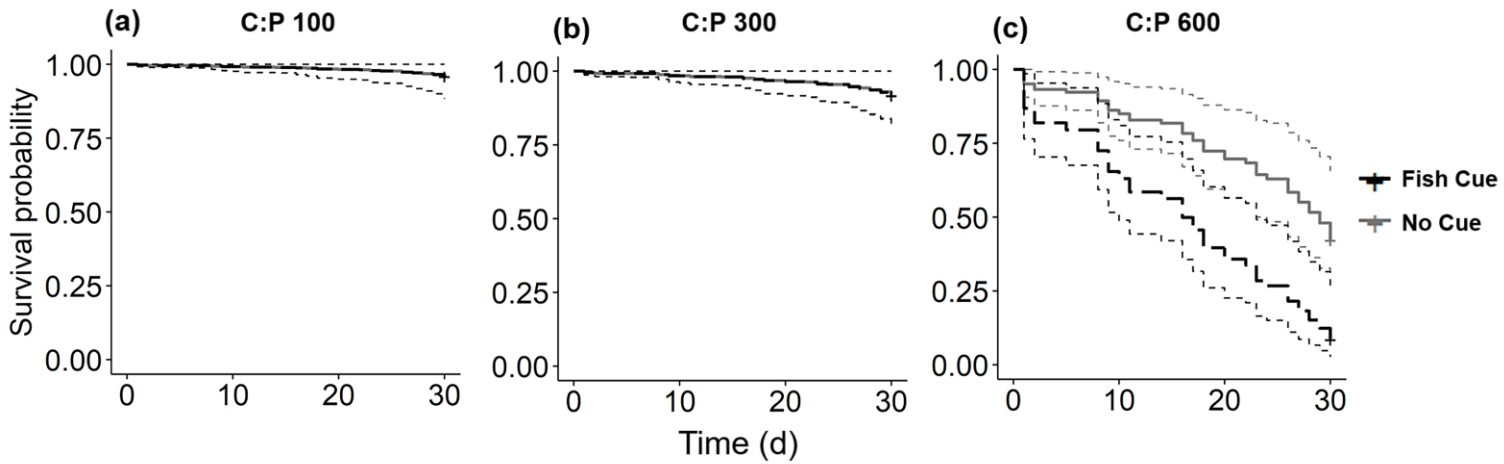
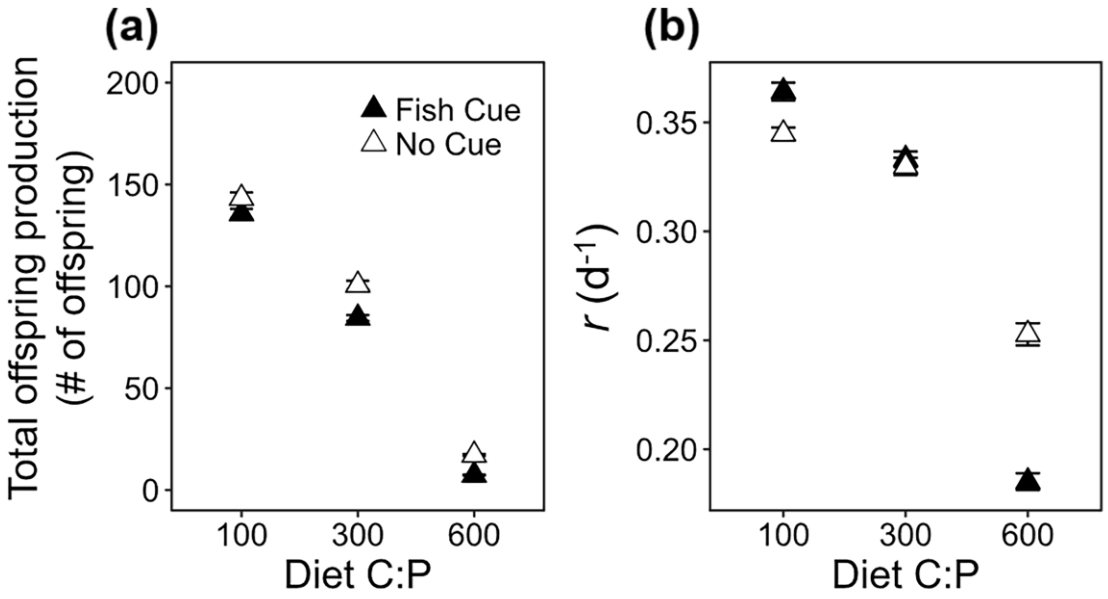

(a)
C:P 100
(b)
C:P 300
(c)
C:P 600
Survival probability
1.00
0.75
0.50
0.25
0.00
0
10
20
30
Time (d)
Fish Cue
No Cue

Figure 4.


(a)
Total offspring production
(# of offspring)
200
150
100
50
0
Fish Cue
No Cue
100
300
600
Diet C:P
(b)
r (d-1)
0.35
0.30
0.25
0.20
100
300
600
Diet C:P