

**STOICHIOMETRIC FOOD QUALITY AFFECTS RESPONSES OF  
*DAPHNIA* TO PREDATOR-DERIVED CHEMICAL CUES**

A Thesis submitted to the Committee on Graduate Studies in Partial Fulfillment of the  
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## Abstract

### Stoichiometric food quality affects responses of *Daphnia* to predator-derived chemical cues

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While both resource quality and predator-derived chemical cues can each have profound effects on zooplankton populations and their function in ecosystems, the strength and nature 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 water flea, *Daphnia pulex*, to predator-derived chemical cues. We compared growth rates, body elemental 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 elemental content of *Daphnia*. Elevated algal C:P ratios reduced growth and body %P, increased respiration, and increased body %C. Most of the effects of predator cues and algal C:P ratios of *Daphnia* were non-interactive. In contrast, the declines in daphnid survival and related population growth rates that arose because of poor food quality 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 trait-dependent and appear connected to animal life-history evolution.

**Keywords:** phosphorus, indirect predator effects, food quality, *Daphnia*, ecological stoichiometry, life history, predator-prey relationships

*Dedicated to*  
*my mother and father*  
  
*-but also to*  
*the Daphnia.*

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There are many people who were involved in making this work what it is. First, I am indebted to my advisors Dr. Paul Frost and Dr. Dennis Murray, who provided invaluable wisdom and generally taught me how to be a better scientist. I would also like to thank Dr. David Beresford for serving on my committee and providing key insight and advice. Dr. Clay Prater, Dr. Nicole Wagner, and Dr. Andrea Conine provided vital guidance throughout my research experience—they have my eternal thanks and respect. I really cannot imagine doing this work without them. Additionally, this work could not have been done without the essential laboratory assistance from Elyse Wakeford, who not only cared for the precious *Daphnia* brood mothers but also helped carry out the seemingly endless and regularly fruitless laboratory experiments. I would additionally like to thank the lab managers Andrew Bradley-Scott and Sarah King for teaching me the ins-and-outs of the lab protocols and keeping the laboratory in working order. To the other members of the Frost-Xenopus lab group, such as Sherri Degasparro, Katrina Cetinik, Dr. Kern Lee, and Sarah D'Amario, I must give thanks for being a reliably helpful and reassuring presence. I am further indebted to both Dr. Tom Hossie and Dr. Liz Kierepka for their invaluable statistical guidance. The 660 residence and its varied inhabitants also deserve credit, particularly Alice “Putu” Pintaric, Pat “Birthday-boy” Heney, Jasper “I’ll trust your judgement” Levitt, Madison “Maddawg” Wikston, and, of course, “Uncle” Lee Campbell. I thank them for the much-needed times of levity and helping me get through this whole ordeal with the possession of my sanity. There are also many in the Murray lab who improved my presentations, broadened my knowledge of ecology, and supplied numerous and productive scientific discussions. To these and others, too many to list

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# **Chapter 1: STOICHIOMETRIC FOOD QUALITY AFFECTS RESPONSES OF *DAPHNIA* TO PREDATOR-DERIVED CHEMICAL CUES**

## **Introduction**

It is understood that predators affect prey populations directly through consumption. Even more, the mere presence of predators can induce effects on prey behavior, morphology, and life history traits (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. Together, predator cues and the availability of food can interactively affect prey reproduction (Pauwels et al. 2010), growth (DeLong and Walsh 2015), and behavior (Heithaus and Dill 2002). While food quantity effects have received past study, less is known about how invertebrate prey responses to predator cues vary with the nutrient content of available food resources.

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 can 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). As exposure to predator cues can produce prey stress responses, including elevated metabolic rates and slower growth, the additional stress of poor nutrition on prey could modify these responses due to constraints on animal energy and material budgets (Hawlena and Schmitz 2010, Dalton and Flecker 2014, Zhang et al. 2016). When present together, the effects of predator cues and poor food quality could amplify or nullify the effects of each other and result in significant interactive effects.

Poor stoichiometric food quality could also influence key life-history responses in prey to predator exposure (Jeyasingh and Weider 2005). *Daphnia* typically respond to chemical cues released from large bodied, size-selective predators by reproducing earlier and at a smaller size, which increases reproductive effort, 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 can generally limit reproductive rates by increasing the age of first reproduction and reducing brood sizes (Weider et al. 2008, Prater et al. 2016). Because poor stoichiometric food quality generally reduces and constrains reproduction, poorly nourished prey species may have reduced abilities to respond to perceived increase in predation-risk. It thus seems likely that the nutritional state of *Daphnia* could interact with predator-induced effects on life history and physiology.

The responses of life-history traits of prey to environmental stress, both in terms of predator exposure and poor food quality, could translate into altered population growth rates. Population growth rates are a function of prey survival and reproduction, as this determines the 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 to offset probable losses of reproductive opportunities (Stearns 1992, Stibor 1992). Predator-induced shifts in life-history traits may require higher investments of resources and thereby could be limited by poor food quality (Zhang et al. 2016). If so, increased population growth rates resulting from exposure to predator-derived cues may be limited by the nutrient content of the prey diet. The nature of interactive effects of predator cues and food quality would depend of the relative strength and timing of effects on reproduction and animal mortality.

This study examined 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 macrochirus*), 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, body elemental content, metabolic rates, reproductive parameters, and survival. We further used reproduction and survival data to examine population growth responses. We expected that responses to predator-derived cues would vary with the diet quality consumed by *Daphnia*, but that the nature and size of these effects would depend on the response variable under examination.

## 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 the alga, *Scenedesmus obliquus* (Canada Physiological Culture Centre, Strain 10). Neonates (<24 hr old) were collected from the 2<sup>nd</sup> – 5<sup>th</sup> broods of these mothers and randomly assigned to different treatment combinations. For all experiments, *Daphnia* were reared individually within 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). 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 spectroscopy (American Public Health Association 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, Mt Laurel, New Jersey). The algal C:N:P ratios were determined on post-mixed food, using the same methods, to ensure 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  $\mu\text{m}$ ) and frozen at  $-20^{\circ}\text{C}$ . 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  $0.1 \text{ fish L}^{-1} \text{ day}^{-1}$ . 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 both P concentration values from 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 and body elemental content of *D. pulicaria* with a 6-day growth experiment. Age-synchronized neonates were collected (<24h old), and subsets of these neonates were randomly assigned into replicate tubes to be later used to quantify growth (n=10), body %P (n=4-5 of 4-6 pooled animals), or body %C and %N (n=3 of 8-16 pooled animals) 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<sup>-1</sup> of either C:P 100, 300, or 600 food every other day. 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  $MSGR = \ln(M_2) - \ln(M_1)/\text{time}$ ; 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 used to measure MSGR were saved for P analysis using the molybdate-blue method, as outlined above. The remaining animals were pooled and weighed in tin cups, which were then used to measure body C and N content.

*Metabolic rates.* To assess metabolic responses of *D. pulicaria*, we measured rates of O<sub>2</sub> consumption of 6-day old animals. 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. After respiration measurements were made, animals were collected, dried for

24 hr and weighed. Respiration rates were calculated by dividing the average corrected oxygen consumption by both total *Daphnia* mass and by time of measurement.

*Life table experiment.* A 30-day laboratory life table experiment was conducted to investigate the 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 every other day for the first 6 d and then 8 mg C for the rest of the experiment. Each tube was checked daily for 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 reproductive effort ( $R_o$ ) and 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. 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 1<sup>st</sup>-3<sup>rd</sup> 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

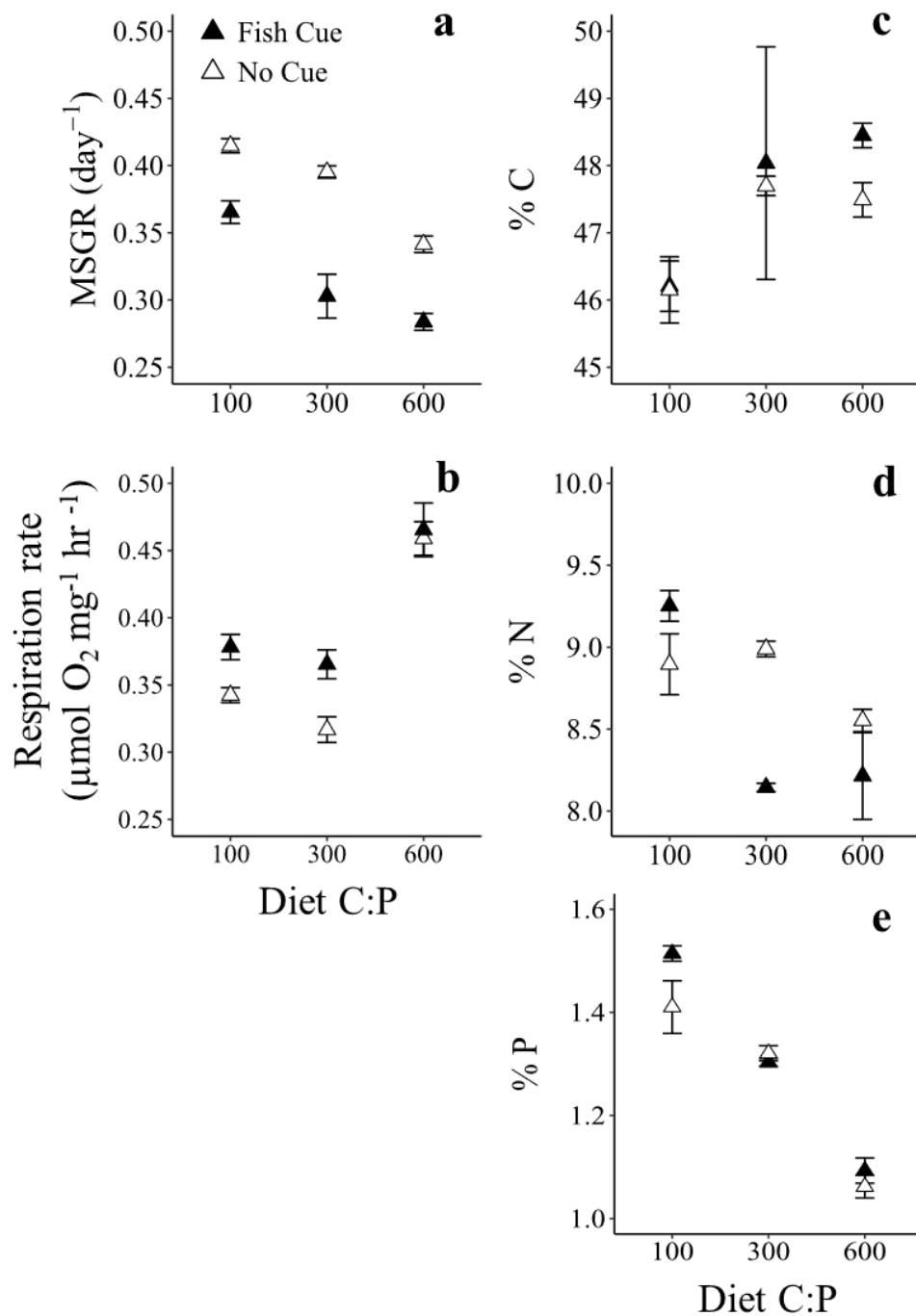
measurement. Photos were taken of the neonates and of the mothers for body length measurements. Daphnid mass was estimated with a length-mass power function which was originally developed from a *D. pulicaria* clone from a nearby lake (Prater 2016). Reproductive investment was calculated as neonate mass/mother mass \*100.

*Statistical analyses.* Effects of food C:P ratios and predator cues on MSGR, body elemental content, respiration, 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, neonate size, and reproductive investment were analyzed using 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 ( $\eta^2$ ), which is a measure of the proportion of variance accounted for by each main effect and interaction term. 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 diet 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  $R_0$  were generated for each treatment combination using a jackknife technique (Meyer 1986); because 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.

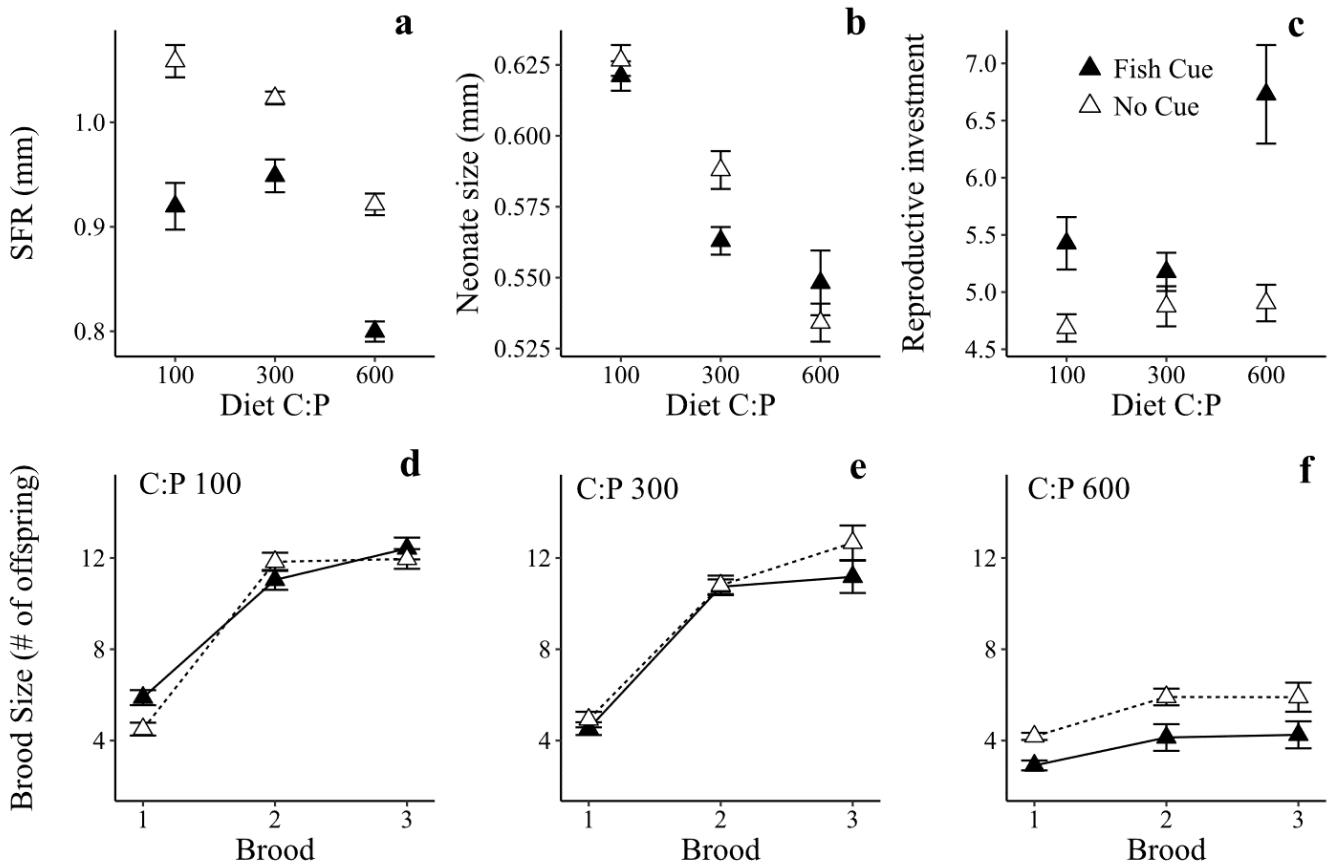
## Results

*Growth, respiration, and body elemental content.* 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 appeared to be largely overshadowed by main effects of slower growth produced by higher food C:P ratios and the presence of cues (Table 1, Fig. 1). We also found effects of treatments on *Daphnia* body sizes over the span of the 30-day experiment; food C:P and predator cues interactive effects were only found on the last day of the experiment, while relatively large main effects of both treatments ( $\eta^2=0.13-0.63$ ) were found on body sizes on animals aged 14, 21, and 30 days (Table 1, Appendix: Fig. 1). No significant interactive effects were found on respiration rates of *Daphnia* (Table 1, Fig. 1). Respiration rates were elevated with increasing food C:P ratios and by exposure to fish cues (Table 1; Fig. 1). No significant interactive effects between food C:P ratios and predator cues were found of body %C or %P content of *Daphnia*. Increasing algal C:P ratios increased body %C and reduced %P in animals, regardless of cue exposure (Fig. 1). In contrast, responses of daphnid body %N to predator cues varied with food C:P ratios (Fig. 1). While body %N was greater in animals exposed to predators in high P conditions, this effect reversed as food C:P ratios increased (Fig. 1).



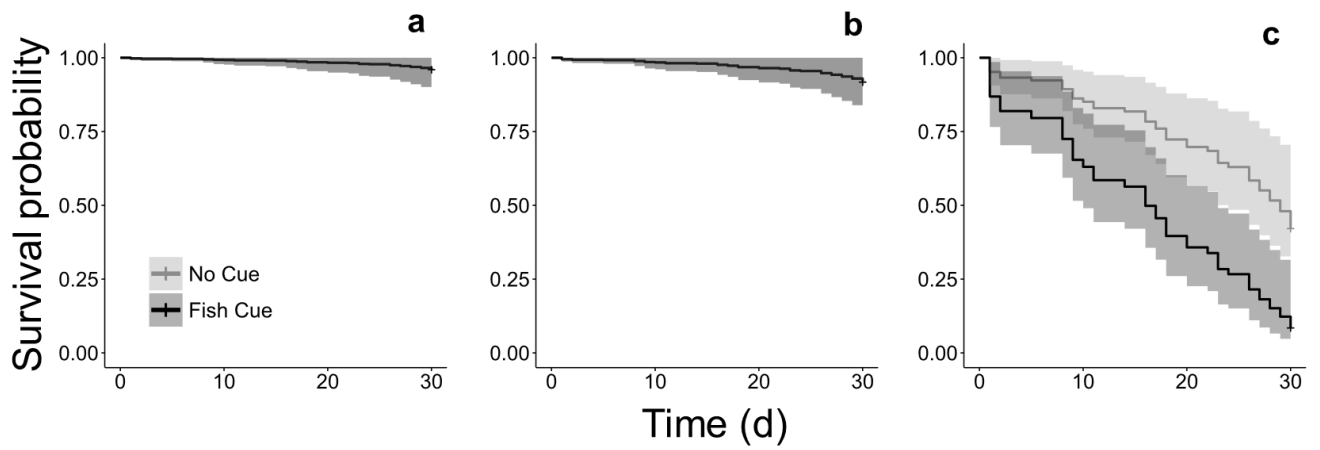
**Figure 1.** (a) Mass specific growth rates, (b) respiration rates, as well as (c) carbon, (d) nitrogen, and (e) phosphorous body content of *Daphnia* fed a gradient of food C:P in the presence (filled triangles) or absence (open triangles) of predator cues. Error bars show  $\pm 1$  SE.

*Life history traits and survival.* While there was no interactive effect of food C:P ratios and exposure to predator cues on SFR (Table 1), SFR was strongly reduced in response to each variable independently (Fig. 2). In terms of neonate size, there was no food C:P ratios  $\times$  cue interaction (Fig. 2) but increasing food C:P ratios consumed by *Daphnia* resulted in smaller offspring (repeated-measures:  $F_{2,239}=39.32$ ,  $p<0.001$ ). There was an interactive effect of food C:P ratios and predator cues on neonate investment, which was measured as neonate mass relative to mother mass (repeated-measures interaction:  $F_{2,297}=4.62$ ,  $p=0.011$ ; Fig. 2). Neonate investment generally increased in response to cues, as the size of this effect appeared much larger for the food C:P ratio 100 and especially for the food C:P ratio 600 (Fig. 2). In our brood size analysis, we found an interactive effect between our two treatments on the first brood; for animals consuming low food C:P ratios, exposure to predator cues increased the number of neonates born compared to the number produced by unexposed animals (Table 1, Fig.2,  $\eta^2=0.12$ ). The main effects of predator cues had minimal influence on brood size of the 2<sup>nd</sup> and 3<sup>rd</sup> broods and accounted for ~1% of the total variation (Table 1).



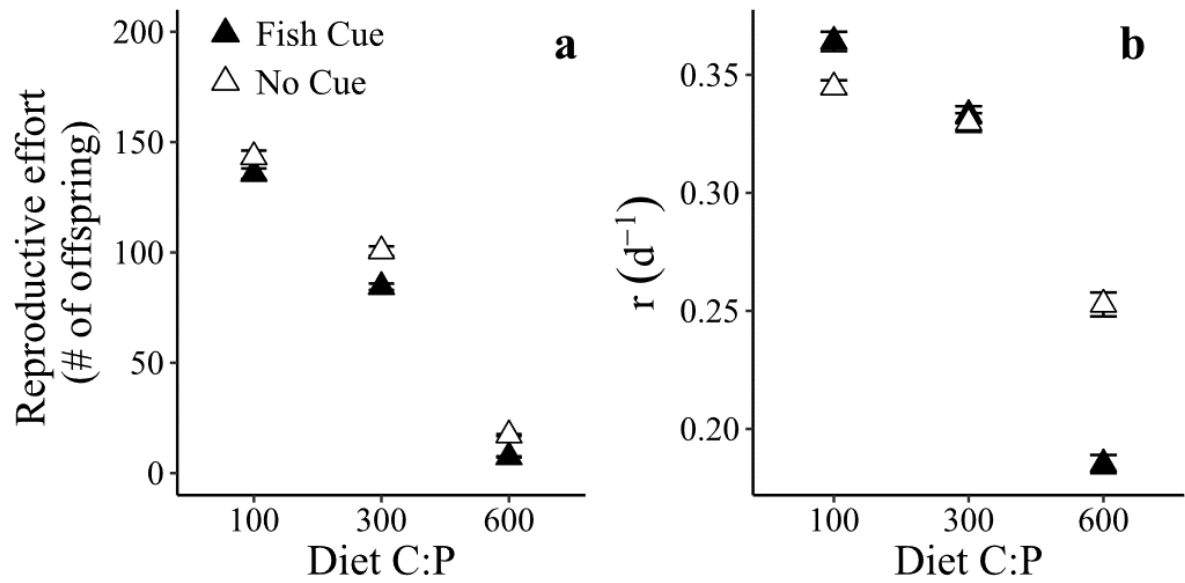
**Figure 2. Effects of diet C:P and fish cues on (a) size of first reproduction, (b) neonate size, and (c) reproductive investment. Neonates used for analysis were from the first 3 broods of mothers raised in each treatment combination. Reproductive investment was calculated as neonate mass/ mother mass \*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 presence (black triangles) or absence (white triangles) of predator cues. Error bars show  $\pm 1$  SE.**

Food quality and cue exposure interacted to affect *Daphnia* survival. Survival in the C:P100 and 300 treatments was high (92-96%) both in the presence and absence of predator cues (Fig. 3). In contrast, 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 was amplified in P-stressed animals that were exposed to fish cues, as we observed near population extinction (~96% mortality) of animals fed P-poor algae over the 30-day experiment (Fig. 3). Cox proportional hazard models showed significant treatment effects on survival, but only in the highest food C:P ratio (600). In the absence of cues, the hazard ratio of animals fed C:P 600 food (HR=20.8, 95% CI=2.7-157.7) indicated that animal mortality risk was increased by ~ 20 times, relative to animals raised on high P. When animals were raised on C:P 600 and additionally exposed to predator cues, the estimated hazard ratio increased to 59.4 (95% CI=7.9-445.5), meaning animals exposed to both treatments had ~ 60 times higher mortality risk relative to unexposed animals consuming high food quality.



**Figure 3. Effects of predator cues on survival of *Daphnia* fed algal food ratios of C:P (a) 100, (b) 300, and (c) 600 for 30 days. Shaded regions show 95% confidence intervals.**

*Reproductive investment and population growth rates.* There was no significant interactive treatment effect on the total number of neonates produced per animal ( $R_o$ ). There were significant main effects of food C:P ratios and predator cues on  $R_o$  (Table 2). As food C:P ratios increased from 100 to 600, there was a reduction of  $R_o$  of over 85% in both cue treatment levels (Fig. 4). Additionally, exposure to predator cues led to reduced  $R_o$  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. 4). In high-P conditions,  $r$  was approximately 5% higher in the fish cue treatment; but as food C:P ratios increased, there was a 27% reduction of  $r$  of *Daphnia* exposed to cues, relative to those in the absence of cues (Fig. 4).



**Figure 4. Effects of diet C:P and fish cues on *Daphnia* (a) reproductive effort and (b) population growth rates ( $r$ ). Each point shows the mean and  $\pm 1$  SD of values generated for each treatment using a jackknife technique.**

**Table 1: Main and interactive effects of food C:P and fish cues on *Daphnia* traits, using two-way ANOVAs.  $\eta^2$  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 $\times$ Cue			
	df	$\eta^2$	F	p	df	$\eta^2$	F	p	df	$\eta^2$	F	p
<b>MSGR</b>	2,53	0.36	53.7	<b>&lt;0.001</b>	1,53	0.42	124.1	<b>&lt;0.001</b>	2,53	0.04	6.51	<b>0.003</b>
<b>Body size</b>												
<i>Day 14</i>	2,44	0.63	92.1	<b>&lt;0.001</b>	1,44	0.13	64.0	<b>&lt;0.001</b>	2,44	0.00	0.68	0.51
<i>Day 21</i>	2,42	0.63	198.3	<b>&lt;0.001</b>	1,42	0.16	98.4	<b>&lt;0.001</b>	2,42	0.00	0.21	0.81
<i>Day 30</i>	2,38	0.62	117.4	<b>&lt;0.001</b>	1,38	0.14	54.4	<b>&lt;0.001</b>	2,38	0.02	3.56	<b>0.038</b>
<b>Respiration</b>	2,24	0.76	57.6	<b>&lt;0.001</b>	1,24	0.06	9.36	<b>0.005</b>	2,24	0.02	1.59	0.23
% C	2,11	0.54	7.27	<b>0.010</b>	1,11	0.04	1.11	0.31	2,11	0.03	0.39	0.69
% N	2,11	0.43	11.0	<b>0.002</b>	1,11	0.07	3.53	0.09	2,11	0.26	7.50	<b>0.009</b>
% P	2,21	0.89	125.2	<b>&lt;0.001</b>	1,21	0.01	3.92	0.06	2,21	0.02	2.62	0.10
<i>SFR</i>	2,47	0.38	49.6	<b>&lt;0.001</b>	1,47	0.35	81.7	<b>&lt;0.001</b>	2,47	0.02	2.78	0.07
<b>Brood size</b>												
<i>1st brood</i>	2,135	0.19	17.9	<b>&lt;0.001</b>	1,135	0.00	0.05	0.82	2,135	0.12	11.5	<b>&lt;0.001</b>
<i>2nd brood</i>	2,128	0.64	122.2	<b>&lt;0.001</b>	1,128	0.01	5.38	<b>0.022</b>	2,128	0.01	1.94	0.15
<i>3rd brood</i>	2,121	0.51	66.7	<b>&lt;0.001</b>	1,121	0.01	2.31	0.13	2,121	0.01	1.82	0.17

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

	Diet C:P		Cue		Diet $\times$ Cue	
	H	p	H	p	H	p
<b><math>R_0</math></b>	5442.6	<b>&lt;0.001</b>	485.1	<b>&lt;0.001</b>	0.63	0.73
<b><math>r</math></b>	5379.9	<b>&lt;0.001</b>	52.4	<b>&lt;0.001</b>	444.9	<b>&lt;0.001</b>

## 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 the multi-generational success of their offspring (Frost et al. 2010, Storm and Lima 2010). Following this, prey that can assess 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 be especially prominent 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, lake zooplankton (i.e., *Daphnia*) 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 change with the nutritional state of prey zooplankton species. Here we assessed whether predator cue-induced changes in animal life-history vary with the stoichiometric quality of the prey's diet.

We manipulated the presence and absence of predator-derived cues using planktivorous fish that had recently consumed daphnid conspecifics. While we were unable to directly verify the presence, quantity, and chemistry of these predator-derived cues, our results nonetheless indicate that we effectively manipulated the presence and absence of cues. Under conditions of high food quantity and quality, we observed slower growth, smaller body size at all ages, higher rates of respiration, smaller size at first reproduction, and increased reproductive effort in animals exposed to cues. Similarly, we

observed strong effects of poor food quality in the absence of cues, which were consistent with past studies of *Daphnia* stoichiometry and their known responses to dietary P-limitation (Sterner and Schulz 1998, Urabe and Sterner 2001). Our results from both predator cue-exposed and poorly P-nourished animals strongly indicates that we generated the needed differences in predator-cues and animal nutrition to assess potential interactive responses of these factors.

From a physiological perspective, interactive responses in prey to the combined stress of predator cues and poor nutrition could emerge if these responses share a common energetic or material basis. For example, poor nutrition by limiting material intake could exacerbate stress created by predator-derived cues. Our results largely failed to match this prediction, as animals exposed to fish cues grew slower, respired faster, and exhibited smaller body size across all ages, regardless of diet. The presence of 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). Reduced growth rates and smaller body size of daphnids appear to result from energy and nutrients being redirected away from biomass accumulation to other purposes, such as reproduction (Stibor 1992), or to meet altered metabolic demands (Slos and Stoks 2008). The lack of strong interactive effects is perhaps not surprising given that slower growth due to predator cues presumably reduces the P-demands of animals needed to sustain high growth rates. The effect of predator-cues on *Daphnia* metabolic rates was not significant in the most P-stressed animals, who also exhibited elevated respiration rates in the absence of predator-cues. This suggests that 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. An alternative explanation for limited interactive effects is that behavior or metabolic changes exhibited by predator cue-exposed prey may primarily affect C-based metabolism, which is not limited or constrained by intake of a P-deficient diet (Hawlena and Schmitz 2010).

*Daphnia* body elemental content also was not strongly affected by interactions between predator cues and food C:P ratios. For these stoichiometric variables, food quality generally explained more variation, as estimated by  $\eta^2$  values, than predator cues, as daphnid body %C and %P was positively related to dietary P supply (Table 1). Although there were no interactive or main effects of predator cues on either body %C or %P, we found that cues increased body %N when *Daphnia* were consuming low food C:P ratios and the reverse when animals consumed high food C:P ratios. The magnitude of these cue effects was relatively small compared to those produced by food quality but nonetheless indicated altered animal metabolism in response to the combined stress of predator cues and food quality. As the biomass of developing *in utero* eggs can have strong effects on animal %N (Frost et al. 2008), changes in the presence or number of eggs in developing mothers may have produced these differential responses across food C:P gradients. Changes in daphnid %N content could also be attributed to increased protein content, commonly observed in daphnid responses to predatory stress (Slos and Stoks 2008, Zhang et al. 2016). However, a more refined study that tracks egg number and animal biochemistry would be needed to better understand the importance and origin of this interactive effect.

Predator-cues are known to result in changes in prey life-history traits related to reproduction (Macháček 1991a, Weider and Pijanowska 1993, Reede 1997). To

compensate for lower lifetime reproductive success that results from reduced longevity, reproductive effort can be increased in younger *Daphnia* exposed to predator cues (Macháček 1991b, Stibor 1992). We found evidence of these changes as interactive effects of predatory cues and food quality influenced daphnid reproductive investment and brood size. *Daphnia* invested more strongly in reproduction when exposed to predator cues, and this effect was greatest under poor food quality suggesting that altered elemental allocation to reproduction could be a generalized stress response used to maximize fitness. The size of the first brood was increased in predator cue exposed animals but only in prey 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 any of the first three broods. It thus appears that predator-stressed animals increase early reproduction but that this increase can be constrained by low supply of dietary P. Consequently, despite changes in animal growth and metabolism, exposure to predator cues yielded little effect on total reproduction. If viewed from the perspective of effort per animal, predator cues increased reproductive effort by *Daphnia* as they maintained reproductive output despite smaller body size. Again, these responses were not altered by animal nutrition, which further indicates the value of continued reproduction even in animals under nutritional and predator-induced stress.

Mortality rates of *Daphnia* were modified by the presence of predator cues and food quality consumed by animals. High survival was found for animals consuming high-P food regardless of predator treatment. Shifting growth and timing of reproduction to younger instars thus did not appear to affect longer-term longevity 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. Sustained P-stress thus appears to exacerbate the trade-offs between sustaining reproduction and maintenance processes underlying survival (McNamara and Buchanan 2005). The significance of such interactive effects in nature is unclear as it may be that the cost of this increased mortality on P-limited *Daphnia* in nature would be minimal given that mortality from actual predator consumption would also be elevated.

For a parthenogenetic invertebrate, such as *Daphnia*, the timing and quantity of early reproduction together with mortality rates 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 with exposure to predator cues, which was largely due to the larger first brood sizes and limited effects on survival. 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 was a function of both smaller brood sizes and significantly higher mortality rates. Our results indicate that *Daphnia* feeding on high quality food and under threat of fish predation can compensate, to some extent, by shifting reproduction earlier (Zhang et al. 2016). In contrast, nutritionally stressed animals exposed to fish cues appear to be constrained in their ability to adjust to higher predation risk, which results in fitness costs due to decreased survival rates. Thus, our results clearly demonstrate that efforts to understand the effects of predators and food quality on prey populations need to account the presence of the other variable.

We have shown how predator cues and food C:P can interact to alter survival and aspects of reproduction, which ultimately affects *Daphnia* population growth rates. As

large differences in predation pressure and food quality occur in lakes across a summer growing season (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 physiological changes seen in prey species (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, the elemental requirements of predator-cue responses at the biochemical level (Elser et al. 2000b) is necessary to understand how poor diet may constrain anti-predator life-history traits. Our results also indicate that subtle 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), 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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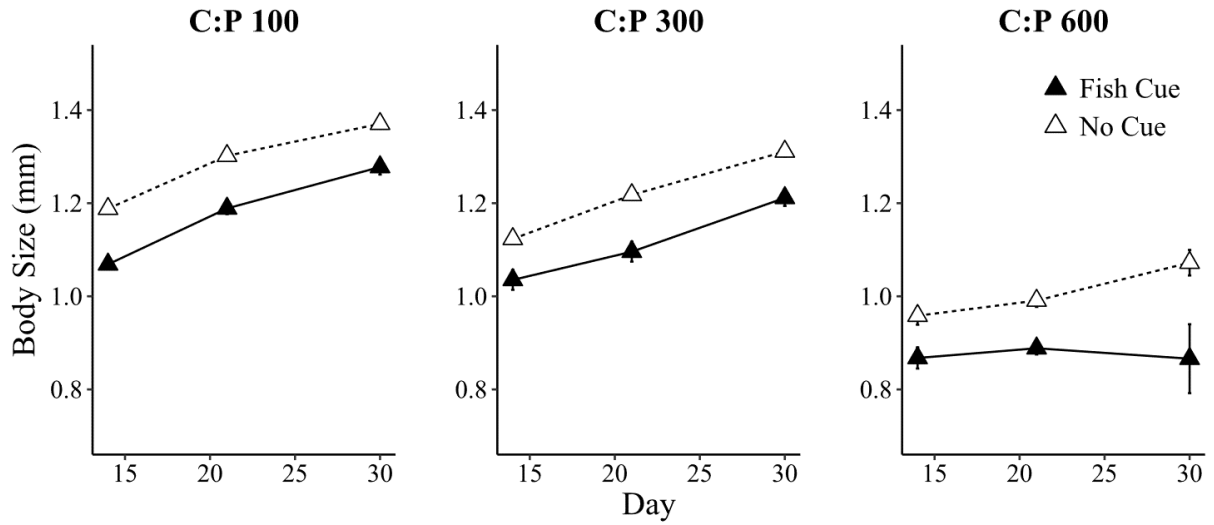
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## Appendix

### Supporting information



**Figure 1.** *Daphnia* body size differences from animals fed *Scenedesmus obliquus* of varying C:P ratios (C:P 100, 300, 600) in the presence (black triangles) and absence (white triangles) over 30 days. Error bars show  $\pm 1$  SE.