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

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24   **Abstract**

25   While resource quality and predator-derived chemical cues can each have profound

26   effects on zooplankton populations and their function in ecosystems, the strength and direction of

27   their interactive effects remain unclear. We conducted laboratory experiments to evaluate how

28   stoichiometric food quality (i.e., algal carbon (C):phosphorus (P) ratios) affects responses of the

29   zooplankter, *Daphnia pulicaria,* to predator-derived chemical cues. We compared growth rates,

30   body P content, metabolic rates, life-history shifts, and survival of differentially P-nourished

31   *Daphnia* in the presence and absence of chemical cues derived from fish predators. We found

32   effects of predator cues and/or stoichiometric food quality on all measured traits of *Daphnia.*

33   Exposure to fish cues led to reduced growth and increased metabolic rates but had little effect on

34   the body %P content of *Daphnia*. Elevated algal C:P ratios reduced growth and body %P, and

35   increased mass-specific respiration rates. While most of the effects of predator cues and algal

36   C:P ratios of *Daphnia* were non-interactive, reduced survival and relatedly reduced population

37   growth rates that resulted from P-poor food were amplified in the presence of predator-derived

38   cues. Our results demonstrate that stoichiometric food quality interacts with anti-predator

39   responses of *Daphnia*, but these effects are largely trait-dependent and appear connected to

40   animal life-history evolution. Given the ubiquity of predators and P-poor food in lake

41   ecosystems, our results highlight the importance of the interactive responses of animals to

42   predator cues and poor nutrition.

43

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

67 decreased body P content and reduced P excretion rates (DeMott et al. 1998), which is consistent

68 with acute P-limitation. Similarly, animals consuming food having high C:P ratios alter C-based

69 metabolic processes that appear to eliminate excess intake of C (Darchambeau et al. 2003; Frost

70 et al. 2005). Due to constraints on animal energy and material budgets imposed by poor

71 elemental nutrition, prey may alter their physiological responses when exposed to predator cues

72 (Hawlena and Schmitz 2010; Dalton and Flecker 2014; Zhang et al. 2016). Poor stoichiometric

73 food quality could also affect key life-history responses in prey to predator exposure (Jeyasingh

74 and Weider 2005). *Daphnia* typically respond to chemical cues released from large bodied,

75 visual predators by reproducing earlier and at a smaller size, which increases reproductive

76 output, at least in younger population cohorts (Stibor 1992; Weider and Pijanowska 2003). These

77 life-history traits are also sensitive to poor stoichiometric food quality, which generally limits

78 reproductive rates by increasing age of first reproduction and reducing brood sizes (Weider et al.

79 2008; Prater et al. 2016). Because poor stoichiometric food quality constrains reproduction,

80 poorly-nourished prey may have reduced abilities to respond to perceived increases in predation

81 risk. When present together, the effects of predator cues and poor elemental food quality could

82 thus amplify or nullify the effects of each other and result in significant interactive effects.

83   The responses of life-history traits of prey to environmental stress, both in terms of

84 predator exposure and poor elemental food quality, could translate into altered population growth

85 rates. Population growth rates are a function of prey survival and reproduction as these rates

86 determine gains and losses of the prey population (Sibly and Hone 2002). As mortality rates

87 increase with the presence of predators, exposed prey generally respond by shifting to earlier

88 reproduction (Stearns 1992; Stibor 1992). Predator-induced shifts in life-history traits may

89 require higher investments of resources at the time of first reproduction, which may be limited by

90   nutrient-poor food (Zhang et al. 2016). If so, low nutrient food may constrain the earlier

91   initiation of population growth, which can result from exposure to predator-derived cues. The

92   nature of such interactive effects of predator cues and elemental food quality, if present, would

93   depend of the relative strength and timing of effects on reproduction and animal mortality.

94   In this study, we examined whether and how predator-induced responses of *Daphnia*

95   *pulicaria* are mediated by stoichiometric food quality. We did so by raising *Daphnia* in the

96   laboratory in the absence and presence of cues released from juvenile bluegill (*Lepomis*

97   *macrochrius*), a common fish predator, and manipulating C:P ratios in their algal food. We then

98   measured physiological and life-history responses of *Daphnia* including mass-specific growth

99   rates, metabolic rates, reproduction, and survival. We further used reproduction and survival data

100   to examine population growth responses. We expected both increased diet C:P ratios and

101   exposure to predator cues to reduce growth rates, increase metabolic rates, and reduce animal

102   survival. We also predicted that animals exposed to predator cues would optimize reproduction,

103   in terms of number and timing of offspring produced, brood size, and neonate size, but that these

104   responses to predator exposure would be limited by higher C:P ratios in the diet. Finally, we

105   predicted exposure to predator cues would reduce population growth rates and that the size of

106   this reduction would increase with food C:P ratios.

107

108   **Methods**

109   *Experimental design and treatments*. We examined responses of differentially P-

110   nourished *Daphnia pulicaria* to fish-predator cues. The daphnid clone used in this study was

111   originally collected from Big Cedar Lake in south-central Ontario (44.606638, -78.171669) and

112   cultured in the laboratory for ~4 years prior to use in our experiments. We collected juvenile

113   bluegill (*Lepomis macrochirus*) from the Otonabee River near Peterborough, Ontario, Canada

114   (44.371780, -78.286718) to produce predator cues.

115   *Daphnia* brood mothers were reared in COMBO media (Kilham et al. 1998) and fed

116   green algae (*Scenedesmus obliquus*, Canada Physiological Culture Centre, Strain 10). Neonates

117   (<24 hr old) were collected from the $2^{nd} – 5^{th}$ broods of these mothers and randomly assigned to

118   different treatment combinations. For all experiments, *Daphnia* were reared individually in 50 ml

119   plastic conical centrifuge tubes, which were maintained in a temperature-controlled growth

120   chamber (20°C) and under low irradiance with a 14:10 light:dark photoperiod.

121   We measured several response variables during a series of full factorial 3×2 laboratory

122   experiments where *D. pulicaria* were exposed to all six treatment combinations of food quality

123   (C:P 100, 300, and 600) and predator cues (absent or present). These algal C:P ratios were

124   selected to fit within the range previously reported for lake ecosystems (Hassett et al. 1997; Elser

125   et al. 2000a). To prepare the experimental diets, we cultured *S. obliquus* with varying media P

126   concentrations and dilution rates in multiple culture flasks. After harvesting, algal suspensions

127   were centrifuged for 10 min at 5000 rpm and re-suspended in N- and P-free COMBO media.

128   Algal P content was determined by persulfate digestion followed by molybdate-blue ascorbic

129   acid colorimetry and spectrometry (APHA 1992), which allowed us to mix algae of different P

130   contents to produce the three food P content levels. We determined the C and N content of algae

131   with an elemental analyzer (Vario EL III, Elementar Incorporated, Mount Laurel, New Jersey).

132   The algal C:N:P ratios were determined on post-mixed food, using the same methods, to ensure

133   that the nominal food types were created.

134   Fish cues were collected from juvenile *L. macrochirus* (80-110mm) housed for 24 hrs in

135   tanks with 4 L of N- and P-free COMBO media. The fish were fed live *Daphnia* during their

136   incubation, so the media contained both predator kairomones as well as conspecific alarm cues.

137   The fish-conditioned media was then membrane filtered (0.40 µm) and frozen at -20°C. Fresh

138   fish cues were harvested and frozen every four days, and before use in experiments the cue

139   media was thawed and diluted to a concentration equivalent to that produced by 0.1 fish L^{-1} day^{-}

140   ^{1}. We selected this concentration of the predator cue based on reported concentrations from past

141   experiments that also examined *Daphnia* responses to fish-predators (Pauwels et al. 2010;

142   Tollrian et al. 2015). While the natural relevance of this concentration is unclear due to

143   differences between lakes and laboratory bottles (e.g., Schindler et al. 1997), the selected fish

144   density (#/L) is moderately higher than that reported for lake littoral zones (Mittelbach 1988).

145   Growth media was refreshed in all the experimental tubes every other day.

146   To account for the effects of additional P from the fish cue on food algal P content, we

147   determined the concentration of soluble reactive phosphorus (SRP) in the cue media prior to food

148   algae preparation. By using P concentration values from both the fish cue and algal food

149   cultures, we mixed food concentrations to the desired dietary C:P ratio (100, 300, and 600) under

150   the assumption that 100% of the fish derived-P would be acquired by the food algae.

151   *Growth and body elemental content.* We examined the effects of food C:P ratios and

152   predator cues on growth rates of *D. pulicaria* with a 6-day growth experiment. Age-synchronized

153   neonates were collected (<24 hr old), and subsets of these neonates were randomly assigned into

154   replicate tubes to be later used to quantify growth rates (n=10) for each treatment combination.

155   Initial neonate mass was determined by collecting three subsets of 20 neonates, which were

156   transferred into pre-weighed aluminum tins, placed into a drying oven (at 60°C) for 24 hr, and

157   reweighed with a microbalance. Experimental animals were reared in treatment conditions for 6

158   days and fed 4 mg C L^{-1} of either C:P 100, 300, or 600 food every other day. These food rations

159   were based on animal mass and feeding rates and provided food quantities in excess of those

160   estimates of daily food requirements for fast-growing daphnids as previously verified in similar

161   experiments (Wagner and Frost 2012). After 6 days of growth, all experimental animals were

162   removed from tubes, rinsed with N and P free COMBO, and dried for 24 hr. Mass-specific

163   growth rates (MSGR) were calculated as:

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

165   where M_2 is the final mass per *Daphnia*, M_1 is the average initial neonate mass, and time is

166   number of days of growth. Animals were saved for P analysis using the same methods as above

167   on persulfate-digested animal bodies. The remaining animals were pooled and weighed in tin

168   cups, which were then used to measure body C and N content; however, due to mortality issues

169   and sample pooling that resulted in reduced sample size, the results of these analyses were not

170   included here but can be found in our supplementary material (Supplementary material Appendix

171   1, Fig. A2).

172   *Metabolic rates.* To assess metabolic responses of *D. pulicaria*, we measured rates of O_2

173   consumption of 6-day old animals following the approach of McFeeters and Frost (2012).

174   Animals were raised following the same procedure as outlined above. Small groups of *Daphnia*

175   (3–6 individuals) were placed in respiration vessels (0.5 ml) containing COMBO and the

176   assigned algal food C:P ratio-fish cue treatment. The vessels were placed in a water bath (21°C)

177   and animals allowed to acclimatize for 10 min. Oxygen consumption was recorded using a

178   micro-oxygen probe (MRCh system; Unisense A/S; Aarhus, Denmark) for 10 min under low

179   light to limit photosynthetic activity. For every five samples, two blank vessels (without

180   *Daphnia*) were measured to determine the effect of algae and fish cues on oxygen

181   concentrations; these baseline values were then used to correct oxygen consumption slopes for

182   each treatment. These baseline changes in O_2 concentration were relatively small (~1%) relative

183   to oxygen changes that occurred when *Daphnia* were in the vessels. After respiration

184   measurements were made, animals were collected, dried for 24 hr, and weighed. Mass-specific

185   respiration rates were calculated by dividing the average corrected oxygen consumption by both

186   total *Daphnia* mass and time of measurement.

187   *Life table experiment.* A 30-day laboratory life table experiment was conducted to

188   investigate differences in life history trade-offs between animals fed different food C:P ratios and

189   in the presence or absence of fish predator cues. For each treatment, 25 *Daphnia* were

190   individually reared in tubes containing 30 mL (age 0–6 day) and then 40 mL (age 7–30 day) of

191   N-and P-free COMBO media. Animals were fed 4 mg C L^{-1} of algal food every other day for the

192   first 6 d and then 8 mg C L^{-1} for the rest of the experiment. These food rations were based on

193   animal mass and feeding rates and provided food quantities in excess of those estimates of daily

194   food requirements for fast-growing daphnids. Each tube was checked daily for animal mortality

195   and reproduction. We measured daily production of offspring, size at reproductive maturity,

196   brood size, and survival. Mortality and survival data were then used to calculate intrinsic rate of

197   increase (r) using Euler's equation,

198
$$1 = \sum_{x=0}^{n} l_x m_x \cdot \exp(-rx)$$

199   where x is age (days), l_x is age-specific survivorship, and m_x is age-specific fecundity.

200   Reproductive output (R_0) was calculated by summing the product of lx and mx from each

201   treatment on each day of the experiment. To measure differences in body size, 10 additional

202   animals of each treatment were reared simultaneously; photos were taken of each animal

203   immediately after the first clutch of neonates was released and then every 7 d for the rest of the

204   experiment. Body length measurements were made from the top of the eye to the base of the tail.

205   *Neonate experiment.* To assess how food C:P ratios and fish cues affected neonate size,

206 neonates were collected from the 1st-3rd broods of animals raised in treatments for 15 days. Ten

207 replicate mother *Daphnia* were raised in each treatment; three neonates from each the first five

208 reproducing animals from each brood were selected randomly for measurement. Photos were

209 taken of the neonates and of the mothers for body length measurements. Daphnid mass was

210 estimated with a length-mass power function which was originally developed from a *D. pulicaria*

211 clone from a nearby lake (Prater et al. 2017). Mass-specific reproductive investment was

212 calculated as neonate mass/mother mass *100.

213   *Statistical analyses.* Effects of food C:P ratios and predator cues on MSGR, respiration,

214 body %P, body size, and brood size were analyzed using 2-way ANOVA, with food C:P ratio

215 and predator cue treatments used as fixed effects. Body size, size at first reproduction, neonate

216 size, and reproductive investment were analyzed with a 2-way repeated-measures ANOVA using

217 linear mixed-effect models with either individual or mother identity as random effects. The effect

218 sizes from the 2-way ANOVA models were estimated using eta-squared (η^2), which is a measure

219 of the proportion of variance accounted for by each main effect and interaction term. Tukey's

220 HSD was used to assess treatment differences for all parametric tests. Differences in survival

221 were assessed using Cox proportional hazards regression models (Cox 1972), using food C:P

222 ratios and predator cue treatments as covariates. The food C:P failed to pass the assumption of

223 proportional hazards, so the levels of each covariate were analyzed individually. To compare

224 population parameters, 1000 values of r and R_o were generated for each treatment combination

225 using a jackknife technique (Meyer et al. 1986). As the residuals violated parametric

226 assumptions of normality, they were statistically compared using a Kruskal–Wallis H test with

227 Scheirer–Ray–Hare extension (Ashforth and Yan 2008, Prater et al. 2016), which is a non-

228 parametric equivalent to a 2-way ANOVA. We used $\alpha = 0.05$ and all data were checked for

229 normality by visually assessing residuals of each model. Homogeneity of variances was assessed

230 by plotting residuals of each model against fitted values. We used R statistical software (version

231 3.3.3 with R studio) for all statistical analyses and image analysis software (IMT i-Solution) for

232 all daphnid body measurements.

233

234 **Results**

235   *Growth and respiration.* We found a significant interactive effect of food C:P ratios and

236 predator cues on the mass-specific growth rates of *D. pulicaria* (Table 1). While this indicates

237 that the response of growth rates to predator cues varied with food C:P ratios, this interactive

238 effect ($\eta^2 = 0.04$) was dwarfed by the main effects of slower growth produced by higher food

239 C:P ratios ($\eta^2 = 0.36$) and the presence of cues ($\eta^2 = 0.42$, Fig. 1a). We also found effects of

240 treatments on *Daphnia* body sizes over the span of the 30-day experiment; a food C:P and

241 predator cue interaction was only found on the last day of the experiment, while relatively large

242 main effects of both treatments ($\eta^2 = 0.13$-0.63) were found on body sizes on animals aged 14, 21,

243 and 30 days (Table 1, Supplementary material Appendix 1 Fig. A1). No significant interactive

244 effects were found on respiration rates of *Daphnia* (Table 1, Fig. 1b). Respiration rates were

245 elevated at the highest food C:P ratios and by exposure to fish cues (Table 1; Fig. 1b). We also

246 did not detect significant interactive effects between food C:P ratios and predator cues on the

247 body %P content of *Daphnia* (Table 1)*;* increasing algal C:P ratios reduced %P in *Daphnia*,

248 regardless of cue exposure (Fig. 1c).

249   *Life history traits and survival.* While there was no interactive effect of food C:P ratios

250 and exposure to predator cues on the size at first reproduction of *Daphnia* (Table 1), this

251 parameter was reduced in response to each treatment independently (Fig. 2a). In terms of neonate

11

252 size, there was no food C:P ratio × cue interaction (Fig. 2b) but increasing food C:P ratios

253 consumed by *Daphnia* resulted in smaller offspring (repeated-measures: $F_{2,58}=37.55$, p<0.001).

254 There was an interactive effect of food C:P ratios and predator cues on mass-specific

255 reproductive investment, which was measured as neonate mass relative to maternal mass

256 (repeated-measures interaction: $F_{2,297}=4.62$, p=0.011; Fig. 2c). While this parameter increased in

257 response to cues, the size of this effect was larger for the food C:P ratio 100 and especially for

258 the food C:P ratio 600 (Fig. 2c). In our brood size analysis, we found an interactive effect

259 between our two treatments with strong effects of cues on animals consuming high food C:P

260 ratios. In these animals, exposure to predator cues increased the number of neonates born in the

261 first brood compared to that produced by unexposed animals (Table 1, Fig. 2d, Food C:P × Cue:

262 $\eta^2=0.12$). In contrast, predator cues had minimal influence on brood sizes of animals eating food

263 C:P ratios of 100 and 300 (Fig. 2d).

264 Cox regression showed food quality and cue exposure interacted to affect *Daphnia*

265 survival (Supplementary material Appendix 1, Table A1). Survival in food C:P 100 and 300

266 treatments was high (92-96%) both in the presence and absence of predator cues (Fig. 3a,b). At

267 food C:P ratio 600, survival of non-cue exposed animals was reduced to approximately 50% of

268 animals at the end of the experiment. This effect in P-stressed animals was amplified by

269 exposure to fish cues, as we observed near population extinction (~96% mortality) of predator-

270 exposed animals fed P-poor algae over the 30-day experiment (Fig. 3c). Cox proportional hazard

271 models showed significant treatment effects on survival, but only in the food C:P 600 treatment.

272 In the absence of cues, the hazard ratio of animals fed food C:P 600 (HR=20.8, 95% CI=2.7-

273 157.7) indicated that mortality risk increased by ~ 20 times relative to animals raised on high P.

274 When animals were raised on food C:P 600 and additionally exposed to predator cues, the

275 estimated hazard ratio increased to 59.4 (95% CI=7.9-445.5), meaning that animals exposed to

276 both cues and high food C:P ratios had ~ 60 times higher mortality risk relative to unexposed

277 animals consuming high food quality.

278 *Reproductive output and population growth rates.* There was no interactive effect of

279 treatments on reproductive output (R_0, Table 2). As food C:P ratios increased from 100 to 600,

280 there was a reduction of R_0 of over 85% in both cue treatment levels (Fig. 4a). Additionally,

281 exposure to predator cues led to reduced R_0 across all diet treatments (Table 2). Observed

282 differences in reproduction and survival translated into differences in the population-level

283 parameter of intrinsic rate of increase (r), which is a measure of population growth and

284 organismal fitness. Food quality and cue treatments had significant interactive effects on r (Table

285 2, Fig. 4b). In high-P conditions, r was approximately 5% in higher in the fish cue treatment, but

286 as food C:P ratios increased, there was a 27% reduction of r of *Daphnia* exposed to predator

287 cues, relative to those in the absence of cues (Fig. 4b).

288

289 **Discussion**

290 Prey species face the difficult task of maximizing fitness in environments having variable

291 predator and nutritional conditions. This challenge is not trivial from a prey's perspective

292 because small changes in their reproduction and survival can yield substantial differences in

293 population growth rates (Frost et al. 2010; Storm and Lima 2010). Following this, prey that can

294 detect and respond to mortality risks should be at an advantage if this leads to altered life-history

295 traits, including reproduction, that account for changes in expected longevity. Such life-history

296 shifts, in terms of reproductive timing and effort, might only be observed in predator-exposed

297 prey if these responses are otherwise costly in the absence of high predator mortality risk

298   (Tollrian 1995; Riessen 2012). Matching these expectations, prey modify their physiology, life-

299   history traits, and morphology when exposed to cues that signal predator presence and a higher

300   risk of mortality (Stibor 1992; Beckerman et al. 2007). It remains unclear, however, whether and

301   how life-history responses to predator exposure would change with the nutritional state of prey

302   species.

303   From a physiological perspective, strong interactive responses to the combined effects of

304   predator cues and poor nutrition could emerge if these responses share a common energetic or

305   material basis. Yet, our individual-based measurements failed to match this prediction, as

306   interactive effects were weak or insignificant and animals exposed to fish cues grew slower,

307   respired faster, and exhibited smaller body size across all ages, regardless of diet. These main

308   effects of predator cues are consistent with previously documented patterns of prey life-history

309   shifts in response to size-selective predation (Stibor 1992; Gliwicz and Maszczyk 2007). In this

310   case, reduced growth rates and smaller body sizes may have resulted from energy and nutrients

311   being redirected away from biomass accumulation and used for other purposes. For example, the

312   maintenance of high body P in our study animals despite reduced growth rates produced by

313   exposure to predator cues suggests that P-rich RNA was instead used for reproduction (e.g.,

314   oogenesis; Markow et al. 2001) or cellular maintenance functions (Bertram et al. 2006). It thus

315   appears that elemental food quality may not strongly modify or constrain physiologically-linked

316   prey responses to predator cues (Jeyasingh and Weider 2005).

317   Similar to life-history traits and body %P, we found no interactive effects of predator

318   cues and food quality on metabolic rates. While predator cues increased *Daphnia* respiration

319   rates in well-fed animals, no apparent effect of cues were observed in the most P-stressed

320   animals, who also exhibited elevated respiration rates in the absence of predator cues. It is

321 unclear why cue exposure did not further increase mass-specific respiration rates beyond that

322 produced by poor food quality. While there may be an upper ceiling to elevated *Daphnia*

323 respiration rates that cannot be exceeded regardless of the combined presence of predator cues

324 and poor food quality, these effects may be partly mediated by direct treatment effects on animal

325 body mass. As our experiments were not designed to separate out the drivers (e.g., mass vs.

326 metabolic reshaping) of elevated mass-specific respiration, future work is needed to better

327 examine how nutrition and predator cues alter metabolic rates of *Daphnia.*

328 Predator cues are known to elicit changes in prey life-history traits related to reproduction

329 (Macháček 1991; Weider and Pijanowska 1993; Reede 1997). To compensate for lower lifetime

330 reproductive success resulting from reduced longevity, reproductive output can be increased in

331 younger *Daphnia* exposed to predator cues (Macháček 1991; Stibor 1992). We found evidence

332 of these changes, with interactive effects of predatory cues and food quality on daphnid mass-

333 specific reproductive investment and brood size. *Daphnia* invested more strongly in reproduction

334 when exposed to predator cues largely due to smaller maternal mass and invariant neonate

335 production. We found that this mass-specific reproductive investment increased the most in

336 animals consuming poor food quality, which indicates this predator cue induced response was

337 not eliminated with acute nutrient stress in *Daphnia*. The number of neonates in the first brood

338 was only increased in animals experiencing increased risk while consuming good food quality.

339 Otherwise, cues either had no effect (for food C:P ratios 100 and 300) or possibly reduced brood

340 size (food C:P ratio 600) on the first three broods. Consequently, despite changes in animal

341 growth and metabolism, exposure to predator cues yielded little effect on total reproduction. The

342 insensitivity of reproductive output (R_0) to predator cue-food quality interactions further

343 indicates its importance even in animals experiencing nutritional and predator-induced stress.

344   Mortality rates of *Daphnia* were modified by the presence of predator cues and by food

345   quality. Highest survival occurred in animals consuming high-P food, regardless of predator

346   treatment. Thus, shifting growth and timing of reproduction to younger instars did not appear to

347   affect short-term survival (~30 days) of well-nourished animals. While predator cues had

348   minimal effects on survival of well-nourished prey, mortality in P-stressed animals increased

349   dramatically with exposure to predator cues. Therefore, sustained P-stress appears to exacerbate

350   trade-offs between sustaining reproduction and maintenance processes underlying survival

351   (McNamara and Buchanan 2005).

352   While we found interactive effects of high food C:P ratios and predator cues on a number

353   of physiological and life-history responses, it is unclear how these effects would change the

354   ecological role of *Daphnia* in lake ecosystems. For example, in terms of respiration, predator

355   exposure increased daphnid respiration only in well-nourished animals. These changes in

356   respiration, if accompanied by increased feeding rates or altered feeding behavior, could alter

357   *Daphnia*-phytoplankton dynamics at a whole-lake scale. Such changes may not appear in lakes

358   with high food C:P ratios where increased respiration rates were found in *Daphnia* regardless of

359   predator-cue exposure. In addition, it is unclear how interactive effects of food quality and

360   predator cues on *Daphnia* population growth (*r*) observed in this lab setting would translate to

361   natural conditions in lakes. This uncertainty rests, in part, on the extent that mortality rates of

362   *Daphnia* are higher in predator-rich lakes due to direct losses to predators beyond that generated

363   simply by exposure to predators. In other words, higher mortality rates observed in P-stressed,

364   predator exposed animals may be lower than those generated by predation itself. Alternatively,

365   direct predator effects on nutrient-limited *Daphnia* populations may be over-estimated if

366   individuals escaping consumption nevertheless experience high mortality from exposure to

367   predator cues. To reduce this uncertainty, future work could focus on *in situ* zooplankton

368   population responses to predators and their cues in lakes with variable food quality and predator

369   densities.

370   For a parthenogenetic invertebrate such as *Daphnia*, the timing and quantity of early

371   reproduction can strongly drive population growth over relatively short, but environmentally

372   relevant (~30 days), time periods (McCauley et al. 1996; Pestana et al. 2013). We found

373   population growth rates in well-nourished *Daphnia* increased modestly with exposure to predator

374   cues, which was driven primarily by higher reproductive investment, larger first brood sizes, and

375   high survival. This indicates that *Daphnia* feeding on high quality food while under threat of fish

376   predation compensate by accelerating reproduction (Zhang et al. 2016). This pattern was

377   opposite in P-deprived *Daphnia*, whose population growth rates were depressed by the presence

378   of fish cues. This reduced population growth rate corresponded with both smaller brood sizes

379   and remarkably higher mortality rates. It thus appears that stress generated by low P food limits

380   the ability of *Daphnia* to maintain early and increased reproduction without sacrificing survival

381   over subsequent time periods. Overall, our findings of strong interactive effects on survival and

382   population growth, despite limited evidence from individual-based parameters, highlight the

383   importance of using integrated parameters such as intrinsic growth rates for understanding the

384   effects of predators and food quality on prey dynamics in natural populations.

385   We have shown how predator cues and food C:P ratios interact to affect survival and

386   some aspects of reproduction, with significant consequences for *Daphnia* population growth

387   rates. As large differences in predation pressure and food quality occur in lakes across summer

388   growing seasons (Sommer et al. 2012), the interactive nature of these effects may combine to

389   influence population dynamics and biomass production in nature. A fuller understanding of these

390   stoichiometric food quality effects on predator-induced responses will partly include deeper

391   knowledge of molecular underpinning of prey physiological changes (Wagner et al. 2013;

392   Mitchell et al. 2017). Such work could include studying how cue detection specifically translates

393   into changes in growth, reproduction, and metabolism. In addition, information on the elemental

394   requirements of predator cue responses at the biochemical level (Elser et al. 2000b) is necessary

395   to understand how poor food quality may constrain anti-predator life-history traits. Our results

396   also indicate that differences in reproduction and survival produced by predator cues may lead to

397   significant effects on population growth rate or organismal fitness. These life-history responses

398   should be incorporated into models of predator-prey interactions, including stoichiometrically-

399   explicit models (Elser et al. 2012; Leroux and Schmitz 2015), to better understand potential

400   impacts of these changes over multiple generations. Similar studies should be conducted on more

401   diverse taxa with other forms of nutrient limitation to determine the nature and ubiquity of

402   stoichiometric constraints on life-history responses to predation risk. Our results on an important

403   zooplankton consumer thus provide insights into a relatively understudied connection between

404   the bottom up effects of food resources and top down effects of predation and point to a need to

405   better connect predator-prey dynamics to emerging issues in nutritional ecology.

406

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	Food C:P				Cue				Food C:P $\times$ 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

569

570

571 **Table 2**: Main and interactive effects of food C:P ratios and fish cues on reproductive output (R_0)

572 and population growth rate (r) using Kruskal–Wallis H test with Scheirer–Ray–Hare extension.

573 Significant effects are in bold.

	Food C:P		Cue		Food C:P $\times$ 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**

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584   **List of Figures**

585

586   **Figure 1.** (a) Mass-specific growth rates, (b) respiration rates, and (c) phosphorus body content

587   of *Daphnia* fed a gradient of food C:P ratios in the absence and presence of predator cues.

588   Asterisks indicate means that are significantly different within each diet treatment ($p < 0.05$,

589   Tukey's HSD). Error bars show ± 1 SE.

590

591   **Figure 2**. Effects of food C:P ratios and fish cues on (a) size of first reproduction, (b) neonate

592   size, and (c) mass-specific reproductive investment. Neonates used for analysis were collected

593   from the first 3 broods of mothers raised in each treatment combination. Mass-specific

594   reproductive investment was calculated as neonate mass/ mother mass *100. Bottom panels

595   show *Daphnia* brood size differences from the first 3 broods of animals fed algal food ratios of

596   (d) C:P 100, (e) C:P 300, and (f) C:P 600 and in the absence (open triangles) or presence (filled

597   triangles) of predator cues. Asterisks indicate means that are significantly different within each

598   diet treatment ($p < 0.05$, Tukey's HSD). Error bars show ± 1 SE.

599

600   **Figure 3**. Effects of predator cues on survival of *Daphnia* fed algal food ratios of (a) C:P 100,

601   (b) C:P 300, and (c) C:P 600. Arrows indicate the relative timing of the first broods and dotted

602   lines show 95% confidence intervals.

603

604   **Figure 4.** Effects of food C:P ratios and fish cues on (a) reproductive output (R_0) and (b)

605   population growth rate (r). Each point shows the mean and ± 1 SD of values generated for each

606   treatment using a jackknife procedure.
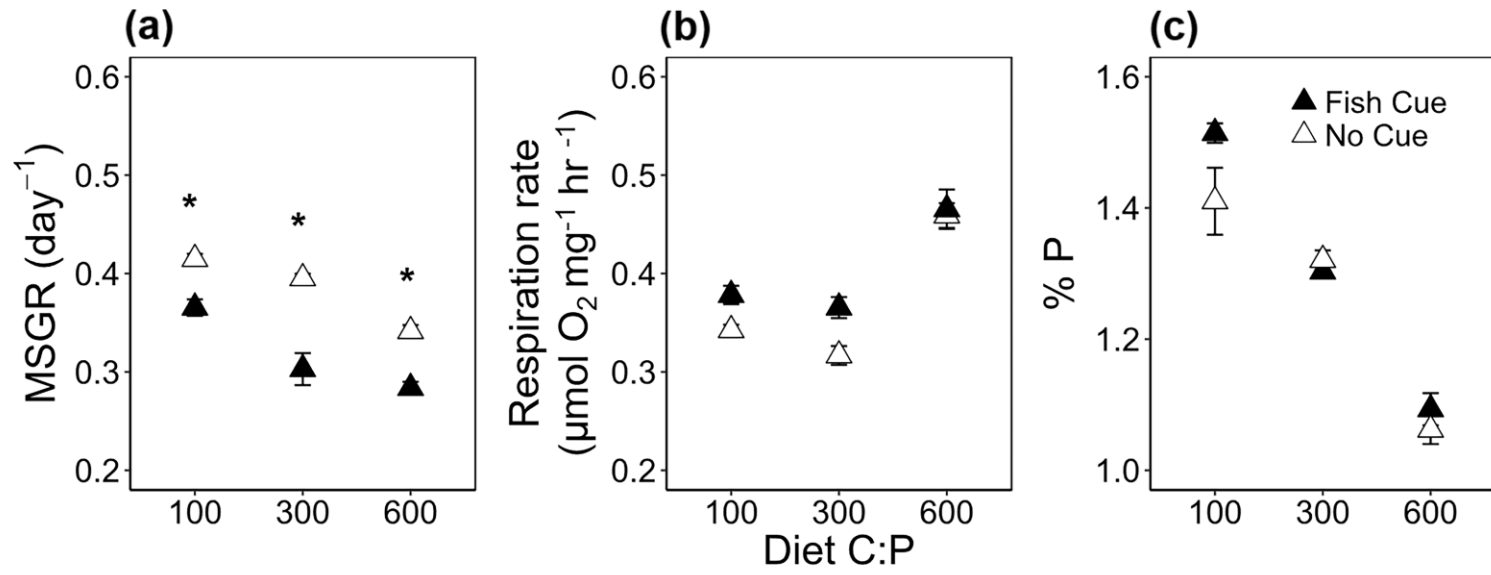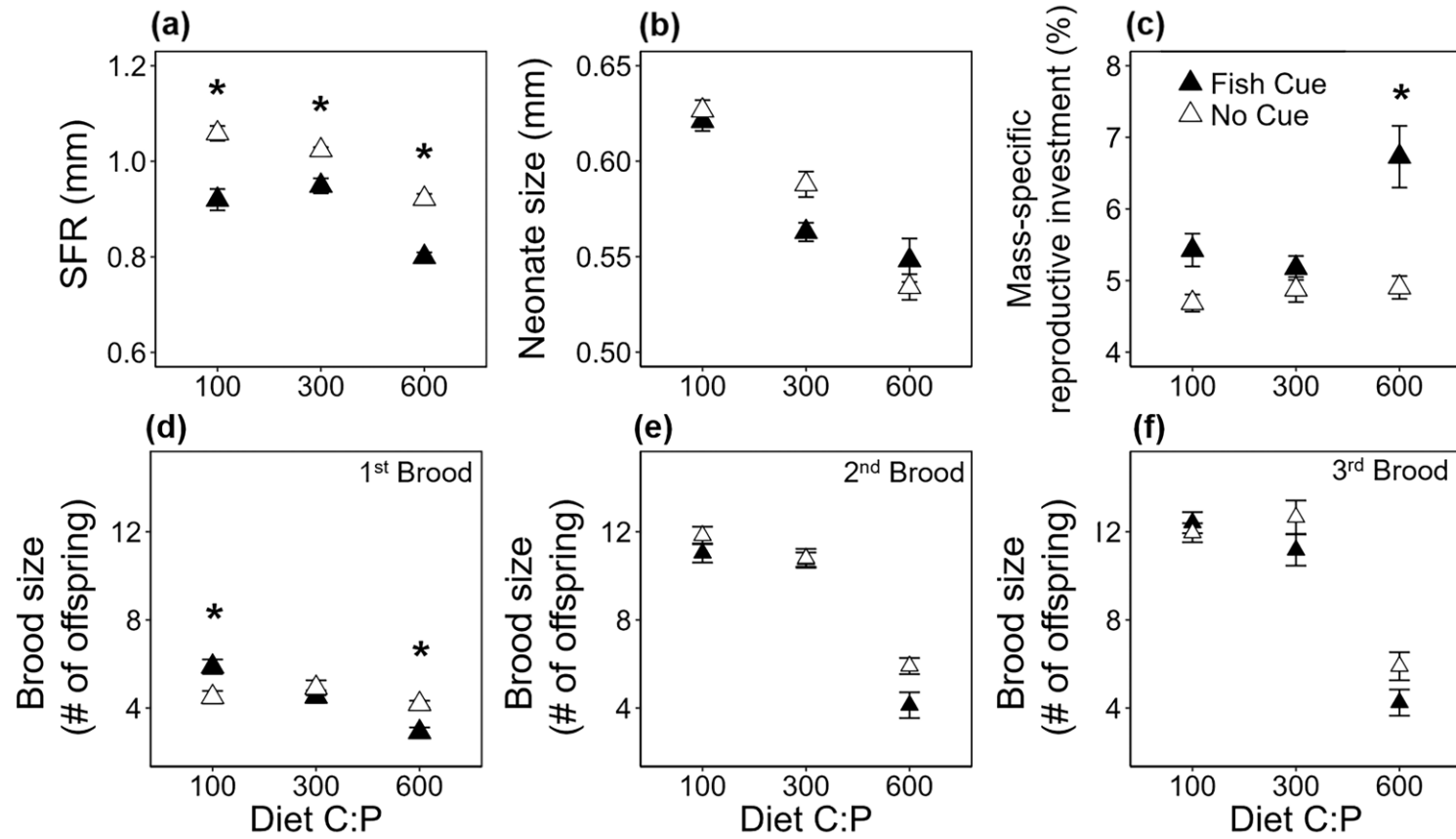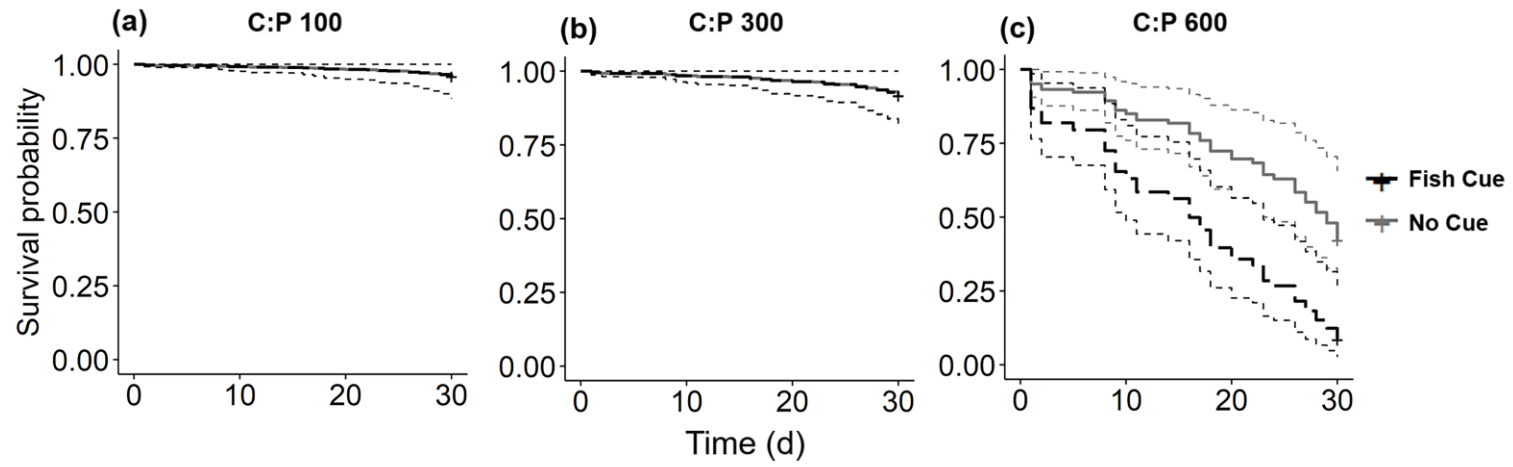
Figure 1.

Figure 2.

Figure 3.

Figure 4.