

## **Main Manuscript for**

# Trait-environment relationships are predictive, but not general across species

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### **Author Contributions**

CMT developed the original idea and experimental system; DNF and AB contributed to experimental design; AB, DNF, and CMT carried out the experiment; DNF ran all analyses; DNF and CMT wrote the first draft and all authors revised it.

## Abstract

Understanding the relationships between organisms and their environments is increasingly important given human impacts on global conditions. However, predicting how community diversity and composition will change in the future remains challenging (Mouquet *et al* 2015). One recent approach is to use traits to mechanistically inform how environmental conditions affect performance (i.e., trait-environment relationships), under the assumptions that these measures relate to each other in predictive and general ways. Unfortunately, results have been inconsistent, ignore phenotypic plasticity, and rely heavily on observational data (Shiple *et al* 2016). We evaluated the predictability and generality of trait-environment relationships in a controlled experimental microcosm system of four daphniid species. We cultured each species along a stressful gradient (conspecific density), measuring performance (fecundity) and traits related to performance (body length, 2<sup>nd</sup> antenna length, eye diameter, relative growth rate, and age at first reproduction). Using structural equation models, we evaluated the role of traits in mediating changes in individual fecundity in response to conspecific density. We built models for each species separately considering within-species trait variation, and for all species together by considering all trait variation across the four species. Results from this controlled system highlight that the relationship between individual traits and the environment (conspecific density) is strong and predictive of performance (fecundity), both within- and across-species. However, the specific trait-environment relationships which predicted fecundity differed for each species and differed from the relationships observed in the interspecific model, suggesting a lack of generality. These results will inform and improve the use of traits as a tool for predicting how changing environments will impact species abundances and distributions.

1 **Main Text**

2

3 **Introduction**

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5 The responses of individuals, populations, and communities to various environmental gradients is  
6 of long-standing interest in ecology [1] and evolution [2]. In an unprecedented era of rapid  
7 anthropogenic change, the ability to describe and predict these responses to rapidly changing  
8 biotic and abiotic conditions is imperative for conservation, restoration, and management [3].  
9 Recent work [4,5] has highlighted the potential to use traits — measurable characteristics that  
10 describe the phenotype, such as morphology, physiology, phenology, and behaviour [6] — to link  
11 environmental conditions and individual performance. Logically, if individual phenotypes reflect  
12 adaptive selection for success in a particular environment [7], then traits should predictably relate  
13 to performance in different environments (i.e., trait-environment relationship) [8,9]. Some studies  
14 have reported moderate to strong correlations between traits and environmental conditions,  
15 particularly at large spatial scales and for broad climatic gradients like temperature and moisture  
16 [10]. On the other hand, studies frequently fail to identify significant or predictive relationships  
17 between traits and the environment [9,11,12]. Drawing general conclusions is complicated by the  
18 large range of spatial scales, species, and methodological approaches considered by  
19 researchers. This complexity must be addressed, to determine if and how trait-environment  
20 relationships can be used to make predictions about the responses of ecological systems to  
21 environmental change. Addressing an independent but related set of questions should clarify our  
22 understanding of trait-environment relationships: What are the functional forms of trait-  
23 environment interactions (i.e., if performance is predicted with a function relating traits and the  
24 environment, what is the function)? Given the functional form, how much variation in performance  
25 does the relationship explain (how predictive is the relationship)? Are these functional  
26 relationships comparable, either between species, and/or across taxonomic groups (how general  
27 is the relationship)?

28

29 Identifying predictive and general trait-environment relationships has not proven to be  
30 straightforward. One problem is that it is difficult to tease apart evidence that these relationships  
31 are not predictive or general across species from the inherent difficulties in measuring trait-  
32 environment relationships. Studies (frequently on plants) of traits and environmental gradients  
33 most often rely on observational data collected at a variety of spatial scales. The presence of an  
34 individual at any given point along a gradient is influenced by multiple processes beyond the  
35 abiotic environment, including dispersal limitation, competition, predation, and mutualisms, all of  
36 which can distort estimates of the underlying trait-environment relationships [13]. Methodological  
37 issues with inferring these relationships from observational data are well-known. The Fourth  
38 Corner problem, for example, refers to the inherent difficulty in quantifying the strength of trait-  
39 environment associations if they are inferred indirectly (see [14]). Analyses of observational data  
40 can also differ greatly in terms of the type of trait data available — some may quantify trait values  
41 directly (field measurements) but it is also common to obtain measures through the use of large  
42 databases (e.g. TRY [15]). Despite the often implicit assumption that trait values should reflect  
43 selection for specific phenotypes in particular environments, they frequently also reflect plastic  
44 responses to the environment [16] and this plasticity may play an important, yet under-considered  
45 role in determining performance. Finally, measures of performance are often approximations of  
46 fitness such as growth, reproduction, survival, or dispersal [8,9,12] which may be imperfect

47 proxies [6,9], or only provide short-term estimates of performance, especially in long-lived  
48 species.

49  
50 In addition to the requirement that trait-environment relationships be predictive, they should also  
51 be general across multiple species and/or taxonomic scales. If an optimal trait value does exist  
52 for a particular environment, then the taxonomic scale at which the trait is measured (among-  
53 individuals, among-populations, among-species, among-communities) should be irrelevant [8,9].  
54 For instance, Vasseur *et al* (2012) showed that there was sufficient variation and similar tradeoffs  
55 within *Arabidopsis thaliana* to produce an intraspecific leaf economics spectrum [17]. This type of  
56 generality has been identified in at least some studies of individuals, populations, and species  
57 [18,19]. But other studies hint at potential inconsistencies in trait-environment relationships both  
58 when compared between species [20] or across ecological or spatial scales [21,22].

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60 Addressing fundamental questions about trait-environment relationships – about the functional  
61 form of these relationships, the predictive ability of this functional relationship, and the generality  
62 of the relationship – is well-suited to work in highly controlled, replicable experimental systems  
63 with short generation times. We use a novel microcosm system containing freshwater daphniid  
64 species (*Daphnia magna*, *Moina micrura*, *Simocephalus vetulus*, *Ceriodaphnia dubia*). These  
65 species are ecologically important consumers and prey, have rapid generation times and simple  
66 morphologies, and many candidate functional traits [23,24]. Based on the wealth of information  
67 on their ecology and life histories [25,26], development and genetics (e.g.,  
68 <https://genome.jgi.doe.gov>), and theoretical ecological models [27], they are excellent model  
69 organisms for ecology and evolution [28]. Using this system, we ask two key questions. 1) Are  
70 there functional relationships between an individual's traits and their performance along an  
71 environmental gradient, and how much variation is explained by this relationship? 2) Are these  
72 functional relationships the same for different daphniid species, or when modelled at different  
73 taxonomic scales (e.g. within- and between-species)? Describing the functional form of these  
74 trait-environment relationships is also in need of further study, but optimally, functional  
75 relationships should be mechanistic and built from fundamental biological principles rather than  
76 statistical or correlational relationships (e.g. see [29,30]). Such an approach is outside of the  
77 scope of this study, but work with daphniids has begun to address this question [27]. For the  
78 purposes of this study, we estimate these functional relationships statistically using first order  
79 approximations.

80  
81 To address these questions, we experimentally manipulated environmental conditions, measured  
82 individual trait values and quantified fecundity. For each species, we varied the density of  
83 conspecific individuals in a given microcosm from 1, 2, 4, 6, to 8 individuals, creating an  
84 increasingly stressful biotic gradient. We expected this gradient to have negative effects on  
85 individual growth, survival, and reproduction. High conspecific densities are associated with  
86 reductions in per capita resource availability as well as crowding, which can lead to changes in  
87 feeding behaviour and reproduction [31,32]. Each day we observed the focal generation's  
88 development, reproduction (counting and removing all juveniles born), and measured fecundity  
89 (the total juveniles produced per adult per microcosm). At senescence, we measured ecologically  
90 relevant traits related to reproduction (body length and age at first reproduction [33]), feeding  
91 (length of 2nd antenna [25,34]), and energy allocation (eye diameter and relative growth rate  
92 [23,35,36]; see Supplementary Materials Table S1 for references and justification of traits). To  
93 explore whether there were functional relationships between the traits and the conspecific density  
94 treatment on performance, and further, if they are predictive or general, we used structural  
95 equation modelling (SEM). This modelling approach allowed us to tease apart the direct and

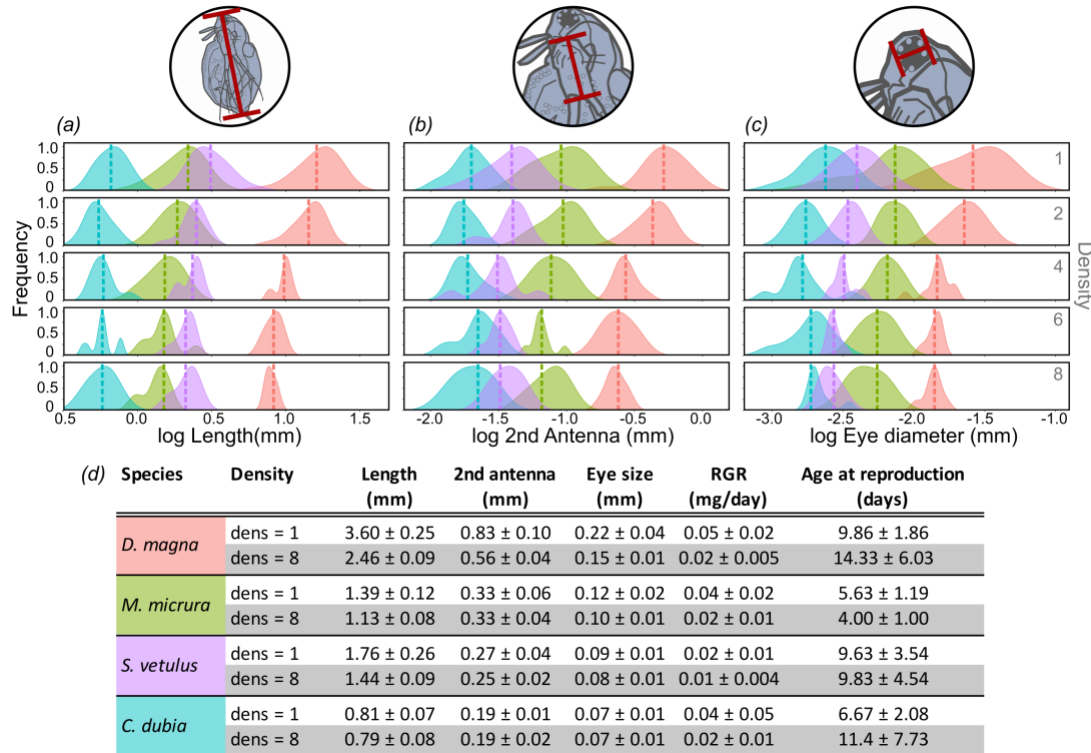
96 indirect (through trait changes) impacts of conspecific density on fecundity and allowed the  
 97 hierarchical structure of the data to be explicitly modeled [37]. We fit the same model structure in  
 98 all cases – one that included all potential direct paths between density, trait values, and fecundity.  
 99 We fit this model for two taxonomic scales (describing within-species or among-species trait-  
 100 environment relationships). This allowed us to test whether there are significant trait-environment  
 101 relationships, and if so, to ask how well they predict fecundity. We also tested whether these  
 102 relationships are general in form for all species, or when compared across the two taxonomic  
 103 scales. We considered a trait-environment relationship as significant when we could identify traits  
 104 which were both significantly impacted by density, which were also significant predictors of  
 105 fecundity.

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## 107 Results

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109 **Effect of density on raw trait values.** Increasing conspecific densities led to notable changes in  
 110 individual phenotypes, including morphological traits (body length, 2<sup>nd</sup> antenna length, and eye  
 111 diameter; Figure 1a-c). The extent of these changes appeared to be species-specific, although



**Figure 1.** Summary results for the effects of conspecific density on species' observed trait measures. a-c) Changes in the frequency distributions of the three morphological traits (body length, 2<sup>nd</sup> antenna, eye diameter) with increases in density (from top row, density = 1, to the bottom row, density = 8). Dashed vertical lines are the average trait value per density treatment. d) Summary of changes in all traits, per species, specifically comparing the trait value at the lowest density to that at the highest density. Values are means ± SD.

112 the direction of change was generally consistent, with higher densities leading to smaller average  
 113 body lengths, eye diameters, and 2<sup>nd</sup> antenna lengths. Similarly, relative growth rate (RGR)

114 decreased with density (except *C. dubia*; Fig. 1d). Variation in trait values tended to decrease  
115 with density (e.g., *D. magna*, *S. vetulus*, and *C. dubia* for eye diameter; Fig. 1).

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### 117 **Intraspecific models of trait-environment interactions for daphniid species**

118 **Model prediction & generality between species.** We fit a structural equation model to the  
119 individual data collected per species. The intraspecific SEMs explained the majority of the  
120 variation in individual fecundity for a species, as a combination of indirect effects of density on  
121 fecundity via traits (all species except *C. dubia*) and direct relationships between trait values and  
122 fecundity (all species, see Fig. 2). For *D. magna*, relative growth rate and age at first reproduction  
123 were significantly affected by density (52% of the variation in RGR is explained by density and  
124 11% of the variation in age at first reproduction), and significant predictors of fecundity ( $p < 0.001$ ;  
125 whole-model  $R_2$  for fecundity was 0.95; Fig. 2). For *S. vetulus* and *M. micrura* significant  
126 relationships between density and fecundity were mediated by body length ( $R_2 = 0.20$  and  $0.22$   
127 respectively), eye diameter ( $R_2 = 0.18$  and  $0.12$  respectively), and relative growth rate ( $R_2 = 0.11$   
128 and  $0.09$  respectively); model  $R_2$  for fecundity were 0.83 and 0.62, respectively. Notably, we did  
129 not find significant trait-environmental relationships for *C. dubia*, although density had a marginal  
130 effect on the age at first reproduction (for the path between density and age at first reproduction,  
131  $p = 0.0589$ ).

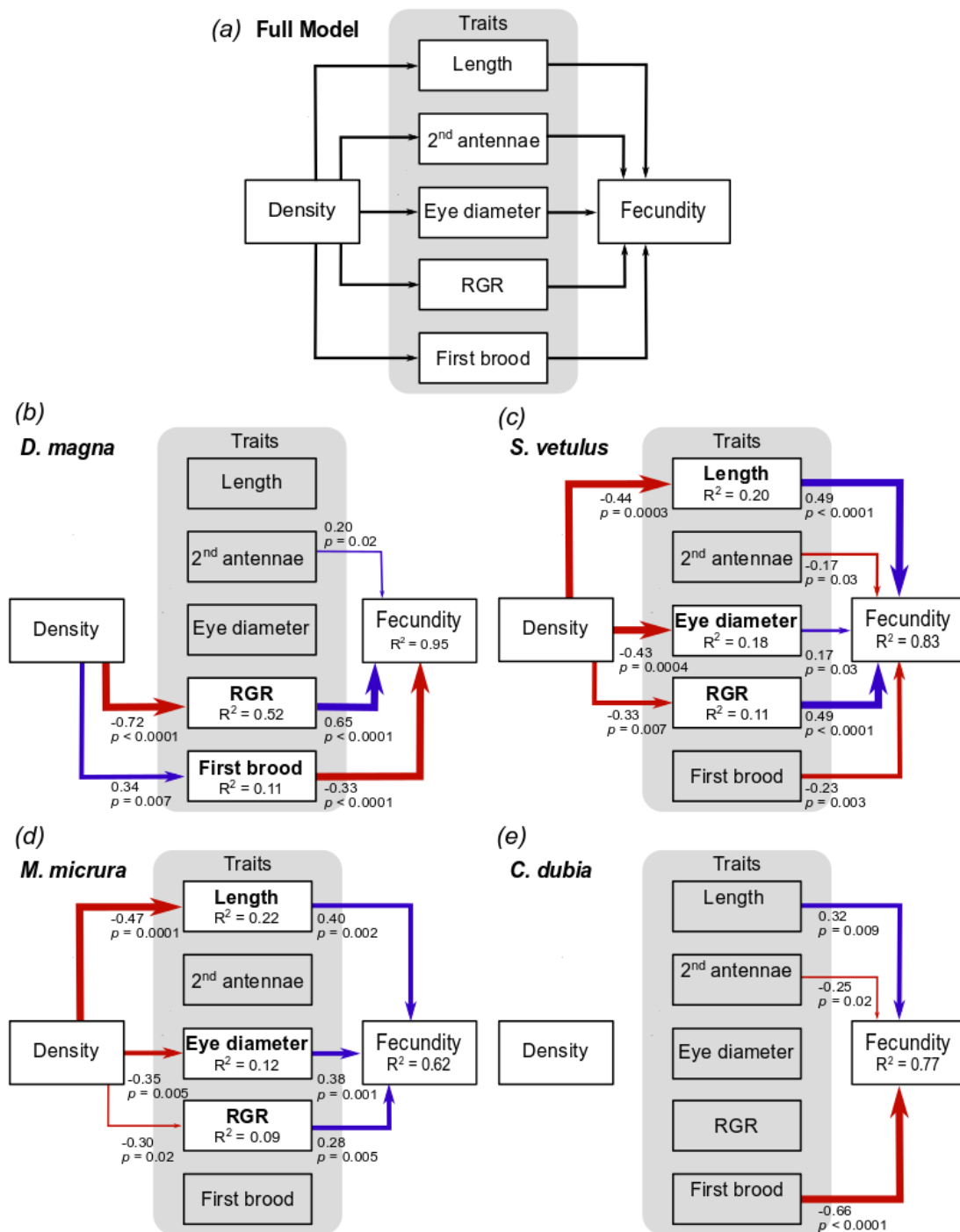
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133 When the structure of these intraspecific models was compared across species, there was a lack  
134 of generality in terms of which traits were significant for each species (although some of the same  
135 traits were significant for several species). Though significant paths varied between species, it is  
136 worth noting that the direction of observed trait-environment relationships was consistent across  
137 species. Increasing densities always led to smaller morphological traits, slower relative growth  
138 rates, and older ages for the onset of reproduction (Fig. 2 b-e). Model fit to observed data is  
139 shown in the Supplementary Materials Fig S1.

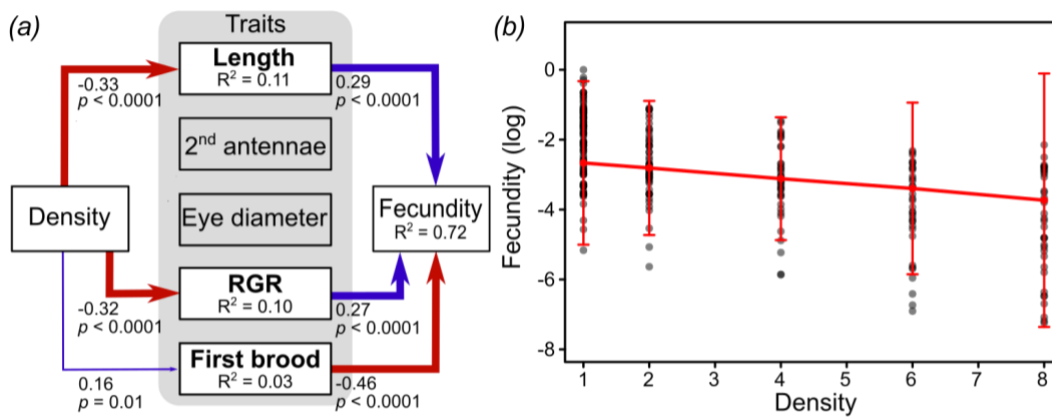
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### 141 **Interspecific models of trait-environment relationships for daphniid species.**

142 **Model prediction & generality across taxonomic scales.** We also asked whether an  
143 interspecific SEM which combined traits from all species could identify general trait-environment  
144 relationships that describe fecundity across all four species. The SEM containing all species data  
145 identified three key traits which were significantly impacted by density (relative growth rate,  $R_2$   
146  $=0.11$ , body length,  $R_2 =0.10$ , age at first reproduction,  $R_2 =0.03$ ), and also have significant  
147 impacts on fecundity ( $R_2$  for fecundity was 0.72; Fig. 3). The good fit of the model's predicted  
148 values to the observed data confirms is shown in Fig. 3. This interspecific scale model appears to  
149 identify general (multi-species) patterns in trait-environment relationships. We also confirmed,  
150 using a multivariate ANOVA, that the multivariate trait values followed a similar trajectory in  
151 response to density, regardless of species. The shift in the mean trait values was significantly  
152 predicted by both the density treatment and species identity ( $p < 0.01$ , Supplementary Material,  
153 Table S3), but the interaction term was small and non-significant (marginal at  $p = 0.05119$ ).



**Figure 2.** Results for the intraspecific structural equation models. *a)* Shows the full model structure fit separately to each species' data. This assumes that all potential paths between conspecific density and fecundity are possible. *b-e)* show the set of significant relationships for *b)* *D. magna*, *c)* *S. vetulus*, *d)* *M. micrura*, and *e)* *C. dubia*. Arrows represent the standardized path coefficients; associated information includes coefficient value and significance. These are also shown visually: arrow width is scaled with significance of the coefficient (wider arrows have smaller  $p$ -values), and blue arrows identify positive path coefficients while red arrows signify negative path coefficients.  $R^2$  values for each linear model are also shown



**Figure 3.** (a) Results for the interspecific structural equation model, calculated using trait values from all four species combined (*D. magna*, *S. vetulus*, *M. micrura*, and *C. dubia*). Arrows represent the standardized path coefficients; associated information includes standardized coefficient values and significance. Arrow width represents the significance of the path coefficient (wider arrows have smaller  $p$ -values), and blue arrows identify positive path coefficients while red arrows signify negative path coefficients.  $R^2$  values for each linear model are also shown. (b) Model fit, showing predicted fecundity, in red, per density treatment. We calculated the mean and standard error of each trait for a given density treatment level and used these to define a normal distribution for each trait combination. We drew randomly from these distributions and used the trait values to calculate fecundity. This procedure was repeated 1000 times for each density. Grey points are the observed values of fecundity. Fecundity is a rate of juveniles produced per adult per day, scaled by the max fecundity across species, and then log-transformed. Error bars are 95% confidence intervals

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## Discussion

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One goal of research into functional relationships between environmental conditions and organism performance is the development of predictive trait-based models of species' distributions and community composition (e.g. [38,39]) Recent works have highlighted limitations in achieving this goal, e.g., traits often appear to have only weak or absent relationships with performance [9]. In this study, we establish tests of the underlying assumptions for trait-environment relationships, including that they should be predictable (that is, variation in trait values in relation to the environment explain significant variation in performance) and general (that is, functional relationships are of similar form among species or across taxonomic scales) in a controlled, experimental system. We found that traits are predictive of fecundity and the structure of these trait-environment relationships is general across the four species, but also that these results are context-dependent in interesting and informative ways. Specifically, we found that a combination of five functional traits (relative growth rate, age of first reproduction, body length, 2<sup>nd</sup> antenna length, and eye diameter) explained the majority of variation in fecundity across a stressful biotic gradient. However, these models of intraspecific trait variation were not always predictive; for one species (*C. dubia*) no traits were significant predictors. Further, the other intraspecific models were not general and differed among species in terms of which traits were significant predictors of fecundity. In contrast, our model of interspecific trait differences was both general and predictive, suggesting traits alone can be sufficient to describe responses to the density gradient.

For all intraspecific models, we found that the five traits measured here explained significant variation in individual fecundity, highlighting the utility of traits for describing performance. The traits we selected have known relationships with daphniid ecology and life-history (e.g., [23,24], see Table S1 for details). For example, body length is associated with growth rate, filtering rate, and survival, and thus with fitness [40].



180 RGR is directly related to resource and energy allocation towards growth [23]. Age at first reproduction is  
181 a trait that commonly trades off with lifespan [33] and in daphniids, earlier maturation is correlated with  
182 higher intrinsic rates of increase [41]. For all species except *C. dubia*, we identified significant axes of trait  
183 variation in response to the environment, specifically related to the pace of life history and body size  
184 which are common responses across multiple groups of species [42]. With increasing stress, fecundity  
185 decreased as a result of declines in body size (length, eye diameter), and/or shifts towards slower growth  
186 rates and delayed onset of reproduction. As shown in Supplementary Fig. S1, model predictions of  
187 fecundity were accurate (and the corresponding  $R_2$  values were high), likely aided by the high temporal  
188 resolution of sampling, and the ability of the microcosm environment to be carefully controlled, including  
189 the age and maternal background of starting individuals as well as non-focal abiotic conditions.

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191 Notably, we found no significant trait-environment relationships for *C. dubia*, although there was a trend of  
192 slower age to first reproduction at higher densities ( $p = 0.0589$ ), and this trait was significantly (negatively)  
193 associated with total individual fecundity ( $p < 0.0001$ ). There are a number of reasons we may not have  
194 identified significant predictors of *C. dubia*'s performance along the conspecific density gradient. Because  
195 of the size differences among the four species, one explanation is perhaps the smaller species (*M.*  
196 *micrura* and *C. dubia*), experienced the crowding aspect of the density gradient more weakly than larger  
197 species (*D. magna* and *S. vetulus*). We performed an additional experiment with a small (*M. micrura*) and  
198 large (*D. magna*) species, where we manipulated food availability rather than conspecific density. This let  
199 us match the gradient of food availability with that in the original experiment, but we fixed the number of  
200 individuals to one. Crowding did not impact the large species (*D. magna*) more than the small, in fact we  
201 found that there was no meaningful difference in the results (i.e., fecundity) from these experiments, for  
202 either of the two species ( $p = 0.49$  and  $p = 0.83$  for *M. micrura* and *D. magna*, respectively;  
203 Supplementary Materials Table S4, Fig. S2). This suggests that food limitation is the primary driver of our  
204 results.

205  
206 When modelling within-species trait variation, we found that even among these four ecologically similar  
207 species, there was no general combination of traits that predicted fecundity. Trait correlations can cause  
208 non-independent responses of traits, and if these correlations are different within-species, they may  
209 constrain individual responses and make generality less likely. Such differences in trait correlations may  
210 reflect underlying developmental, physiological, evolutionary, ecological, and genetic constraints, and  
211 therefore selective pressures [43]. Among-species, and at higher taxonomic scales, consistency in trait  
212 correlations is perhaps more likely [44], perhaps explaining the greater generality for the among-species  
213 model. The trait differences among species, related to the pace of development (RGR, age at first  
214 reproduction) and body size (length) responded to the density gradient in a predictable and general  
215 fashion. Not only did interspecific trait values mediate interactions between the environment and density,  
216 they explained 72% of the overall variation in fecundity without requiring species-specific terms (Fig. 3).  
217 That we did not find a significant interaction between density and species identity highlights the general  
218 ability of these five traits to describe multi-species fecundity without incorporating a species-specific  
219 response to the density gradient.

220  
221 It is important to note that the changes in trait values observed here are primarily the result of phenotypic  
222 plasticity, in addition to genotype sorting due to differential mortality. Plasticity is widespread in nature and  
223 can impact demographic rates, life history, and species interactions [45]. Though trait-environment  
224 relationships are often conceptualized in terms of adaptation or species sorting, plasticity can also  
225 produce strong trait-environment relationships (see [46] for an example where light conditions determine  
226 leaf structure through plasticity). Zooplankton species are known to exhibit an exceptional range of  
227 adaptive plasticity [47]. Thus, predicting the response of zooplankton species to environmental change  
228 almost certainly requires that plasticity (and differences in plasticity among species or traits) be

229 incorporated as a mechanism by which trait-environment relationships can develop [47]. Differences in  
230 plasticity across species and traits are likely common in nature and could explain some of the observed  
231 variation in trait-environment relationships, however, further study is necessary. Work with controlled  
232 experimental systems may be ideal for manipulating and measuring plasticity and genetic variation in  
233 order to place plastic changes in the context of trait-environmental relationships.

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235 We tested two fundamental questions related to the utility of using relationships between traits and the  
236 environment to understand performance. We identified, consistent with other works (e.g., [10]), important  
237 explanatory or predictive relationships between traits and performance for multiple species. We found  
238 less support for the assumption that trait-environment relationships are general across taxonomic scales  
239 or among species. Hopefully these results will inform and improve the use of traits as a tool for predicting  
240 how changing environments and human impacts will affect species abundances and distributions in the  
241 future.

242

## 243 **Materials and Methods**

244

245 **Experimental conditions.** We used four daphniid species that are typically found in freshwater ponds  
246 and lakes: *Daphnia magna*, *Moina micrura*, *Ceriodaphnia dubia*, and *Simocephalus vetulus*. We  
247 established microcosms in 125 mL glass jars cultured under standard laboratory conditions with five  
248 density treatments per species, and each treatment was replicated at least 10 times. Microcosms were  
249 fed to maintain species-specific concentrations (cells/mL media) of *Chlamydomonas moewusii* three  
250 times weekly. From populations with standardized conditions, we collected pre-reproductive females,  
251 added them to 125 mL jars (1 female per jar), and allowed them to grow and reproduce. We selected 1-  
252 day old juveniles from their first brood to start the experimental microcosms, adding either 1, 2, 4, 6, or 8  
253 same-age juveniles depending on the density treatment. Once established, we checked each microcosm  
254 daily (except on Sundays) and recorded demographic information including the number of juveniles born  
255 and adult mortality for each day. Juveniles were then removed, maintaining the initial treatment density;  
256 adults that died were not replaced since mortality is a meaningful outcome of high-density conditions. The  
257 experiments lasted for a single generation, and individuals were collected when they reached  $\sim\frac{3}{4}$  of their  
258 average life expectancy. This length of time varied by species (*M. micrura* = 12 days, *C. dubia* = 16 days,  
259 *D. magna* = 20 days, *S. vetulus* = 30 days). Collecting individuals before they naturally senesce is  
260 essential, as deaths are unpredictable and decomposition occurs rapidly, making it difficult to measure  
261 traits accurately. Collected adults were euthanized with 90% ethanol, photographed, and then dried for  
262 24h in a 60° C oven and weighed.

263

264 **Daphniid traits.** In order to understand the independent contribution of each morphological trait (body  
265 length, 2nd antenna, and eye diameter) to a functional relationship between the density gradient and  
266 performance, beyond their strong correlations with total body mass, we detrended these traits for body  
267 mass (dry mass, mg, see Table S1 for values by species). We fit an allometric model to each  
268 morphological trait (per species) as a function of body mass:

269

$$270 \log(\text{trait}) \sim \log(a) + b \cdot \log(\text{mass}),$$

271

272 where *a* is a normalization constant and *b* the scaling term (e.g.[48]). For each individual measurement,  
273 the difference between the observed and predicted trait value was used as the new body mass-detrended  
274 trait value. To calculate individual relative growth rate (RGR), we used independent data which included,  
275 for all species, biomass measurements for  $\sim 20$  replicate individuals per day, for a 20-day time series. We  
276 fit a Weibull function to each species' data, which describes body mass as a saturating function of time,  
277 and retained the species-specific estimates of minimum individual mass. Then, for each experimental

278 replicate, we parameterized a Weibull function with the species-specific estimates of minimum body  
279 mass, set maximum intraspecific body mass as the maximum size observed in that respective density  
280 treatment, and then used observed body mass to solve for the remaining variable – RGR of that  
281 individual. For microcosms with multiple surviving individuals, we used the average trait values for the  
282 replicate. Finally, we used fecundity as the measure of performance, calculated as the average per capita  
283 rate of juvenile production (juveniles/adult/day). For each treatment with more than one adult individual,  
284 we divided the total number of juveniles born by the weighted mean number of adults present in a jar over  
285 the course of the experiment.

286  
287 **Statistical Analyses.** We wanted to know which traits interact with the conspecific density gradient to  
288 determine individual performance. To improve normality, we log-transformed length, 2nd antenna, eye  
289 diameter, and RGR, and then standardized all traits by unit variance. Standardization was applied within-  
290 species (intraspecific) for single species analyses, and across-species for the multi-species (interspecific)  
291 analyses. We used the R package *piecewiseSEM* (version 2.0.2, [49]) to fit structural equation models  
292 (SEMs). We first created the full SEM which incorporated all possible links between density and the  
293 measured traits (body length, 2nd antenna, eye diameter, age at first reproduction, and relative growth  
294 rate), and between these traits and fecundity (Fig. 2a). Each path in the SEM was represented by a single  
295 model. Conspecific density was treated as an exogenous variable, and potential paths between traits and  
296 density were fit with linear models. For the intraspecific model, individual fecundity was scaled by the  
297 maximum fecundity for each species and then log-transformed to increase normality. In the interspecific  
298 SEM, fecundity was scaled to the maximum fecundity value across species, then log transformed. We  
299 initially considered both linear and quadratic forms and used AIC to determine if a quadratic model should  
300 be retained; as we found no support for retaining the quadratic terms, all SEM functions are linear.

301  
302 We evaluated all unspecified claims for non-independence using tests of directed separation and ensured  
303 that all significant pathways were included. Goodness-of-fit was assessed using these tests of directed  
304 separation, which gives a Fisher's C statistic that is  $\chi^2$  distributed. Large  $p$ -values ( $> 0.05$ ) associated with  
305 Fisher's C indicate that the model represents the data well. We also fit the data to a second model in  
306 which we explicitly tested for a direct link between fecundity and density, however, in every case, this  
307 model was not favored over the model in which these parameters are treated as correlated errors  
308 ( $\Delta AIC \leq 2$ ). After fitting the full model per species, we also fit a nested version containing only the  
309 significant links identified in the full model to confirm, using AIC, that the full model is not more likely than  
310 the reduced model, given the data. We defined significant trait-environment relationships as those for  
311 which both the path from density to the trait, and from the trait to fecundity were significant ( $p < 0.05$ ). For  
312 all species, the nested SEM was supported over the full model ( $\Delta AIC \gg 2$ ); however, we report the  
313 results of the full models so that direct comparisons of path coefficients and model structure can be made  
314 across species. Nested model results are in the Supplementary Materials (Table S2).  
315 Additionally, to ask whether there are general changes in trait values associated with the density gradient  
316 across all species, we applied a permutational MANOVA (using *RRPP*, [50]) of the form:

317  
318  $c(\text{Length, 2nd Antenna, Eye Diameter, RGR, age at 1st reproduction}) \sim \text{Density} * \text{Species}.$

319  
320 All analyses were performed in R (version 3.5.2, R Core Team 2018).

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322  
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328

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